ESHG

ABSTRACTS COLLECTION

Abstracts from the 58th European Society of Human Genetics (ESHG) Conference: ePosters

European Journal of Human Genetics (2025) 33:110–507; https://doi.org/10.1038/s41431-025-01934-6

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Volume 33 | Supplement S1

Milan, Italy

May 24–27, 2025

Sponsorship: Publication of this supplement was sponsored by the European Society of Human Genetics. All content was reviewed and approved by the ESHG Scientific Programme Committee, which held full responsibility for the abstract selections.

Disclosure Information: In order to help readers, form their own judgments of potential bias in published abstracts, authors are asked to declare any competing financial interests.

Contributions of up to EUR 10,000 (Ten thousand Euros, or equivalent value in kind) per year per company are considered "Modest". Contributions above EUR 10,000 per year are considered "Significant".

Presenting author names **are bold** in the contributor lists.

EP01 Cancer Genetics

EP01.001 Genetic testing clinical characteristics and outcomes in *NF2* related Schwannomatosis patients

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Background: Different genetic scores have been proposed to predict the phenotype of *NF2* related Schwannomatosis (NF2 -SCHW MIM # 101000), a rare t familial cancer syndromes caused by pathogenic variants of the *NF2* gene.

Material and methods: 81 patients (median age 41) were identified. The median follow-up was 14 yrs. Clinical diagnosis was established following the Updated diagnostic criteria for neurofibromatosis type 2 and schwannomatosis". All patients underwent *NF2, SMARCB1* and *LZTR1* mutational screening by NGS and MPLA analysis using blood DNA or the tissue of two different tumors when available.*NF2* gene gpathogenic variants were found in 57 subjects (70%), in most cases *NF2* point mutations, but in 5 cases a whole gene deletion was observed, in 2 a ring *NF2* chromosome 22 was identified and in 7 cases a mosaic *NF2*-SCH was found. In 24 (30%) patients no pathogenic variant was found in 24 (30%) patients no pathogenic variant was found. We assessed NF2 clinical phenotype in relation to the UK GSS and FGSS to validate their use.

Results: The disease phenotype differed significantly depending both on clinical and genetic factors. GSS and FGSS showed significant correlation with several measures, allowing stratification of patients with severe and mild disease but not with moderate phenotype. Large cohorts of NF2-SCHW patients are needed to identify more accurate scores.

C.O.I. Disclosure:

R. Paterra: None. **N. Rosati:** None. **M. Ibba:** None. **M. Moscatelli:** None. **I. Vetrano:** None. **V. Cojazzi:** None. **V. Nazzi:** None. **M. Eoli:** None.

EP01.002 Optimization of the evaluation of the drug therapy method for aggressive types of cancer using the D-VC drug based on cfDNA

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Background: Solid tumors(ST) are difficult to treat due to the molecular heterogeneity of the tissue. RECIST1.1 criteria are the assessing standard for the therapy efficacy, but studies show the feasibility of using cfDNA for real-time monitoring. A phase 1/2 clinical trial of Anti-EGFR therapy for ST, including CRC, with a combination of ATO/D-VC drugs ic being conducted in Kazakhstan. The aim is to optimize the method of ATO/D-VC therapy monitoring using cfDNA.

Material and methods: Patients with CRC who have undergone a phase 1 clinical trial and patients without D-VC treatment in KazIOR were included in the study. Plasma was collected in PAXgene and K2EDTA tubes. A portion of plasma was frozen at

−80 °C. DNA was isolated from 1–10 ml of plasma using QIAamp MinElute ccfDNA Kits. The quality of. cfDNA checked spectrophotometrically and fluorometrically. Statistical comparison was performed with t-criterion ($p < 0.05$). vejvnfjn

Results: The concentration of cfDNA from fresh plasma was higher than that from plasma stored up to 2 weeks in PAXgene at RT. cfDNA from frozen plasma (7 days) also had a higher concentration than from samples stored at −80 °C for a month. Comparison of cfDNA concentrations in the first D-VC treated group and the controls showed no significant difference ($t = 1.6$, $p = 0.1$). It is likely that cfDNA concentration diversity between groups was not achieved due to short treatment duration.

Conclusion: cfDNA can be detected in patients with ST regardless of therapy. This method can be used for disease progression assessment and monitoring of drug therapy in cancer patients.

C.O.I. Disclosure:

T. Kadenova: None. **N. Tynyshtykbayeva:** None. **A. Aitkulova:** None. **A. Akilzhanova:** None. **D. Sarbassov:** None.

EP01.003 PRSS57 gene expression in myeloid leukemia cells treated with tyrosine kinase inhibitors and all-trans retinoic acid

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Background: Myeloid leukemia is classified into two categories: chronic myeloid leukemia (CML) and acute myeloid leukemia (AML). CML arises from the uncontrolled proliferation of myeloid lineages due to *BCR-ABL1* fusion gene, while AML is characterized by the expansion of immature myeloid lineages with impaired differentiation. *PRSS57*, arginine-specific serine protease, has been found to be highly expressed in myeloblasts-promyelocytes. *PRSS57* has been associated with early molecular response failure in chronic-phase CML. Its function in AML remains unclear.

Material and methods: The present study aims to investigate *PRSS57* expression following treatment with tyrosine kinase inhibitors (TKIs) in CML and all-trans retinoic acid (ATRA) in AML. K562 cells were treated with imatinib, nilotinib, dasatinib, while AML cell lines (THP1, NB4, HL60) were treated with ATRA. Half-maximal inhibitory concentrations (IC50) of the TKIs were determined via MTT assay followed by the application of these drug concentrations to the cells. *PRSS57* expression changes were examined via RT-qPCR at 24h, 48h, 72h.

Results: Compared to the control group, imatinib, nilotinib, and dasatinib increased *PRSS57* expression in K562 cells by 10.29-, 5.81-, and 23.34-fold at 24h and 3.29-, 2.94-, and 20.11-fold at 48h, respectively. In THP1 cells, *PRSS57* expression increased 4.5-, 18.54-, and 14.52-fold at 24h, 48h, and 72h, respectively, but no significant change was observed in HL60 and NB4 cells.

Conclusion: Cell type-specific regulations in AML cells may cause differences in expression profile. TKIs may induce *PRSS57* expression in K562 cells, potentially activating cell death pathways and playing a role in cell death.

C.O.I. Disclosure:

B. Kasap: None. **S. Sirma Ekmekci:** None. **N. Abaci:** None.

EP01.004 Novel heterozygous variants in *BMPR1* and *SMAD4* genes in patients with juvenile polyposis syndromes: genetic and clinicopathological data

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Background: Juvenile polyposis syndrome (JPS) is rare genetic disorder, caused by the mutations in SMAD4 or BMPR1 genes. The incidence of JPS in Europe is near 1:100,000 births. Risk for gastrointestinal cancer ranged from 11% to 86%.

Material and methods: The positive diagnosis of JPS was based on JASS and WHO guidelines clinical criteria, endoscopic examination and NGS of DNAs extracted from blood.

Results: Among 41 probands with polyposis in 2 patients were confirmed mutations in BMPR1 gene and one has mutation of SMAD4 gene. In 22-year-old women it was identified a heterozygous missense c.1229C>T(p.Pro410Leu) variant in BMPR1 gene in exon 11. She has multiply polyps in colon, stomach, fibromas and congenital abnormality of aortic valve. Her mother had chronic anaemia, fibromas and papilomas. In 20-year-old men one pathogenic heterozygous variant c.1267del (p.Gln423Serfs*13) in BMPR1 gene caused by premature translational stop signal in exon 11 was identified. Endoscopic examination showed multiply polyps in colon. In 20-year-old women with multiply polyps of colon, polyneuropathy, breast fibroadenoma, polycystic liver disease was confirmed pathogenic variant (p.Ser485Valfs*9) creates premature translational stop signal in SMAD4 gene.

Conclusion: Among patients with polyposis from western regions of Ukraine JPS was confirmed in 7.31% individuals. The novel variants of BMPR1 and SMAD4 genes were not present in population databases.

C.O.I. Disclosure:

M. Lozynska: None. **L. Lozynska:** None. **I. Shymanska:** None.

EP01.005 OvCanalysis (beta-version)—a tool for machine learning based analysis of copy number variations in ovarian cancer

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Background: High grade ovarian cancers (HGOC) frequently exhibit genomic instability, resulting in multiple copy number alterations (CNA) accompanied by alterations in zygosity. The genomic instability in HGOC has various causes, with homologous recombination deficiency (HRD) as the clinically most important, but not only one. CNAs are thought to be random events. Clonal selection of "favourable" combinations of CNAs takes place during tumor development. *OvCanalysis* is a developmental stage tool for cumulative whole cohort qualitative CNA and clinical parameter analysis in HGOC for a better understandig of tumor development and resulting clinicopathologically distinct patient subgroups.

Material and methods: CNAs of 161 HGOC tumors (separated in a training and a control cohort) with detailed clinical information (26 parameters) underwent DNA-array analysis. Resulting data was standardized and subsequently processed by *OvCanalysis*.

OvCanalysis allows integrated analysis of whole cohort CNA and clincal data by dimension reduction algorithms (e.g. PCA, LLE) followed by automated clustering (k-means). Mean CNA profiles (CNAs incl. B-allel frequency) of clusters are visualized and a spreadsheet with clustered tumors incl. clinical parameters can be exported.

Results: A functional beta-version of *OvCanalysis* was established and is currently tested. Preliminary machine learning based

CNA-analysis allowed clustering of patients with distinct clinicopathological features beyond HRD-determination.

Conclusion: Our work demonstrates the feasibility of automated CNA-analysis for disclosure of characteristic CNA events that allow identification of clinicopathologically distinct patient subgroups (e.g. age at diagnosis).

C.O.I. Disclosure:

B. Murauer: None. **J. Laschtowiczka:** None. **H. Fiegl:** None. **A.G. Zeimet:** None. **J. Zschocke:** None. **S. Schnaiter:** None.

EP01.006 A case report of a fetal neck immature teratoma

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Background: Teratoma is an extremely rare tumor that arises from pluripotent germ cells (GCTs). The specific causes of the development of teratoma are not known, it is hypothesized that its onset is due to the autonomous development of some embryonic cells, which become independent from the rest of the organism following a genetic alteration.

Material and methods: Cultures of cells derived from lung, epidermal, superficial and deep tumour tissue were performed. CGH-SNP Array, Whole-Exome Sequencing (WES) and transcriptomic analyses were performed on these three tissues.

Results: We report a case of malignant immature fetal teratoma with numerous ependymal rosettes and neuroblasts. Ultrasound examination of the foetus at 20 + 4 weeks' gestation showed a hypoechogenic neck mass 3 cm in diameter and poorly vascularised. Its rapid growth indicated the aggressive nature of the tumour. CGH-SNP analysis and WES analysis were not informative. Transcriptome analysis identified 37 genes in the lung-deep tumour pairing, of which 17 genes are down-regulated and 20 genes are up-regulated, and identified 19 genes in the lung-surface tumour, of which 9 genes are down-regulated and 11 genes are up-regulated. A gene pathway analysis with KEGG and GO enrichment was performed. KEGG enrichment resulted in 3 pathways for the first pair (Calcium signalling pathway, Cytoskeleton in muscle cells, Neuroactive ligand-receptor interaction) and 2 pathways for the second pair (Complement and coagulation cascades, Pertussis

Conclusion: Our studies suggest that it may be useful to perform transcriptome analyses on teratoma cases to better understand the pathogenicity and mechanisms of occurrence of these rare tumors

C.O.I. Disclosure:

R. Zuntini: None. **V. Vaccari:** None. **V. Toto:** None. **B. Melli:** None. **V.Y. Cusenza:** None. **A. Vezzani:** None. **S.G. Caraffi:** None. **S. Boiardi:** None. **M. Pati:** None. **M.P. Bonasoni:** None. **L. Garavelli:** None.

EP01.007 Anti-inflammatory effect of Nanopiperine treatment in head and neck cancer

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Background: Genetic, environmental and inflammatory factors can increase the risk of head and neck cancer. In this context, we highlight the central inflammation regulator gene, *Prostaglandin-Endoperoxide Synthase 2 (PTGS2)*, which can be inhibited by anti-inflammatory agents. We evaluated a phytochemical, piperine (extracted from *Piper nigrum*), which has anti-tumorigenic properties and, due to its low bioavailability, it was necessary to use nanotechnology. Thus, the objective of the study was to analyze the effect of PLGA nanoparticles, loaded with piperine (nanopiperine), as a potential antitumor agent, on head and neck squamous cell carcinoma (HNSCC).

Material and methods: Assays including proliferation, cytotoxicity, migration, apoptosis and gene expression were verified in HNSCC (HEp-2 and CAL-27) treated with nanopiperine, with and without cisplatin, for 4, 24 and 48 hours.

Results: The results obtained indicated reduction in cell number after 24 hours of treatment in groups treated with the combination of nanopiperine and cisplatin. Signals of apoptosis were observed, such as *blebs* formation and reduction in the cytoplasm size of the cells, results confirmed by flow cytometer assays. Our data show a decrease in cell migration process, probably due to a decrease in the expression of *MMP2/9* genes. In addition, nanopiperine modulated *PTGS2* and *PTGER4* genes expression, which are related to inflammation process.

Conclusions: Taken together, our data show that nanopiperine has antiproliferative, antimigratory and anti-inflammatory effects and could be a potential phytoterapic medicine for the complementary treatment of head and neck carcinoma, opening up new possibilities for innovative therapies in this disease.

C.O.I. Disclosure:

F.C. Rodrigues-Lisoni: None. **L.F. Zanusso Cunha:** None. **S.O. Sousa:** None. **J.O. Martinho:** None. **A.R. Campassi:** None. **E.O. Souza:** None. **S.R. do Amaral:** None. **F.L. Primo:** None. **C.R. Bonini-Domingos:** None. **S.M. Oliani:** None.

EP01.008 Genomic profiling of pancreatic ductal adenocarcinoma

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Background: Kirsten rat sarcoma (KRAS) mutation is an oncogenic driver of pancreatic ductal adenocarcinoma (PDAC). KRAS mutations are predominantly found in pancreatic cancer and are identified in nearly all (95%) PDAC.

Material and methods: We performed mutation analysis of the second and third exons of the KRAS gene in patients with pancreatic cancer using Sanger sequencing. Genomic DNA was extracted from 8 frozen pancreatic tumor samples using Qiagen and Promega kits, according to the manufacturer's protocols. All extracted genomic DNA samples met the quality requirements for sequencing. Sequencing was carried out according to the standard protocol using the BigDye Terminator v.3.1 sequencing kit. Sequencing was performed on the 3500 Genetic Analyzer (Applied Biosystems).

Results: Of the 8 samples, 5 contained a mutation in the second exon of the KRAS gene. In 3 samples, the G12D mutation was identified, while in the remaining two samples, the mutations G13D and G12V were detected.

Conclusion: Thus, we demonstrate that mutations in the second exon of the KRAS gene are the most common in pancreatic cancer, with the G12D mutation being the most frequently observed.

C.O.I. Disclosure:

B. Yermekbayeva: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; BR24992841 Optimization of the therapy targeting aggressive cancers by the D-VC drug combination developed in Kazakhstan, National Laboratory Astana, Nazarbayev University. **D. Samatkyzy:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; BR24992841 Optimization of the therapy targeting aggressive cancers by the D-VC drug combination developed in Kazakhstan., National Laboratory Astana, Nazarbayev University.

EP01.009 Pathogenic DICER1 mutation in a child with pleuropulmonary blastoma: a case report

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Background: Pleuropulmonary blastoma is a very rare and aggressive pediatric lung tumor, usually diagnosed <4 years of age and accounting for less than 1% of all primary malignant lung tumors. DICER1 germline mutations are discovered in a majority of PPBs as part of a familial cancer predisposition syndrome. Its association with other neoplasms requires close genetic surveillance of all family members.Here we present the case of a 1.5-year old child, presenting with signs of respiratory distress and an intrathoracic mass, arising from the right middle lobe.

Material and methods: Sequence analysis and copy number variation analysis on DNA from buccal swab was performed using the Blueprint Genetics (BpG) Hereditary PediatricCancer Panel, encompassing 71 genes.

Results: The targeted molecular-genetic analysis identified a heterozygous nonsense variant DICER1 c.1966C>T, p.(Arg656*), classified as pathogenic. This variant generates a premature stop codon in exon 12 (of a total of 27 exons), which leads to a loss of normal protein function. Genetic testing was recommended for all family members considering the autosomal-dominant inheritance. Moreover, the central pathology review of the tumor tissue revelaed areas of P53 overexpression suggesting an underlying TP53 mutation.

Conclusion: Genetic testing for pediatric cancer patients is of utmost importance in relation to screening, prevention and treatment. When it comes to PPB the transition from type I to the more aggressive types II/III can be detected in at-risk individuals using the published guidelines for follow-up. The standardized care for patients with cancer predisposition syndromes has a positive impact on their overall survival rates.

C.O.I. Disclosure:

R.E. Georgieva: None. **M. Beltcheva:** None. **H. Hristozova:** None.

EP01.010 Two cases of patients with papillary thyroid carcinoma and pathogenic variants in DICER1 gene

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Background: In 2001, DICER1 was identified as a member of the RNase III family of nucleases. Pathogenic variants in the *DICER1* gene present a known cause of Pleuropulmonary blastoma. Additionally, DICER1- related disorders are a familial tumor susceptibility syndrome that confers increased risk for ovarian sex cord-stromal tumors and thyroid gland neoplasia- multinodular goiter, adenomas, or different thyroid cancer.

Material and methods: The first case considers a 38 year old patient with thyroid papillary carcinoma. The diagnosis was made at 12 years of age. The father of the proband has unknown thyroid condition and no other specific data is available. Whole Exome Sequencing was performed and a panel of 60 genes related with thyroid cancer was analyzed. The second case is a 29 year old man with papillary thyroid carcinoma, whose mother and sister have thyroid cancer. Whole Exome Sequencing was performed.

Results: Regarding the first patient a heterozygous pathogenic variant c.4407_4410delTTCT (NM_001271282.2) was detected in the gene *DICER1*. It causes a frameshift at position 1469 in the amino acid sequence of DICER1. The variant is predicted to cause loss of function of the affected copy of the gene. In the second case- a likely pathogenic variant c.145-2A>G (heterozygous) in the *DICER1* gene was established.

Conclusion: The reported variants in the gene *DICER1* present a possible cause for the clinical manifestations observed in the patients.

C.O.I. Disclosure:

R. Budinova: None. **M. Glushkova:** None. **S. Atemin:** None. **M. Sleptsova:** None. **S. Galeva:** None. **A. Todorova:** None.

EP01.011 Investigating the relationship between hub genes and LNCRNAS involved in the signaling pathway and effective drugs in acute myeloid leukemia (AML)

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Background: Acute myeloid leukemia (AML) is an aggressive malignancy of white blood cells that results in symptoms related to bone marrow failure and organ infiltration. AML is a universally fatal condition, and life-threatening complications can develop rapidly in asymptomatic patients.

Material and methods: For this research, a gene expression data set based on microarray from the public database NCBI Gene Expression Omnibus (GEO) was found from the gene extension numbers for the obtained data set, which keywords include acute myeloid leukemia (AML), tumor, and normal. Examples are the Microarray 2.0 platform, A robust multi-array RNA interface for Log2 transformation, normalization, and background correction that was preprocessed for analysis by array quality metrics was realized in R.

Results: We investigated the construction of the regulatory network related to the signaling pathway and the relationship between the expression of hub genes, lncRNA, effective drugs, cytogenetics, and molecular genetics related to acute myeloid leukemia (AML), which had a significant relationship.

Conclusion: According to the studies conducted on acute myeloid leukemia (AML) cancer, it was observed that there is a significant decrease in the tumor tissue compared to the tumor margin, considering 5>LogFC >-5 and P-value < 0.05.

C.O.I. Disclosure:

S. Fakher: None. **S. Sabouri:** None. **F. Zeinali*:** None.

EP01.012 Diverse phenotypic expressivity of a familial ETV6 mutation

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Background: Germline mutations in the ETV6 gene are involved in familial thrombocytopenia and leukopenia, predisposing individuals to hematologic malignancies. These inherited mutations impair hematopoiesis, are often associated with thrombocytopenia and leukopenia, particularly in those with a relevant family history and may result in bone marrow failure, dysplasia, and an elevated leukemia risk.

Case and methods: We present a 28-year-old male with past medical history of leukopenia, worsening thrombocytopenia, bleeding manifestations, 5% blasts in his bone marrow (BM) biopsy, and a family history of thrombocytopenia, raising suspicion for myelodysplastic syndrome (MDS) or inherited bone marrow failure syndrome (IBMFS). Molecular investigation on BM aspiration sample was performed using the Archer® VariantPlex® Myeloid Panel through Next-Generation Sequencing.

Results: The patient is characterized as having low-risk MDS based on the IPSS-M criteria. Molecular testing identified a known pathogenic heterozygous germline mutation in ETV6 c.641C>T. Family screening confirmed the presence of the same mutation in his mother and sister. Due to the clinical characteristics and molecular findings of the patient, along with the presence of ETV6 mutation in the family, the patient is a candidate for matched unrelated donor (MUD) stem-cell transplantation.

Conclusion: This case underlines the importance of genetic testing in diagnosing familial platelet disorders and the risk for development of acute leukemias, especially in patients with unexplained hematologic symptoms, lifelong cytopenias, and BM abnormalities. Especially in the presence of a germline ETV6 mutation, familial screening and genetic counselling is essential to identify any at-risk relatives, guide management, and enable early intervention.

C.O.I. Disclosure:

A. Elpidoforou: None. **A. Mitsidou:** None. **K. Kaouranis:** None. **A. Miltiadous:** None. **Y. Kyprianou:** None. **G. Sianiou:** None. **C. Zartila:** None. **C. Ioannou:** None. **A. Christofi:** None. **M. Johnson:** None. **M. Kanezou:** None. **E. Georgiou:** None. **C. Avgousti:** None. **L. Koumas:** None. **K. Nikolaou:** None. **P. Gerasimou:** None. **J. Chi:** None. **P. Costeas:** None.

EP01.013 *BRCA* gene mutations in the Bangladeshi population: a narrative review

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Background: Breast cancer ranks as the second most common type of cancer and is rising at an alarming rate among females in Bangladesh. Approximately 5–10% of breast cancer cases are hereditary and are associated with mutations in the *BRCA1* and *BRCA2* genes. Despite their utility, *BRCA* genetic tests are rarely done in Bangladesh, a least-developed country, prompting this review to summarize the *BRCA* gene mutations in this population to raise awareness among researchers, healthcare providers, policymakers, genetic testing companies, and patients.

Methodology: We conducted a literature search to examine the spectrum of *BRCA1* and *BRCA2* gene mutations in the Bangladeshi population. We identified and narratively summarized published literature on this topic.

Results: Six articles were included for data extraction. The studies included patients and healthy controls from a single case to 310 patients with breast cancer. Methods used to detect mutations included polymerase chain reaction-restriction fragment length polymorphism, targeted sequencing using an Illumina Miniseq sequencer, and validation through Sanger sequencing. *BRCA1* (rs80357713, rs80357906) and *BRCA2* (rs11571653) polymorphisms were found to be mostly associated with breast cancer in this population. 13.95% of samples contained pathogenic mutations. In exon2, insertions, deletions and single nucleotide substitution and in exon11, three novel mutations were observed in the *BRCA1* gene.

Conclusion: Cancer treatment increasingly emphasizes personalized medicine, with the FDA approving drugs tailored to specific genetic mutations. However, low-resource countries like Bangladesh face challenges due to limited resources, facilities, and qualified personnel to conduct genetic testing, which hampers the critical care that cancer patients require.

C.O.I. Disclosure:

M. Hasan: None. **N. Tabassum:** None. **A. Maruf:** None.

EP01.014 Streamlined analysis of DNA methylation and its functional consequences from FFPE tumor samples

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Methylation of DNA sequences is an important mechanism for regulating gene expression, in normal cell physiology and in diseases. To detect and analyze the effects of methylation, multiple assays must be performed; however, in clinical research sample amounts are often limiting. The multifactorial analysis of consequences of methylation therefore depends on efficient utilization of precious samples. We show efficient methods for characterizing links between methylation, mismatch repair gene expression, and microsatellite instability from small amounts of colon tumor samples. DNA and RNA were isolated from ten 5 µm FFPE preserved colon tumor samples using an automated DNA/RNA extraction method. From the extracted genomic DNA, two samples were found to have high microsatellite instability. Mismatch repair enzyme gene expression was measured using a 5-plex panel of qPCR assays that analyzed mismatch repair (MMR) enzyme expression. Using the extracted RNA, *MLH1* was found to be significantly decreased in the two samples that demonstrated MSI instability. This was confirmed using dPCR with the same MMR multiplex panel. Twelve potentially methylated CpGs in a region of the *MLH1* promoter were analyzed using Sanger sequencing of the extracted DNA. Samples with MSI had significant methylation in all CpGs in this region. The fraction of genomic DNA methylated was determined by dPCR; both samples with high MSI had greater than 50% of the DNA methylated. Together, these results show how DNA methylation and its impact on the MMR pathway can be analyzed from precious samples using fast, simple, and cost-effective laboratory assays.

C.O.I. Disclosure:

S. Jackson: A. Employment (full or part-time); Significant; Thermo Fisher Scientific - Carlsbad, CA.

EP01.015 The fundamentals of cancer-transcription factors as mutation triggers and the proliferation stage

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Background: The cellular microcosm's essential order is profoundly disrupted by cancer, which manifests as a rebellion against biological balance. It is a story of transformation, in which the fundamental nature of cellular identity and function is compromised by genetic mutations, epigenetic changes, and molecular aberrations. A key component of this rebellion is the dysregulation of transcription factors (TFs), which are essential regulators of genomic expression and cellular fate.

Material and methods: We used an in silico approach that combined bioinformatics analysis and computational modelling to unravel complex the condition. We explored the chaos caused by dysregulated TFs in cancer by mapping transcriptional regulatory networks in an effort to identify the molecular roots of carcinogenesis.

Results: Our data highlight that the breakdown of DNA replication fidelity, the persistent drive of cell division, and the enhancement of genomic instability are the significant effects of TF dysregulation. In addition to bringing about carcinogenesis, these dysfunctional cascades create tumor heterogeneity, which shapes an adaptive and constantly changing cancer.

Conclusion: Re-establishing a correct TF activity is an effective strategy in the field of cancer biology. Our study demonstrates how targeted therapies can recalibrate faulty TFs, opening the door to more personalized treatment approaches. A future in which cancer is treated with interventions that are specific to its complex and dynamic nature, rather than with general medicines, is becoming closer thanks to a deeper comprehension of these molecular storylines.

C.O.I. Disclosure:

M. Chetta: None. **A. Cammarota:** None. **N. Bukvic:** None. **A. Rosati:** None.

EP01.016 Insights into in vitro effects of halogenated boroxine on osteosarcoma cells

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Background: Osteosarcoma, a highly aggressive bone malignancy, exhibits significant resistance to current therapeutic strategies, underscoring the urgent need for novel approaches. Halogenated boroxine (HB) has shown antitumor potential in a series of in vitro and in vivo studies, but its effects on osteosarcoma cells remain unexplored. This study aimed to evaluate the antiproliferative effects of HB on osteosarcoma cells.

Material and methods: MG-63 osteosarcoma cells were treated with HB at final concentrations of 50, 75, 100, 150, 200 and 400 ug/mL. Cytotoxic activity was assessed using WST-1 assay, and reactive oxygen species (ROS) levels were measured using the cell-permeable ROS probe 2',7'-dichlorofluorescein diacetate. Cytotoxicity was evaluated at two time points (24 hours and 72 hours post-treatment), whereas ROS levels were measured at 24 hours post-treatment.

Results: HB treatment at higher concentrations significantly inhibited MG-63 cell proliferation at both time points. Proliferation inhibition observed 24 hours post-treatment was accompanied by altered ROS levels, suggesting a potential link between oxidative stress and HB-mediated cytotoxicity.

Conclusion: HB demonstrates potent antiproliferative effects on osteosarcoma cells, with oxidative stress likely contributing to its mechanism of action. These findings highlight HB's potential as a candidate for further investigation in the development of novel therapeutic strategies for osteosarcoma.

C.O.I. Disclosure:

N. Tomic: None. **S. Esmkhani:** None. **H. Yazici:** None. **A. Dinc:** None. **B. Saric Medic:** None. **J. Ramic:** None. **N. Lojo-Kadric:** None. **L. Pojskic:** None.

EP01.017 The critical importance of reporting clinically unclassified variants in cancer predisposing genes

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Background: Several genes are associated with hereditary predisposition to breast and/or ovarian cancer. However, the level of gene specific cancer risk for these malignancies may markedly differ.

Material and methods: We present the case of two siblings, a male (I1) with prostate adenocarcinoma and a female (I2) with metachronous bilateral breast cancer, with family history of breast cancer. I2 underwent multigene panel testing at another hospital, that identified a pathogenic variant in *BRIP1*. To current knowledge, germline pathogenic variants in *BRIP1* are associated with an increased risk of ovarian cancer, but not of breast and prostate cancer. Therefore, when I1 referred to our centre, we opted for an independent multigene panel test, considering the particular family history.

Results: In addition to the familial *BRIP1* variant, our analysis identified a heterozygous variant (c.2596G>T) in *PALB2*. Targeted sequencing detected the presence of this variant also in I2. Pathogenic variants in *PALB2* have been shown to be associated with a high risk of breast cancer. However, the c.2596G>T variant is reported in ClinVar with conflicting classifications of pathogenicity, preventing its clinical use. In *silico* analysis predicts that the variant induces abnormal RNA splicing. A transcript analysis is ongoing to verify the pathogenic role of the variant.

Conclusion: This case highlights the critical importance of reporting clinically unclassified variants even in the presence of co-occurring established pathogenic variants, taking into account the specific phenotype of the family under scrutiny, in order to enhance the accuracy of hereditary cancer risk assessment for clinical management.

C.O.I. Disclosure:

S. Cannova: None. **E. Cicchetti:** None. **G. Tolva:** None. **C. Pesenti:** None. **P. Radice:** None. **C. Foglia:** None. **M. Colombo:** None. **R. Villa:** None. **L. Fontana:** None. **M.R. Miozzo:** None.

EP01.018 Diagnostic doubt and the crucial role of cytogenetic analysis in complex hematologic malignancies

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A 66 year man arrive at our attention with dubious clinical indication of myeloid or lymphoid neoplasia. Clinical history reveals anemia and thrombocytopenia, severe asthenia, fever, and weight loss for which a treatment was required so bone marrow (BM) evaluation was performed. The BM aspirate revealed a hypocellular sample, absence of tissue fragments and megakaryocytes, and immunophenotype analysis didn't reveal alterations. Cytogenetic analysis was also performed [1].

For karyotype analysis and second FISH investigation, on BM sample, a 24-hour unstimulated culture cell was performed. FISH was performed with TP53 deletion probe and, MYC, NUP98, KMT2A, ETV6, BCL2, and RUNX1 break-apart probes.

Karyotype analysis revealed an abnormal clone characterized by a pseudotriploid chromosomal pattern ranging between 68 and 73 chromosomes, with both numerical and structural alterations. FISH analysis identified an extra der(17) with loss of one copy of *TP53* gene, an extra 11q isochromosome, *MYC* duplication and *BCL2* and *RUNX1* amplification. All these alterations were observed in metaphases but not in interphase nuclei, which appear normal.The investigated genes are involved in both lymphoid and myeloid neoplasms and interestingly and unexpectedly these alterations are detected exclusively in FISH metaphases, while interphase nuclei remain normal.

Although cytogenetic investigation wasn't conclusively favouring one lineage over the other, it was the first informative analysis, only later BM biopsy investigation given diffuse large B-cell lymphoma diagnosis. This case pinpoints the importance and the crucial role of cytogenetic in the diagnostic workflow of hematologic malignancies identifying promptly therapeutic target even if a diagnosis is not yet defined.

C.O.I. Disclosure:

S. Albertazzi: None. **M. Lista:** None. **F. Estersini:** None. **K.E. Ayissou:** None. **F. Bagnoli:** None. **R. Caselli:** None.

EP01.019 Influence of galanin receptor 2 and presence of tertiary lymphoid structures in patients with head and neck squamous cell carcinoma

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Background: Galanin receptor 2 (GALR2) is a G-protein coupled receptor involved in immune modulation, with conflicting evidence suggesting immunosuppressive or tumor suppressive effects. Tertiary lymphoid structures (TLSs) are immune aggregates outside secondary lymphoid organs, associated with better survival in cancers. This study investigates the correlation between GALR2 expression and TLS presence in head and neck squamous cell carcinoma (HNSCC).

Material and methods: Thirty-five tumor samples from HNSCC patients were collected after surgical resection. Conventional immunohistochemistry (IHC) was performed using CD20, GALR2, and RAC1 antibodies, with additional CD8 and CD4 IHC in 17 samples. Staining was quantified using ImageJ. Real-time PCR was conducted for CXCL13, CCL19, and CCL21, cytokines important for TLS formation. Statistical analyses were performed using GraphPad-9.0 ($p \leq 0.05$).

Results: GALR2 and CD20 expressions were grouped by tumor size (T1/T2, T3/T4) and lymph node invasion (N0, N1+). Significant difference in CD20 expression were found between N0 and N1+ groups ($p = 0.0064$**). A non-significant positive correlation was found between GALR2 and RAC1 expression ($r = 0.2119$, $p = 0.2443$), with RAC1 indicating GALR2 activation. Samples with CD20 overexpression also show higher GALR2 expressions, although not significant ($p = 0{,}4363$). CD20 expression showed non-significant positive correlations with CCL19 ($r = 0.0901$, $p = 0.5908$) and CCL21 ($r = 0.05088$, $p = 0.7616$), and a negative correlation with CXCL13 ($r = -0.0927$, $p = 0.6019$). Higher GALR2 expression was associated significantly with increased CD8 and CD4 expression (CD8:$p = 0.0369$*, CD4:$p = 0.0447$*).

Conclusion: These findings suggest that GALR2, in the presence of CD20 (TLS marker) related to better prognosis, may function as a tumor suppressor.

C.O.I. Disclosure:

V.S. Possebon: None. **C.I. Cuzziol:** None. **L.B. Figueiredo:** None. **A.S. Vila:** None. **V.S. Júnior:** None. **M.M. Castanhole-Nunes:** None. **I.B. Reis:** None. **É.C. Pavarino:** None. **J. Maniglia:** None. **C.R. Júnior:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; FAPESP. **E.M. Bertollo:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; CNPq. B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; FAPESP.

EP01.020 Mosaic form of the von Hippel-Lindau syndrome: case report

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Background: von Hippel-Lindau syndrome (VHLS) is a hereditary cancer syndrome with CNS hemangioblastoma (HB), clear cell renal carcinoma (CCRC), pheochromocytoma, retinal angiomas, and other manifestations. VHLS is caused by a mutation in the *VHL* gene. However, some cases of VHLS develop de novo, and among them there are rare patients with a mosaic form of the disease. Genetic testing in mosaic patients is prone to false-negative results due to the low copy number of a mutant allele in DNA isolated from the blood.

Material and methods: we describe a 39-year-old patient with multiple CNS HBs and CCRC. He underwent *VHL* testing in blood DNA using Sanger sequencing and MLPA twice with negative results. Then mutation analysis was performed in asynchronous primary tumors and NGS of blood DNA with a high coverage for

the mutant position. To obtain an amplification library of the short target region, the Prep&Seq™ U-target module system (Parseq Lab, Russia) was used.

Results: we have detected the pathogenic variant c.481C>T (p.Arg161Ter) in the *VHL* gene by Sanger sequencing in DNA from both FFPE tumor samples: CCRC and HB. Targeted sequencing of the blood DNA sample on the Illumina NextSeq platform allowed us to achieve coverage on the position of interest as x261029. The proportion of the mutant T allele was determined to be 2%.

Conclusion: the patient was diagnosed with a mosaic form of VHLS. Timely diagnosis of VHLS is important for monitoring the mutation carrier, surgical treatment options and targeted therapy with belzutifan.

C.O.I. Disclosure:

D.S. Mikhaylenko: None. **N.B. Kuryakova:** None. **A.V. Efremova:** None. **I.V. Volodin:** None. **S.I. Kutsev:** None. **D.V. Zaletaev:** None. **V.V. Strelnikov:** None.

EP01.021 A rare intragenic deletion in*DICER1*: novel association with mesoblastic nephroma

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Background: *DICER1* syndrome is a rare genetic disorder caused by germline mutations in the *DICER1* gene, which encodes an RNase III enzyme essential for post-transcriptional gene regulation. Individuals with *DICER1* syndrome are predisposed to developing a spectrum of neoplasms.

Material and methods: Genetic testing, including NGS analysis of a 154-gene panel for hereditary cancer predisposition and MS-MLPA analysis of the *11p15* locus for Beckwith-Wiedemann syndrome. Subsequently, CNV detection from NGS data was performed out using CNVkit (v0.9.9). CNV detection was achieved by comparing test sample read depths to a pooled reference of over 200 samples from our Institute. CNVs were annotated using AnnotSV (v3.0.7).

Results: We report a child with macrocephaly, dysmorphic facial features, and mesoblastic nephroma. First-tier genetic testing yielded normal results. Given the presence of mesoblastic nephroma, sharing phenotypic overlap with cystic nephroma, a tumor frequently associated with *DICER1* syndrome, along with the patient's clinical features, a second-tier analysis was carried out. This analysis led to the identification of heterozygous intragenic microdeletions involving exons 4, 19, 22, and 29, classified as likely pathogenic by ACMG rules. MLPA analysis is currently underway to validate the microdeletion.

Conclusion: This case underscores the diagnostic complexity of *DICER1* syndrome and the critical role of comprehensive CNV assessment in suspected cases. The distinctive clinical presentation prompted further genetic evaluation, leading to the identification of a suspected *DICER1* intragenic deletion, a finding observed in fewer than 10% of *DICER1* patients. This represents the first reported association between mesoblastic nephroma and *DICER1* syndrome.

C.O.I. Disclosure:

E. Minale: None. **A. Gambale:** None. **A. Buonagura:** None. **S. Martone:** None. **F. Acquaviva:** None. **R. Marra:** None. **A. Iolascon:** None. **M. Capasso:** None. **I. Andolfo:** None. **R. Russo:** None.

EP01.022 Identification of germline variants in pediatric patients with suspected cancer predisposition syndromes

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Background: The most recent studies suggest that at least 10% of paediatric cancer patients carry a germline mutation associated with an inherited cancer syndrome. The aim was to identify hereditary cancer predisposition syndromes in pediatric cancer patients using whole exome sequencing (WES).

Material and methods: The Ripperger criteria were taken into account to select those pediatric patients with suspected hereditary cancer predisposition syndromes. WES was performed using the NovaSeq6000 (Illumina). A clinical exome was performed by filtering for human phenotype oncology and known pediatric cancer predisposition genes.

Results: Twelve patients (6 males/6 females) diagnosed with different types of pediatric cancer were included. Two patients had been diagnosed with two or more different tumors; six were included because of the type of neoplasm (five medulloblastomas, and one fibromatosis). Three patients suffered multiple relapses. One patient had family history of colon cancer. Nineteen variants were identified in nine patients: One pathogenic, two likely pathogenic and 16 variants of uncertain significance (VUS). The pathogenic and likely pathogenic variants were identified in the same patient, who was diagnosed with leukemia and four years after with Ewing sarcoma. These variants were identified in CTC1, GJB2 and NRAS genes.

Conclusion: WES is a useful and fast tool for detecting germline alterations in patients with suspected hereditary cancer predisposition syndromes. There is still a lack of knowledge about the genes associated with pediatric cancer predisposition and many of the variants identified are VUS. Functional studies are needed to investigate the pathogenicity of the variants.

C.O.I. Disclosure:

P. Alba-Pavon: None. **L. Alaña:** None. **A. Diaz-Jorge:** None. **C. Garcia-Barcena:** None. **I. Astigarraga:** None. **O. Villate:** None.

EP01.023 Epigenetic biomarkers in HPV-associated precancerous lesions: advancing early detection and prognosis

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Background: HPV challenges are expanding beyond cervical cancer to include other lesions, such as anal and oropharyngeal cancers, which are becoming more prevalent and are not routinely screened. This study explores the emerging role of epigenetic biomarkers in understanding HPV-associated precancerous lesions, focusing on how episignatures can improve early detection and inform disease progression, leading to novel prevention and treatment strategies.

Material and methods: A review of recent literature examined epigenetic biomarkers in predicting the progression of HPV-associated precancerous lesions. PubMed and Google Scholar were searched for studies published in the last five years, focusing on DNA methylation and other biomarkers in early detection, diagnosis, and prognosis of HPV-related lesions.

Results: HPV, a group of over 200 DNA viruses, includes high-risk types like HPV 16 and 18, which cause most HPV-related cancers. While persistent infections lead to 95% of cervical cancers, over 25% of HPV-related cancers occur in men. HPV infection alone is not sufficient for malignant transformation; factors such as viral integration, genotype, and epigenetic changes play a role. A combined analysis of biomarkers helps predict high-risk lesions. Epigenetic changes, including DNA methylation and RNA dysregulation, are crucial in HPV carcinogenesis, with markers like FAM19A4 and miR-124-2 linked to disease progression. HPV-positive head and neck cancers also exhibit distinct miRNA profiles.

Conclusion: DNA methylation episignatures and markers like FAM19A4 and miR-124-2 show promise for predicting the progression of HPV-associated precancerous lesions.

C.O.I. Disclosure:

B. Abdelmoula: None.

EP01.024 Early-onset renal phenotype in an Indian patient with Birt-Hogg-Dubé Syndrome

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Background: Birt-Hogg-Dubé syndrome (BHDS) is an autosomal dominant disorder characterized by fibrofolliculomas, a heightened risk for renal neoplasms, and spontaneous pneumothorax. East Asian studies have shown that the lung-related manifestations are more prevalent (87.3%) compared to skin lesions (36.7%) and kidney cancer (7.2%).

Material and methods: A 23-year-old female presented with abdominal pain and a large unilateral left upper polar renal mass, raising concern for a malignant cause. She had a family history of abdominal cancer in paternal grandmother, details were unavailable. She underwent left radical nephrectomy, histopathology confirmed clear cell renal cell carcinoma (RCC) with a TNM stage of T1bN0M0. Suspecting familial cancer syndrome, next-generation sequencing was offered.

Results: A pathogenic (PVS1, PM2,PP5), heterozygous single base pair duplication in exon 9 of the *FLCN*gene (p.Ala310SerfsTer80) was detected leading to BHDS. Reverse phenotyping revealed fibrofolliculomas over face but absence of lung cyst on CT chest. This variant has not been reported in the Indian population to date. The patient is under 25 years old and female, presents with atypical features compared to previously reported cases. Variable expressivity is known which explains asymptomatic parent of proband. Patient is currently under surveillance for pulmonary cysts, recurrence of malignancy in contralateral kidney and colonic polyps.

Conclusion: Our findings highlight renal presentation, early age and female gender in Indian family with BHDS as compared to previous studies. To make an early diagnosis and minimize the severity of complications, careful observation, and timely genetic examination of the *FLCN* gene is essential.

C.O.I. Disclosure:

S.K. Goyal: None. **S. Gupta:** None. **D. Gupta:** None.

EP01.025 Could *CHEK2* and *BRIP1* germline variants be associated with the risk of gastrointestinal neuroendocrine tumors?: a case report

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Introduction: Gastrointestinal neuroendocrine neoplasms (GI-NENs) are rare tumors, most commonly found in the small intestine, rectum, stomach, and pancreas. While typically sporadic, germline predisposition can also contribute to NEN development. We report a rectal NEN case with pathogenic germline variants in two tumor suppressor genes.

Case: A 52-year-old female presented with rectal bleeding, where a polypoid rectal tumor was identified. Immunohistochemical analysis confirmed a grade 1 well-differentiated NEN. The oncology team referred her for germline predisposition evaluation. Her medical history was unremarkable, but her family history included multiple solid organ carcinomas. A hereditary cancer panel of 36 genes revealed heterozygous pathogenic variants in *CHEK2* (NM_007194.4): c.846+4_846+7del and *BRIP1* (NM_032043.3):c.608del (p.N203Tfs*71). Genetic counseling and segregation analysis were recommended, along with somatic panel analysis on paraffin-embedded tissue to identify potential driver genes.

Discussion: *CHEK2* and *BRIP1* are DNA damage repair genes linked to breast adenocarcinoma and other solid cancers. While some reports associate *CHEK2* variants with GI-NENs, no prior studies have linked *BRIP1* variants to GI-NENs. Further research is needed to clarify these genes' roles in gastrointestinal and site-specific NEN development. A deeper understanding could enhance preventive strategies and screening protocols for patients and at-risk relatives.

C.O.I. Disclosure:

C. Leblebici: None. **S. Sayır:** None. **I. Kuzu:** None. **G. Utkan:** None. **S. Ekinci:** None.

EP01.026 BRCA carriers identified through National population screening: reactions and attitudes in the real-world experience

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Background: The high *BRCA1/BRCA2* pathogenic variant frequency in Ashkenazi-Jews led Israel to implement the first National BRCA-population screening (PS) worldwide. Aim: To evaluate by in-depth qualitative analysis the PS process, carriers' experience and emotional outcomes in the PS real-world setting.

Methods: We sampled carriers attending our institution's carrier surveillance clinic, identified in 2021–2024. We compared carriers identified through PS vs. genetic counseling (GC). Semi-structured interviews were analyzed for recurring themes using an inductive-grounded theory approach.

Results: We interviewed 21 PS- and 12 GC-carriers. PS-carriers' mean age was 38.9y similar to 42.1y in GC-carriers. 40% had significant cancer family-history, vs. 67% among GC-carriers. Pre-test: The main drive for testing in both PS- and GC-carriers was knowledge of cancer risk. Unique to PS-carriers was being tested

merely because it was "on a checklist", without seeking further information. PS-carriers suggested improving general awareness and pre-testing information to achieve higher uptake with better decision-making process. Reaction to results: In both groups carriers reported fear of cancer and sadness. PS-carriers uniquely reported an initial reaction of shock. They emphasized the need to improve result disclosure by treating physicians or by pre-scheduled phone calls, enabling preparedness. Dealing with being a carrier: carriers had high satisfaction and positive attitudes toward PS. They criticized the limited knowledge and support among community care-givers.

Conclusion: PS is associated with less attention at testing and higher initial shock. Nonetheless, the process was viewed favorably, for cancer prevention. PS-carriers' perspective may be useful for improving PS in Israel and future PS elsewhere.

C.O.I. Disclosure:

S. Lieberman: None. **S. Armon:** None. **A. Tomer:** None. **E. Koller Volkow:** None. **P. Mor:** None. **E. Levy-Lahad:** None. **N. Srebnik:** None. **R. Michaelson-Cohen:** None.

EP01.027 Molecular characterization of solid tumors: A comprehensive analysis of fusion events

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Background: Fusion genes, which result from aberrant fusion of two genes and are involved in tumourogenesis, are being investigated as diagnostic/prognostic biomarkers and therapeutic targets. This study investigated the frequency of novel fusion genes in solid tumours and the limitations of detection methods.

Material and methods: Between November 2022 and 2024, 728 solid tumour patients referred to the Medical Genetics Clinic were evaluated. Next-generation sequencing (NGS) analysis was performed on the Illumina Nextseq 2000 system using the Archer FusionPlex CTL panel.

Results: A total of 36 different fusion genes were identified in the study, 14 of which contain novel partners not previously described in the literature. In 227 lung cancer patients, 8% fusion genes were detected. Fusions such as *BRAF::AGK*, *EML4::ALK* (9 patients), *LOC101929418::ALK* (2 patients), *NAV3::RET*, *RELCH::RET* were found. *MET* exon 14 skipping mutation was detected in six patients and *SS18::SSX2* fusion in synovial sarcoma. Novel fusions detected in our study in other solid tumours are detailed in the table.

Conclusion: Fusion genes show variety in solid tumours and novel fusion gene partners are common. These findings are valuable for molecular characterisation and personalised therapy. Methods for detecting fusions differ with respect to the number of genes analysed, the methodology and the target molecule. The increasing identification of biomarkers has favoured the development of comprehensive profiling. While NGS methods offer the advantage of comprehensive analysis, they have limitations such as high RNA input demands and PCR-induced biases.

C.O.I. Disclosure:

A. Deniz: None. **H.B. Erdem:** None. **T. Bahsi:** None.

EP01.028 Lynch syndrome screening in endometrial cancer

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Background: Endometrial cancer is one of the cancers that female patients with Lynch syndrome have a high risk of developing. In 2023 immunohistochemistry testing of the MMR proteins of all endometrial cancers diagnosed in the southern healthcare region in Sweden began. Here the experience from the first 18 months is described.

Material and methods: Immunohistochemistry on the tumors was performed and when MMR deficiency (dMMR) was detected a recommendation of genetic testing of a blood sample was issued. The genetic analysis was performed with a KAPA-Twist hybrid capture panel. The analysis included *EPCAM, MLH1, MSH2, MSH6, PMS2* and *PTEN*.

Results: Eightyseven patients with dMMR were referred to genetic testing and in 10 patients a pathogenic variant was detected (11 %). Sixtyfour patients had absent expression of MLH1 and PMS2. In this group one pathogenic MLH1-variant was detected (2 %). Eight had isolated absence of PMS2, one individual in that group had a pathogenic PMS2-variant (13 %). Five had absence of MSH2 and MSH6, one of these patients had a pathogenic variant in MSH2 (20 %). Two tumors displayed absence of MLH1, PMS2 and MSH6 and in that group one pathogenic MSH6 variant was detected. Eight tumors had isolated absence of MSH6 and in that group six patients had a pathogenic MSH6-variant (75 %).

Conclusion: The diagnostic yield when testing patients with endometrial tumors displaying dMMR is high however absence of MLH1 and PMS2 in an endometrial tumor is associated with a relatively low chance of detecting a constitutional genetic cause.

C.O.I. Disclosure:

M. Hellberg: None.

EP01.029 Investigation of Stem cell markers in Bortezomibe-resistant Multiple Myeloma cell line

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Background: The accumulation of malignant plasma cells characterizes multiple myeloma(MM).MM remains incurable due to high rates of relapse and drug-resistant disease after first-line treatment.Evidence regarding MM stem cells(MMCSCs), which are known to play a role in drug resistance in MM, is insufficient.This study aims to contribute to research on drug resistance in MM therapies by examining stem cell marker gene expressions in both sensitive and resistant MM cell lines.

Material and methods: The bortezomib-resistant RPMI8226/7B cell lines were kindly provided by Dr. Javier Naval (Universidad de Zaragoza, Saragossa, Spain).MM cells were maintained in RPMI1640 medium, with 1%L-glutamax,10% FBS and 1% penicillin/streptomycin at 37 °C and 5% CO2. ABCG2 (BCRP), BAX, BCL2, MDR1(ABCB1), CD24, NANOG, Oct4, Sox2 and GAPDH genes were analyzed by RT-qPCR method in BTZ-sensitive RPMI8226 and BTZ-resistant RPMI8226/7B cell lines.

Results: BTZ-resistant RPMI8226/7B cell line compared to sensitive cell lines; ABCG2 (BCRP), which is associated with drug resistance, and stem cell markers NANOG, OCT4 and BCL2, CD24 genes were found to be expressed higher.Expression of BAX, MDR1 and SOX2 genes was not observed in BTZ-sensitive and resistant cell lines.

Conclusion: In this study, the effectiveness of the Cancer Stem Cell phenotype in BTZ-resistant cell lines was investigated; While the highest expression of OCT4 and BCRP genes was observed in resistant cells, the expression of Nanog, CD24, and BCL-2 genes was also increased in these cells. These preliminary data suggest

that the expression of stemness and anti-apoptotic genes in BTZ-resistant MM RPMI7B cell lines is responsible for resistance.

C.O.I. Disclosure:

N. Abdullayeva: None. **K. Gayret:** None. **T. Ozkan:** None. **A. Sunguroglu:** None.

EP01.030 BCR::ABL positive cell-free ssDNA triggers inflammatory pathways and affects RNA exosome complex activity in chronic myeloid leukemia

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Background: Chronic myeloid leukemia (CML) is characterized by the formation of the BCR::ABL gene, which encodes a constitutively active tyrosine kinase that promotes cell proliferation and survival. BCR::ABL disrupts RNA processing, stability, and accumulation by affecting RNA metabolism. The RNA exosome complex (ExoC) is a ribonuclease complex responsible for processing stable RNAs, unstable RNA degradation, and genome stability. We hypothesized that cell-free single-stranded DNA (ssDNA) triggers inflammation in CML and that ssDNA-induced inflammation disrupts cell homeostasis by modulating the activity of ExoC.

Material and methods: ssDNA was isolated from the culture supernatant of K562 cells (K562-ssDNA) and cord blood (CB) plasma (CB-ssDNA). The CB mononuclear cells (MNCs) were co-cultured with 2ng-ssDNA for 48hours(h) after 30 min of treatment with a TLR9 inhibitor (300nM). Relative gene expression in MNCs was analyzed by RT-PCR.

Results: K562-ssDNA confirmed to contain a BCR::ABL by PCR analysis and induced the expression of *TLR9, cGAS, STING, NF-κB, IFNG, HMGB1*, and *ZFP36L1* in MNCs. *DIS3*, an ExoC subunit with both exo- and endonuclease activities, is sensitive to ssDNA-mediated inflammation and was dramatically overexpressed (~8fold)($p < 0.0001$) after induction. Both gene and protein expression of EXOSC1 in MNCs, which is reported to be involved in ssDNA cleavage, increased in MNCs cultured with K562-ssDNA. TLR9 inhibitor application to MNCs cultured with K562-ssDNA resulted in the downregulation of *TLR9, NF-κB, IFNG, ZFP36L1*, and *HMGB1* expression. In addition, *DIS3*, which is overexpressed during inflammation, is suppressed.

Conclusion: ExoC activity is affected by inflammation, and TLR9 inhibitors show promise for preventing inflammation in CML.

C.O.I. Disclosure:

E.N. Demirtaş: None. **S.F. Toprak Yalçın:** None. **S. Sozer:** None.

EP01.031 Descriptive analysis of breast cancer in Albania, exploring receptor status and familial history

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Background: Over the past twenty years, research on breast cancer has led to remarkable progress in our knowledge of the disease and more effective treatments. Our study aims to assess the descriptive data of breast cancer in the Albanian population during the past few years and the importance of receptor status and familiar history in this pathology.

Material and methods: Data is collected from the medical records of patients with breast cancer admitted to the oncology department of Mother Teresa University Hospital between 2021 and 2024. IBM SPSS Statistics is used to process the data.

Results: The study includes 252 female participants. The over-45 age group has the highest diagnostic rate (92.5%) compared to the under-45 age group (7.5%). Her2+ is detected in 14.7% of the subjects, PR+ in 80.6%, ER+ in 85.3%, TN in 11.1%, and non-TN in 88.9% of the subjects overall. 13.9% (35/252) of the subjects were identified with a family history of cancer, and in a sample of individuals with familial connections, we detected the heterozygous 40nt deletion in BRCA1 exon11. The association between receptor status and age group is statistically significant ($p = 0.02$). A statistically significant correlation ($p < 0.001$) exists between the receptors' state and the disease's stage. The majority of breast cancer patients were grade II (69.8%), with ductal carcinoma being the most prevalent form (81.3%).

Conclusion: Our study's results underscore the importance of receptor profiling and age-specific screening in breast cancer management. Further research is needed to explore the genetic factors contributing to familial cases.

C.O.I. Disclosure:

A. Hasa: None. **M. Xhetani:** None. **F. Marku:** None. **B. Laze:** None. **R. Dishi:** None. **H. Nina:** None.

EP01.032 Retrospective study of genetic testing results reveals pathogenic variants beyond *BRCA1/2* in hereditary breast and ovarian cancer cases in New Brunswick: Implications for future care

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Background: In Canada, founder variants in breast cancer susceptibility genes have been identified in populations residing in Québec and Newfoundland, thus demonstrating the value in characterizing the genetic profile of local populations for better clinical management. New Brunswick has a diverse, yet genetically unexplored population that includes founder Irish and Acadian ancestry, among others, and we hypothesized that this population could demonstrate potential enrichments for variants in breast cancer genes.

Material and methods: Health records were retrospectively analyzed for 445 cases referred to the genetics clinic in Moncton, New Brunswick, their molecular results were summarized and compared to allele frequencies from similar studies in Canada.

Results: No ethnic or age-related correlation for specific variants could be identified. However, BRCA1/2 variant frequency was lower than expected in the study group and variants in other susceptibility genes such as ATM and CHEK2 were higher when compared to similar studies.

Conclusion: This study demonstrates a distinct profile in hereditary breast cancer genetics in a previously uncharacterized population, thus adding to existing knowledge of population genetics in Atlantic Canada.

C.O.I. Disclosure:

K. David: None. **K. Gauvin:** None. **V. Allain:** None. **N. Bouhamdani:** None. **C. Williams:** None. **Y. Saheb:** None. **C. Savoie:** None. **L. Macrae:** None. **K. Hodson:** None. **Y. Zhu:** None. **E. Allain:** None. **M. Ben Amor:** None.

EP01.033 A pediatric Epithelioid Glioblastoma with a rare TRIM24::NTRK2 fusion; comprehensive characterization and targeted treatment

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Background: High-grade gliomas are one of the most lethal cancers and the leading cause of cancer-related mortality in children. Standard treatment typically involves resection, followed by chemotherapy and radiation, yet these interventions offer limited improvement in survival rates. Targeted therapies are emerging and have specifically shown effect in solid tumors harboring tyrosine-kinase activating fusion genes. Here, we present a thorough characterization of an epithelioid glioblastoma in a six-year-old patient, who experienced four relapses and a variety of different treatment strategies.

Material and methods: By whole genome sequencing we identified a rare TRIM24::NTRK2 fusion, which enabled targeted treatment with TRK-inhibitor. We performed in-vitro functional analyses of the downstream pathway effects of the fusion gene, and extensive genomic, methylation, and mRNA expression profiling of tumor tissue from all recurrences and different locations. Also, examination of various treatment strategies in patient-derived cell cultures was conducted.

Results: Our findings show that the *TRIM24::NTRK2* fusion initially had oncogenic abilities, but this became less imperative as the tumor evolved. Molecular profiling inferred parallel development of two tumor subclones with distinct genomic alterations. The only pathogenic variants recurrent in all operations and clones was the *TRIM24::NTRK2* fusion and a *CDKN2A/B* homozygous deletion. A general drug resistance was documented over time, and later relapse clones also acquired resistance mutations in *MTOR* and *NTRK2*, bypassing TRK-inhibition.

Conclusion: This study addresses the complex and adaptive nature of pediatric glioblastomas and highlights the need for continues molecular profiling from relapses and various tumor regions to enable multitarget treatment approaches.

C.O.I. Disclosure:

S. Keane: None. **I. Sjöberg:** None. **L. Deland:** None. **A. Lindlöf:** None. **T. Olsson:** None. **T. Kling:** None. **M. Blomstrand:** None. **H. Fagman:** None. **J. Nilsson:** None. **D. Nilsson:** None. **M. Sabel:** None. **H. Carén:** None. **F. Abel:** None.

EP01.034 Investigating the expression of cyclin-dependent kinase 1 (CDC1) and genes related to lncRNAs involved in tumor tissue and tumor margins in hepatocellular carcinoma (HCC)

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Background: Liver cancer is one of the most common malignancies worldwide. HCC, the predominant pathological type of liver cancer, accounts for about 75–85% of all liver cancers. Metabolic lipid has emerged as an important feature of HCC.

Material and methods: By referring to the GEO database, based on searching keywords such as liver cancer, tumor and normal, the raw data of GSE84005 was implemented as CEL using the GEOquery package, then using the limma package The R environment was used to perform quality control, preprocessing and statistical data analysis. To identify the set of differentially expressed genes, the combined criterion of adj.P-value < 0.05 and log2 fold change >1 was used after creating a suitable design matrix in limma. Finally, the results of the most important module were entered into the Mcode software based on Cytoscape to find hub genes and create a gene network within the module.

Results: Based on the research conducted after the loss of HAMP expression in SMMC-7721 cells of tumor tissues, the expression of cyclin-dependent kinase 1 (CDC1) is significantly increased. The expression of STATA3 and B. phosphor-stat3 is significantly increased, respectively, considering LogFC >- 5 and P-value < 0.05.

Conclusion: According to the studies conducted on LncRNA in the tumor tissue and the tumor margin of hepatocellular carcinoma patients, according to the data, the tumor tissue has an increased expression compared to the tumor margin.

C.O.I. Disclosure:

Z. Ashrafipour: None. **Y. Ghaderi:** None. **F. Zeinali*:** None.

EP01.035 Association of Gastric Adenocarcinoma And Neuroendocrine Tumors: A Case Study

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Background: A 61-year-old male patient was admitted to the oncology service in May/24, with complaints of epigastric pain, melena and weight loss. He underwent endoscopy in March/24, with a vegetative lesion in the antrum and biopsy showing a well-differentiated adenocarcinoma. Immunohistochemistry revealed suspected overexpression of the Her-2 oncogene and microsatellite instability, confirmed with the loss of nuclear expression of MLH1 and PM52. The upfront approach was chosen due to obstructive neoplasia, with subtotal gastrectomy with D2 lymphadenectomy. The anatomopathological revealed mixed carcinoma with components of gastric adenocarcinoma, large cell neuroendocrine carcinoma and well-differentiated neuroendocrine tumor. Pathological staging according to AJCC was pT3pN1M0: gastric adenocarcinoma - moderately differentiated G2 (25%) / Large cell neuroendocrine carcinoma - poorly differentiated G3 (50%) / Well-differentiated neuroendocrine tumor - G2 (Ki67 15%) - (35%). The

patient is currently undergoing follow-up. It was decided to start adjuvant chemotherapy, however interrupted due to toxicity.

Discussion: Patient admitted to the service with a diagnosis of gastric adenocarcinoma, with locally advanced lesion, indicating upfront surgery. Well-differentiated neuroendocrine tumors are more common, while large cell tumors are rarer and generally more aggressive.

Conclusion: The coexistence of adenocarcinoma and neuroendocrine tumors is rare and can be observed in patients with atrophic gastritis and in the context of Zollinger-Ellison syndrome. Chronic inflammation can favor the transformation of cells of both types of neoplasia. Further studies are needed to better understand the relationship between these types of cancer and the clinical implications of their coexistence.

C.O.I. Disclosure:

C. Bonini-Domingos: None. **A. Bonini Domingos:** None. **S.R. Orrico:** None. **A.R. da Silva:** None. **F.G. Parra:** None. **E. Campos Jr:** None.

EP01.036 The importance of cytogenetics in clinical diagnosis: Transformation of paroxysmal nocturnal hemoglobinuria to T cell acute lymphoblastic leukemia

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired disorder. Bone marrow cytogenetic analysis can point the main diagnosis of a patient whose other tests are not diagnostic.An adult male patient who was diagnosed with PNH six years ago but did not continue his follow-up, re-applied with complaints of recent weight loss and sweating. The diagnosis process leading to the identification of T-ALL in the patient who underwent bone marrow aspiration biopsy due to clinical progression will be discussed.

Material and methods: Chromosome analysis was performed after 24-hour bone marrow cell culture without mitogen.

Results: t(9;14)(p21;q11) was detected in four of the 20 metaphases analyzed. The translocation detected in the patient is in *CDKN2A/TRA* region. This rearrangement is related with adult T-lineage acute lymphoblastic leukemia.

Conclusion: Hematopoietic stem cells and their progenies have reduced or absent glycosylphosphatidylinositol anchored proteins on the cell surface. That leads to the premature death and impaired production of blood cells and mainly affects adults with a 1–10/1million prevalence. Nearly all patients are symptomatic with associated with anemia, neutropenia and/or thrombocytopenia related findings. Rarely leukemia can emerge from PNH associated MDS. Conventional cytogenetic examination maintains its importance in bone marrow disorders. Although labor intensive, cytogenetic analysis can help the management of newly diagnosed and relapsed patients easier and more accurate.

C.O.I. Disclosure:

S.-. Altiner: None. **A. Vicdan:** None.

EP01.037 *GATA2*mutations in Tunisian myelodysplastic syndrome patients: a preliminary study

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Background: *GATA2* is a zinc finger transcription factor important for the production and maintenance of hematopoietic stem cells. Mutations in *GATA2* can be either inherited or acquired. Germline *GATA2* mutations cause cellular deficiencies, and different locations of *GATA2* mutations are associated with distinct phenotypes and leukemogenic mechanisms. Since there is limited data on the North African population, this study was carried out to investigate the *GATA2* mutation in a cohort of myelodysplastic syndrome (MDS) Tunisian patients.

Material and methods: A retrospective study was carried out in the Hospital University of Sfax, south of Tunisia, including six MDS patients. A comprehensive clinical, hematological, and genetic assessment of the *GATA2* mutation was performed. Sanger sequencing in the *GATA2* gene (NM_032638.4) using a high-fidelity DNA polymerase was applied.

Results: *GATA2* mutations were detected in two patients with a confirmed diagnosis of MDS.

Conclusion: Our data on the Tunisian population highlights GATA2 mutation is frequent and can be a useful marker. A larger study is mandatory to elucidate prognosis impact of GATA2 mutation and to identify the genetic landscape of MDS and specific individual disease outcome

C.O.I. Disclosure:

R. Frikha: None. **Z. Boujelben:** None. **R. Fakhfakh:** None. **M. Charfi:** None. **H. Kamoun:** None.

EP01.038 Clinicopathological profile of breast cancer patients harboring germline pathogenic variants in*BRCA* genes - a single center experience

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Background: Pathogenic (PVs) germline variants in *BRCA1* and *BRCA2* genes are a determining factor in breast cancer (BC) development and progression and can influence personalized case management. Our study aimed to establish a set of correlations between genotypes and clinicopathological features of patients who were referred to our center for *BRCA1/2* testing.

Material and methods: The study involved patients with personal or familial history of BC who tested positive for *BRCA1/2* PVs at the Dolj Regional Centre for Medical Genetics, over a three-year period. An immunohistochemical panel was used to define molecular subtypes of breast tumors. AmpliSeq for Illumina *BRCA* Panel was used to evaluate germline variants in the two genes.

Results: A total of 9 patients were evaluated, 6 of which carried *BRCA1* PVs and 3 had *BRCA2* PVs. *BRCA1* carriers were mostly over 40 years and often diagnosed with a breast lesion by clinical examination and biopsy. The lesion was almost always malignant and was usually a triple-negative tumor. *BRCA2* carriers were all older than 40 and were often diagnosed with a breast lesion by clinical examination coupled with a surgical procedure, followed by histopathological examination. The lesion was always malignant and was usually of luminal A-type.

Conclusion: *BRCA1* PVs were associated with aggressive phenotypes of malignant proliferations while *BRCA2* PVs were associated with lower grade proliferations. Thus, *BRCA1/2* testing could be more efficiently implemented in cost-effective screening and risk-reducing strategies, contributing to the genetic epidemiology of BC and providing optimal therapeutic options for patients harboring germline PVs.

C.O.I. Disclosure:

R. Pleșea: None. **A. Riza:** None. **I. Gavrilă:** None. **&. Andrei:** None. **A. Barbu:** None. **F. Burada:** None. **I. Streață:** None.

EP01.039 Ethnic Background Matters: Evaluating the Impact of the *CHEK2**1100delC Germline Variant on Breast Cancer Susceptibility in Saudi Women with a Comparison Across Ethnic Groups

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Background: Effective breast cancer management requires an understanding of population-specific genetic etiology. Different genes influence cancer development. Rare studies have investigated the clinical relevance of the common *CHEK2**1100delC variant in Saudi females. This study aimed to investigate its implications for Saudi female with breast cancer and compare the results with other ethnic groups.

Materials and methods: The *CHEK2**1100delC variant was studied in a cohort of 268 female Saudi patients with breast cancer. The age of the participants ranged from 19 to 70 years. Our patients fulfilled the National Comprehensive Cancer Network criteria for hereditary breast and ovarian cancer syndrome testing. Exome sequencing using the Bioscientia Hereditary Breast and Ovarian cancer gene panel had been carried out in 168 patients, while Sanger sequencing was performed for the remaining 100. We screened this variant in the whole exome data of 150 healthy Saudi individuals in the control group, and we did not find it. We also described the clinical data of Saudi female patients.

Results: This study included 268 female Saudi patients with breast cancer. No cases of *CHEK2**1100delC pathogenic variants were identified. This suggests that this variant is either rare or nonexistent in the Saudi population. Interestingly, more than 40% of the cases presented with disease onset before 36 years of age.

Conclusion: The absence or rarity of *CHEK2**1100delC pathogenic variant in the Saudi population underlines the importance of ethnic background in genetic testing. The high percentage of early onset disease in this cohort demands attention when designing a screening program.

C.O.I. Disclosure:

W.T. Habhab: None. **S. Ekram:** None. **S. Bayashut:** None. **S. Sindi:** None. **S. Alhazmi:** None. **S. Hassan:** None. **M. Ganash:** None. **N. Alburae:** None. **H. Alkhatabi:** None. **A. Elaimi:** None. **K. Algothmi:** None. **G. Jadkarim:** None. **D. Aljeaid:** None. **N. Shaik:** None.

EP01.041 Novel variants in medium and low penetrance predisposing genes in a Hungarian malignant melanoma cohort with increased risk

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Background: Both germline and somatic variants contribute to the genetic background and pathogenesis of melanoma.Germline variants include the presence of rare pathogenic or likely pathogenic variants of high, medium, and low penetrance melanoma-predisposing genes. Rare variants of high penetrance melanoma-predisposing genes are associated with melanoma development, whereas the medium and low penetrance predisposing genes can significantly increase melanoma risk. In this study, we clarified the germline genetic background of a Hungarian melanoma cohort (n = 17).

Methods: Using a gene panel of 30 melanoma-predisposing genes, germline genetic variants were identified in 10 of the 17 patients (58.82%).

Results: A novel, likely pathogenic, missense variant (p.Y143C) in a medium penetrance melanoma-predisposing gene, *melanocortin 1 receptor gene (MC1R)*, and two novel, likely pathogenic nonsense variants in low penetrance genes, p.Q218Ter in caspase 8 *(CASP8)* and p.Q40Ter in the fat mass- and obesity-associated *(FTO)* gene were detected.

Conclusions: This study highlights the importance of elucidating the germline genetic background of melanoma, which may improve prediction of individual risk and the risk of family members and to optimize preventive, screening, and therapeutic measures for each patient and melanoma-prone families.

C.O.I. Disclosure:

B. Bokor: None. **A. Abdolreza:** None. **F. Kaptás:** None. **M. Pál:** None. **Z. Battyani:** None. **M. Széll:** None. **N. Nagy:** None.

EP01.042 Novel *FANCI* and *RAD54B* variants and the observed clinical outcomes in a Hungarian melanoma cohort

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Background: Accumulating evidence suggests that inherited melanoma is not rare and approximately 1 in 7 individuals with melanoma has clinically relevant hereditable cancer predisposing and/or susceptibility variant(s). Concerning its germline genetic background, genetic screening aims to identify either variants of predisposing genes with high penetrance or variants of susceptibility genes with medium or low penetrance. In addition, there is some evidence suggesting that germline variants of genes involved mainly in DNA repair mechanisms (*BRCA2, POLE, WRN, FANCI, PALB2* and *RAD54B*) have been implicated in rendering melanoma patients more susceptible to tumor progression and therapy resistance, leading to poor prognosis and survival.

Material and methods: We aimed to investigate whether the germline genetic background of a Hungarian melanoma malignum cohort (n = 17) contains any pathogenic or likely pathogenic variants of the *BRCA2, POLE, WRN, FANCI, PALB2* and *RAD54B* genes, using a 6-gene virtual genepanel based on NGS sequencing, and if the presence of these variants correlate with the clinical findings of the patients.

Results: We identified three novel variants in the *FANCI* gene, one likely pathogenic and two variants of unknown significance, and one novel variant in the *RAD54B* gene classified as a variant of unknown significance. We detected rapid disease progression, unfavourable outcome, and therapeutic resistance in the patient carrying the likely pathogenic *FANCI* variant.

Conclusion: Our study highlights the importance of screening germline variants of genes influencing melanoma progression, therapy resistance and survival to better estimate individual prognosis and find the best treatment options for melanoma patients.

C.O.I. Disclosure:

B.A. Bokor: None. **A. Abdolreza:** None. **F. Kaptás:** None. **M. Pál:** None. **Z. Battyani:** None. **M. Széll:** None. **N. Nagy:** None.

EP01.043 Clinical and molecular characterization of retinoblastoma in Colombian patients: Insights into genetic mutations

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Background: Retinoblastoma (Rb) is the most common malignant eye tumor in children, with a global incidence of 1 in 18,000 live births annually. Approximately 40% of cases are hereditary, resulting from germline mutations in the *RB1* gene. In developing countries, early diagnosis is often delayed due to limited healthcare resources, compromising both survival and ocular preservation. This study aimed to analyze the clinical and molecular characteristics of Rb in Colombian patients.

Material and methods:A total of 54 Rb patients were evaluated. *RB1* gene sequencing was performed using Next-Generation Sequencing (NGS) in 53.7% of cases and a combination of NGS and Multiplex Ligation-dependent Probe Amplification (MLPA) in 46.3%. Clinical and genetic data were analyzed retrospectively.

Results: The median age at clinical diagnosis was 23 months, while genetic testing was performed at a median age of 40 months. Pathogenic or likely pathogenic variants were identified in 31% of patients, with 88% classified as loss-of-function and 11.8% as missense variants. A previously unreported variant, c.191T>A, was detected in *RB1*. No copy number variations were identified by MLPA.

Conclusion: This study provides insight into the genetic and clinical profile of Rb in Colombia, underscoring the importance of comprehensive molecular testing for accurate diagnosis. Notably, all identified variants were detected by NGS, while MLPA yielded no findings. The discovery of a novel *RB1* variant expands the genetic landscape of Rb and highlights the need for further investigation.

C.O.I. Disclosure:

M. Galvez: None. **J. Geles:** None. **M. Pana:** None. **A. Rodriguez:** None.

EP01.044 Distinct Pathways: Exploring Rare Clinical and Genetic Presentations in Breast Cancer Associated Hereditary Cancer Syndromes

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Background: Hereditary cancer syndromes present at an earlier age and require tailored management that is usually different from non-hereditary cancers; and can have unique presentations observed in clinical practice.

Material and methods: A retrospective, observational study was conducted, rare case presentations were selected from cancer patients at the Hereditary Cancer Clinic at the Aga Khan University Hospital in Karachi, Pakistan.

Results: A total of 13 patients' data (from 10 families) were included in the study. Cases fell into two broad categories: patients with (pathogenic/likely pathogenic) P/LP variants in multiple genes and patients with rare presentations of cancers, those not associated with cancers they presented with. *BRCA1* variants (50%) were most common, and others included *MSH6, PMS2, FANCM, PALB2* and *CHEK2*. Among those with P/LP variants in multiple genes, one patient had P/LP variants in *BRCA1* and *CHEK2*, presented with breast (31y) and secondary biliary cholangiocarcinoma. In another case, a P/LP *ATM* variant was observed in a daughter with breast cancer (31y) and her mother (62y) with renal clear cell carcinoma. Another consanguineous family showed monoallelic P/LP variant in *MUTYH* in mother with breast cancer (48y), whose two children had the similar but biallelic variant with metastatic colon carcinoma in early thirties.

Conclusion: Our cases highlight the importance of multi gene panel testing, the need for tailored surveillance strategies for patients and family members as well as further exploration of unique cancer predisposition gene-gene interaction leading to unique disease presentation which are not currently well elucidated, subsequently translating into improved patient care.

C.O.I. Disclosure:

F. Akbar: None. **M. Kazmi:** None. **A. Arif:** None. **A.T. Wali Mohammad:** None. **Y. Rashid:** None. **A. Sattar:** None. **S. Kirmani:** None.

EP01.045 Breast Cancer: Impact of Protumoral Dietary Components and Patterns on MicroRNA Regulation

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Background: MicroRNAs (miRs) regulate gene expression and are specifically dysregulated in cancer (Oncogenic and Tumor Suppressor-miRs). Considering the potential role of dietary habits in cancer development, this study investigated the impact of protumoral dietary patterns on miR expression commonly dysregulated in breast cancer.

Material and methods: The effects of fructose (F), palmitic acid (PA), and their combination (F + PA) were evaluated in MCF7 breast cancer cells and Carcinoma-Associated Fibroblasts F88 (CAF-F88). Additionally, miR expression was analysed in a murine breast cancer model exposed to diets high in fructose (PBA), fat (PCS), or both (PBA + PCS). miRs were extracted from cultured cells, serum, and tumor tissues. Expression levels of Oncogenic-miRs (miR-21, miR-155, miR-10b, and miR-210) and Tumor Suppressor-miRs (miR-let7a and miR-195) were assessed using reverse transcription and quantitative PCR (RT-qPCR) with Taqman-specific primers and probes. Biological triplicates and reaction triplicates were used in all experiments. Statistical analysis included one-way ANOVA and Bonferroni's test ($P < 0.05$) with GraphPad Prism 9.00 software.

Results: Results showed overexpression of Oncogenic-miRs and reduced Tumor Suppressor miRs across models. In MCF7 cells, F + PA induced significant changes, while in CAF-F88 cells, PA increased Oncogenic miRs, and F + PA reduced Tumor Suppressor-miRs. In the murine model, the PBA + PCS diet significantly increased miR-21 and decreased miR-let7a expression in serum and tumor samples.

Conclusion: These findings highlight the role of miRs in breast cancer progression, suggesting they may mediate tumor-promoting effects of protumoral dietary patterns.

C.O.I. Disclosure:

O.S. Alessandroni: None. **A. Garzón:** None. **T.S. Gareis:** None. **L. Sosa:** None. **M.E. Pasqualini:** None. **A. Quintar:** None. **A.R. Pérez de Rosas:** None. **M.M. Stroppa:** None.

EP01.046 CHEK2 variants detected in single centre hereditary cancer panels evaluation according to ACMG classification

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Background: CHEK2 gene poses an intermediate risk for hereditary cancer when compared with other hereditary cancer-associated genes. It is especially associated with breast, ovarian and colon cancers. We aimed to evaluate the variants we detected in the HC panels studied in our centre according to the acmg classification and according to the population frequency.

Material and methods: 2162 hereditary cancer panels and 2591 other panels studied in our centre were retrospectively screened for CHEK2 variants. Variants found were classified according to ACMG criteria. Clinical histories of positive patients were compiled.

Results: 141 cases (6.9%) in HCS and 15 (0.6%) in other panels, chek2 variants were detected. 104 of them were pathogenic and likely pathogenic. 13 different null variants were identified. 31 different missense variants were identified. 22 of them were evaluated as vus.CHEK2:c.470T>C p.Ile157Thr 1427C > T p.Thr476Met and 592 + 3A > T 499G > A p.Gly167Arg (NM_007194.4) pathogenic variants' frequency were found to be 0.59%, 0.68%,0.3% and 0.39%, respectively.

Conclusion: We suggest that a comprehensive study of variant distribution and clinical correlation of such a gene with moderate cancer effect will contribute to hereditary cancer genetics.

C.O.I. Disclosure:

L. Parali: None. **A. Ece Solmaz:** None. **A. Durmaz:** None. **A. Aykut:** None. **H. Akin:** None.

EP01.047 Specific EGFR mutation analysis in NSCLC patients using liquid biopsy: comparison to tumour tissue

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Background: EGFR mutation analysis in circulating cell free-tumour DNA (cf-DNA) from plasma constitutes a convenient and less invasive method in cases without adequate tumour material for molecular analysis or monitoring of progression.

Material and methods: We analysed 310 cf-DNA samples from patients with NSCLC at time of diagnosis in treatment naïve patients. In parallel we detected EGFR mutations in 90 cases with matched FFPE tissues using Cobas® EGFR mutation test v2 (IVD).

Results: The presence of mutations in plasma (from patients with NSCLC) reached 8% (23/310). Exon 19 deletions were the most frequent mutations (61%), followed by point mutations in exon 21 (35%) and point mutation in exon 18 in one case. Insufficient tumor cells were encountered in 16/90 (18%) FFPE samples whereas an invalid cobas test in 15/90 (12%). 1/31 FFPE cases in total showing invalid test result displayed EGFR mutation in cf-DNA. 5 FFPE cases with EGFR mutations showed no EGFR mutation in cf-DNA. The overall concordance of EGFR mutation status in plasma and tumor samples was 90% and the sensitivity 58%, specificity 100%, PPV 100%.

Conclusion: EGFR mutation testing in cf-DNA using Cobas IVD test showed significant concordance with the result in tissue samples and an optimal specificity and PPV. However, the sensitivity of the test has to be further improved indicating that a biopsy should be optimally analyzed for patients with an EGFR mutation-negative cf-DNA test.

C.O.I. Disclosure:

M. Michelli: None. **E. Roupou:** None. **N. Prekete:** None. **N. Alevizopoulos:** None. **N. Kavantzas:** None. **A. Saetta:** None.

EP01.048 POLE genotyping in Slovenian endometrial cancer patients

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Background: Endometrial cancer accounts for 90% of uterine cancer cases and is among the most common gynaecological malignancies. Its classification relies on immunohistochemistry and molecular diagnostics, with prognosis and treatment decisions influenced by molecular subtypes. Patients with *POLE*-pathogenic variants have the best outcomes, while those with abnormal TP53 protein expression have the worst. As a result, various international guidelines now recommend molecular classification as part of the diagnostic process. This study aimed to develop a simple, time-efficient method to identify *POLE*-pathogenic variants.

Materials and methods: Our study group consisted of 479 patients diagnosed with endometrial cancer between 2022 and 2024. Tumour genotyping was performed on DNA from FFPE samples, using an in-house qPCR method utilizing custom-designed TaqMan genotyping assays to detect the six most common and clinically significant *POLE*-variants (Table 1). For 97 patients NGS was performed using Illumina TruSight Oncology 500 panel.

Results: Among the 387 endometrial cancer patients tested with qPCR, 32 (8.3%) were identified with clinically significant *POLE*-variants. In 97 patients who underwent NGS sequencing, two additional pathogenic *POLE*-variants were identified outside the six commonly tested hot-spot variants (Table 1).

Conclusions: Among Slovenian patients with endometrial cancer, 8.1% were found to have *POLE* mutations, which aligns with published data reporting a prevalence of 7.0–9.0%. Compared to sequencing methods, qPCR targeting the six most common and clinically significant *POLE*-variants offers a simple, straightforward and time-efficient approach for identifying patients with a *POLE* molecular profile.

Table 1: *POLE* genotyping in Slovenian endometrial cancer patients using in-house qPCR and NGS sequencing.

POLE **pathogenic variant**	***POLE*** **mutated EC patients using qPCR and custom designed TaqMan genotyping assays (387 tested patients)**	***POLE*** **mutated EC patients using NGS sequencing and TruSight Oncology 500 (97 tested patients)**
c.857C>G p.(Pro286Arg) P286R*	14	4
c.890C>T p.(Ser297Phe) S297F*	0	0
c.1231G>T p.(Val411Leu) V411L*	11	1
c.1231G>C p.(Val411Leu) V411L*	2	0
c.1366G>C p.(Ala456Pro) A456P*	4	0

POLE pathogenic variant	*POLE* mutated EC patients using qPCR and custom designed TaqMan genotyping assays (387 tested patients)	*POLE* mutated EC patients using NGS sequencing and TruSight Oncology 500 (97 tested patients)
c.1376C>T p.(Ser459Phe) S459F*	1	0
c.1331T>A p.(Met444Lys) M444K	not included in genotyping	1
c.1381T>A p.(Ser461Thr) S461T	not included in genotyping	1
	32 *POLE*-mutated EC (8.3%)	**7 *POLE*-mutated EC (7.3%)**
	All together 39 *POLE*-mutated EC among 479 EC patients (8.1%)	

EC - endometrial cancer; *clinically significant *POLE*-variants included in qPCR genotyping.

C.O.I. Disclosure:
P. Škerl: None. **V. Stegel:** None. **V. Šetrajcic Dragoš:** None. **G. Klancar:** None. **A. Bombac:** None. **A. Zagožen Klasinc:** None. **G. Hertl:** None. **N. Rihar:** None. **T. Samardzic:** None. **L. Kunc:** None. **S. Novakovic:** None.

EP01.049 Clinical Comparison of Cobas IVD Test and Sanger Sequencing for Detecting PIK3CA Mutations in Breast Cancer Patients

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Background: The PI3K pathway plays a crucial role in tumorigenesis, and mutations in the PIK3CA gene serve as important therapeutic biomarkers. This study compares the effectiveness of the Cobas IVD test and Sanger sequencing in detecting hot spot PIK3CA mutations to determine the most reliable method for identifying these mutations in HR + /HER2- advanced breast cancer, which may guide treatment decisions.

Material and methods: 113 DNA samples were extracted from FFPE tissues of breast cancer patients. They were analysed using the Cobas test (exons 1,4,7,9,20) versus Sanger sequencing to detect and identify PIK3CA mutations. To ensure consistency, the same DNA extract was used for both methodologies.

Results: Regarding the Cobas test that does not discriminate mutations at the same codon, 58 cases (52.7%) were mutant in exons 4,7,9,20, and 52 cases (47.3%) were normal, whereas three samples (2.6%) were invalid. Sanger sequencing was applied to identify mutations in exons 7,9, and 20, except for two cases that were not sequenced due to limited DNA. The most prevalent mutation was p.E545K (43% of the mutant cases).

Conclusion: Both Cobas test and Sanger sequencing are effective in detecting PIK3CA mutations as they showed 100% concordance in normal and mutant cases. Although Sanger sequencing was capable of providing detailed mutation profiles, it faced challenges in amplifying and sequencing at least 4 different exons and in cases with limited samples. These findings suggest that Cobas may be preferred for rapid and reliable routine clinical applications, while Sanger sequencing remains valuable for in-depth analysis when necessary.

C.O.I. Disclosure:
N. Prekete: None. **E. Roupou:** None. **M. Michelli:** None. **V. Chaidou:** None. **A. Nonni:** None. **N. Kavantzas:** None. **A. Saetta:** None.

EP01.050 *SPP1* and *P4HA1* as stage-specific biomarkers for metastatic progression in colorectal cancer

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Background: Colorectal cancer (CRC) is frequently diagnosed at an advanced stage, often with metastatic spread and limited therapeutic options. Hypoxia and epithelial-to-mesenchymal transition (EMT) play pivotal roles in CRC progression. Investigating genes involved in these processes may provide insights for developing effective therapies. This study identifies and validates *SPP1* and *P4HA1* as key markers of metastatic progression particularly in stage IV disease.

Methods: The study cohort included 86 primary tumors, 25 liver metastases and 31 non-tumor controls. Differential gene expression (DGE) analysis was performed on 12 liver metastases and 3 adjacent non-tumor epithelial tissue, using a targeted RNA panel of 395 cancer-related transcripts. Data analysis was conducted with DRAGEN differential expression tool. *SPP1* and *P4HA1*, identified as significantly differentially expressed, were validated via quantitative real-time PCR across the cohort and evaluated using ΔΔCt method and t-test, and correlated across CRC stages I-IV.

Results: Both *SPP1* and *P4HA1* were significantly overexpressed in primary tumors and liver metastases compared to control ($p < 0.001$). Expression further increased in liver metastases relative to primary tumors ($p < 0.001$) and progressively rose across CRC stages, peaking in stage IV patients ($p < 0.001$).

Conclusion: The overexpression of *SPP1* and *P4HA1* in advanced CRC underscores their potential as biomarkers for metastatic progression and highlights their relevance for future therapeutic research strategies.

C.O.I. Disclosure:
E. Turyova: None. **P. Mikolajcik:** None. **M. Kalman:** None. **D. Loderer:** None. **M. Grendar:** None. **M. Slezak:** None. **J. Marcinek:** None. **M. Skerenova:** None. **T. Burjanivova:** None. **J. Strnadel:** None. **J. Miklusica:** None. **Z. Lasabová:** None.

EP01.051 Are mutations found in ovarian cancer germline or somatic?

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Background: Ovarian cancer arises as a consequence of genetic mutations, which can be either somatic or germline. Germline mutations are inherited and present in all cells, while somatic mutations are acquired during a patient's lifetime and confined to tumor tissue. Identifying whether somatic mutations are also germline is crucial for personalized treatment and family risk assessment.

Material and methods: Twenty-five ovarian cancer samples were previously analyzed using targeted next-generation sequencing and comprehensive genomic profiling (CGP) to detect mutations in tumor tissue. After confirming the presence of mutations in ovarian tissue, blood samples were tested by Sanger sequencing to determine whether these mutations were germline. Variant allele frequency (VAF) from CGP report was also considered during analysis.

Results: In 25 ovarian cancer samples, it was found 27 pathogenic variants in *BRCA1*(19), *BRCA2*(3), *ATM*(1), *TP53*(2), *MUTYH*(1) and *RB1*(1) genes. Seven variants in genes *BRCA1*(4), *TP53*(2) and *RB1*(1) were not confirmed as germline. Some variants detected in tissue using the CGP method had a VAF high (between 61 to 95%) but were not found in blood, indicating they were only somatic. On the other hand, variants with VAF low (between 18 to 31%) have been proven to be inherited.

Conclusion: According clinical guidelines, targeted therapy for ovarian cancer is based on BRCA1/2 genetic testing in tumor tissue, because mutations in BRCA1/2 genes can be germline and somatic. Confirming germline mutations is crucial for family monitoring, refining genetic counseling, assessing hereditary risk and guiding cancer prevention.

C.O.I. Disclosure:

P. Anić: None. **M. Čikotić:** None. **M. Popović:** None. **N. Dedić Plavetić:** None. **I. Rako:** None.

EP01.052 Dedifferentiated Liposarcoma in a Patient with Neurofibromatosis Type 1: Case Report

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Background: Neurofibromatosis type 1 (NF1, OMIM #162200) is an autosomal dominant neurocutaneous disorder caused by pathogenic variants in the NF1 gene, encoding the tumor suppressor neurofibromin. While malignant peripheral nerve sheath tumor (MPNST) is the most common NF1-associated malignancy, dedifferentiated liposarcoma (DDLPS) represents a crucial differential diagnosis to MPNST with liposarcomatous transformation. DDLPS is characterized by transition from well-differentiated liposarcoma to pleomorphic, non-lipogenic tissue with spindle cells. Diagnosis relies on histopathological features and MDM2 and CDK4 gene amplification. DDLPS has rarely been associated with neurofibromatosis. Hereby, we report the first case of DDLPS in a patient with NF1 in Latin America.

Material and methods: A 33-year-old female with hypothyroidism and left inguinal DDLPS was referred for genetic evaluation due to café au lait macules. The patient fulfilled NF1 clinical criteria. Given the liposarcoma and family history, a hereditary cancer next-generation sequencing (NGS) panel was performed. The tumor tissue underwent immunohistochemical analysis.

Results: A pathogenic variant c.7970+1G > A p.? was identified in the NF1 gene. Histopathology revealed a spindle cell tumor of difficult classification. Immunohistochemistry showed partial reactivity for MDM2, CDK4, DESMIN, and P16, with negativity for CK, CD34, STAT6, progesterone receptor, and SOX10, confirming DDLPS.

Conclusion: This represents the first reported case of NF1 with DDLPS in Latin America. For inconclusive histopathology and immunohistochemistry cases, MDM2 amplification by FISH is recommended to confirm DDLPS diagnosis.

C.O.I. Disclosure:

L.A. Melo-Porras: None. **L.A. Trujillo-Moreno:** None. **G. Contreras-García:** None. **C.I. Vargas-Castellanos:** None.

EP01.053 *MIR-142* deregulation mechanisms in Diffuse Large B-cell Lymphoma

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Background: The purpose of our study was to perform the analyses of *MIR-142* deregulation mechanisms by mutations in Diffuse Large B-cell Lymphoma (DLBCL) tumor tissue.

Material and methods: The biopsies of tumor lymph nodes of 121 DLBCL patients was examined. Mutations in this gene *MIR-142* were evaluated by direct Sanger sequencing. Localization of the mutations on the secondary stem-loop structure of the miR-142 precursor was predicted with mfold Web Server.

Results: 19/120 (15.8%) cases of DLBCL with mutations in the gene *MIR-142* were detected. The distribution of mutations by type was as follows. Mutation in the "seed" sequences miR-142-5p and miR-142-3p have been identified in 1 and 2, respectively; in other regions of the mature miR-142-5p and miR-142-3p sequence - in 4 and 2, respectively; in the pre-miR-142 sequence - in 8 cases, of which 2 are in the DROSHA recognition site. There are 2 cases in the pri-miR-142 sequence. Note that in 2 cases two mutations (n.13C/T and n.68G/A, as well as n.23A/U and n.25C/U) and in 1 - three (n.35G/A, n.39G/A and n.52C/U) mutations in the gene *MIR-142* occurred simultaneously.

Conclusion: *MIR-142* is the most frequently mutated microRNA gene in DLBCL. Mutations are widely distributed in the gene sequence. They are disrupt the interactions with targets, change the thermodynamic properties and disrupt the stability of the microRNA molecule. In the case of multiple mutations, the most profound change in the secondary structure of the microRNA precursor is observed.

C.O.I. Disclosure:

E. Voropaeva: None. **S. Minnih:** None. **O. Seregina:** None. **M. Voytko:** None. **T. Babaeva:** None. **V. Maksimov:** None. **T. Pospelova:** None.

EP01.054 Identification of a Rare e8a2 *BCR-ABL* Transcript in a Chronic Myeloid Leukemia patient: a Molecular and Cytogenetic CharacterizationIdentification of a Rare e8a2 *BCR-ABL* Transcript in a Chronic Myeloid Leukemia Patient: A Molecular and Cytogenetic Characterization

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Background: Chronic Myeloid Leukemia (CML) is characterized by the BCR-ABL fusion gene, typically presenting with e13a2 or e14a2 transcripts. Rare alternative transcripts can impact disease progression and therapy. We report a CML case with an atypical e8a2 BCR-ABL transcript, emphasizing the need for comprehensive molecular analysis in atypical presentations.

Material and methods: Karyotyping was performed to detect the Philadelphia chromosome. RT-PCR assays targeted common BCR-ABL transcripts (p210 and p190). An unexpected band in the p190 assay prompted further investigation using nested PCR for rare variants, including e8a2. Results were validated in two independent samples. Sanger sequencing determined the fusion gene breakpoint.

Results: Karyotyping confirmed 46,XY,t(9;22)(q34;q11.2)[20]. RT-PCR did not detect expected transcripts but revealed an aberrant band in the p190 assay. Nested PCR amplified an e8a2 BCR-ABL transcript. Sanger sequencing identified a fusion between BCR exon e8 and ABL exon a2, incorporating a 46 bp sequence from BCR intron 8 at the junction, forming an in-frame e8a2 BCR-ABL fusion.

Conclusion: Expanded molecular testing is indispensable < !-- EndFragment--> when standard RT-PCR fails to detect expected transcripts. This atypical transcript has been reported in CML patients achieving a major molecular response to imatinib (PMID: 16270035). Identifying rare BCR-ABL variants has diagnostic and therapeutic implications, underscoring the importance of advanced sequencing in ambiguous cases.

C.O.I. Disclosure:

T.F. Silva: A. Employment (full or part-time); Significant; CGC genetics by Unilabs. **D. Fernandes:** A. Employment (full or part-time); Significant; CGC genetics by Unilabs. **S. Teixeira:** A. Employment (full or part-time); Significant; CGC genetics by Unilabs. **A. Oliveira:** A. Employment (full or part-time); Significant; CGC genetics by Unilabs. **C. Pereira:** A. Employment (full or part-time); Significant; CGC genetics by Unilabs. **A. Pereira:** A. Employment (full or part-time); Significant; CGC genetics by Unilabs. **R. Cerqueira:** A. Employment (full or part-time); Significant; CGC genetics by Unilabs. **J. Sá:** A. Employment (full or part-time); Significant; CGC genetics by Unilabs. **I. Salgado:** A. Employment (full or part-time); Significant; CGC genetics by Unilabs.

EP01.055 KRAS mutated colorectal cancer cell phenotype is altered by nanopolystyrene and nanopolyethylene exposure

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Background: Nanopolystyrene (NPS) and nanopolyethylene (NPE) are employed in many industrial and biomedical fields, but their spread usage in common items favored their environmental dispersion. The goal of our research is to determine how people with genetic predisposition to diseases may respond to NPS and NPE exposure. Currently we are focusing on human colon-rectal cancer both in vivo and in vitro. We previously demonstrated that NPS induces genomic instability and clastogenic effects on HCT116 cell line using the micronuclei assay. These results were confirmed in vivo on *Drosophila melanogaster* intestine with an adapted comet assay protocol on the flies gut.

Materials and methods: We employed a label-free method of live cell imaging using 3D holotomography microscopy (Nanolive) to visualize NPS and NPE internalization and viability in HCT 116 cell line (ATTC, 5% CO2, 37 °C, DMEM). Standard protocols were performed for the alkaline comet assay and acridine orange staining (apoptosis).

Results: We demonstrated that internalized NPE reduces cell viability presumably via apoptosis while NPS stimulates it. Other phenotypes such as organelles integrity, nuclei morphology and cellular dimensions did not result altered. A transcriptomic analysis is currently ongoing to correlate phenotypic changes to molecular pathways.

Conclusion: Our results showed that a genetic predisposition to a disease can be aggravated by NPS and NPE exposure. In addition, a polymer-specific genotoxicity was detected complicating the topic even more. Future studies are needed to deep down the correlation between nanoplastics and molecular pathways regulating cell proliferation and homeostasis.

C.O.I. Disclosure:

M. Aloisi: None. **S. Colafarina:** None. **O. Zarivi:** None. **A.M. Poma:** None.

EP01.056 Monitoring of BCR-ABL1 p210 with treatment outcomes in cohort of Ukrainian CML patients

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Background: Chronic myeloid leukemia (CML) is caused by a translocation between chromosomes 9 and 22, forming the BCR-ABL1 oncogene. About 90% of CML patients have e13a2 or e14a2 transcripts, producing the BCR-ABL1 p210 oncoprotein. Tyrosine kinase inhibitors (TKIs) have improved survival, but 20–30% of patients still experience therapy failure. RT-qPCR is essential for monitoring BCR-ABL1 transcripts. We analyzed a Ukrainian CML cohort to address gaps in ethnic and epidemiological data.

Material and methods: Monitoring of BCR-ABL1 p210, isolated RNA from whole blood, was performed by RT-qPCR standardized at the International Scale (BCR-ABL1 IS).

Results: We monitored 167 CML patients with confirmed BCR-ABL1 p210 transcript, treated with TKIs for at least one year. The overall male-to-female ratio ranges from 1.3 to 1.8; however, in our cohort, the ratio is 0.84 (CI 95%, 0.62–1.13, P-Value = 0,27). The median age at diagnosis was 54 years, which is comparable to other populations (52 ± 12 years). According to the European Leukemia Network (ELN) guidelines, the optimal response threshold at 12 months is BCR-ABL1 IS < 0.1% (major molecular response, or MMR). In our cohort, 70.66% of patients achieved MMR, with 50.3% reaching deep molecular response (DMR, BCR-ABL1 IS < 0.01%), which is crucial for treatment-free remission. However, 29.34% had a warning response (BCR-ABL1 IS 0.1–1%) or treatment failure (BCR-ABL1 IS > 1%).

Conclusion: Our cohort showed a slightly lower male-to-female ratio but a comparable median age. About 71% achieved an optimal response, while 29% struggled, consistent with TKI resistance data in the literature.

C.O.I. Disclosure:

S. Levandivska: None. **O. Korolchuk:** None. **I. Yurchyshak:** None. **H. Makukh:** None.

EP01.057 The role of *HEYL* and *HEY1* single nucleotide polymorphisms in laryngeal squamous cell carcinoma development

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Background: Laryngeal squamous cell carcinoma (LSCC) is the most common cancer of the head and neck region. Despite the great research effort, the role of potential behavioral and genetic risk factors remains unclear. Therefore, it is essential to search for genetic factors that could contribute to earlier diagnostics and help improve patient survival.

Material and methods: 172 men with diagnosed LSCC were enrolled in this retrospective case-control study with the control group of 220 healthy men. Genomic DNA was extracted from EDTA-preserved peripheral venous blood samples from all individuals. Genotyping of selected polymorphisms *HEYL* rs41264499 and rs11206478, *HEY1* rs2467789 and rs1046472 using real-time PCR was performed at the Oncology Research Laboratory of Oncology Institute at the Lithuanian University of Health Sciences. Statistical data analysis was performed using SPSS v29.0.

Results: Our study results show that the *HEYL* rs41264499 CG genotype carriers had a 1.874-fold increased risk of LSCC (OR = 1.874, CI 95% (1.057–3.323), p = 0.031). Also, it was determined that the G allele carriers of *HEYL* rs41264499 had an increased risk of LSCC (OR = 1.838, CI 95% (1.055–3.203), p = 0.032). Further, *HEY1* rs1046472 TT genotype carriers (compared to GG genotype) had an increased odds of LSCC by 2.527-fold (OR = 2.527, 95% CI (1.028–6.212), p = 0.043). *HEY1* rs1046472 G allele carriers had a reduced likelihood of LSCC compared to the control group (OR = 0.395, 95% CI (0.163–0.955, p = 0.039).

Conclusion: *HEYL* rs41264499 and *HEY1* rs1046472 polymorphisms have significant association with LSCC development.

C.O.I. Disclosure:

A. Mikulskienė: None. **R. Vadeikienė:** None. **E. Padervinskis:** None. **R. Ugenskienė:** None.

EP01.058 Evaluating the impact of microsatellite instability status and association of mismatch repair gene mutations on resistance to targeted therapy in patients with metastatic colorectal cancer

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Microsatellite Instability (MSI) is a new key biomarker for targeted therapy (TT) of colorectal cancer. Mutations in key genes of the MMR system lead to genetic hypermutability, or MSI. In this study, we investigated the association between the mutations of MMR genes and MSI status in CRC patients resistant to TT.

MSI status and MMR genes mutations were analyzed from targeted next-generation sequencing data of 14 patients with metastatic CRC resistant to bevacizumab TT using DRAGEN TSO500 v 4.3.6 Software.

MSI-H status was detected in 100% of all patients. Genetic analysis revealed the presence of missense mutation c.655A>G and intronic mutation c.1039-8T > A in the *MLH1* gene in 5 (36%) of 14 patients. Patient #4 had a somatic frameshift variant c.2733_2734insCTTC in the *PMS1* gene and deletion c.706-4del in the intronic region of the *PMS2* gene. This deletion was also detected in samples #6 and 14. Missense mutation c.2197G>A in the *MSH2* gene was detected in sample #2. Intronic mutation c.211+9C > G in the *MSH2* gene was detected in 7 of 14 patients (50%). It is noteworthy that missense mutations c.2846A>G and c.3133G>A in the *MSH3* gene were detected in 13 out of 14 cases. Patient #1 was a carrier of the previously known pathogenic mutation c.3103C>T in the *MSH6* gene. In addition, patient #4 was found to have a frameshift insertion c.4068_4071dup in the *MSH6* gene.

Our results show a correlation of genetic mutations in MMR system genes with high MSI status and a phenotype resistant to targeted therapy.

C.O.I. Disclosure:

A. Aitkulova: None. **N. Tynyshtykbayeva:** None. **T. Kadenova:** None. **T. Rogounovitch:** None. **A. Akilzhanova:** None. **D. Sarbassov:** None.

EP01.059 Effects of Origanum majorana on the expression of self-renewal genes in the Human Immortalized Ovarian Surface Epithelium (IHOEC) Cells Aged with H_2O_2

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Background: It has been known that oogonial stem cells (OSCs) regulate the ovarian reserve and are effective on the renewal of the follicle pool, which has an important role in ovarian aging. Female fertility declines with age, influenced by multiple genes, including Nrf2, a key transcription factor that enhances cellular lifespan by protecting against oxidative stress. H_2O_2 induces oxidative stress, leading to premature aging and telomere shortening. Origanum majorana (OM), a perennial plant of the Lamiaceae family, possesses antioxidant properties. The aim of this study is to determine the effects of OM oil extract on OSC or stemness genes and apoptosis in the aging process with H_2O_2 in IHOEC cells.

Material and methods: IHOEC cells were maintained in RPMI 1640 medium supplemented with 10% FBS, 1% Streptomycin-Penicillin, and L-glutamine at 37 °C and 5% CO_2. The MTT assay was used to determine the IC50 concentrations of OM and H_2O_2 when applied alone or in combination. Gene expressions of antioxidative NRF2 gene, BAX, BCL2 apoptotic genes and SOX2, OCT4 stemness gene expressions were analyzed by qRT-PCR.

Results: In this study, for the first time, OM was shown to significantly increase the expression of stem cell markers SOX2 and OCT4 genes when applied alone or together with H_2O_2 on IHOEC cells.

Conclusion: This study suggests that Origanum majorana L. oil extract exhibits anti-aging effects in the female reproductive system by counteracting oxidative stress. OM enhances cell viability by upregulating Bcl-2 via Nrf2 and promotes stem cell properties, increasing their regenerative potential.

C.O.I. Disclosure:

E. Dener Kılıc: None. **N. Farhangzad:** None. **T. Ozkan:** None. **A. Gürbüz Bolkan:** None. **A. Sunguroglu:** None.

EP01.060 A novel *BMPR1A* mutation causing juvenile polyposis syndrome

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Background: Juvenile Polyposis Syndrome (JPS) is rare autosomal

dominant condition predisposing to gastrointestinal (GI) hamartomatous polyps. JPS is associated with increased risk of GI malignancy, mainly in the colon, with cumulative lifetime risk of cancer from 38.7% to 86.2%. JPS is genetically and clinically heterogeneous, with strong genotype-phenotype correlation. Most cases of JPS are caused by disease causing variants of genes *SMAD4* and/or *BMPR1A*. The *BMPR1A* gene (10q23.2) comprises 11 coding exons, encoding type 1 receptor of the TGFβ superfamily that mediates BMP intracellular signalling through *SMAD4* and is involved in colonic epithelial growth.

Material and methods: Patient's blood samples were collected and genomic DNA was subjected to Whole exome sequencing on NovaSeq6000 platform (Illumina). Variants in polyposal-associated genes were analyzed using systematic bioinformatics pipeline and ACMG criteria. Candidate variants were additionally validated by Sanger sequencing.

Results: We report 14-year-old boy with multiple benign colon polyps, anaemic, weight reduction, hematemesis which led to high transfusion dependence and high risk of malignancy. We detected in *BMPR1A* gene novel pathogenic frameshift mutation c.747G>A (p.Trp249Ter), exon 9, leading to stop codon and truncated protein. Moreover, variant of unknown significance found in *CDH1*, c.353C>G (p.Thr118Arg), functionally tested to lower the cell adhesion and increase EGFR activity.

Conclusion: Our systematic approach reported new *BMPR1A* mutation and shed more light in genetic causes of JPS. Mutations in *CDH1* are related to hereditary cancer-predisposing syndrome and despite its unknown significance, there is a chance the variant highly influence the clinical phenotype.

C.O.I. Disclosure:

G.S. Stancheva: None. **K. Mihova:** None. **V. Petkova:** None. **M. Georgiev:** None. **I. Boronsuzov:** None. **M. Jordanova:** None. **R. Kaneva:** None.

EP01.061 Microsatellite Instability Analysis in Cancer Patient Samples: Real world data from a single centre

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Background: Microsatellite instability (MSI) is commonly observed in various cancers, particularly colorectal, endometrial and gastric. MSI detection has significant clinical implications, as a predictive and prognostic biomarker, informing eligibility for immune checkpoint inhibitors.

Material and methods: A total of 229 samples, mainly colorectal cancers (146 cases), were analysed for MSI using two different assays. First, High Resolution Melting Analysis (HRMA) was employed for BAT26, BAT25, NR21 and NR24 mononucleotide microsatellite markers (LDT). Second, the commercially available MicroSight test which is based on HRMA was applied to assess MSI for mononucleotide microsatellite markers: BAT25, BAT26, NR22, NR24 and MONO27.

Results: The cases were classified as MSI-H (20 cases / 8.7%) and MSS (209 cases / 91.3%). 14/20 MSI-H cases displayed MLH1 loss. To define the mutational profile of the MSI-H cases, KRAS and BRAF mutations were studied. The most common KRAS mutations were p.G12D and p.G13D in patients 62–86 years of age, whereas p.V600E was the only BRAF mutation found in patients of 60–82 years of age.

Conclusion: MSI analysis is currently widely implemented for treatment decisions regarding immunotherapy. Given the probability for Lynch syndrome, genetic counseling based on MSI results can guide appropriate surveillance strategies for affected individuals and their family members. Establishing a multidisciplinary molecular tumor board will enhance the precision of cancer care and enable the identification of novel therapeutic targets for patients with MSI-associated cancers.

C.O.I. Disclosure:

A. Saetta: None. **N. Prekete:** None. **I. Giannopoulou:** None. **E. Roupou:** None. **M. Michelli:** None. **N. Kavantzas:** None.

EP01.062 Detection of clinically significant gene variants in Ukrainian breast cancer samples using a cancer hotspot panel

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Background: At present, the prevalent genetic screening for breast cancer (BC) in Ukraine is PCR for seven common mutations in *BRCA1* gene. In this study, we have investigated more efficient strategy that utilises targeted next-generation sequencing (NGS) of 50 cancer-driving genes hotspot regions to identify clinically relevant gene variants.

Material and methods: The material for the study comprised 57 female BC patients' tumour tissue, collected during surgical treatment (mean age 54.2 ± 13.2 years). Ethics approval and informed consent were obtained. Genomic DNA was amplified using the custom Ion AmpliSeq™ cancer hotspot panel. The DNA libraries were sequenced using an Ion S5 Plus sequencer. The results were then filtered using Franklin by Gennox to identify clinically significant variants.

Results: A total of 48 clinically significant variant were found in 9 genes. The largest number of mutations was identified in four genes: *TP53*, *PIK3CA*, *PTEN* and *VHL* (19, 18, 3 and 3, respectively). Two variants were identified in *NRAS* and one variant in *CDH1*, *FGFR2* and *FGFR*. Five mutations were identified in more than one sample, *PIK3CA* c.3140A>G, *PIK3CA* c.1633G>A, *TP53* c.742C>T, *TP53* c.646G>A and *TP53* c.817C>T (11, 4, 3, 2 and 2, respectively). The prevalence of Tier 1 gene variants was found to be approximately 83%. In addition, six novel mutations were identified, including *PTEN* c.64G>A, whith potential therapeutic level A.

Conclusion: Variants with high therapeutic (level A-B) significance were detected in 32 BC samples (56%). This data suggests a high level of clinical efficacy of the NGS cancer hotspot approach.

C.O.I. Disclosure:

A.M. Bezverkhiy: None. **R.V. Gulkovskyi:** None. **G.V. Gerashchenko:** None. **M.V. Tomiak:** None. **O.E. Lobanova:** None. **Z.I. Rossokha:** None. **N.G. Gorovenko:** None. **Z.Y. Tkachuk:** None. **V.I. Kashuba:** None. **M.A. Tukalo:** None.

EP01.063 Revisiting the impact of allele dropout on therapy/somatic testing: a case study of ovarian tumor misclassification as BRCA-proficient

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Background: Allelic dropout in BRCA1 somatic testing is a significant concern in the context of breast and ovarian cancers, particularly due to its implications for accurate mutation detection and subsequent therapeutic decisions. By means of a case study, we raise awareness to this uncommon but nevertheless existent challenge.

Methods: An ovarian cancer diagnosis was made for a 51-year-old female patient. Germline analysis for BRCA was performed using Illumina sequencing-by-synthesis method and MLPA. Further somatic testing was carried out using Oncomine BRCA Assay GX and was continued with Oncomine Comprehensive Assay Plus to assess the HRD status (Thermo Fisher Scientific).

Results: The germline analysis of BRCA1 and BRCA2 revealed no clinically significant variants. The Oncomine BRCA Assay GX for somatic variants was negative, the reason for which testing has escalated to Oncomine Comprehensive Assay Plus, where a pathogenic variant was detected in BRCA1: NM_007294.4:c.4327C>T, with an allelic frequency (VAF) of 73.47%. Therefore, we are in front of two discordant sequencing results. Both somatic panels utilize AmpliSeq chemistry (amplicon-based enrichment), making allelic dropout the most likely explanation for the discordant result. This phenomenon is dependent on the primer design in the respective kits, potentially leading to the failure to amplify certain alleles and resulting in false negative results.

Conclusion: A negative result does not exclude a genetic alteration. It is essential to understand the limitations of each test to ensure accurate genetic counseling, enabling informed decision-making regarding further testing, risk assessment, and potential treatment options.

C.O.I. Disclosure:

I.E. Simina: D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Modest; Sanofi, Oncohelp, Komtur Pharmaceuticals. **I.T. Perva:** None. **C.V. Munteanu:** None. **D. Popovici:** None. **A.P. Trifa:** None.

EP01.064 Non-canonical TRAIL pathway is significantly deregulated in breast cancer

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Background/Objectives: Dysregulation of TRAIL-mediated apoptotic pathway has been associated with carcinogenesis. Our aim was to investigate the possible predictive value of three components of the TRAIL non-canonical pathway (*IAP1, RIPK1, TRAF2*) in breast cancer.

Materials and methods: Relative mRNA levels of TRAIL pathway genes (*IAP1, RIPK1, TRAF2*) were examined in 103 breast cancer tissues by RT-PCR/ΔΔCt. Statistical analysis for clinico-pathological associations and per pair analysis was performed by SPSSv.25 software.

Results: Most cases (66–71%) presented reduced mRNA levels of *RIPK1* and *TRAF2*, that percentage being 31% for *IAP1*. Elevated mRNA levels ranged between 25–34% of the cases for all three genes. Statistically significant correlations were recorded between: a) the expression levels of all three genes and oestrogen receptor expression b) the expression of the *IAP1* or *RIPK1* genes and the age of diagnosis, c) *IAP1* mRNA levels and *PIK3CA* mutations d) *TRAF2* mRNA levels and pPrognostic stage. Last, co-expression patterns of the analysed genes (per pair analysis) showed moderate and strong correlations.

Conclusion: The observed high frequency of altered expression levels implies significant deregulation. Correlations of mRNA levels with the age of diagnosis and ER expression might suggest a hormone-dependent regulation. Elevated *IAP1* mRNA levels correlated negatively with mutations in the *PIK3CA* gene, possibly implying a tumour suppressive mechanism. The positive correlation between *TRAF2* expression and the pPrognostic stage could indicate activation of the non-canonical pathway in late stages. In conclusion, the above correlations suggest that these genes should be further evaluated as potential predictive biomarkers for TRAIL targeting therapies.

C.O.I. Disclosure:

M. Konsolaki: None. **E. Roupou:** None. **M. Michelli:** None. **N. Kavantzas:** None. **N. Michalopoulos:** None. **A. Saetta:** None.

EP01.065 Next Generation Sequencing for the identification of predisposition syndromes to pediatric medulloblastoma

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Background: Medulloblastoma (MB) is the most common malignant brain tumor in pediatrics, with different histological and molecular variants, each with distinct characteristics and variable prognoses. Among the subgroups, Sonic hedgehog (SHH) pathway-activated MB is the most frequently associated with a higher risk of tumor development due to cancer predisposition syndromes. The aim of this study was to identify pathogenic variants in cancer predisposition genes in a retrospective cohort of 24 pediatric patients diagnosed with SHH-MB.

Material and methods: A customized Next Generation Sequencing (NGS) panel was used for the mutational study of blood and tumoral samples. We included 17 genes (*CTNNB1, APC, TP53, PTCH1, SUFU, SMO, BOC, ELP1, GPR161, PALB2, BRCA2, CREBBP, NBN, NF2, SDHA, VHL* y *PTEN*) for the search of germline and somatic abnormalities in 25 patients diagnosed with medulloblastoma.

Results: Mutations were found in 16 out of the 25 patients included in the study, in *PTCH1*, *TP53*, *SMO*, *PTEN*, *CREBBP, ELP1* and *GPR161* genes. Five of these mutations, in *TP53* (3), *CREBBP* (1) and *GPR161* (1) genes, were detected in germline and somatic tissues of five patients. Mutations were predicted to be pathogenic and likely pathogenic with Varsome, ClinVar databases and Franklin predictor. These mutations were related with Li-Fraumeni syndrome for *TP53* and Rubinstein-Taybi for *CREBBP*. In case of *GPR161*, the name of the syndrome it causes is still unknown.

Conclusion: NGS analysis of tumoral tissue contributes to the identification of genes associated with cancer predisposition syndromes in medulloblastoma, but germline analysis are needed for diagnosis confirmation.

C.O.I. Disclosure:

L. Alaña Rodrigo: None. **A. Díaz Jorge:** None. **L. Zaldumbide:** None. **L. Mosteiro:** None. **I. Martin-Guerrero:** None. **O. Villate:** None. **P. Alba-Pavón:** None. **I. Astigarraga:** None. **M. García-Ariza:** None.

EP01.066 Oxidative DNA damage and radiotherapy response in ductal carcinoma in situ

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Background: Ductal carcinoma in situ (DCIS) is a non-invasive type of breast cancer commonly treated with surgery and adjuvant radiotherapy (RT). RT induces DNA damage directly or indirectly through increasing oxidative stress in cells. Our aim was to determine if RT affects concentrations of oxidative DNA damage biomarker 8-hydroxy-2-deoxyguanosine and if single-nucleotide polymorphisms (SNPs) in DNA repair genes *XRCC1* and *OGG1* are associated with plasma 8-hydroxy-2-deoxyguanosine levels in DCIS patients.

Material and methods: Our pilot study included 54 DCIS patients treated with adjuvant RT. We isolated genomic DNA from blood samples and genotyped all patients for *XRCC1* rs2682585, rs1799782, and rs25487 and *OGG1* rs159153, rs1052133, and rs293795 SNPs using competitive allele-specific PCR. Oxidative DNA damage was measured in plasma before and after RT with competitive enzyme-linked immunosorbent assay (ELISA) for 8-hydroxy-2-deoxyguanosine. Nonparametric tests were used in statistical analysis.

Results: Concentration of 8-hydroxy-2-deoxyguanosine in plasma did not change significantly after RT ($p = 0.157$). Carriers of two polymorphic *OGG1* rs1052133 alleles had lower 8-hydroxy-2-deoxyguanosine concentration before RT compared to other DCIS patients ($p = 0.028$). In carriers of at least one polymorphic *OGG1* rs159153 ($p = 0.012$) and rs293795 ($p = 0.033$) allele, 8-hydroxy-2-deoxyguanosine concentration increased more after RT compared to carriers of two normal alleles. *XRCC1* polymorphisms were not associated with 8-hydroxy-2-deoxyguanosine concentration.

Conclusion: Our results suggest *OGG1* polymorphisms are associated with plasma oxidative DNA damage levels and might serve as a biomarker of radiosensitivity in DCIS.

C.O.I. Disclosure:

M. Marincic: None. **E. Serianz:** None. **V. Dolžan:** None. **T. Marinko:** None. **K. Goričar:** None.

EP01.067 Weighted gene co-expression network analysis unveils key pathways and hub genes driving colorectal cancer progression and clinical outcomes

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Background: Gaining insight into the molecular mechanisms of colorectal cancer (CRC) is essential for identifying novel therapeutic targets and prognostic markers. This study aims to uncover key pathways and their driving genes linked to CRC clinical features through robust downstream analysis.

Material and methods: We performed Weighted Gene Co-expression Network Analysis (WGCNA) to analyze RNA-seq data from 131 matched tumor (CRC) and healthy (NCT) tissues. Signed correlation networks were constructed from both CRC+NCT and CRC-only samples, and gene modules were identified. Clinical traits, including gender, age of onset, tumor stage, grade, localization, lymphovascular and perineural invasion, tumor-infiltrating lymphocytes (TILs) score, pathological TNM staging and survival rate were correlated to key modules in CRC-only network. Gene set enrichment analysis (GSEA) was carried out using the MSigDB database to explore biological pathways enriched in the significant modules.

Results: The CRC+NCT network highlighted gene modules strongly correlated with CRC, enriched in activated pathways such as E2F and MYC gene targets, G2M checkpoint and mitotic spindle. Conversely, functional processes involved in fatty acid metabolism, hypoxia, oxidative phosphorylation, myogenesis, response to ultraviolet (UV) radiation and heme metabolism were suppressed. CRC-only network enabled the detection of modules correlated to clinical characteristics. Analysis of significant modules revealed hub genes within pathways exhibiting crucial roles in cancer progression.

Conclusion: We successfully identified genes and pathways closely related to CRC and its clinicopathological features, providing valuable insights for screening potential biomarkers and drug targets. These findings may contribute to developing personalized treatments and improving patient outcomes in CRC.

C.O.I. Disclosure:

V. Rallo: None. **M. Massidda:** None. **P. Forabosco:** None. **S. Onano:** None. **M. Deiana:** None. **A. Maschio:** None. **A.M. Scanu:** None. **M.R. De Miglio:** None. **A. Angius:** None.

EP01.068 Ansell's mole-rat as a novel furred model of skin anti-tumorigenesis

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Background: The naked mole-rat (*Heterocephalus glaber*) is a long-lived mammal that has been studied for genomic and cellular anti-tumorigenic mechanisms, but it is challenging to exclude species-specific features when using only mouse, human, and naked mole-rat for comparative analyses. Furthermore, naked mole-rat's baldness limits the ability to study many skin cancers, which can originate from hair follicle stem cells. We introduce the Ansell's mole-rat (*Fukomys anselli*) as a novel cancer-resistant model to address these challenges, which is more closely related to naked mole-rat than mouse but has hair follicles.

Material and methods: We performed DMBA/TPA-induced skin carcinogenesis on Ansell's mole-rat and collected single-cell RNA-seq and histology data from skin. We also collected long-read and short-read whole genome sequencing, Hi-C, and bulk RNA sequencing from nine organs to assemble a robust genome.

Results: Ansell's mole-rat skin shares commonalities with both naked mole-rat and mouse skin upon exposure to potent carcinogens. For instance, like naked mole-rat, Ansell's mole-rat

exhibits anti-tumorigenesis. However, Ansell's mole-rat shows an intermediate number of resident T-cells and immune infiltration similar to mouse. We are able to leverage our new genome and single-cell data to understand the transcriptional drivers of anti-tumor mechanisms across cell types.

Conclusion: Ansell's mole-rat is a valuable model for studying anti-tumorigenic mechanisms and clarifying which biological differences across mammals confer cancer resistance. With its new highly contiguous and robustly annotated genome, Ansell's mole-rat can also serve as a valuable resource in cross-species genomic research.

C.O.I. Disclosure:

V.F. Busa: None. **M. Bekavac:** None. **R.T. Coimbra:** None. **M. Behm:** None. **S. Begall:** None. **A. Goncalves:** None. **D.T. Odom:** None. **M. Frye:** None.

EP01.069 Aberrant splicing due to ITK splice-site variant in a pediatric patient with secondary plasmablastic lymphoma: a case study

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Background: Inherited immunodeficiencies are a heterogeneous group of rare disorders with a predisposition to lymphoma. Interleukin-2 inducible T-cell kinase (*ITK*) plays a crucial role in T-cell signaling, and its alterations have been linked to immune dysregulation and lymphoproliferative disorders. This report highlights the clinical course of a homozygous pathogenic *ITK* variant within a family.

Methods: Germline WES was performed in a consanguineous family (n = 5) with an affected child who developed primary Burkitt's lymphoma at age 12 and later secondary plasmablastic lymphoma (PBL). Sanger sequencing was used for variant segregation. *ITK* expression was evaluated in patient's cDNA and control cell line. Splice site effect of the variant was verified by using primers targeting exon 6 and exon 7.

Results: A novel homozygous likely pathogenic *ITK* variant (NM_005546.4: c.647+1G>T), was identified, predicted to disrupt protein function. Segregation analysis revealed that one of the patient's three sisters was wild-type, one heterozygous, and one homozygous. Patient cDNA analysis revealed a lack of normal transcript formation.

Conclusion: Immunodeficiency-associated lymphoproliferations vary in severity and may contribute to lymphoma recurrence and therapy resistance. Herein, we identified a novel *ITK* variant that may contribute to lymphoma development. On the other hand, the asymptomatic homozygous sibling highlights the complexity of genotype-phenotype correlations. Understanding the molecular consequences of the ITK variants is valuable in elucidating their role in tumorigenesis in the context of secondary lymphoma.

C.O.I. Disclosure:

T. Sudutan: None. **Y. Erbilgin:** None. **R. Kebudi:** None. **S. Kalayoglu Besisik:** None. **M. Sayitoglu:** None.

EP01.070 The potential of resveratrol to sensitize MCF-7 cells to radiotherapy

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Background: Radiotherapy is frequently used in the treatment of breast cancer. However, radioresistance remains the primary disadvantage of this therapeutic approach. Therefore, the search of chemicals, which could induce radiosensitivity is of great importance. One of these chemicals is resveratrol (RSV). Several investigations revealed RSV's ability to inhibit the expression of cancer-specific genes, induce changes in the cell cycle, and activate apoptosis. Our study aimed to investigate the effects of RSV on radiosensitivity and the expression of *BCL2* gene in the breast cancer.

Material and methods: An X-ray linear accelerator was used to irradiate cells with 2 or 4 Gy doses. The anti-proliferative effect of RSV in MCF-7 cells was determined by colony formation assay. The apoptosis level and changes in the cell cycle were measured using the Muse Cell Analyzer. Real-time PCR was used to quantitatively determine *BCL2* gene expression.

Results: Our results indicated, that RSV decreased MCF-7 cell viability. When a combination of RSV and radiation therapy (IR) was used, the anticancer effects on cells were noticeably stronger. It was demonstrated that RSV + IR increased the proportion of cells undergoing apoptosis and the number of cells in the G2/M phase. Based on RT-PCR results, RSV + IR combinations statistically significantly reduced *BCL2* gene expression compared to RSV-alone or IR-alone treatment.

Conclusion: According to the findings of our study, resveratrol is a potential radiosensitizer of MCF-7 breast cancer cells. RSV and IR combinations decreased cell proliferation, which was associated with the induction of apoptosis and reduced expression of the anti-apoptotic *BCL2* gene.

C.O.I. Disclosure:

D. Laukaitiene: None. **R. Vadeikiene:** None. **A. Vaitkus:** None. **R. Ugenskiene:** None. **E. Juozaityte:** None. **A. Inciura:** None.

EP01.071 Incidence of Low-Penetrance *BARD1*, *MUTYH*, and *RAD50* Variants in Hereditary Cancer Syndromes: A study of 1200 samples

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Background: Hereditary cancer syndromes (HCS) arise from germline variants that increase cancer susceptibility. Over time, several low-penetrance genes have been proposed to be associated with HCS, but their role in cancer predisposition remains unclear. This study analyzes the low-penetrance genes *BARD1*, *MUTYH*, and *RAD50* in our population.

Material and methods: We analyzed 1,200 blood samples from patients with suspected HCS referred from genetic counseling centers across the Castile and León region. NGS was performed

using a 35-gene predisposition panel. Genomic DNA was extracted from peripheral blood, and library preparation was performed using the Ion Chef System (ThermoFisher), followed by sequencing on the Ion S5 platform. Variant analysis was conducted with IonReporter Software.

Results: Pathogenic variants (PV) in at least one of the three genes were identified in 57 samples (4.75%): 51 in *MUTYH* (4.25%), 4 in *RAD50* (0.33%), and 2 in *BARD1* (0.16%). We detected 10 distinct PVs in *MUTYH*, 2 in *RAD50*, and 1 in *BARD1*.Additionally, 10.5% of samples carried at least one Variant of Uncertain Significance (VUS): 39 in *MUTYH* (3.25%), 66 in *RAD50* (5.5%), and 21 in *BARD1* (1.75%), corresponding to 12 VUS in *MUTYH*, 29 in *RAD50*, and 15 in *BARD1*.

Conclusion: Our study identifies pathogenic variants and VUS in *BARD1*, *MUTYH*, and *RAD50*, with *MUTYH* being the most frequently altered gene. The high prevalence of *RAD50* VUS highlights the need for further research to determine their clinical significance. These findings may impact genetic counseling and risk assessment in hereditary cancer.

C.O.I. Disclosure:

M. Arranz Ledo: None. **N. Rojo Calvo:** None. **E. Lastra Aras:** None. **L. Mateo Otero:** None. **A. Olaverri Hernández:** None. **L. Hernández Sanz:** None. **N. Martínez Martín:** None. **M. Infante Sanz:** None. **M. Durán Domínguez:** None.

EP01.072 Multigene panel testing for hereditary cancer predisposition in portuguese breast and ovarian cancer patients. Experience of SYNLAB Genética Médica Porto

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Background: Next-generation sequencing (NGS) allows for a more cost-effective and quickly detection of pathogenic and likely pathogenic variants. Identification of these variants in breast and ovarian cancer allows for early detection and surgical decision, prediction of the response to PARP inhibitors, and allows genetic counselling.

Methodology: To determine the pathogenic/likely pathogenic (P/LP) variants prevalence in hereditary and sporadic breast-ovarian cancer cases, we performed a retrospective review of 2920 samples received at SYNLAB Porto. All samples were tested by a multigene panel, that included the following genes: *ATM, BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1, CHEK2*, *CTNNA1*, *EPCAM*, *MLH1*, *MSH2*, *MSH6, NBN*, *PALB2*, *PMS2*, *PTEN*, *RAD51C*, *RAD51D*, *STK11* and *TP53*. NGS was performed using a custom Twist kit (Twist Bioscience).

Results: Our study revealed 238 P/LP variants in all analysed genes (238/2920 = 8,2%). However, we noticed that in the subgroup with breast or ovarian cancer and family history of HBOC (n = 612) the prevalence is higher (9,4%), while in the subgroup with no family history (n = 2158) the prevalence is 7,6%. In the subgroup of healthy individuals with family history of breast and ovarian cancer (n = 150) only 5,3% had P/LP variants. Within the P/LP variants, 41% of the variants were identified in *BRCA1* and *BRCA2*.

Conclusion: This retrospective review supports the relevance of using multigene panels as a standard approach for breast and ovarian cancer patients. It was also important to highlight the genetic heterogeneity found in these patients, with the majority of P/LP variants detected in non-*BRCA1/2* genes.

C.O.I. Disclosure:

L. Castro: None. **J. Gonçalves:** None. **M. Cardoso:** None. **M. Dantas:** None. **C. Ferreira:** None. **C. Ferreira:** None. **A. Conceição:** None. **N. Salgueiro:** None. **M. Reis-Lima:** None.

EP01.073 High-throughput sequencing: a game changer in diagnosing hereditary colorectal cancer

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Background: Colorectal cancer (CRC) arises in 2% to 5% of cases in the setting of well defined inherited syndromes including Lynch syndrome (LS), familial adenomatous polyposis, MUTYH-associated polyposis, and certain hamartomatous polyposis conditions. We aimed to present our department's experience in diagnosing and managing hereditary CRC syndromes.

Material and methods: Our retrospective descriptive study included 10 patients referred to our oncogenetics consultation for suspected CRC predisposition. They underwent genetic investigation, clinical assessment and molecular analysis by gene panel sequencing.

Results: All patients came from unrelated families and three had consanguinous parents. Seven patients were referred for CRC (7/10), one patient for endometrial cancer (1/10), and one for cervical cancer (1/10). All patients had a significant family history of cancers. Molecular study identified pathogenic variants in several genes: in a heterozygous state in MLH1 (3/10), MSH2 (3/10) and APC (1/10) and in a homozygous state in MLH1 (1/10) and MUTYH (2/10). This confirmed LS in six patients (6/10), CMMRD in one patient (1/10) and MUTYH-associated polyposis in two patients (2/10). Genetic counseling was provided, and cascade screening was proposed for their relatives. This screening in the CMMRD patient enabled us to identify the same variant in a heterozygous state in his father, his mother, his brother, and unrelated sister-in-law. These individuals were therefore predisposed to developing cancers associated with LS, and appropriate management was initiated.

Conclusion: High-throughput sequencing has revolusionized CRC management. It facilitates rapid diagnosis, personalized risk assessment, guiding preventative measures and tailored treatments for patients and their families.

C.O.I. Disclosure:

S. Gabteni: None. **S. Ben Yahia:** None. **R. Meddeb:** None. **A. Achour:** None. **F. Akrout:** None. **K. Meddeb:** None. **Z. Sghayer:** None. **A. Ben Safta:** None. **A. Zanati:** None. **S. Bouchoucha:** None. **M. Trabelsi:** None. **R. Mrad:** None. **N. Belguith:** None.

EP01.074 The landscape of somatic variants in patients diagnosed with NSCLC: a public funded cancer program in Romania

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Background: Lung cancer is the leading cause of cancer death worldwide. Non-small cell lung cancer (NSCLC) is one of the major histological types of lung cancer and includes adenocarcinoma, squamous cell carcinoma, large cell carcinoma. Genomic alterations in the following genes: *EGFR, ALK, BRAF, ROS1, RET, NTRK1, NTRK2, NTRK3, MET, KRAS, ERBB2* are frequently found in NSCLC and can be targeted with approved therapies. In Romania the implementation of a public funded cancer program helped with the enrolment and mass genomic testing for patients with NSCLC.

Material and methods: Clinical targeted sequencing of 50 genes, using Oncomine Precision Assay Panel on Ion Torrent Genexus System, was performed with DNA and RNA extracted from formalin-fixed paraffin-embedded (FFPE) tumor tissue samples collected from 726 patients. Sequencing data was analyzed using Genexus Software and somatic variants were curated manually. Pathogenic and likely pathogenic somatic variants were reported according to guidelines.

Results: The analysis of 726 samples detected at least one somatic variant in 79.9% of the patients. The most frequent somatic variants were found in the actionable genes: *EGFR* (15.5%), *KRAS* G12C (12.6%), and *BRAF* V600E (2.2%); fusions (including one rare variant: *CCDDC6::RET*) were detected in 8.4% of the positive cases. *TP53* somatic variants were found in 44.3% of the cases and 3.5% of these had 2 pathogenic variants.

Conclusion: The detection of these alterations in actionable genes with targeted sequencing in NSCLC patients was possible due to the implementation of the program, which provides access to the latest treatments.

C.O.I. Disclosure:

G. Stancu: None. **A. Tecuceanu-Vulpe:** None. **M.A. Irofei:** None. **A.M. Brezeanu:** None. **M.I. Spirea:** None. **A.G. Matache:** None. **M.I. Bugeanu:** None. **C.M. Ardeleanu:** None.

EP01.075 Clinical significance of germline variants in BRCA2 gene and their association with prostate cancer risk in Polish man

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Background: Prostate cancer (PC) is a complex disease that affects millions of men globally. We tested the association of germline variants in BRCA2 with PC risk and estimated their impact on disease clinical course, including overall survival time in Polish men with localized prostate cancer qualified for radical treatment.

Material and methods: DNA from 97 men with PC from all over Poland and DNA from 100 men - volunteers, healthy at the time of the study. NGS, Sanger sequencing.

Results: The 5 variants of BRCA2 gene were detected in single PC patients. There were four substitutions (c.8010G>C, c.682-32A > G, c.9257-75G > C, c.516+17G > C) and one deletion (c.6393_6396del). Among detected variants, there were: 1 pathogenic, 1 variant of uncertain significance (VUS), and three likely benign. The c.8010G>C was a new variant of uncertain signification. In carrier of c.6393_6396del pathogenic variant, PC was diagnosed at T3 stage and the patient survived only 48 months after prostate cancer confirmation (the date of biopsy). Bioinformatic analysis of all changes was performed using Franklin database.

Conclusion: The presence of BRCA2 c.6393_6396del pathogenic variant was probably associated with clinical features of the disease (GS and TNM) and shorter survival of patient with localized prostate cancer qualified for radical treatment. We also suppose that multi-organ cancer aggregation in a family, including prostate cancer aggregation in close relatives, and young age at cancers onset should be taken under consideration by clinicians as an indication to refer patients to molecular testing.

Grant: This study was supported by the AKRS.480.3.511.2023 grant of the CM UMK.

C.O.I. Disclosure:

M. Heise: None. **P. Jarzemski:** None. **A. Bąk:** None. **O. Haus:** None.

EP01.076 Searching for germline mutations in the *RUNX1* gene in patients with myelodysplastic syndrome (MDS) and de novo acute myeloid leukemia (AML) - preliminary studies

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Background: Germline *RUNX1* mutations lead to familial platelet disorder with associated myeloid malignancy (FPDMM), characterized by thrombocytopenia, abnormal bleeding, and an elevated risk of developing myelodysplastic neoplasia (MDS) and acute myeloid leukemia (AML) at a young age. It is not known why or how germline carriers of *RUNX1* mutations have a particular propensity to develop myeloid hematologic malignancies, but it is known they can cause their development at an earlier age, confer a higher risk of relapse or predispose to secondary leukemias, including therapy-related leukemias.

Material and methods: 70 unrelated consecutive patients with MDS and 70 unrelated consecutive patients with de novo AML were enrolled in the study. We performed sequencing of bone marrow cells and buccal swabs of DNA to detect germline pathogenic mutations in the *RUNX1* gene.

Results: This study aimed to detect and compare the quality and frequency of mutations in the *RUNX1* gene in patients with MDS, AML, and the control group. In the investigated group, 31 variants in the *RUNX1* gene were detected. In MDS patients, we detected 2 pathogenic, 10 benign, and 2 VUS. In de novo AML patients, we detected 3 pathogenic, 10 benign variants, and 4 VUS. The variants identified in the study were missense, frameshift, silent, and intronic.

Conclusion: Finding MDS and AML susceptibility genes may help fully understand the pathogenesis of the disease and identify persons at high risk. The search for germline mutations in MDS and AML is also a very promising approach for discovering new treatment options.

C.O.I. Disclosure:

A. Bąk: None. **S. Potoczek:** None. **M. Czyżewska:** None. **A. Jaśkowiec:** None. **O. Haus:** None. **M. Pasińska:** None.

EP01.077 Composition of potentially significant gene variants in Ukrainian bladder cancer samples explored by cancer hotspot panel

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Background: Bladder cancer is distinguished by genomic heterogeneity and high tumour mutation burden. That gives potential for identification of gene variants that may serve as targeted therapies response predictors or markers for bladder cancer detection and surveillance. In this study, we have used 50 cancer-driving genes hotspot regions targeted next-generation sequencing (NGS) to identify potentially relevant variants.

Material and methods: The material for the study comprised 38 bladder cancer patients' tumour tissue, collected during surgical treatment. Ethics approval and informed consent were obtained. Genomic DNA was amplified using custom Ion AmpliSeq™ cancer hotspot panel. The DNA libraries were sequenced using an Ion S5 Plus sequencer. The results were then filtered using Franklin by Gennox to classify variants.

Results: A total of 30 significant variant were found in 9 genes. The largest number of mutations was identified in four genes: *TP53*, *FGFR3*, *PIK3CA* and *FRAF* (10, 7, 4 and 3, respectively). Two variants were identified in *ERBB2* and one variant in *AKT1*, *HRAS*, *KRAS* and *PTEN*. Four mutations were identified in more than one sample, *FGFR3* c.746C>G, *PIK3CA* c.1633G>A, *FGFR3* c.1118A>G and *BRAF* c.1406G>C (4, 3, 2 and 2, respectively). In addition, two novel mutations were identified, *ERBB2* c.2305G>T and *PTEN* c.759C>G.

Conclusion: Of the variants that have been identified, *FGFR3* mutations is thus far the only alteration for which a therapeutic function has been validated, with targeted agent erdafitinib. NGS cancer hotspot approach clinical efficacy was approximately 18,3%. These data might help guide targeted therapies selection for future clinical trials.

C.O.I. Disclosure:

B.O. Vladymyrets: None. **R.V. Gulkovskyi:** None. **A.M. Bezverkhiy:** None. **G.V. Gerashchenko:** None. **M.V. Tomiak:** None. **O.S. Mankovska:** None. **A.I. Honcharenko:** None. **V.O. Haviaz:** None. **O.A. Kononenko:** None. **Z.Y. Tkachuk:** None. **V.I. Kashuba:** None. **M.A. Tukalo:** None.

EP01.078 Utilizing a multi-method approach in France to identify obstacles and solutions for prevention in rare tumor risk syndromes (PREVENTABLE Project): The example of Li-Fraumeni syndrome

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Background: The European PREVENTABLE project focuses on addressing the underappreciation of prevention in healthcare, especially in rare tumour risk syndromes (RTRS). A large part of the project assesses the clinical impact of prevention, but the social and financial impacts are also studied.

Material and methods: Various methods were employed to gather data on the social and financial impacts of prevention for RTRS. These included interviews with geneticists and oncologists, and two anonymous surveys for patients and clinical teams to identify barriers and facilitators in implementing prevention pathways. Three roundtable discussions ("PREVENTALK") were held to foster collaboration between public authorities and academic institutions. Videos featuring RTRS patients and families offered personal insights.

Results: In France, 22 clinicians and 51 patients participated in surveys to identify the top barriers and facilitators to implementing prevention pathways for RTRS. The 2nd PREVENTALK took place in Rouen and featured key stakeholders like Sophie Deveaux (project manager, Institut National du Cancer), Dr Judith Balmaña (clinician, Vall d'Hebron Institute of Oncology), Pr Jean-Yves Blay (president of Unicancer), and Louis Charlet (financial officer, CHU Rouen), who discussed improvements in patient care, prevention, and policy recommendations. Additionally, the project promoted the translation of a children's book on Li-Fraumeni syndrome into French, enhancing awareness among affected families and the public.

Conclusion: The next step will be to organise 3 separate focus groups with patients, their social networks and clinicians. The results and awareness-raising material will be disseminated via communication tools (educational campaign, press releases, social media) to patients and stakeholders.

C.O.I. Disclosure:

M. Rolain: None. **P. Faure:** None. **M. Marques:** None. **F. Machado:** None. **M. Moreto:** None. **L. Mathey:** None. **R. Papp:** None. **D. Cviticanin:** None. **I. Lopes:** None. **S. Setiawan:** None. **B. Dhoro:** None. **M. Clément Le Choismier:** None. **M. Branchaud:** None. **N. Parodi:** None. **S. Baert-Desurmont:** None. **E. Kasper:** None. **G. Bougeard:** None. **M. Renaux-Petel:** None. **T. Vermeulin:** None. **R. Amorim:** None. **S.B. Pereira:** None. **L. Sousa:** None. **C. Oliveira:** None. **J. Théry:** None. **C. Houdayer:** None.

EP01.079 potential prognostic value and clinical significance of NRBP1 in glioma

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Background: Glioma is the most common primary intracranial malignant tumor. NRBP1 is a gene encoding protein containing 535 amino acids, which is regarded as pseudokinase. Previous studies have shown that there are differences in the expression of NRBP1 in breast cancer, bladder cancer, prostate cancer and rectal cancer, and the prognosis is related. But it has not been reported in detail in glioma.

Material and methods: In this study, 111 genes closely related to the prognosis of glioma patients were screened by bioinformatics analysis of China glioma genome map database (CGGA), and NRBP1 was selected as the target gene through literature retrieval, and survival analysis, clinical correlation analysis, GSEA enrichment analysis and immune cell infiltration analysis were carried out. Finally, NRBP1 was evaluated as a potential prognostic marker in glioma.

Results: There are differential expressions of NRBP1 in glioma, and the high expression is positively correlated with the poor prognosis of glioma patients. NRBP1 can be used as an independent prognostic factor in patients with glioma, and it has good predictive performance. NRBP1 is positively correlated with metabolism, P53 signaling pathway and the infiltration of immune cells such as dendritic cells.

Conclusion: NRBP1 can be used as a reliable marker for prognosis evaluation of glioma patients.NRBP1 is closely related to the infiltration of immune cells in glioma, and may play an important role in many biological pathways. This means that NRBP1 is a potential immune regulatory target, which provides new ideas and methods for the treatment of glioma.

C.O.I. Disclosure:

H. duan: None. **T. Duan:** None. **C. Hao:** None.

EP01.080 Exploring ER Stress-Related Genes in PDAC Under Gemcitabine Exposure

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Background: Pancreatic cancer is the fourth leading cause of cancer-related deaths globally, with pancreatic ductal adenocarcinoma (PDAC) being the most prevalent subtype, accounting for over 90% of cases. Prognosis remains poor due to early metastasis and late-stage diagnosis. Conventional chemotherapeutic regimens, primarily gemcitabine (GEM), show limited efficacy due to resistance mechanisms. Identifying novel biomarkers and molecular targets is critical for improving therapeutic outcomes. Disruption of endoplasmic reticulum (ER) homeostasis, leading to ER stress, offers a potential therapeutic avenue by triggering cell death.

Material and methods: This study investigates ER stress-related factors in PANC-1 cells, a PDAC model, under GEM treatment. Fold changes in expression (FC) of GADD45B, SERPINE1, OCT3/4, ATF4, and CHOP genes were analyzed via qRT-PCR in GEM-treated (GEM+) and untreated (GEM-) groups. GEM-treated cells were further stratified using CD24/CD44 surface markers.

Results: GADD45B was upregulated (FC-GEM + = 1.34, FC-CD24 + /CD44 + = 1.8; $p = 0.04$). ATF4 was downregulated (FC-GEM + : 2.33 and FC- CD24 + /CD44 + = 1.33; $p = 0.004$). Cohen's d values showed large effects for ATF4 and CHOP ($d = -3.37$ and -3.92) and significant increases for and GADD45B and SERPINE1 ($d = 2.34$ and 2.58). Other genes showed more moderate effects.

Conclusion: These results highlight the potential roler of GADD45B and SERPINE in chemoresistance and suggest ATF4 and CHOP involvement in stress adaptation. This study underscores the importance of ER stress modulation in PDAC and identifies and potential biomarkers for improving therapeutic strategies.

C.O.I. Disclosure:

N. Ferhadi: None. **M. Poda:** None. **I. Sangu:** None. **D. Semra:** None.

EP01.081 Search for mutations in the PIK3CA gene using digital PCR in breast cancer

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Background: Breast cancer is one of the most common oncological diseases among women. Somatic mutations play a key role in the pathogenesis of cancer and influence therapeutic approaches. Liquid biopsy, a minimally invasive method, is used for analyzing circulating tumor cells and cell-free tumor DNA (cfDNA). Droplet digital PCR (ddPCR) is a highly sensitive and specific method for detecting cfDNA at low concentrations.

Material and methods: Blood and tumor tissue samples were collected from 30 patients with early-stage luminal breast cancer undergoing neoadjuvant/adjuvant chemotherapy with follow up over 18 months (6 points of collection). The study design included two phases: (1) identification of somatic mutations in tumor tissue samples using NGS and (2) detection of mutations in liquid biopsy samples using ddPCR.

Results: Of the 146 samples 13 (9%) showed the PIK3CA mutation p.Glu542Lys. The main points of mutation detection were points 1 and 2: before the start of neo/adjuvant therapy and after the first treatment phase. Mutation PIK3CA: p.Glu542Lys were detected in cfDNA samples with high specificity. The ddPCR platform demonstrated reliable amplification with minimal false positive results. The findings indicate the potential of this method for early detection and monitoring of therapy in breast cancer.

Conclusion: The ddPCR method offers a promising approach for somatic mutation analysis in liquid biopsy, enabling personalized therapy and reducing reliance on invasive procedures. Further clinical studies are need to validate its routine use in diagnostics and treatment.

C.O.I. Disclosure:

A.A. Golodushkin: None. **I.S. Abramov:** None. **M.S. Blinova:** None. **I.V. Baratova:** None. **A.S. Chegodar:** None. **L.G. Zhukova:** None. **T.M. Ibragimova:** None. **D.A. Filonenko:** None. **N.A. Bodunova:** None.

EP01.082 The neurofibromin-lysosome axis in breast cancer: implications for mTOR regulation and tumor progression

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Background: Neurofibromin (NF1) loss-of-function mutations are linked to poor breast cancer prognosis. NF1 loss leads to constitutive mTORC1 activation via Ras-MAPK signaling. Moreover NF1 interacts with LAMTOR1, a key lysosomal protein for mTORC1 activation. Alterations in lysosomal function and spatial distribution are closely associated with cancer progression, and TFEB (Transcription Factor EB) plays a pro-tumorigenic role in breast cancer by enhancing lysosomal function and autophagy.

Material and methods: We generated NF1-KO breast cancer cell lines (SKBR3, BT474, HCC1954) using CRISPR-Cas and analyzed mTOR signaling, lysosomal phenotype, and TFEB nuclear translocation using high-content imaging and biochemical approaches.

Results: In all three breast cancer cell lines, mTOR activity remains partially active under nutrient deprivation and increases

upon nutrient refeeding. Instead, NF1 depletion causes a further increase of the mTORC1 activity in both conditions. Additionally, NF1-KO cells show increased and condensed lysosomes compared to controls, but TFEB translocation rates remain unchanged, suggesting an NF1-TFEB independent regulation of mTOR and lysosomes.

Conclusion: NF1 loss leads to persistent mTOR activation and lysosomal alterations in breast cancer, and our preliminary experiments suggest that it acts independently of TFEB. Understanding NF1's role in lysosomal metabolism and mTOR regulation may provide new insights for cancer progression and therapeutic strategies.

C.O.I. Disclosure:

A. Delicato: None. **P. Chacon-Millan:** None. **B. Achutti-Duso:** None. **A. Castiglioni:** None. **E. Messuti:** None. **S. Galavotti:** None. **P. Pelicci:** None. **D.L. Medina:** None. **L. Mazzarella:** None. **M. Morleo:** None.

EP01.083 Clinical implementation of genome-wide DNA methylation profiling for pediatric CNS tumors, insights from a Polish population-based study

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Background: DNA methylation profiling has emerged as a crucial molecular diagnostic tool for classifying central nervous system (CNS) tumors. It complements, supports and refines traditional histopathological assessments, particularly in cases with ambiguous features, by offering high-resolution molecular classification. This study evaluates the clinical utility of DNA methylation profiling in pediatric CNS tumor diagnostics, assessing its impact on diagnosis, classification refinement, and treatment strategies.

Material and methods: A cohort of 120 pediatric patients with CNS tumors underwent prospective genome-wide DNA methylation profiling using high-throughput platforms. Tumor samples were analyzed using the Heidelberg classifier and t-distributed stochastic neighbor embedding (t-SNE) clustering. Methylation-based classification results were compared with initial histopathological diagnoses to assess concordance and clinical implications.

Results: DNA methylation profiling confirmed the initial histopathological diagnosis in 17.5% of cases. It refined (45,8%), or changed (18,3%) the classification in 64,2% of cases. In 18.3% of cases, despite the use of methylation profile assessment, the diagnosis could not be established. Cases with uncertain histological classification benefited from improved stratification with tailored treatment approaches. Furthermore, methylation profiling enabled the identification of novel or unrecognised tumour subtypes.

Conclusion: Integrating DNA methylation profiling into routine work-up significantly improves the accuracy of CNS tumor classification, facilitating precise molecular subtyping and personalized treatment decisions. This study underscores the necessity of implementation of methylation-based diagnostics as a frontline tool in neuro-oncology, particularly for complex and diagnostically challenging cases.

KK, EN and JT contributed equally to this work.

C.O.I. Disclosure:

K. Kwaśniak: None. **E. Niecikowska:** None. **W. Grajkowska:** None. **M.K. Ciołkowski:** None. **P. Kowalczyk:** None. **O. Rutynowska-Pronicka:** None. **B. Dembowska-Bagińska:** None. **J. Trubicka:** None.

EP01.084 Comprehensive genomic profiling of pediatric gliomas for a precision medicine

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Background: Gliomas are the most common type of pediatric central nervous system tumors. They consist of WHO histological grade 1–2, low-grade gliomas (pLGG) and grade 3–4, high-grade gliomas (pHGG). In general, pLGGs have more than 90% overall survival rate; on the contrary, pHGGs have less than 10% long-term survival rate, Among pLGGs, the most common alterations are BRAF alterations, followed by FGFR1 lesions, whereas in pHGGs, TP53 and H3F3A mutations dominated a more heterogeneous genomic landscape.

Material and methods: we performed a comprehensive genomic profiling of 74 pediatric gliomas (44 pLGG and 30 pHGG) using the Oncomine Comprehensive Assay Plus panel (OCA Plus, ThermoFisher Scientific), a 500+ gene targeted oncology research panel, that evaluates DNA variants, RNA fusions and other biomarkers such as the tumor mutational burden (TMB), microsatellite instability (MSI), and homologous recombination repair deficiency (HRD) and genomic instability (GM).

Results: 89% of gliomas had at least 1 mutation of Tier I class (variants predictive of response to therapies or variants with predictive, prognostic or diagnostic value); 8 tumors were found with high TMB; all tumors were MSS stable and had a low GIM. A rare driver fusion of FGFR1 (FGFR1/TACC) was detected in one pLGG. 55% of pHGG harbored a pathogenic mutation of TP53. All BRAF alterations,, that are the most common driver mutations of pediatric gliomas, previously identified with gold standard methods, were captured by OCA plus panel, demonstrating high sensitivity.

Conclusion: This study provide valuable insights into the molecular and genetic features of pediatric gliomas.

C.O.I. Disclosure:

P. De Marco: None. **M. Di Duca:** None. **M. Ognibene:** None. **K. Mazzocco:** None. **V. Vellone:** None. **F. Zara:** None. **G. Gaggero:** None. **C. Milanaccio:** None.

EP01.085 Investigation of germline variants in patients with luminal A and triple-negative breast cancer

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Background: Pathogenic and likely pathogenic germline variants in genes associated with breast cancer (BC) play an important role in the development, progression, and response to therapy. Therefore, the identification of these variants can provide valuable insights into the mechanisms of BC pathogenesis and improve early disease detection, treatment strategies, and patient outcomes.

Material and methods: Whole-exome sequencing (WES) was performed on 52 women diagnosed with luminal A or triple-negative BC at the Hospital of Lithuanian University of Health Sciences Kaunas Clinics. The study used genomic DNA extracted from peripheral blood samples. WES data were analyzed using the Franklin platform by Genoox. Pathogenic and likely pathogenic variants were assessed in 81 genes, as defined by the ACMG Secondary Findings V3.2 (2023), and in 18 genes associated with BC.

Results: A total of ten pathogenic and four likely pathogenic germline variants were identified in the study group. Of these, five variants were detected in the *BRCA1* gene, three in the *BRCA2* and *CHECK2* genes, and one each in the *NBN*, *ATM*, and *MUTYH* genes. Overall, all variants were found in 19 patients, each of whom had only one clinically significant variant. Nine different variants were identified in patients with luminal A breast cancer, and four - in triple-negative breast cancer. Only one pathogenetic variant was identified in both groups.

Conclusion: In conclusion, 14 clinically significant germline variants were identified in patients with luminal A or triple-negative breast cancer that may potentially affect tumor pathogenesis.

C.O.I. Disclosure:

J. Jurevice: None. **A. Bartnykaite:** None. **E. Korobeinikova:** None. **J. Gudaitiene:** None. **R. Ugenskiene:** None. **E. Juozaityte:** None.

EP01.086 Application of comprehensive genomic profiling in breast cancer

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Background: Somatic profiling of malignant tumors allows to personalize patient treatment, provides monitoring and prediction of disease progression.

Material and methods: We analyzed 30 paired DNA samples isolated from initial tumor biopsies and germline DNA from patients with luminal A/B BC. DNA libraries were performed using the SENTIS Pan-Cancer+Discovery panel (BGI). The panel offers 816 cancer-related genes and interrogates the most common types of alterations, including SNVs, indels, CNVs and fusions in solid tumors. NGS was performed on the DNBSEQ-G400 (MGI).

Results: 33 altered genes were identified. 86 clinically significant variants in 30 patients were detected. 23 pathogenic variants of the nucleotide sequence in 19 patients (63%) in genes *AKT1*, *CDH1*, *GATA3*, *HNF1A*, *HRAS*, *PIK3CA*, *PTEN*, *RB1* and *TP53* were found. Three patients showed a combination of two mutations previously described as pathogenic: rs1962456814 *CDH1* and rs104886003 *PIK3CA*; rs1593060859 *HNF1A* and rs121913301 *RB1*; rs121913343 *TP53* and rs104894230 *HRAS*. A combination of 3 pathogenic variants: c.2299-2A > G *CTNNA1*, rs1593060859 *HNF1A* and rs121913301 *RB1* - and >5 likely pathogenic variants were found in 1 (3%) patient. Likely pathogenic variants were also identified: *GATA3* - 8 (17%); *KMT2C* - 3 (6%); *MAP3K1* - 3 (6%); *TP53*-3 (6%); *RUNX1* - 2 (4%).

Conclusion: Potential targets for therapy (mutations in *PIK3CA*, *AKT*, *PTEN*) were identified in 12 (40%) patients. Despite the high cost, CGP with NGS can help to better understand the biology of BC, identify new biomarkers and developing of new drugs.

C.O.I. Disclosure:

I. Abramov: None. **A. Danishevich:** None. **S. Nikolaev:** None. **A. Osipova:** None. **I. Baratova:** None. **D. Mineeva:** None. **A. Chegodar:** None. **M. Blinova:** None. **T. Ibragimova:** None. **D. Filonenko:** None. **N. Bodunova:** None. **L. Zhukova:** None.

EP01.087 The frequency of gene fusions of diagnostic and therapeutic importance in solid tumors

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Background: Gene fusions represent an important class of somatic alterations in malignant tumors, playing a critical role in oncogenesis. Some are used in routine therapeutic procedures as prognostic and diagnostic factors and, most importantly, as targets in molecularly targeted therapies. The study aimed to analyze the frequency of gene fusions with documented and potential importance in personalized medicine in solid tumors.

Material and methods: The tested material was collected from 453 patients (487 samples in total), diagnosed and/or treated at the MSC National Research Institute, Gliwice Branch. RNA was isolated using Maxwell RSC RNA FFPE or Maxwell RSC miRNA Tissue Kits (Promega). Fusion detection was performed by next-generation sequencing using the FusionPlex CTL and Sarcoma v2 kit (Archer), and the obtained data were analyzed with Archer Analysis, STAR, STARFusion, fgbio, and VarDictJava software.

Results: Data analysis was available for 77% of the studied samples (374/487). Gene fusions were detected in material from 19 patients with the following diagnoses: thyroid cancer, lung cancer, brain tumor, intrahepatic bile duct cancer, prostate cancer, malignant tumor of the parotid gland, myxoid chondrosarcoma. The following gene fusions were detected: *IRF2BP2::NTRK1*, *SQSTM1::NTRK3*, *CCDC6::RET*, *MKRN1::BRAF*, *ETV6::NTRK3*, *KIAA1549::BRAF*, *NCOA4::RET*, *KIF5B::MET*, *LUC7L2::BRAF*, *SRGAP3::RAF1*, *FGFR2::UFD1*, *EML4::ALK* and *EWSR1::NR4A3*. *BRAF* gene fusions were most frequently observed, accounting for 32% of all detected fusions.

Conclusion: Although the overall gene fusion frequency in solid tumors is not high, detected fusions are targets for molecularly targeted therapies, are part of clinical trials, and/or essential for differential diagnosis of cancer.

C.O.I. Disclosure:

D. Rusinek: None. **M. Kowalska:** None. **A. Pfeifer:** None. **M. Cieslicka:** None. **P. Tudrej:** None. **T. Tyszkiewicz:** None. **A. Zajkowicz:** None. **A. Pawlaczek:** None. **E. Chmielik:** None. **W. Bal:** None. **E. Nowicka:** None. **J. Krajewska:** None. **R. Suwinski:** None. **R. Cyplinska:** None. **M. Oczko-Wojciechowska:** None.

EP01.088 Biogenic Silver Nanoparticles: A Novel Anticancer Approach Against Colorectal Cancer

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Background: Using a green synthesis approach, biosynthesized silver nanoparticles (A-AgNPs) from aronia extract and assessed their cytotoxic and apoptotic effects on HT29 and Caco-2 colorectal cancer cell lines.

Material and methods: The characterisation of nanoparticles synthesised from aronia extract was carried out by various analyses. MTT assay was used to evaluate the cytotoxic effects of A-AgNPs and extract. Annexin V-FITC staining was performed to assess apoptotic effects.

Results: A distinct color change from light pink to dark brown marked the successful formation of nanoparticles, as verified by UV-Vis spectroscopy, which displayed a strong absorption peak at 427 nm. The structural evaluation performed by TEM analysis demostrated that A-AgNPs had an irregular spherical structure and the particle sizes ranged from 14.95 to 28.30 nm. The presence of elemental silver was corroborated by EDX spectroscopy, which identified a distinct peak at 3 keV. The IC_{50} values indicated that A-AgNPs showed a dose-dependent cytotoxic effect on HT29 and Caco-2 cells, 5.59 and 3.09 µg/mL, respectively. These suggest that A-AgNPs possess a strong antiproliferative activity against cancer cells. A-AgNPs significantly increased early apoptotic cell populations in both cell lines. In HT29 cells, apoptosis was accompanied by DNA damage, confirmed by elevated levels of p-H2AX protein. Aronia extract, however, exhibited a milder apoptotic effect and did not increase DNA damage.

Conclusion: A-AgNPs synthesized via the green method demonstrated strong antiproliferative and apoptotic effects on colorectal cancer cells. The findings suggest that A-AgNPs could serve as a potential anticancer agent, highlighting their therapeutic potential in cancer treatment.

C.O.I. Disclosure:

S. Yesilot: None. **D. Satana:** None. **S. Pehlivanoglu:** None. **S. Celik:** None. **C. Aydin Acar:** None.

EP01.089 Analysis of Macrophage Spatial Distribution and Tumor Microenvironment in Esophageal Squamous Cell Carcinoma Using Stereo-ClTE-seq

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Background: Esophageal cancer ranks as the sixth leading cause of cancer-related mortality worldwide, with 90% diagnosed as esophageal squamous cell carcinoma (ESCC). The tumor microenvironment (TME) in esophageal cancer is crucially linked to treatment outcomes and patient survival. Macrophages, key immune cells within the TME, are classified into M1 and M2 phenotypes. M1 macrophages are involved in antitumor activity, while M2 macrophages contribute to tumor progression. High densities of tumor-associated macrophages (TAMs) in esophageal cancer correlate with shorter survival. This study employs spatial multi-omics (Stereo-CITE-seq) to investigate macrophage phenotypes in the ESCC tumor invasive margin.

Material and methods: We developed the Stereo-CITE-seq workflow by integrating CITE-seq and Stereo-seq technologies, capturing antibody-derived tags and mRNAs on the same tissue section. We analyzed one ESCC sample and validated findings in three additional samples using Stereo-seq V1.3, employing tools like Scanpy, Stereopy, and Seurat for cell type identification and distribution analysis.

Results: Spatial multi-omics identified macrophages at the tumor invasive margin, revealing three subpopulations: CD86 + CD163+ (tumor core), CD86 + CD163- (tumor edge), and CD86-CD163+ (tumor periphery). High PD-L1 expression was detected at the invasive margin, correlating with CD86+ expression and T cell exhaustion markers. Pathway analysis showed enrichment in immune regulation and antigen presentation pathways, indicating macrophage subtypes influence TME and immune suppression.

Conclusion: Stereo-CITE-seq reveals spatial distribution of macrophage phenotypes at the ESCC invasive margin, highlighting their role in TME and immune suppression. These findings suggest TAMs may serve as potential prognostic indicators and therapeutic targets for ESCC.

C.O.I. Disclosure:

J. Yi: None. **X. Shentu:** None. **Z. Li:** None. **Y. Heng:** None. **X. Xu:** None. **X. Feng:** None. **T. Chen:** None. **D. Jia:** None. **W. Guan:** None. **S. Liao:** None. **A. Chen:** None. **J. Li:** None.

EP01.090 Genomic profiling of triple-negative breast cancer to predict response to neoadjuvant therapy

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Background: The variability of pathological complete response (pCR) rates after neoadjuvant chemotherapy (NACT) in non-metastatic triple-negative breast cancer (TNBC) and the challenge of treating residual disease highlights the need for improved therapeutic strategies. This study utilized FoundationOne®CDx (F1CDx) genetic testing to predict TNBC response to NACT.

Material and methods: Genomic profiling with NGS-based F1CDx was performed on diagnostic biopsies (N = 93) of patients with stage II-III TNBC who were subsequently treated with NACT. Ordinal logistic regression was applied for multifactorial data analysis. The centrality analysis was conducted using STRING, while pathway enrichment analysis was performed with PANTHER.

Results: Mutations of 134 genes of the F1CDx were detected in 93 TNBC tumors. Structural variants (SVs) were the major mutation type, accounting for 62% of all mutations registered. Amplifications predominated, accounting for 81% of SVs. Large amplicons, including 8p11.23, 8q24, 11q.13, 9p24.1, 12p.13, markedly influenced the mutation profile. A key network node, mutated TP53, was detected in 95% of tumors. Of the 23 *BRCA1* or *BRCA2* mutations detected in the tumor cells, 15 cases were confirmed as germline. The multivariate analysis confirmed *BRCA*1/2 mutation as a significant predictor of favorable NACT response, while CCNDs co-mutations with FGFs predicted poor NACT response.

Conclusion: The identification of frequent co-amplification loci in TNBC highlights their potential role in aggressive phenotype development and therapy resistance. Our study emphasizes the value of early comprehensive genetic testing in understanding

TNBC biology and highlights the potential of F1CDx in predicting NACT response in TNBC.

C.O.I. Disclosure:

S. Jarmalaite: None. **M. Drobniene:** None. **D. Breimelyte:** None. **I. Sadzeviciene:** None. **R. Valkiuniene:** None. **R. Meskauskas:** None. **D. Dabkeviciene:** None.

EP01.091 Patients with multiple pathogenic variants in hereditary cancer genes

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Background: Next-generation sequencing (NGS) has improved genetic diagnosis in hereditary cancer, allowing the identification of multiple genes altered in the same individual and opportunistic testing of other cancer-related genes.

Material and methods: NGS cancer panel has been used for genetic testing in clinical practice in our Hospital since 2016. Personal and family cancer history is used to select the virtual gene panel to be analyzed in each patient. For all patients, opportunistic testing of *BRCA1, BRCA2, MLH1, MSH2*, and *MSH6* is offered when not included in the specific virtual gene panel. Those probands in which two pathogenic or likely pathogenic variants (PV) in different genes were selected for this study. Homozygous or compound heterozygous patients (i.e. *MUTYH, CHEK2*) were not considered for this study.

Results: Of the more than 6000 probands tested from 2016 to 2024 in our Hospital, 15 carried two PV in different genes (Table 1). Half of them carried PV in genes related to the same cancer syndrome, while the other half carried PV in genes related to different cancer syndromes.

Conclusion: The identification of double PV carriers represents a small percentage of cases, but genetic counseling and cascade screening is extremely important in those families, to offer a personalized follow-up. In families with a known PV, upon the diagnosis of new cancers that could lead to suspicion of another cancer syndrome, additional genetic testing should be recommended.

Table 1. Double pathogenic variant carriers

Patient	Personal and/or Family Cancer history	Variant 1	Variant 2
P_01	Basal cell carcinoma (Gorlin syndrome), breast and brain cancers	*SUFU* DEL E3-12	*ATM* c.2098C>T p.(Gln700Ter)
P_02	Melanoma, ovarian and breast cancers	*CDKN2A* c.301G>T p.(Gly101Trp)	*ATM* c.8981_8987+4del
P_03	Breast cancer	*BRCA2* c.3264dupT p.(Gln1089SerfsTer10)	*MSH6* c.3725G>A p.(Arg1242His)
P_04	Breast, pancreas and prostate cancers	*PALB2* c.2964delA p.(Val989Ter)	*ATM* c.802C>T p.(Gln268Ter)
P_05	Breast cancer	*PALB2* c.757_758delCT p.(Leu253IlefsTer3)	*BARD1* DEL E5-E6
P_06	Breast cancer	*PALB2* c.1240C>T p.(Arg414Ter)	*CHEK2* c.1100delC p.(Thr367MetfsTer15)
P_07	Melanoma, ovarian and breast cancer	*BARD1* c.1061C>A p.(Ser354Ter)	*MITF* c.952G>A p.(Glu318Lys)
P_08	Ovarian cancer	*RAD51D* c.1A>T p.(?)	*MLH1* c.731G>A p.(Gly244Asp)
P_09	Ovarian cancer	*RAD51D* c.901C>T p.(Gln301Ter)	*CHEK2* c.846+4_846+7delAGTA
P_10	Breast cancer	*PALB2* c.196C>T p.(Gln66Ter)	*MLH1* c.306+5G > A
P_11	Breast and ovarian cancers	*BRCA1* c.5054C>T p.(Thr1685Ile)	*BRCA2* c.6275_6276delTT p.(Leu2092ProfsTer7)
P_12	Breast and ovarian cancers	*BRCA2* DUP E17-E18	*CHEK2* c.1100delC p.(Thr367MetfsTer15)
P_13	Breast cancer	*PALB2* DEL E1-E10	*CHEK2* c.349A>G p.(Arg117Gly)
P_14	Breast cancer and melanoma	*BRCA1* c.5123C>A p.(Ala1708Glu)	*BRCA2* c.3264dup p.(Gln1089SerfsTer10)
P_15	Colorectal and ovarian cancers	*APC* c.3747C>A (p.Cys1249Ter)	*PMS2* c.2276-113_2245+159del

C.O.I. Disclosure:

M. Potrony: None. **M.I. Alvarez-Mora:** None. **L. Moreno:** None. **E. Grau:** None. **J.L. Villanueva-Cañas:** None. **A. Montalban:** None. **E. Verdura:** None. **C. Arnau-Collell:** None. **T. Ramon y Cajal:** None. **P. Aguilera:** None. **J.A. Puig-Butillé:** None. **J. Oriola:** None. **A. Sanchez:** None. **C. Badenas:** None.

EP01.092 Integrated genomic and transcriptomic analysis reveals a 15-gene signature for breast cancer diagnosis and prognosis

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Introduction: Breast cancer (BC) is the most common malignancy among women and a leading cause of morbidity and mortality. Its incidence is rising sharply, particularly among young females, often with aggressive disease presentation. Identifying robust biomarkers for early diagnosis and prognostic estimation is crucial to improving patient survival and reducing the disease burden.

Material and methods: We performed an integrated analysis of global transcriptomic profiles and copy number alterations (CNAs) in Middle Eastern women with BC. The identified gene panel was validated using independent datasets comprising over 4,000 BC patients with detailed clinical information. Machine learning models and survival analyses were performed to evaluate its diagnostic and prognostic potential. Functional enrichment, gene interaction networks, and pathway analyses were performed.

Results: Our multi-omics analysis identified a 15-gene signature. The SVM classifier achieved over 93% accuracy in distinguishing BC patients from healthy controls. The risk score derived from the 15-gene signature demonstrated a strong association with poor disease outcomes across over 4,000 BC patients and was

significantly higher in ER-negative and high-grade tumors. In multivariate analysis, the risk score remained an independent predictor of disease outcome, regardless of clinicopathological factors such as age, tumor grade, lymph node status, ER status, and tumor size ($p < 0.0001$).

Conclusion: Our study presents a 15-gene signature with high diagnostic accuracy and prognostic value. These findings support its potential as a gene panel for early detection and risk stratification, may lead to personalized treatment strategies and improved clinical outcomes.

C.O.I. Disclosure:

D. Colak: None. **I.H. Kaya:** None. **O. Al-Harazi:** None. **A.S. AlZahrani:** None. **N. Kaya:** None.

EP01.093 3D models of intratumor mutational heterogeneity of precancerous colorectal lesions as a possible tool for studying the development of colorectal cancer

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Background: Considering the heterogeneous clinical manifestation of colorectal cancer (CRC), intratumor mutational heterogeneity (ITH) of premalignant colorectal lesions is a promising area of research aimed at better understanding of how CRC originates and develops, in order to optimize its diagnosis and/or therapy. In this work, we present a unique set of 44 large adenomas in 3D models illustrating their mutational heterogeneity.

Material and methods: ITH in 44 precancerous colorectal lesions > 10 mm obtained during screening colonoscopy from 36 patients was studied. The lesions were cut into 3–8 parts and embedded in paraffin. Each part was further divided into samples corresponding to a 5 mm^3 of tissue. DNA was isolated from each sample followed by analysis of 8 mutation hotspot exons in 5 genes (KRAS, BRAF, TP53, APC, PIK3CA). The resulting data were processed into 3D models reflecting the occurrence and amount of individual mutant clones.

Results: 3D models of 4 hyperplastic polyps, 15 serrated adenomas, 5 tubular adenomas, 16 tubulovillous adenomas and 4 adenomas with intramucosal carcinoma structures were constructed. The highest degree of IHT showed serrated lesions, especially those in advanced stages (2 to 5 mutational clones). IHT was seen not only in terms of the type and number of different mutations, but also in the spatial representation within the lesion and the fraction of mutated alleles.

Conclusion: IHT models of precancerous lesions can be a useful tool in studying the process of CRC development.

C.O.I. Disclosure:

T. Halkova: None. **K. Blechova:** None. **S. Suchanek:** None. **E. Traboulsi:** None. **T. Grega:** None. **M. Minarik:** None. **J. Bures:** None. **K. Hejcmanova:** None. **O. Ngo:** None. **O. Majek:** None. **M. Zavoral:** None. **L. Benesova:** None.

EP01.094 Rearrangement of *EPS15::KLF2* in childhood acute lymphoblastic leukaemia

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Background: Recent genomic studies have identified a wide range of novel alterations that have substantially increased our knowledge of the biology of acute lymphoblastic leukemia (ALL) and defined new subtypes with prognostic and therapeutic relevance. Next-generation sequencing approaches have identified new cryptic fusions have been described, such as the *ATP5L::KMT2A* fusion, described by Gestrich et al. Similarly, we found *EPS15::KLF2* fusion in 16 of 139 ALL cases that we examined with targeted NGS. This unexpected high frequency of *EPS15::KLF2* prompted us to extend the evaluation of these 16 ALL cases for other molecular and clinical findings.

Material method: Total amount of 50–200 ng RNA was used as an input material. Sequencing libraries were generated using targeted NGS assay The Archer® FusionPlex® ALL Panel kit following the manufacturer's recommendations to detect fusions, splice variants, SNVs. After library preparation, appropriate volume of pool was loaded into Illumina MiSeq platform. ArcherDX, Version 7.2.1-1 platform was used to streamline the detection, analysis, and interpretation of genomic variants.

Results: All sixteen samples, aged 1–18 years, showed the *EPS15* exon1- *KLF2* intron2 rearrangement. Seven cases had known driver mutations and four cases had different genetic changes. (Table 1)

Conclusion: The *EPS15::KLF2*, which is probably resulted from cryptic deletion in 19p13.11, has not been previously reported to be associated with ALL. However, since *KLF2* has been shown to be necessary for T cell trafficking, further investigation in large patient series is needed to elucidate its effect on leukemogenesis.

Case	Age	Gender	*EPS15::KLF2* VF	Driver mutation	Driver mutation VF	Other genetic alteration (OGA)	OGA VF
1	15	F	66.67%	IKZF1 exon skipping	24.47%		
2	2	M	45%	CRLF2:c.695T>G	68.78%	JAK2:c.2049A>C	13.19%
3	14	M	70.59%				
4	1	M	83.33%	PAX5::JAK2	35.26%		
5	5	F	60%			NRAS c.34G>A	39.08%
6	2	M	58.82%				
7	17	M	87.50%			NRAS c.35G>C	10.01%
8	18	M	59.09%				
9	13	M	53.85%	ETV6::RUNX1	3.92%		
10	6	M	56.25%				
11	4	M	57.69%	KMT2A::MLLT1	50.16%		
12	3	F	19.12%	ETV6::RUNX1	46.83%		
13	12	F	43.75%				
14	11	F	66.70%				
15	7	F	69.57%	ETV6::RUNX1	35.83%		
16	4	F	21.88%			PTPN11:c.1508G>C	38.07%

C.O.I. Disclosure:

I. Akin: None. **B. Baysefer Çelik:** None. **M. Sezgin Evim:** None. **Y. Düzenli Kar:** None. **Y. Kizilirmak:** None. **M. Sonmez:** None. **U.C. Mete:** None. **A. Kaynak:** None. **G. Giran:** None. **A.M. Güneş:** None. **A. Tükün:** None.

EP01.095 Combined effects of KRAS-G12V and KRAS-LCS6 alterations on tumor progression and metastasis in HNSCC using a xenograft mouse model

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Background: Head and neck cancer (HNC) is the seventh most common cancer worldwide, with KRAS mutations playing a key role. The G12V mutation of *KRAS* is one of the most commonly identified and has been associated with tumor progression and aggressive disease. Additionally, T > G alteration (rs61764370) in LCS6 (let-7 complementary site 6) in the *KRAS*-3'UTR, which is the binding site of miRNA let-7, has been found to promote cancer development, metastasis, and treatment resistance.

Material and methods: Laryngeal cancer HEp2 cells, transfected with plasmid constructs carrying the *KRAS*-G12V mutation and the combined G12V + LCS6 mutations were transplanted subcutaneously (SC) or intraperitoneally (IP) into CD-1 nude mice with non-transfected control cells. The mice were euthanized on the 40th day post-transplantation and examined macroscopically and histopathologically.

Results: Tumors developed in all cell-transplanted mice. The G12V mutation significantly promoted tumor growth ($p < 0.001$), while G12V + LCS6 further accelerated progression ($p < 0.0001$). No metastases occurred in SC-injected mice, but IP-injected mice with KRAS mutations developed metastases. G12V alone led to liver metastases (50%), whereas G12V + LCS6 resulted in metastases in the liver (100%), lung (75%), kidney (25%), and paraaortic lymph nodes (75%). No brain metastases were observed in any group.

Conclusion: This study shows that while the KRAS-G12V mutation enhances HNSCC tumor growth and metastasis at relatively low rates, its combination with the LCS6 alteration significantly accelerates both. These findings suggest that KRAS-3'UTR mutations contribute to cancer aggressiveness and could serve as potential therapeutic targets.*This research was partially supported by TUBİTAK (project no 112S498).

C.O.I. Disclosure:

H. Acar: None. **M.B. Kaplan:** None. **V.B. Yenigün:** None. **Y.S. Acar:** None. **K. Ozturk:** None.

EP01.096 Identification of an immune-related breast cancer associated DNA methylation profile, using machine learning

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Background: There is currently a need for precise biomarkers for the early and non-invasive detection of breast cancer. The aim of this study was to identify blood-based DNA methylation biomarkers associated with breast cancer.

Material and methods: Using germline DNA from peripheral blood samples, we performed DNA methylation profiling using the Infinium MethylationEPIC array on 524 Asian individuals (256 breast cancer patients and 268 age- and ethnicity-matched healthy controls). Feature selection was applied to 649,688 CpG sites in the training set. The selected CpGs were used to train machine learning models. The performance of the predictive models was evaluated on an independent test set. Additionally, enrichment analysis was done to identify transcription factors binding to regions associated with the selected CpG sites, as well as pathway analysis for genes located nearby.

Results: A 51-CpG methylation profile was identified that effectively distinguishes breast cancer patients from healthy controls, achieving an AUC of 0.823 on an independent test set. Importantly, this profile outperformed all four previously reported breast cancer-associated methylation profiles. Enrichment analysis revealed the enrichment of genomic loci associated with the binding of immune modulating AP-1 transcription factors. Pathway analysis of nearby genes showed an overrepresentation of immune-related pathways associated with IL-12, IL-21, T helper 17 cell lineage commitment, and natural killer cell activation.

Conclusion: Through a machine learning approach, we have identified an immune-related breast cancer-associated methylation profile which can potentially be used for early breast cancer detection.

C.O.I. Disclosure:

N. Lee: None. **M. Hum:** None. **G. Tan:** None. **P. Ong:** None. **P.T. Kin:** None. **C. Lim:** None. **J. Samol:** None. **N.T. Tan:** None. **H. Law:** None. **M. Tan:** A. Employment (full or part-time); Significant; Lucence Diagnostics. **S. Lee:** None. **P. Ang:** None. **A. Lee:** None.

EP01.097 Aberrant DNA methylation of *BRCA1* and *RAD51C* in ovarian tumors and blood of cancer patients

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Background: In addition to genetic variants, promoter hypermethylation (PHM) — both constitutional and acquired somatic — represents an alternative mechanism for transcriptional silencing and gene inactivation. We examined the prevalence of PHM in genes associated with genetic predisposition to ovarian cancer (OC) to assess the potential utility of aberrant methylation as a risk factor for OC development.

Material and methods: Methylation array ($n = 14$) and matched RNA-seq ($n = 4$) datasets deposited in GEO and TCGA were analyzed to compare the methylation status of *BRCA1/2*, *BRIP1*, *RAD51C/D*, *ATM*, *NBN*, *CHEK2*, and *HOXA11* in ovarian tumors ($n = 1107$) versus normal tissue ($n = 285$) and its impact on gene expression. Additionally, PHM of genes in whole blood from 230 OC cases and 150 healthy controls was assessed using targeted deep bisulfite sequencing.

Results: Ovarian tumors demonstrated significant enrichment of PHM in *BRCA1*, *RAD51C*, and *HOXA11* compared to normal tissue. The hypermethylated status of samples correlated with lower expression levels of *BRCA1* and *RAD51C*, but not *HOXA11*.

Furthermore, PHM was detected in *BRCA1* in considerable fractions of blood samples from OC patients but was rare in controls. PHM in *RAD51C* was also detected in blood but with comparable frequency in both groups.

Conclusion: Although most of the observed methylation changes in ovarian tumors are likely somatic events, representing a consequence rather than a cause of neoplastic transformation, some may represent constitutional changes also observed in normal tissues (as seen in blood) and provide evidence for the relevance of aberrant methylation, particularly of *BRCA1*, to OC risk.

C.O.I. Disclosure:

M. Suszynska: None. **T. Kocamanoglu:** None. **D. Kuznicki:** None. **J. Dabrowska:** None. **M. Ratajska:** None. **A. Mostowska:** None. **P.P. Jagodzinski:** None. **P. Kozlowski:** None.

EP01.098 Comparison of CNV Data for BRCA1/2 Genes in an NGS-Based Multigene Panel with MLPA Analysis Based on Our Cohort Data

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Background:MLPA plays a critical role in identifying significant deletions and duplications in predisposition genes for cancer, such as BRCA1 and BRCA2. This study aims to analyze the concordance and clinical utility of CNV results obtained from an NGS-based multigene panel by comparing them with MLPA results.

Material and methods: Peripheral blood samples were collected from 1026 patients admitted to our clinic with various indications for hereditary cancer testing. Patient selection conformed to NCCN guideline indications, including subjects with a family and/or individual history of breast, ovarian, prostate, and other relevant malignancies. All subjects underwent NGS-based multigene panel testing and MLPA testing for BRCA1/2 gene analysis.

Results: Among the 1026 patients analyzed, MLPA testing detected 20 BRCA1 deletions, 1 BRCA2 deletion, and 1 BRCA1 duplication. Of these, 10 had a family history, 9 had breast cancer, 2 had ovarian cancer, and 1 had both. CNV analysis missed 4 BRCA1 deletions confirmed by MLPA.

Conclusion: This study highlights the critical role of MLPA in detecting BRCA1 and BRCA2 deletions and duplications, particularly since we observed that the accuracy of NGS CNV data can remain around 80%, consistent with the literature. The observed discrepancies emphasize the necessity of incorporating MLPA as a complementary tool to enhance diagnostic accuracy. Our findings underscore the importance of integrating multiple methodologies to ensure the reliable detection of clinically significant variants in hereditary cancer testing.

C.O.I. Disclosure:

S. Ay: None. **E. Yilmaz Güleç:** None. **F. Ozen:** None.

EP01.099 Circular RNAs originating from *MDM2* are upregulated in liposarcomas with 12q amplification

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Background: A subgroup of liposarcomas is characterized by *MDM2* amplification. Still, different subtypes show varying degrees of aggressiveness: atypical lipomatous tumors (ALTs) are relatively benign, while well-differentiated liposarcomas (WDLPs) and dedifferentiated liposarcomas (DDLPs) are malignant. The expression and oncogenic role of *MDM2* circular RNAs (circMDM2) across ALTs, WDLPs, and DDLPs were analyzed to unravel a possible contribution to tumor aggressiveness.

Material and methods: Total RNA-seq and RT-qPCR were performed on ALTs, WDPLs, and DDLPs ($n = 41$), as well as on liposarcoma cell models (93T449, 94T778, GOT-3) with circMDM2 siRNA knockdown.

Results: RNA-seq showed the overexpression of three circMDM2 (hsa_circ_0099106, hsa_circ_0027491, hsa_circ_0002770) in WDLPs vs. ALTs. RT-qPCR in a larger patient cohort confirmed their higher expression in WDLPs/DDLPs than in ALTs. circMDM2 knockdown was performed in liposarcoma models to investigate their potential oncogenic roles. RNA-seq and RT-qPCR indicated the overexpression of *MET*, *TAPT1*, and *LZTFL1* genes in all the cell lines knocked down for all circMDM2 molecules. Functional enrichment analysis revealed that *LZTFL1* and *TAPT1* participate in processes related to cilium function, while *MET* is involved in cell adhesion, oxidative stress response, and Ras, PI3K-Akt, MAPK, and STAT3 signaling pathways. Furthermore, *MDM2* showed decreased expression.

Conclusion: Our results show the overexpression of circMDM2 molecules in malignant liposarcomas, suggesting a potential role of these transcripts in the progression of these tumors, possibly through their impact on primary cilia, which are crucial for adipocyte differentiation.

C.O.I. Disclosure:

D. Tolomeo: None. **G. De Martino:** None. **A. Ashadi:** None. **J. Nilsson:** None. **L. Magnusson:** None. **I. Cifola:** None. **M. Severgnini:** None. **F. Mertens:** None. **C. Storlazzi:** None.

EP01.101 Silencing of *EGFR* increased the sensitivity of TNBC cells to chemotherapeutics

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Background: Triple negative breast cancer (TNBC) is characterised by overexpression of the epidermal growth factor receptor (*EGFR*), which is present in 60% of all cases of this cancer type. *EGFR* increases cellular proliferation and contributes to cancer metastasis. It also plays a role in resistance to therapies. This raises the need to develop carriers to target pro-cancer genes as a more effective anticancer therapy. Therefore, we asked whether using DOPE/TAP/R9-chol liposomes to transport siRNAs targeting *EGFR* could sensitise TNBC cells to chemotherapy.

Material and methods: The effect of liposomes in complex with siRNA were investigated by western blot, real time-PCR, flow cytometry and confocal microscopy. Cytotoxicity was tested by Alamar Blue and LDH assay techniques.

Results: The tests confirmed the penetration ability of liposomal DOPE/TAP/R9-chol:anti*EGFR* siRNA complex into TNBC cells. Western blot, RT-PCR and confocal microscopy images proved the inhibition of EGF receptor expression. After a 3-day

incubation with liposome:anti*EGFR* siRNA complexes, the cells were treated with chemotherapeutics at different concentrations. Cytotoxicity assays performed showed increased sensitivity of the cells to the chemotherapeutic agents.

Conclusion: Silencing of the *EGFR* gene affects the sensitisation of TNBC cells to chemotherapeutics.

C.O.I. Disclosure:

P. Bialecki: None. **K. Gronkowska:** None. **S. Braccia:** None. **R. Bellavita:** None. **A. Falanga:** None. **E. Pedziwiatr-Werbicka:** None. **S. Galdiero:** None. **A. Robaszkiewicz:** None.

EP01.102 Familial uveal melanoma and other tumours in 35 families with monoallelic germline *MBD4* variants

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Background: Monoallelic germline pathogenic variants (PV) in *MBD4* are involved in the predisposition to uveal melanoma (UM)[1]. They have also been reported in breast cancers (BC) and myxofibrosarcomas[2]. We aimed to explore tumour spectrum in monoallelic germline PV carriers.

Material and methods: 12 carriers of a monoallelic germline PV in *MBD4* with UM have been identified in previous projects[1]. We sequenced *MBD4* in 621 consecutive patients treated for UM at Institut Curie and in 3240 patients who underwent genetic analyses for suspected predisposition to BC[3].

Results: 35 carriers of a monoallelic germline PV in *MBD4* were identified, 28 with UM and 7 with BC. Among patients with UM, 4 had at least one documented other choroidal naevus and one had one naevus and 2 independent UM of the right eye at ages 20 and 26, developed on pre-existing naevi. Two patients also presented another cancer: a papillary thyroid carcinoma and an in situ BC. Several types of cancers were reported among relatives, including 11 BC and another UM in a proband's sister who also carried the familial *MBD4* PV. The *MBD4* PVs co-segregated with tumours in 2 families with BC aggregation and sarcoma. Moreover, we found a positive association between BC and *MBD4* PVs comparing with a control population (gnomAD 2.1.1, $p = 0.0281$).

Conclusion: Monoallelic germline PVs in *MBD4* are associated with multiple and familial UM. They are also associated with other tumours, especially BC and possibly sarcomas. Carriers' identification is crucial to precise the tumour spectrum and estimate tumour risks.

C.O.I. Disclosure:

M. Villy: None. **A. Le Ven:** None. **M. Le Mentec:** None. **J. Masliah-Planchon:** None. **A. Houy:** None. **I. Bièche:** None. **S. Vacher:** None. **J. Sauge:** None. **C. Dubois d'Enghien:** None. **M. Schwartz:** None. **S. Piperno-Neumann:** None. **A. Matet:** None. **D. Malaise:** None. **V. Bubien:** None. **A. Lortholary:** None. **A. Ait Omar:** None. **M. Cavaillé:** None. **D. Stoppa-Lyonnet:** None. **N. Cassoux:** None. **M. Stern:** None. **M. Rodrigues:** None. **L. Golmard:** None. **C. Colas:** None.

EP01.103 From community to clinic: the benefits of identifying individuals at lower risk of disease in the population

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Background: Genetics can be used to identify individuals at lower risk of disease. Benefits include reallocating resources to those at increased risk and avoiding unnecessary screening. However, genetic screening programs currently do not return results for low disease risk. Our aim is to assess the impact of identifying those at low risk of breast cancer in the general population.

Material and methods: This retrospective study utilizes Helix Research Network™ data from 144,852 participants. A person is classified as low genetic risk if they meet two criteria: (i) do not have a pathogenic or a rare variant of uncertain significance, and (ii) have a low polygenic risk score. Clinical data came from electronic health records.

Results: 4.9% of women were labeled at lower genetic risk of breast cancer (no pathogenic variants in 11 breast cancer genes). They were at lower risk of breast cancer compared to those at average risk, hazard ratio = 0.45 (0.36–0.57). Importantly, USPSTF recommends biennial mammography from age 40 to catch or prevent early cancers. Of women at average risk, 0.89% had a breast cancer diagnosis by age 45, a rate not reached until age 51 for women classified as low genetic risk. Keeping the same threshold of residual risk, this screening strategy can save up to 3 mammogram screenings per person for 4.9% of the population.

Conclusion: Our results illustrate the potential benefits of identifying individuals at lower disease risk and returning these results.

C.O.I. Disclosure:

A. Bolze: A. Employment (full or part-time); Significant; Helix Inc. **C. Hajek:** None. **K. Schiabor Barrett:** None. **M. Levy:** None. **N. Telis:** None. **W. Lee:** None. **S.F. Powell:** None. **D. Stoller:** None. **C. Chapman:** None. **A.A. Chahal:** None. **D.P. Judge:** None. **D.A. Olson:** None. **J.J. Grzymski:** None. **E.T. Cirulli:** None.

EP01.104 A novel recurrent fusion circRNA from the *KMT2A::AFF1* fusion gene in B-ALL patients and cell lines harboring the t(4;11)(q21.3-q22.1;q23.3) translocation

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Background: *KMT2A::AFF1* fusion-circular RNAs (f-circRNAs) were identified by bioinformatics analysis in B-cell Acute Lymphoblastic Leukemia (B-ALL), though their leukemogenic contribution has not yet been explored. Here, we identified a novel recurrent

KMT2A::AFF1 f-circRNA and studied its functional role in leukemia cell models.

Material and methods: *KMT2A::AFF1* f-circRNA was investigated by PCR approaches and Sanger sequencing in RNAse R-treated/untreated RNA from B-ALL t(4;11) patients, cell lines and their derived extracellular vesicles (EVs) as well as from t(12;21) patients and control samples. Total RNA-seq profiling, confocal microscopy, and electronic microscopy were used to disentangle the functional role of *KMT2A::AFF1* f-circRNA in a DsiRNA knockdown (KD) cell line model.

Results: RT-PCR/Sanger sequencing revealed a back-splicing junction between *AFF1* exon 8 and *KMT2A* exon 2 (AK_8_2) in RNAse R-treated/untreated samples. The AK_8_2 f-circRNA, with a preferential cytosolic localization, was recurrent in B-ALL t(4;11) cases while absent in t(12;21) and normal karyotype patients. Notably, relapse samples of two patients showed AK_8_2 decrease and lack of expression, respectively. RT-qPCR disclosed the presence of AK_8_2 in cell line-secreted EVs. RNA-seq analysis of KD cells highlighted up-regulated genes involved in oxidative stress response and apoptosis regulation. Concordantly, KD cells showed ROS over-production, increased mitochondrial membrane potential and dysmorphic mitochondria.

Conclusion: AK_8_2 is a novel f-circRNA, recurrent in B-ALL t(4;11) cases. Results in KD cells indicate a possible function of AK_8_2 in the leukemic cell mitochondrial metabolism. Further investigations aim to disentangle its biological role and its potential function as liquid biopsy biomarker, considering its secretion in EVs.

C.O.I. Disclosure:

D. Tolomeo: None. **G. Fazio:** None. **S. Venuto:** None. **L. Calamo:** None. **G. Visci:** None. **A. Lonoce:** None. **I. Cifola:** None. **M. Severgnini:** None. **M. Mastrodonato:** None. **D. Semeraro:** None. **M. Bardini:** None. **L. Valsecchi:** None. **M. Mauri:** None. **S. Serrati:** None. **A. Azzariti:** None. **A. Ferrari:** None. **G. Cazzaniga:** None. **C. Storlazzi:** None.

EP01.105 Functional characterization of BRCA1 and BRCA2 variants of unknown significance through semi-automated analysis of replication fork stability

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Background: Pathogenic variants in *BRCA1* or *BRCA2* predispose individuals to breast and ovarian cancers with clear clinical management implications. Variants of unknown significance (VUSs) do not result in clinical action and therefore require functional assessment. BRCA1 and BRCA2 maintain genomic integrity through homology-directed repair (HDR), replication fork protection (RFP) and gap suppression. HDR has been employed for variant classification since a predominant role of HDR over RFP in tumorigenesis is postulated. Nevertheless, separation-of-function variants impairing RFP, but not HDR, are linked to cancer. Furthermore, some tumours of *BRCA1* or *BRCA2* mutation carriers exhibit haploinsufficiency for RFP but not HDR. Thus, here we assessed whether the extent of RFP can be employed for variant curation.

Material and methods: Here, we genetically engineered *BRCA1* and *BRCA2* variants in untransformed cells and analysed the extent of RFP using a DNA fibre assay for which we developed a programming script allowing for semi-automated assessment.

Results: We found a significant impairment of RFP in the cells harbouring heterozygous pathogenic variants versus the cells harbouring hetero- or homozygous benign variants. The extent of RFP of several VUSs was also measured and none of them were predicted to be pathogenic. Additionally, mutational signatures were extracted, and the extent of RFP was correlated with other functional and in silico assays. A significant negative correlation was found between the extent of RFP with the AlphaMissense score.

Conclusion: Together, our findings suggest that the extent of RFP could be a useful tool for variant curation of BRCA1 and BRCA2 VUSs.

C.O.I. Disclosure:

S.E. van Gijn: None. **V. Sabatino:** None. **M. Mocera:** None. **I. Ivanovski:** None. **D. Kraemer:** None. **A. Rauch:** None.

EP01.106 HMGB1-Mediated Effects of Exosomes Derived from Triple-Negative Breast Cancer Cells on Healthy Breast Epithelial Cells

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Background: Breast cancer is one of the most common malignancies threatening women's health worldwide. *HMGB1*, known to play a role in cancer development, also has critical functions in various biological processes such as inflammation and cytokine production. Its significance in breast cancer makes it a key focus of research aimed at understanding the disease's underlying mechanisms. This study examines the effects of exosomes secreted by the triple-negative breast cancer cell line (MDA-MB-231) on the healthy breast epithelial cell line (MCF10A), with a specific focus on *HMGB1* and its related biological processes.

Material and methods: Exosomes were isolated from the 96-hour culture medium of MDA-MB-231 cells and added to the MCF10A-cells. After 48 hours of incubation, MCF10A-cells and culture-supernatants were collected separately for analysis. The relative *HMGB1* expression change in MCF10A-cells were evaluated using qRT-PCR, while apoptosis was assessed with 7AAD-AnnexinV staining by flow cytometry and cytokine profiles were analyzed using the LEGENDplex™ELISA method.

Results: MCF10A cells treated with MDA-MB-231-derived exosomes showed a ~ 2-fold increase in relative *HMGB1* expression. Apoptosis analysis revealed a statistically significant increase in cell death($p < 0.0001$), while cytokine profiling revealed a significant increase in IL-6 levels ($p < 0.0001$).

Conclusion: The findings suggest that exosomes from triple-negative breast cancer cells influence inflammation, apoptosis and cytokine production potentially by increasing *HMGB1* expression. Moreover, tumor-derived exosomes may contribute to breast cancer progression by altering the function of healthy cells. Future studies is needed to investigate these mechanisms and develop novel therapeutic strategies targeting *HMGB1* and exosomes.

C.O.I. Disclosure:

G. Tuna: None. **B. Yaşa Çevik:** None. **S. Sözer Tokdemir:** None.

EP01.107 Scaling population-based BRCA1/BRCA2 testing: A pilot initiative for the Jewish population in Canada

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Molecular Oncology, University of British Columbia, Vancouver, BC, Canada.

Background: British Columbia, Canada's third-largest province, is home to over 30,000 individuals of Jewish ancestry, including Ashkenazi and Sephardic populations. Ashkenazi Jews face a 1/40 risk of carrying pathogenic *BRCA1/BRCA2* variants, while rates in Sephardic Jews may reach 1/140. Inspired by initiatives such as the NHS's population BRCA testing program, we launched a 3-year pilot offering funded *BRCA1/BRCA2* testing to Jewish adults in British Columbia. Our program aims to recruit 1,050 participants and employ a tailored, digital pre-test counselling tool, the Genetics Adviser.

Material and methods: This initiative was developed in collaboration with a local, patient-led BRCA advocacy organization to create an education campaign and ensure community needs are met. We also consulted local clinical specialists to assess the impact of identifying additional carriers on local surveillance and prevention programs.

Results: Since its launch in March 2024, the Jewish BRCA Testing Program has enrolled over 800 participants, with nearly 500 already tested and nearly 300 results returned. Family history assessments revealed that over a quarter of participants qualified for broader, provincially funded multi-gene panel testing, highlighting the program's potential to address unmet needs in genetic services delivery.

Conclusion: This pilot program addresses critical gaps in access to genetic testing for individuals of Jewish ancestry. By leveraging innovative digital tools for education and counselling, it offers a scalable model for population genetic testing. The results from this initiative could inform broader implementation strategies for population-based BRCA testing, contributing valuable insights to the growing international efforts in cancer prevention.

C.O.I. Disclosure:

S. Shickh: None. **A. Fok:** None. **M. Asrat:** None. **J. Nuk:** None. **R. Cheifetz:** None. **S. Sun:** None. **K. Schrader:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Research funding from AstraZeneca and Merck. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Genetics Adviser. F. Consultant/Advisory Board; Modest; AstraZeneca, Precision RxDx. F. Consultant/Advisory Board; Significant; Genetics Adviser. Other; Significant; Co-Founder, Evident Genomics, Co-Inventor, patent application for parent-of-origin determination methodology held by PHSA.

EP01.108 The Impact of DHRS2 Overexpression on DNA Repair Mechanisms in NSCLC

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Background: Non-small cell lung carcinoma (NSCLC) is among the most common and deadly cancers globally. The Tp53 prevents cancer progression by arresting the cell cycle or inducing apoptosis in response to cellular stress, with its activity negatively regulated by MDM2. The BRCA1-BARD1 complex is crucial for DNA repair and genomic stability, supporting tumor suppression through TP53-dependent apoptotic pathways. Recent studies show the role of the DHRS2 gene in pathobiology. This study aims to investigate the association between DHRS2 and DNA repair-related genes in NSCLC and explore the underlying regulatory mechanism.

Material and methods:DHRS2 overexpression was induced in A549 (p53 wild-type), H1299 (p53-null), and BEAS-2B (non-tumorigenic) cells using an expression vector, with overexpression confirmed by qPCR. Subsequently, BARD1, BRCA1, MDM2, and p53 expression levels were compared across DHRS2-overexpressing cell lines using qPCR.

Results: In A549 cells, MDM2 expression significantly increased (up to 2-fold), while p53, BARD1, and BRCA1 expressions decreased. In BEAS-2B cells, MDM2 expression remained stable, and p53 slightly decreased, while both BARD1 and BRCA1 expressions were notably reduced. In H1299 cells, MDM2 remained unchanged, and p53 expression was absent. BARD1 showed a slight increase, while BRCA1 expression moderately increased.

Conclusion: This study highlights the role of DHRS2 in modulating DNA repair mechanisms. DHRS2 overexpression in A549 cells affected DNA repair processes and cellular responses by regulating key genes like MDM2, p53, BARD1, and BRCA1. These findings suggest that DHRS2 plays an important role in modulating genomic stability, particularly those involving p53, BARD1, and BRCA1, providing potential therapeutic targets for NSCLC treatment.

C.O.I. Disclosure:

V. Zarerajabi: None. **B. Salman yaylaz:** None. **N. Abaci:** None. **S. Sirma ekmekci:** None.

EP01.109 Diagnostics of chronic myeloid leukemia in Lviv region of Ukraine

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Background: The Philadelphia chromosome, resulting from the t(9;22)(q34;q11) translocation, is present in 98.5% of chronic myeloid leukemia (CML) cases. This translocation generates the BCR-ABL1 fusion gene, encoding an abnormal tyrosine kinase protein.

Methods: The research was conducted from January 2023 to October 2024 at the scientific medical genetic center "LeoGEN", adhering to the ISCN 2020. We analyzed peripheral blood (PB) and bone marrow (BM) samples from 39 patients with suspected CML using FISH (LSI BCR/ABL dual-color Cytocell probes), GTG karyotyping, and RT-PCR. The diagnosis of CML was established on the basis of clinical and hematological analysis.

Results: FISH analysis of 35 PB samples confirmed the BCR-ABL1 translocation. We observed nuc ish(*BCR*x3)(*ABL1*x3)(*BCR*con*ABL1*x2) in 34 patients. One case showed an atypical pattern suggesting an additional chromosome of unknown origin (nuc ish(*BCR*x3)(*ABL1*x3)(*BCR*con*ABL1*x1)). In BM cells of 11 out of 39 individuals, the GTG method identified: in 6 - t(9;22)(q34;q11); in 1 - 49;XX, + 8, + 12,t(9;22)(q34;q11),+der(22)t(22;9)(q34;q11); in BM cells of 4 - 46;XX and 46;XY were found. Cytogenetic methods confirmed the presence of changes characteristic of CML or the absence of changes post-treatment. In PB cells of 12 treated individuals RT-PCR was conducted: in 2 - EMR was identified; in 9 - MR3/MR4; and in 1 - loss of response.

Conclusion: Cytogenetic methods like FISH and GTG are essential for diagnosing CML, identifying complex translocations.

RT-PCR is highly sensitive for monitoring known transcripts but lacks the ability to detect uncommon breakpoints. A combined approach ensures accurate diagnosis and effective disease monitoring.

C.O.I. Disclosure:

M. Melnyk: None. **M. Valchuk:** None. **S. Levandivska:** None. **Y. Karol:** None. **N. Goron:** None. **R. Nazar:** None. **N. Huleyuk:** None.

EP01.110 PHF19-207 might be long non-coding RNA with regulatory role in colon cancer

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Background: Recent data from a pan-cancer transcriptome analysis demonstrated differential activity of alternative *PHF19* promoters. The upregulated promoter in colon cancer gives rise to the PHF19-207 transcript, indicating its possible tumor-promoting role. The aim of this study was investigation the function of PHF19-207 using an in silico and in vitro approach.

Material and methods: Prediction of miRNA binding, localization and coding potential was performed using bioinformatic tools. RNA from colorectal cell lines cultured in 3D (non-malignant - HCEC-1CT, malignant - HCT116, DLD1 and SW620) was sequenced on Illumina's NovaSeq6000 platform and subsequently analyzed by qRT-PCR. The expression profile of PHF19 was analyzed in the nuscleus, cytoplasm and extracellular vesicles of HCEC-1CT and SW620 and in the HCEC-1CT cell line over-expressing *KRAS*.

Results: The expression of PHF19-207 was increased in all malignant cell lines compared to HCEC-1CT. The expression of PHF19-207 was induced by *KRAS* dysregulation and its presence was exclusively observed in extracellular vesicles of SW620.

Conclusion: The expression analysis confirms that PHF19-207 may play a tumor-promoting role and could be used as a diagnostic biomarker. The key genetic event for the overexpression of this transcript is the *KRAS* mutation, indicating the potential of this transcript for screening and early diagnosis. Its presence in vesicles of SW620 cells suggests that this transcript may play a role in intercellular communication. Binding of miRNA was predicted in its retained intron, which could act as a molecular sponge. Future studies should focus on investigating the role of PHF19-207 in processes relevant to carcinogenesis.

C.O.I. Disclosure:

D. Pavlovic: None. **T. Babic:** None. **S. Dragicevic:** None. **A. Nikolic:** None.

EP01.111 Identification of *WDR11* and *BMF* Variants in a Patient with Invasive Ductal Carcinoma of Breast

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Background: Invasive ductal carcinoma (IDC) is one of the most common histologic types of breast cancer posing a major threat to women's health worldwide. IDC emerges in the milk ducts of the breast and then invades the surrounding tissue.

Material and methods: A 54-year-old female patient diagnosed with invasive ductal carcinoma has admitted to our center for genetic consultation and hereditary cancer screening.

Results: Next generation sequencing analysis revealed variants of heterozygous likely pathogenic c.880-1_880insC (p.Val294Argfs8), c.885_889del (p.Ile295Metfs5), c.880-26_880-2del and a variant of unknown significance (VUS) c.891C>G (p.Cys297Trp) in *WDR11* (NM_018117.12) gene. Moreover, a heterozygous VUS variant of c.3G>T (p.Met1?) and c.3_4delinsTC (p.Met1?) in *BMF* (NM_001003940.2) gene were detected.

Conclusion: The interactome analysis of *WDR11* and *BMF* showed functional enrichment in vesicle trafficking related proteins and apoptosis. Notably, *WDR11* interacting with autophagy, carcinogenesis and proliferation associated genes on one hand, *BMF* interacting with antiapoptotic genes (e.g. BCL2) on the other hand, might hold the explanatory potential to the carcinoma development of the patient. Here we for the first time report coexisting variations of the *BMF* and *WDR11* genes in a patient with IDC.

C.O.I. Disclosure:

C. Yücel: None. **A. Özketen:** None. **I. Sunar:** None. **M. Solak:** None. **I. Akalın:** None.

EP01.112 Luminal A Breast Cancer-Derived Cell-Free DNA Regulates*RASSF1*Tumor Suppressor Gene Expression in Healthy Breast Epithelial Cells

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Background: RASSF1, a key tumor-suppressor gene regulating the cell cycle and apoptosis, is crucial in breast cancer development and genomic instability. This study investigated the effects of cell-freeDNA(cfDNA) obtained from Luminal A breast cancer patients on *RASSF1* gene expression and cellular behavior in healthy breast epithelial cells (MCF10A).

Material and methods: cfDNA was isolated from pre and postoperative blood samples of 13 Luminal A breast cancer patients and primary culture of postoperative tissue. *RASSF1* expression was assessed by qRT-PCR 48hours after cfDNA passive transfection into MCF10A cells. Functional analyses of MCF10A cells passive transfected with cfDNA were performed including cell migration by scratch assay and AnnexinV/7AAD staining by flow cytometry. Additionally, the concentrations of cfDNA isolated from the patients pre- and post-surgery were compared.

Results: Passive transfection of cfDNA obtained from pre-operative blood samples and tissue cultures significantly increased *RASSF1* expression (~2fold)($p < 0.05$). However, the effect of cfDNA on *RASSF1* expression before and after surgery was not statistically significant($p > 0.05$). In functional analyses, no change was observed in apoptosis($p > 0.05$), whereas a significant increase in cell migration was observed($p < 0.001$). Comparisons were made with MCF10A that was not passively transfected. Postoperative cfDNA concentration was shown to be significantly decreased compared to preoperative($p < 0.001$).

Conclusion: Regulation of the methylation-regulated *RASSF1* gene with Luminal A cfDNA demonstrates the epigenetic regulation of cfDNA. It is also emphasized that cancerous cfDNA supports cell migration in MCF10A. Removal of tumor tissue by surgery decreases cfDNA concentration. Further studies on cfDNA

and its sequences will help elucidate the mechanisms of cancerogenesis.

C.O.I. Disclosure:

B. Yasa Çevik: None. **G. Tuna:** None. **F. Trabulus:** None. **S. Sözer Tokdemir:** None.

EP01.113 Investigating the correlation of genes and LNCRNAS involved in tumor tissue and tumor margins of ovarian cancer (OC)

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Background: Cancer is one of the leading causes of death worldwide. Ovarian cancer (OC) has the highest mortality rate among female tumors, which threatens the health and life of women. Most cases (70%) are diagnosed at an advanced stage because the clinical manifestations of early OC are hidden or nonspecific.

Material and methods: In general, the WGCNA method can identify gene modules with correlation. This method reconstructs co-expression networks using the correlation criterion, as a measure of similarity between a pair of genes. From the network of genes obtained from the desired module with Cytoscape and Mcode software, we identified 5 genes in connection with each other, and the important cluster of these genes was given to us by Mcode, which includes MMP7, MMP2, IL13, LCN2, MSLN was obtained with Confidence = 0.400.

Results: Studies conducted on the IL13 gene showed that the production of TH2 cytokine IL-13 was induced by AP-125, and this indicated the selective HDAC6 inhibition of IL-13, Which is the same as our analysis. We investigated the construction of the regulatory network related to the signaling pathway and lncRNA, the effective drugs related to ovarian cancer (OC), which had a significant relationship.

Conclusion: According to the investigations conducted on ovarian cancer (OC), it was observed that there is a significant decrease in the tumor tissue compared to the tumor margin, considering 5>LogFC >-5 and P-value < 0.05.

C.O.I. Disclosure:

M. Nasiri Yekan: None. **S. Sabouri:** None. **F. Zeinali*:** None.

EP01.114 Homozygous ATR variation in T-cell leukemia and T-cell lymphoma siblings in the absence of dysmorphic features

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Background: Leukaemia/lymphoma can develop due to like germline variants in cancer predisposition genes (CPG). Monoallelic variants of the ATR gene, which plays a role in DNA repair, are linked to cancer predisposition, while biallelic are associated with Seckel syndrome. Here we report a T-ALL case with homozygous ATR variation without Seckel Syndrome.

Material and methods: A 7-year-old T-ALL boy, with related parents and later developed low-grade glioma. His T-cell lymphoma sibling died due to toxicity. The index peripheral blood (PB) was studied by whole-exome sequencing (WES) and analyzed on Gennext platform. The index's buccal swab (BS), the FFPE tumor sample of the brother, parents and unaffected siblings were studied by Sanger sequencing.

Results: We detected a novel homozygous VUS in ATR (p.Gly1758Ala), a heterozygous LP in NOTCH1 (p.Gly977Arg) and a homozygous VUS in BIRC6 (p.Asn2042Asp). The BS of the index and brother's FFPE were homozygous for the ATR variant, and parents were heterozygous. The NOTHC1 was heterozygous in the FFPE and the father. The BIRC was heterozygous in the mother, but FFPE was insufficient for confirmation.

Conclusion: The homozygous VUS ATR and heterozygous LP NOTCH1 were positive in a T-ALL case and his T-cell lymphoma sibling without ant syndromic features. The amino acid change in ATR was mild, which may explain the non-syndromic but cancer predisposing effect of ATR. NOTCH1 was shown to overcome BRCA1 deficiency via ATR-CHK1 signaling. Here deregulation of NOTCH1-ATR-CHK1 cascade might lead to malignancy. Evaluation of the combined effects of these variants is ongoing.

C.O.I. Disclosure:

G. Onder: None. **O. Dogru:** None. **S. Aras Gurcan:** None. **O. Ozdemir:** None. **B. Ergun:** None. **K. Yalcin:** None. **U. ozbek:** None. **N. Agaoglu:** None. **O. Hatirnaz Ng:** None.

EP01.115 PREVALENCE OF SOMATIC MOSAICISM IN THE *APC* GENE AS THE CAUSE OF ADENOMATOUS POLYPOSIS IN SLOVENE POPULATION

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Background: Patients with adenomatous polyposis of the colon present with a large number of colorectal (CR) polyps. In cases of hereditary adenomatous polyposis, the development of these polyps is caused by pathogenic variants/likely pathogenic variants (PV/LPV) in various genes. Hereditary adenomatous polyposis is the cause of approximately 1% of CR cancers. A genetic alteration occurring after the first zygotic division results in the presence of different genetic variants in different cells of the body, a state called somatic mosaicism. As per available literature, as much as 20% of adenomatous polyposis cases with no family history are likely to be caused by somatic mosaicism for variants in the *APC* gene.

Material and methods: Patients presenting with ≥10 adenomatous polyps in whom we have detected no germline variants associated with adenomatous polyposis were tested for the

presence of somatic mosaicism in the *APC* gene at Institute of Oncology Ljubljana. Testing was performed on two polyp samples from different colon segments. DNA was extracted from FFPE and NGS was performed using Illumina TruSight Tumor 170.

Results: In 2024 we tested 45 patients for the presence of somatic mosaicism in the *APC* gene at Institute of Oncology Ljubljana. Somatic mosaicism was detected in 7 patients (16%). Analysis was not successful in 5 patients (11%), most likely due to poor sample quality.

Conclusion: Our results, which appear comparable to estimates in available literature, confirm that a significant portion of adenomatous polyposis cases with no family history are caused by somatic mosaicism in the *APC* gene.

C.O.I. Disclosure:

A. Novaković: None. **V. Šetrajčič Dragoš:** None. **A. Bombač:** None. **P. Škerl:** None. **G. Klančar:** None. **V. Stegel:** None. **M. Krajc:** None. **A. Blatnik:** None. **K. Strojnik:** None. **I. Opalič:** None. **B. Stojanov:** None. **S. Novaković:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Institute of Oncology Ljubljana.

EP01.116 Detection of *BRCA1/2* likely pathogenic/pathogenic variants in *BRCA*-associated hereditary cancer: a single-center study (2018–2025)

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Introduction: *BRCA1* and *BRCA2* are tumor suppressor genes that play a crucial role in DNA repair through homologous recombination. Germline pathogenic variants in these genes are associated with an increased risk of hereditary breast, ovarian, pancreatic, and prostate cancers.

Methods: The mutation spectrum and clinical findings of patients with cancer who were found to have germline mutations in the *BRCA1/2* genes at the Ege University Department of Medical Genetics over the past seven years were analyzed retrospectively.

Results: A total of 183 patients diagnosed with cancer and found to have *BRCA1/2* likely pathogenic/pathogenic variants were evaluated at the Ege University Medical Genetics outpatient clinic between 2018 and 2025. Among these patients, 126 (69%) had breast cancer, 50 (27%) had ovarian cancer, 4 had pancreatic cancer, and 3 had prostate cancer. Among the 126 breast cancer patients, *BRCA1* variants were detected in 65 (51%) and *BRCA2* variants in 61 (49%). In breast cancer patients, invasive ductal carcinoma was predominantly identified. Within the 50 ovarian cancer patients, *BRCA1* variants were found in 36 (72%) and *BRCA2* variants in 14 (28%). Serous carcinoma was the most common histological subtype in ovarian cancer patients. The most frequently observed mutations were *BRCA1* c.5266dup (p.Gln1756Profs) and *BRCA2* c.67+1 G > A.

Conclusion: This study aims to present our seven-year experience with *BRCA*-associated hereditary cancers at a single center. We provide a comprehensive analysis of the prevalence and mutation spectrum of *BRCA1/2* variants in Turkish patients with *BRCA*-associated hereditary cancers.

C.O.I. Disclosure:

A.S. Can: None. **A. Ece Solmaz:** None. **F. Uguzdogan:** None. **H. Akın:** None.

EP01.117 The role of *DHRS2* gene expression in modulating epithelial mesenchymal transition related genes in breast cancer

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Background: Breast cancer (BC) is the most frequently diagnosed cancer among women worldwide. Despite generally high survival rates, prognosis significantly worsens when the cancer metastasizes to other tissues. Previous studies have reported alterations in the expression of the *DHRS2* gene in breast cancer and suggested its potential link to epithelial-mesenchymal transition (EMT). This study investigates the impact of increased *DHRS2* gene expression on EMT-associated genes in the estrogen receptor-positive breast cancer (MCF7) and control breast (MCF10A) cell line.

Material and methods: In this study, *DHRS2* gene expression was upregulated in MCF7 and MCF10A cells using liposomal agent to deliver vector-based constructs. Different time points (24, 48 and 72 hours) were incubated to determine the time at which *DHRS2* high expression was most significant. Gene expression changes in *DHRS2* and five EMT-associated genes were evaluated using qPCR in *DHRS2*-overexpressing and control cells.

Results: The results demonstrated that increased *DHRS2* expression led to decreased *VIM, CDH1*, *SNAI1*, *TWIST* and *TMED5* expression in both MCF7 and MCF10A cell lines. Notably, a contrasting expression pattern was observed in *SNAI1* and *CDH1* genes between breast cancer cells and controls, suggesting that these two genes may have important effects in *DHRS2* influencing EMT.

Conclusion: This study highlights the potential role of *DHRS2* in regulating EMT-associated gene expression, thereby influencing the metastatic process in breast cancer. These findings emphasize the significance of *SNAI1* and *CDH1* genes as key mediators of EMT affected by *DHRS2* expression, offering novel insights into the molecular mechanisms underlying breast cancer metastasis.

C.O.I. Disclosure:

H. Karaca: None. **B. Salman yaylaz:** None. **S. Punar:** None. **V. Zarerajabi:** None. **S. Sirma ekmekci:** None. **N. Abaci:** None.

EP01.118 Demonstrating multivariate statistical methods in genomic research: correlating endometrial cancer-related genes obtained from whole exome sequencing of adenomyosis patients with bleeding symptoms

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Background: Adenomyosis, a benign gynecological condition with endometrial glands found the myometrium, is can be detected in hysterectomy specimens of endometrial cancer (EC) cases. This study aimed to utilize redundancy analysis (RDA) to correlate adenomyosis bleeding symptoms with EC-related genetic variants identified through whole exome sequencing (WES) of pathologically confirmed sporadic adenomyosis cohort.

Material and methods: Adenomyosis was confirmed via histopathological examination following hysterectomy. Standardized protocols from the World Endometriosis Research

Foundation-Endometriosis Phenome Harmonisation Project (WERF-EPHect) were followed. WES was performed on genomic DNA utilizing Illumina NovaSeq6000. Bioinformatic analysis and variant filtering were conducted on the Pairend platform. Relationships between biotic (EC-related genetic variants) and abiotic (adenomyosis bleeding symptoms) variables were analyzed via RDA with Monte Carlo tests ($p < 0.05$) on R. Spearman's rank correlation assessed variable associations, while Bray-Curtis clustering and normalized Euclidean distances evaluated patient profile similarities in Primer-v6.

Results: 33 women diagnosed with adenomyosis were enrolled. Bleeding symptoms, assessed via WERF-EPHect questionnaire, revealed menorrhagia in 15(45%), metrorrhagia in 11(33%), and menometrorrhagia in 10(30%) patients. WES revealed 45 previously identified EC-related genetic variants. RDA indicated that EC-related variants explained the largest proportion of variance, with significant positive correlations between specific variants and bleeding symptoms.

Conclusion: This study is the first to demonstrate genomic-phenotypic correlations in adenomyosis utilizing multivariate analysis, highlighting the potential role of EC-related genetic variants in symptom manifestation. Future research should expand on these findings by incorporating multivariate analyses with broader parameters to further elucidate the complex interplay between genetic and clinical factors in adenomyosis.

C.O.I. Disclosure:

S. Aydin: None. **M. Balci:** None. **N. Eser MA:** None. **N. Topbas Selcuki:** None. **E. Oral:** None. **F. Tuncer:** None.

EP01.119 Cancer Early detection in Li-Fraumeni Syndrome - can cfDNA concentration alone signal cancer development and rationalise sequencing methods?

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Background: Li Fraumeni Syndrome (LFS) is a hereditary cancer predisposition syndrome with high risk of aggressive cancer (80% by 70yrs). Early detection strategies in LFS rely on imaging surveillance which may give false positive/negative findings. Cell-free DNA (cfDNA) fragment length (FL) has been shown to be a potential biomarker of early cancer in LFS however, could cfDNA concentration measured following whole blood extraction also signal cancer development?

Material and methods: We undertook cfDNA analyses in 49 adult LFS individuals [sequential samples = 39, total samples = 115 [cancer status: never = 44 (G1); current stage1/2 = 2 (G2a); current stage 3/4 = 4 (G2b); previous cancer, currently cancer free (G3) = 65 and a combined active cancer stage 1–4 = 6 (Gall)].

Results: We found reduction in mean FL between cancer naïve (G1) and active cancer (Gall) groups and between the previous cancer (G3) and active cancer (Gall) groups (Mann Witney U = 18, P = 0.001 and Mann Witney U = 33, P = 0.0002 respectively). Mean FL comparison demonstrated variance across all clinical groups (Kruskal-Wallis statistic = 12.16, P = 0.0069) but not between early and late-stage cancer samples (t = 0.9233, df = 4, P = 0.4081). CfDNA concentration rise (>1ng/ul) was measured prior to cancer diagnosis in 4/4 patients whom developed cancer.

Conclusion: We show significant reduction in cfDNA FLs in those with cancer compared to individuals without. Further data are required but our initial data provides further evidence to cfDNA FL being a biomarker of early cancer in LFS patients. Preliminary data also suggests cfDNA concentration rise may signify cancer development and allow for the cost-effective rationalisation of sequencing; even showing early promise as a stand-alone biomarker in its own right.

C.O.I. Disclosure:

P. Sodde: None. **G. Rice:** None. **J. O'Sullivan:** None. **I. Donaldson:** None. **S. Ng:** None. **M. Rawson:** None. **G. Evans:** None. **E. Woodward:** None.

EP01.120 Acute myeloid leukemia with t(7;21)(p22;q22) resulting in fusion of *RUNX1 (AML1)* with the ubiquitin-specific protease gene *USP42*

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Background: Acute myeloid leukaemias (AMLs) are heterogeneous diseases with genetic abnormalities, morphology, immunophenotypes and variable clinical outcomes. Some genetic abnormalities correlate with the clinical phenotype. Finding fusion genes helps with diagnosis, prognosis and therapy. Conventionally, fusions are identified using standard cytogenetic tests, FISH and RT-PCR. These are sometimes inadequate, so using NGS technologies (analysing many genes in a single test) can help detect unexpected partner genes and rare breakpoints. The *RUNX1* gene (21q22) is involved in more than 50 translocations and several mutations in AML, ALL, and MDS. The rare cryptic translocation t(7;21)(p22;q22) involves a rearrangement with the *USP42* gene, appears to be specific and recurrent. The coexistence of t(7;21) and 5q segment loss has been proposed as a non-random finding in AML patients.

Material and methods: We describe a case of AML secondary to MDS with t(7;21)(p22;q22) translocation with *RUNX1::USP42* fusion gene, not associated with del(5q).

Results: At onset in 2021 the patient had MDS and standard karyotype, FISH for 5q, 7q and 20q deletions were normal. In 2024, the diagnosis was AML secondary to MDS. The bone marrow sample showed a poor karyotype; interphase FISH showed *RUNX1* rearrangement in 82% of nuclei, negative for *RUNX1::ETO* and *ETV6::RUNX1* translocations. Metaphase FISH showed t(7;22) in 2/7 cells. Another cell line (55% of nuclei) showed chromosome 17 loss (lacking P53, D17Z1 and PML).

Conclusion: NGS analysis using the Oncomine Myeloid Research Panel showed *RUNX1::USP42* fusion. The loss of chromosome 17 in patients with the *RUNX1::USP42* fusion gene is a new finding.

C.O.I. Disclosure:

F. Cambosu: None. **S. Tranquilli:** None. **A. Oggiano:** None. **G. Soro:** None. **F. Farina:** None. **D. Cambedda:** None. **M.E. Petretto:** None. **F. Longu:** None. **F. Zorzetto:** None. **G. Piras:** None. **P.M. Campus:** None.

EP01.121 Evaluation of a case study on oncohaematological patients at the Cytogenetics Laboratory of the AOU of Sassari

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Background: According to the WHO, cytogenetic analysis is essential in the initial evaluation of haematological malignancies and should be repeated to assess disease progression and response to therapy. FISH can also detect cryptic abnormalities, metaphases that cannot be analysed, or the absence of mitosis. The present study is an evaluation of case histories related to the analysis of patients, with various diagnoses, affected by haematological malignancies from 2018 to 2024.

Material and methods: 2569 samples (bone marrow and peripheral blood) were analysed. The percentage of males was 56% compared to 44% females, while the ratio of myeloid to lymphoid pathology was about 1:1. Short-term cultures were set up (24h and/or 24/72h synchronised and with the addition of oligonucleotides and interleukin-2 for CLL) followed by cytogenetic analysis in QFQ bands and FISH analysis.

Results: Approximately 23% karyotypes had numerical and/or structural abnormalities sometimes complex or composite, ineligible samples and those undergoing analysis were excluded.

Conclusion: Two factors can explain the high number of normal karyotypes. Firstly, in the context of myeloid pathology, the number of samples, not patients, since the analyses include the onset (frequently pathological) and post-therapy control (often normal). In lymphoid diseases, this is due to cells of the lymphoid lineage not undergoing spontaneous mitosis in culture, so most cells come from non-neoplastic cells (e.g. in MM, proliferating neoplastic cells are found in the advanced stages of the disease, whereas in CLL, using oligonucleotides and IL2, produce greater proliferation and better evaluation of neoplastic metaphases).

C.O.I. Disclosure:

S. Tranquilli: None. **P.M. Campus:** None. **G. Soro:** None. **D. Cambedda:** None. **M.E. Petretto:** None. **A. Oggiano:** None. **R. Orizi:** None. **F. Farina:** None. **A.C. Montella:** None. **F. Cucca:** None. **F. Cambosu:** None.

EP01.122 The significant germline structural variants in Russian patients with solid tumors: whole genome sequence results

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Background: The prevalence of germline structural variants (SVs) in cancer patients cohorts is poorly understood due to technical limitations of NGS panels and exome sequencing.

Material and methods: objective: to analyze the significant (pathogenic/likely pathogenic - PV/LP) SVs in cancer patients with suspected hereditary cancer syndromes (HCS). 2500 patients >18 y.o. with solid tumors and suspected HCS whole genome sequencing (WGS) were performed. WGS: 30x, DNBseq-T7, EVOGEN LLC; bioinformatics analysis accelerators: EVA Pro, EVOGEN, Russia.

Results: 502 significant variants (PV/LP) in cancer-associated genes were identified (20,1%) including 30 SVs. The table shows some previously undescribed SVs identified. Table. Selected novel SVs.

SVs position (hg38)	Morbid genes	SVs type	SVs size	Cancer cases
chr18:51047298-51054779	*SMAD4*	deletion	7481 b.p., exons 3–4, introns 2–4	Male, 59 y.o., colorectal cancer, polyposis
chr18:50778738-53834227	*SMAD4, DCC*	deletion	~3Mb	Male, 25 y.o., colorectal cancer, polyposis
chr2:47796532-47803196	*MSH6*	deletion	6664 b.p., exons 4, introns 3–4	Male, 38 y.o., colorectal cancer
chr13:32370570-32380320	*BRCA2*	deletion	9750 b.p., exons 20–24, introns 19-23	Female, 39 y.o., breast cancer
chr17:43068355-43070981	*BRCA1*	deletion	2627 b.p., exon, intron 15	Female, 42 y.o., breast cancer
chr17:43138933-43140891	*BRCA1*	deletion	1958 b.p.	Female, 58 y.o., breast cancer
chr2:47439298-47450442	*MSH2*	deletion	11 145 b.p., exon 8, introns 7–8	Female, 45 y.o., endometrial cancer

Conclusion: WGS makes it possible to identify SVs, including large gene deletions and copy number variations, which is a significant contribution to understanding the HCS etiology.

C.O.I. Disclosure:

M. Makarova: None. **M. Nemtsova:** None. **M. Byakhova:** None. **A. Danishevich:** None. **O. Sagaydak:** None. **M. Woroncow:** None. **M. Belenikin:** None. **A. Krinitsina:** None. **A. Semenova:** None. **N. Bodunova:** None. **I. Khatkov:** None. **V. Galkin:** None.

EP01.123 Hereditary cancer predisposition genes in childhood solid tumors: a cohort study

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Background: Pediatric tumors are primarily influenced by hereditary genetic factors rather than environmental exposures. Pathogenic and likely pathogenic (P/LP) variants in cancer predisposition syndrome (CPS) genes have been reported in 8–18% of pediatric cancer patients. Identifying these variants is crucial for reducing morbidity and mortality in patients and their families.

Material and methods: We analyzed hereditary cancer panels in a cohort of 103 patients diagnosed with solid tissue tumors

before the age of 20. The cohort included primarily brain tumors (n = 27), sarcomas (n = 26), and neurofibromas (n = 11). P/LP variants in CPS genes were categorized as copy number variations (CNVs), missense, loss-of-function (LOF), or novel variants.

Results: P/LP variants in CPS genes were identified in 36.8% (37/103) of patients. Two patients carried two P/LP variants each. Among the variants, 3 CNVs, 11 missense, 25 LOF, and 8 novel. The most frequently affected genes were *NF1* (n = 9), *VHL* (n = 4), and *TSC1*, *TSC2, RB1*, *BRCA1*, *MUTYH*, and *SAMD9* (n = 2 each). The observed higher prevalence of P/LP variants compared to previous studies may be explained by the inclusion of patients with tumors (not limited to cancer) and the frequent presence of family histories in the cohort.

Conclusion: This study demonstrates a high prevalence of P/LP variants in CPS genes among pediatric solid tumor patients. It underscores the importance of genetic testing for early diagnosis. Such testing can help tailor management and improve outcomes for patients and their families.

C.O.I. Disclosure:

Ö. Güngör: None. **B. Ak:** None. **A. Ece Solmaz:** None. **H. Akın:** None.

EP01.124 The importance of tumor cell fraction in determining homologous recombination deficiency in an ovarian cancer patient - a case study

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Background: Homologous recombination repair deficiency (HRD) is a frequent feature of high-grade serous ovarian, fallopian tube, and peritoneal carcinoma (HGSC) and is associated with sensitivity to PARP inhibitor (PARPi) therapy. Here, we present a case demonstrating that the proportion of tumor cells in the sample used for DNA extraction is crucial for accurately determining HRD status.

Material and methods: FFPE tumor sample of female patient with HGSC was referred for tumor DNA genotyping to evaluate eligibility for PARP inhibitor therapy. Initially, sample with 20% tumor cells was tested using NGS with the Illumina TruSight Oncology 500 to detect clinical important variant in homologous recombination genes. Subsequently, the same sample was tested for HRD status using the Illumina TruSight Oncology 500 HRD. Due to inconclusive results, the same tumor sample was manually macrodissected by a pathologist to increase the tumor cell proportion to 90%. The sample was then genotyped and retested for HRD status.

Results: Genotyping of the tumor sample containing 20% tumor cells identified the BRIP1:c.2492+1G > C p.? pathogenic variant, which could potentially impact HRD status. However, subsequent HRD testing on this sample yielded a negative result. Given the possible clinical significance of the BRIP1 pathogenic variant, the enriched tumor sample was reanalyzed, revealing a positive HRD status (GIS 46). As a result, the patient became eligible for PARPi therapy.

Conclusion: This case study demonstrates that increasing the proportion of tumor cells in the sample allowed for accurate HRD status determination, ensuring precise tumor classification and guiding appropriate therapy selection.

C.O.I. Disclosure:

G. Klancar: None. **P. Skerl:** None. **V. Stegel:** None. **V. Setrajcic Dragos:** None. **S. Novakovic:** None.

EP01.125 Uveal melanoma is part of the Lynch syndrome tumor spectrum

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Background: Uveal melanoma (UM) is the most common intraocular malignancy in adults. Germline mutations of two genes have been identified to predispose to UM: *BAP1*, responsible for the *BAP1* tumor predisposition syndrome, and *MBD4*, which alterations are associated with tumor predisposition, specific tumor mutational signature and response to immunotherapy. However, only a fraction of familial and bilateral forms of UM can be explained by these germline alterations, suggesting the existence of other predisposing factors.

Material and methods: We analyzed a panel of 122 genes on germline DNA from a prospective cohort of 381 UM patients.

Results: We identified 20 pathogenic variants (PVs) in genes that are relevant for genetic counselling (7 in *MBD4*, 3 in *BAP1*, 3 in *BRCA2*, 1 in *MLH1*, 1 in *MSH2*, 2 in *MSH6* and 4 in *PMS2*). The frequencies of the 3 MisMatch Repair (MMR) genes *MLH1*, *MSH2* and *MSH6* were significantly higher than in the gnomAD (v2.1.1) non-Finnish European database as a matched control sub-cohort (p = 0.00073, OR: 10.4 [2.8–27.5]). *PMS2* was excluded from this statistical analysis because its pseudogene prevents the interpretation of public data. One UM was characterized from the patient carrying the germline *MLH1* PV. This UM displayed biallelic inactivation of *MLH1*, loss of MLH1 and PMS2 expression, and a prominent Substitution Mutational Signature 6 (SBS6) associated with MMR deficiency.

Conclusion: This study confirms MMR genes as predisposing factors in UM, as suggested by two previous case reports (Lobo et al. Virchows Arch 2017;470:347; Abdel-Rahman et al. Ophthalmology 2020;127:668).

C.O.I. Disclosure:

A. Le Ven: None. **M. Villy:** None. **A. Bortolini Silveira:** None. **A. Houy:** None. **J. Masliah Planchon:** None. **M. Warcoin:** None. **M. Le Mentec:** None. **F. Simaga:** None. **A. De Pauw:** None. **B. Buecher:** None. **T. Verrier:** None. **K. Merchadou:** None. **V. Renault:** None. **A. Vincent-Salomon:** None. **J. Sauge:** None. **S. El Zein:** None. **C. Dubois D Enghien:** None. **S. Piperno-Neumann:** None. **A. Matet:** None. **D. Malaise:** None. **N. Cassoux:** None. **L. Lumbroso-Le Rouic:** None. **D. Stoppa Lyonnet:** None. **M. Stern:** None. **M. Rodrigues:** None. **L. Golmard:** None. **C. Colas:** None.

EP01.126 STUB1 expression as a diagnostic biomarker and its association with drug resistance in multiple myeloma

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Background: Multiple myeloma (MM) is a hematological malignancy in which paraprotein production disrupts cellular protein homeostasis. In our transcriptome study, we identified the STUB1 gene as one of the upregulated genes in MM. STUB1 regulates the

degradation of misfolded proteins and maintains homeostasis through its ubiquitinase activity. It also plays a role in tumourigenesis, metastasis, and drug resistance. This study investigates STUB1 expression in MM patients and its association with drug resistance in MM cell lines.

Materials and methods: PBMCs were isolated from bone marrow samples of 34 MM patients, 14 individuals with monoclonal gammopathy of undetermined significance (MGUS), and 13 healthy controls. STUB1 gene expression levels in MM, MGUS, and controls were quantified by qRT-PCR. Statistical significance was assessed using the Kruskal-Wallis test, followed by the Mann-Whitney-U test for pairwise comparisons. Drug-resistant MM cell lines (btz-RPMI8226 and btz-U266) were generated by inducing bortezomib resistance, and STUB1 expression levels were assessed by qRT-PCR in both untreated and resistant cells.

Results: STUB1 expression was significantly higher in MM patients compared to controls and MGUS patients ($p < 0.01$). ROC analysis demonstrated the diagnostic potential of STUB1 (AUC = 0.841). Correlation analysis revealed positive associations between STUB1 expression and RBC, HCT, urea, and creatinine levels, while a negative correlation was observed with eGFR ($p < 0.05$). Furthermore, STUB1 expression was significantly increased in btz-RPMI8226 and btz-U266 cells compared to their respective control cell lines ($p < 0.05$).

Conclusion: These findings suggest that STUB1 expression levels could serve as a diagnostic biomarker for MM and are strongly associated with bortezomib resistance.

C.O.I. Disclosure:

S. Punar: None. **M. Sariman:** None. **M. Ayer:** None. **I. Suer:** None. **K. Cefle:** None. **S. Ozturk:** None. **S. Sirma ekmekci:** None. **N. Abaci:** None.

EP01.127 Evidence of *JAK2*V617F Mutation Transfer Through cell-free DNA and exosomes in Polycythemia Vera

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Background: Myeloproliferative neoplasms(MPNs) are associated with genetic alterations that contribute to disease progression.cfDNA and exosomes released by mutant cells may play a role in the horizontal transfer of oncogenic mutations into healthy cells, converting them into cancerous cells.This study aimed to investigate the presence and potential transmission of *JAK2V617F* mutation via HEL cell-derived cfDNA and exosomes to healthy cells.

Material and methods: cfDNA and exosomes were isolated from the supernatant of HEL cells cultured for 96 hours(h) and confirmed to carry *JAK2V617F* by allele-specific nested PCR.Subsequently, cfDNA and exosomes were individually applied to healthy cord blood mononuclear cells (MNCs)for 48 h, and target MNCs were analyzed for the presence of *JAK2V617F* mutation.Changes in the involvement of nucleic acid sensing pathway-related genes, including *TLR9*, *cGAS*, and *STING*, were assessed by RT-PCR in response to exposure.

Results: Following the culture of HEL-derived cfDNA and exosomes with healthy MNCs, cells acquired *JAK2V617F* in both heterozygous and homozygous states.Gene expression analysis indicated a significant upregulation(2-fold) in *TLR9* and *cGAS* compared to the control group, whereas changes in *STING* expression were not statistically significant.

Conclusion: These findings confirm that HEL cell-derived cfDNA and exosomes carry the JAK2V617F mutation and can be transferred to healthy cells, leading to the acquisition of the mutation.The observed activation of TLR9 and cGAS suggests a potential role of cfDNAs and exosomes in facilitating the uptake of genomic materials via nucleic acid sensing pathways.This experimental model supports the concept of genometastasis and underscores the importance of targeting extracellular genetic material to prevent further genetic alterations.

C.O.I. Disclosure:

S.F. Toprak yalçin: None. **E. DemİrtaŞ:** None. **S. Sözer tokdemİr:** None.

EP01.128 Utility of Polygenic Risk Scores (PRS) in predicting pancreatic cancer; A systematic review and meta-analysis of common variant and mixed scores

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Background: Pancreatic cancer remains among the top five causes of cancer-related mortality. Only 18% of patients present with early-stage, potentially curable disease. Patient risk stratification is critical to increase the number of individuals eligible for surgery. Polygenic risk scores (PRSs), combined with clinical risk factors, offer a promising approach to assess the lifetime risk of pancreatic cancer. This systematic review synthesizes the results of all published PRSs and mixed models for pancreatic cancer.

Material and methods: We systematically searched the MEDLINE, Embase, Ovid and Web of Science databases. The OR reported between quintiles were transformed to an OR per SD increase in risk score. Reported AUCs were synthesized and compared between the PRSs and mixed models.

Results: Twenty-seven studies were identified for synthesis. The PRSs yielded an OR of 1.40 (1.28–1.53) for pancreatic cancer. Mixed models incorporating clinical risk factors showed a higher OR of 1.58 (1.34–1.88). The AUC for the PRSs was 0.61 (0.58–0.65), compared to 0.70 (0.61–0.80) for the mixed models. The predictive power of PRS models was positively correlated with the number of SNPs included in the studies. Ancestry significantly influenced prediction accuracy, with an OR of 1.42 (1.30–1.56) in Caucasian populations compared to 1.21 (0.77–1.90) in Asian populations.

Conclusion: Adding clinical risk factors to PRS models significantly increases their predictive capability. Further research is needed to identify a comprehensive set of risk SNPs, enhance accuracy across diverse populations and incorporate rare genetic variants. These advancements have the potential to boost the predictive accuracy of risk models.

C.O.I. Disclosure:

G. Verras: None. **Z. Hamady:** None. **A. Collins:** None. **W. Tapper:** None.

EP01.129 Germline mutational spectrum in patients with advanced pancreatic cancer

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Background: Pancreatic cancer remains a leading cause of cancer-related deaths, with most cases diagnosed at advanced stages. Genetic factors contribute to 5–20% of cases, making genetic testing crucial for personalized treatment approaches and early detection strategies.

Material and methods: We conducted a retrospective cohort study of 356 patients with advanced pancreatic ductal adenocarcinoma (IIb-IV stages). Germline DNA sequencing was performed targeting 17 cancer susceptibility genes. Variants were classified according to ACMG-AMP guidelines and clinical characteristics were compared between carriers of pathogenic/likely pathogenic (P/LP) variants and non-carriers.

Results: P/LP variants were detected in 9.27% of patients, predominantly in *BRCA1* (2.81%), *BRCA2* (2.53%), and *ATM* (2.25%). *BRCA1/2* carriers were diagnosed at a significantly younger age (median 55 vs. 67 years, $p = 0.0001$) and were more likely to present with metastatic disease at diagnosis. No significant associations were found between carrier status and family history of cancer.

Conclusion: Our findings confirm the importance of germline genetic testing in advanced pancreatic cancer. The high prevalence of *BRCA1/2* P/LP variants in younger patients and their association with metastatic presentation emphasizes the need for targeted screening strategies.

C.O.I. Disclosure:

J. Galego-Carro: None. **A. Marcón-González:** None. **M. Santamariña:** None. **A. Blanco-Pérez:** None. **O. Fuentes-Ríos:** None. **A. Crujeiras-González:** None. **C. Coedo-Costa:** None. **C. Lopez-Pleguezuelos:** None. **M. E. Aguado-Barrera:** None. **A. Vega-Gliemmo:** None.

EP02 Reproductive Genetics

EP02.001 Male infertility linked to myotonic dystrophy type 1: a case report

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Background: Myotonic Dystrophy Type 1 (DM1) is a multisystemic genetic disorder caused by the expansion of CTG repeats in the DMPK gene. It is characterized by myotonia, progressive muscle weakness, and endocrine abnormalities, including male infertility. In this case presentation, we discuss an infertile couple referred to the Yazd Research and Clinical Center for Infertility, where the male partner, aged 34, presented with infertility lasting several years, accompanied by progressive weakness in the distal extremities. After genetic counseling and familial history assessment, it was found that the mother and sister and two of the proband cousins also have muscular disorders and myotonia, suggesting a genetic predisposition to neuromuscular conditions. The hormonal evaluation showed an increase in the levels of LH and FSH and a decrease in the testosterone level. Semen analysis and sperm processing after an appropriate abstinence period rarely show motile and immotile sperm, making the case suitable for ICSI.

Material and methods: Genomic DNA was extracted from whole blood. The PCR-based assay was used to amplify CTG-repeat-containing regions of the DMPK gene.

Results: According to the test results, the patient had one normal allele (13 repeats) and one full mutation allele (>50 repeats) in the expansion of the CTG repeat, thus confirming classic DM1.

Conclusion: The expansion of CTG in DM1 affects neighboring genes, particularly DMR N9, which may regulate spermatogenesis. This highlights the need for comprehensive genetic counseling and testing to inform treatment options and familial risks in male infertility cases.

C.O.I. Disclosure:

S. BakhshandehBavarsad: None. **N. GhasemiFiroozabadi:** None. **S. Asadollahi:** None. **Z. Dashti:** None. **N. Karami:** None. **M. MosadeghMehrjardi:** None.

EP02.003 Preimplantation genetic testing, PGT-M, for Familial Amyotrophic Lateral Sclerosis, ALS

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Background: Genetic factors are known to underlie the etiology of amyotrophic lateral sclerosis (ALS), particularly in familial cases, but in sporadic cases as well. More than 50 different genes have been associated with ALS, therefore, genetic evaluation of ALS and ALS-FTD patients and at-risk family members has become part of the routine workup, often depending on ethnicity, age of onset and family history. Many of these genes are characterized by incomplete penetrance and late onset. As such, prenatal diagnosis for these conditions is ethically questionable. Conversely, pre-implantation genetic testing (PGT-M) is considered more acceptable for these conditions, either by direct mutation testing or by exclusion testing. We describe our experience with PGT-M for ALS.

Material and methods: Four ALS patients requested PGT-M following the identification of causative mutation in the following genes: C9orf72 PFN1, and VCP. Four PGT-M cycles were performed on single blastomeres of day 3 or 5 embryos using a multiplex nested PCR protocol for direct mutation testing for PFN1 and VCP. For C9orf72, diagnosis was based on polymorphic markers flanking the repeat region.

Results: There are currently two ongoing pregnancies, both in C9orf72 cases. To the best of our knowledge, this is the first report of PGT-M for this condition.

Conclusion: Prevention of late-onset neurodegenerative diseases such as ALS raises significant ethical dilemmas. PGT-M provides a safe and an ethically acceptable option. With expanding diagnostic options and increased awareness we expect a further increase in demand for PGT for monogenic ALS, mainly in familial cases.

C.O.I. Disclosure:

D. Barel: None. **M. Malcov:** None. **T. Frumkin:** None. **A. Reches:** None. **H. Baris Feldman:** None. **Y. Yaron:** None.

EP02.004 First cytogenetic study on disorders of sex development at the genetics department of CHU Mohamed VI, Marrakech: Diagnostic and clinical implications

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Background: Disorders of sex development (DSDs) constitute a group of congenital conditions affecting chromosomal, gonadal, or anatomical sex development. These conditions result from chromosomal aberrations or genetic mutations that disrupt the normal sexual differentiation process. Clinically, DSDs present a wide spectrum of manifestations. The analysis of sex chromosomes is essential for establishing a diagnosis, improving the understanding of these disorders, and optimizing patient management.

Material and methods: This is a retrospective descriptive study conducted at the Medical Genetics Department of CHU Mohamed VI, Marrakech, including 234 patients. The study includes all patients referred for a disorder of sex development and who underwent constitutional cytogenetic analysis, molecular cytogenetics (fluorescence in situ hybridization, FISH), or molecular analysis (PCR-based detection of the SRY gene).

Results: Cytogenetic analysis of patients with disorders of sex development (DSD) revealed a significant proportion of chromosomal rearrangements involving the sex chromosomes. Among the identified abnormalities, some syndromic conditions were predominant, particularly those affecting ovogenesis and spermatogenesis such as Turner and Klinefelter, while others were characterized by complex mosaicisms, suggesting considerable phenotypic variability. From a clinical perspective, the most frequently observed anomalies included primary amenorrhea, azoospermia, micropenis, cryptorchidism, and the absence of palpable gonads. These manifestations were sometimes associated with facial dysmorphisms or other clinical markers suggestive of specific syndromes.

Conclusion: Cytogenetic analysis of sex chromosome abnormalities in patients with DSDs is crucial for refining the diagnosis and improving patient management. A multidisciplinary approach, involving clinicians, surgeons, geneticists, and psychiatrists, remains essential for a better understanding and management of these conditions

C.O.I. Disclosure:

F. Bouzid: None. **G. draiss:** None. **R. el quadiry:** None. **Y. oulad siyed:** None. **M. bouskraoui:** None. **F. ansari:** None. **N. Aboussair:** None.

EP02.005 Efficacy of Preimpiantation Genetic Testing for Monogenic diseases in fertile couples

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Background: Preimplantation Genetic Testing (PGT) aims to determine the presence of genetic and/or chromosomal defects in embryos produced by IVF. What is the probability for fertile couples at risk of transmitting genetic disease to have a healthy child in arms by undergoing PGT-M + PGT-A?

Material and methods: Results of IVF, genetic/chromosomal analyses of biopsied trophectoderm cells and clinical from 64 fertile and 53 infertile couples undergoing PGT-M combined with PGT for embryonic aneuploidy (PGT-A) were compared. Embryo biopsy was performed on Day 5. DNA was tested for monogenic disease and chromosomal content through NGS.

Results: The number of oocytes needed to obtain one blastocyst to biopsy, one transferrable blastocyst after PGT-M and one euploid blastocyst after PGT-M + A was statistically inferior for fertile couples compared to infertile couples (one blastocyst to biopsy : 2.3 oocytes for fertile couples versus 2.5 oocytes for infertile couples; one transferrable blastocyst: 3.7 versus 4.0; one euploid transferrable blastocyst: 6.3 versus 7.4). Live birth rates after thawing and embryo-transfer were respectively 53.8% and 49.2% for fertile and infertile couples. Each oocyte adds of 14.7% the probability of having a save child for fertile couples. This value is 11.2% for infertile couples.

Conclusion: By knowing more precisely the probability of having a healthy child after PGT-M, women can decide to avoid the dilemma of abortion after positive prenatal diagnosis. Fertile couples need at least 7 metaphase II oocytes to micro-inject in ICSI to maximize the probability of having a save child after PGT-M + PGT-A.

C.O.I. Disclosure:

S.M. chamayou: None. **M. sicali:** None. **A. guglielmino:** None.

EP02.006 The 3β-HSD Deficiency CAH in a Multidisciplinary Approach - The Clinical Analysis with Literature Review

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Introduction: Congenital adrenal hyperplasia (CAH) due to 3β-hydroxysteroid dehydrogenase type 2 deficiency (3β-HSD2D) is an exceptionally rare disorder affecting adrenal steroidogenesis, leading to variable clinical presentations. This study aims to highlight the phenotypic variability and management challenges associated with 3β-HSD2D through the analysis of three pediatric cases.

Methods: We retrospectively reviewed three patients diagnosed with 3β-HSD2D at the Pediatric Endocrinology Department of the University Children's Hospital in Krakow. Clinical features, laboratory findings, genetic analyses, and management strategies were evaluated.

Results: Case 1: A female neonate presented with adrenal insufficiency, electrolyte imbalances, hyperpigmentation, and congenital heart defects. Genetic testing revealed a novel homozygous missense variant c.760T>G (p.Tyr254Asp) in the HSD3B2 gene.

Case 2: A male infant exhibited atypical genitalia without salt-wasting crises. Compound heterozygous variants c.760T>G (p.Tyr254Asp) and c.308-6G > A in HSD3B2 gene were identified. He remains asymptomatic without glucocorticoid therapy.

Case 3: A female infant with salt-wasting crises and virilization was diagnosed with 3β-HSD2D. She later developed gallstones and gastrointestinal symptoms. A pathogenic c.849del variant in homozygosity in HSD3B2 gene was detected.

Conclusions: These cases underscore the clinical heterogeneity of 3β-HSD2D and the necessity for comprehensive genetic evaluation. Novel mutations in the HSD3B2 gene contribute to diverse phenotypes, complicating diagnosis and management. Multidisciplinary approaches are crucial for optimal outcomes, emphasizing the role of genetic testing in guiding therapy and prognostication.

C.O.I. Disclosure:

E. Preizner-Rzucidlo: None. **D. Janus:** None.

EP02.007 Frequencies of KIR alleles and genotypes in a cohort of Romanians with fertility problems

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Background: Killer cell immunoglobulin-like receptors (KIR) represent a family of genes clustered in one of the most variable regions of the human genome. KIR receptors show the highest degree of diversity at the level of NK cells and have a stimulatory or inhibitory effect on these cells. The main purpose of the study is to determine the KIR alleles' and genotypes' frequencies in the analyzed Romanian patients.

Material and methods: The current study includes 266 unrelated Romanian patients with fertility problems which underwent genetic testing for 16 KIR genes. The genotyped KIR alleles were KIR 2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DL1, 3DL2, 3DL3, 3DS1, 2DP1, 3DP1. KIR genotyping was performed by the PCR-SSO technique, using DNA extracted from whole blood collected on EDTA.

Results: The most frequent KIR genotype observed in our patients was the AB genotype (58.6%), followed by AA (28.1%), BB being the least frequent genotype (13.1%). The AA genotype was assigned for patients carrying the KIR haplotype A associated genes (KIR2DL1, KIR2DL3, KIR2DS4 and KIR3DL1). Patients lacking one of these four alleles were associated with the BB genotype. Other combinations of KIR genes are considered to be genotype AB.

Conclusion: The results of the current study reveal the frequencies of KIR genes and genotypes found in the analyzed Romanian cohort. Further studies are needed for the confirmation of the results.

C.O.I. Disclosure:

R. Ursu: None. **A. Caragea:** None. **A. Constantinescu:** None. **I. Constantinescu:** None. **L. Bohiltea:** None.

EP02.008 A Novel Loss of Function Mutation in The MCMDC2 Gene is Associated With Male Infertility

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Background: Approximately 30–40% of male infertility cases are idiopathic. It is believed that some of them are caused by genetic defects.

Recently, homozygous mutations in the MCMDC2 gene were associated with azoospermia in humans.

Material and methods: Whole Exome Sequencing of peripheral blood DNA and bioinformatic analysis, as well as sanger sequencing.

Results: Using bioinformatic analysis for homozygous deletions and SNP's variants we identified a novel homozygous frameshift mutation in the MCMDC2 gene (p.L86Vfs*10) in a 28-year-old Ashkenazi Jew male suffering from non-obstructive azoospermia with complete maturation arrest.

It was proposed that MCMDC2 enables ATP and DNA binding activities and be involved in double-strand break repair via break-induced replication. Three missense and three nonsense variants in the MCMDC2 gene had been reported in humans, in relation to male infertility. MCMDC2 knockout mice present male infertility characterized by spermatogenesis arrest and azoospermia, as well as female infertility.

Conclusion: Based on these findings, we propose that this variant is probably the leading cause of the patient's infertility. Additionally, these results highlight the importance of genetic testing in idiopathic male infertility cases which may provide better infertility consulting.

Ori cohen and Florence Abou contributed equally.

C.O.I. Disclosure:

O. Cohen: None. **F. Abou:** None. **N. Waldenberg:** None. **M. Gershoni:** None. **N. Steiner:** None. **R. Parvari:** None. **S. Barak:** None.

EP02.009 Exploring the role of long non coding RNAs as a molecular marker of endometriosis

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Background: Endometriosis is a chronic gynecological condition affecting women of reproductive age, characterized by the presence of endometrial-like tissue outside the uterus. Recent advances in molecular biology suggest that long non-coding RNAs (lncRNAs) may play a significant role in disease mechanisms. This study aims to analyze lncRNA expression profiles to identify potential molecular markers associated with endometriosis.

Material and methods: LncRNA expression was analyzed in patients with endometriosis ($n = 24$) and endometriosis-free controls ($n = 24$) using the Expression v3 Microarray Kit, $8 \times 60K$ (Agilent, Perlan). Bioinformatics and statistical analyses are currently in progress to identify differentially expressed lncRNAs with potential diagnostic relevance.

Results: The laboratory phase of the study, including RNA isolation and microarray analysis, has been completed. Initial data processing is underway to evaluate lncRNA expression patterns and their potential as molecular markers of endometriosis. The next phase will focus on statistical validation and functional analysis of the identified lncRNAs.

Discussion: This study investigates whether specific lncRNA expression patterns could serve as molecular markers for endometriosis. Identifying such markers may contribute to the development of improved diagnostic tools and a deeper understanding of the disease at the molecular level. Further research will be essential to confirm these findings and explore their potential for clinical implementation.

C.O.I. Disclosure:

U. Wysocka: None. **A. Kucinska:** None. **T. Szaflik:** None. **K. Szyłło:** None. **A. Gach:** None.

EP02.010 Cytogenetic studies in reproductive function disorders: a retrospective analysis

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Background: Fertility disorders are the most common problems of reproductive medicine. Chromosomal abnormalities (CA) play a crucial role in reproductive failure (RF). The main purpose of our study was to determine the types and frequency of CA in couples with history of infertility.

Material and methods: Karyotyping was performed in 652 couples (1304 individuals) with RF between 2004 and 2022, including 203 couples (406 individuals) with primary infertility (PI), 240 couples (480 individuals) with recurrent pregnancy loss (RPL) and 209 (418 individuals) - with sporadic pregnancy loss (SPL).

Results: Numerical and structural CA were detected in 37 individuals (2.84 %), most frequently in group with PI - 3.4 % and RPL - 3.3 %. Karyotype abnormalities were detected in 3.1 % woman and 2.6 % men. The most common CA (26 cases; 2 %) were translocations, reciprocal (RT) - 18 cases (1.4 %) and Robertsonian (RobT) - 8 cases (0.6 %). 15 out of 23 chromosomes participated in RT. Chromosomes 2, 3, 8, 6, 9, 10, 5, 12 and 17 were most often involved. The RobT were detected as der(13;14) (most often, 3 cases), der(13;15), der(14;22), der(15;21). Numerical and structural gonosomal abnormalities were detected in 9 individuals (0.7 %), supernumerary marker chromosome - in 2 (0.14 %).

Conclusion: Karyotype abnormalities are more common in individuals with PI and RPL and occur with the same frequency among women and men. Translocations are the predominant CA in individuals with RF. Numerical gonosomal abnormalities were detected only as mosaic form.

C.O.I. Disclosure:

N. Huleyuk: None. **H. Bezkorovaina:** None. **I. Tkach:** None. **D. Zastavna:** None.

EP02.011 Effect of busulfan on cell viability and mitochondrial dynamics gene expression in rat sertoli cells

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Background: Mitochondria are dynamic organelles essential for cellular homeostasis through fusion and fission, which are crucial for spermatogenesis. Busulfan, a chemotherapeutic agent, disrupts spermatogenesis by inducing mitochondrial dysfunction. While its effects on germ cells are established, its impact on Sertoli cells is less clear. Sertoli cells are more resistant to busulfan-induced apoptosis, but high doses or prolonged exposure may still affect their function, including mitochondrial dynamics, oxidative stress, and viability. Although busulfan may not cause significant Sertoli cell death, it can alter mitochondrial dynamics. The effects on mitochondrial dynamics-related gene expression remain understudied.

Material and methods: The effects of busulfan on Sertoli cell viability and mitochondrial dynamics were assessed in vitro. Rat Sertoli cells were treated with busulfan (0.01 nM to 2000 µM), and cell viability was evaluated using the MTT assay 24 and 48 hours. Mitochondrial fission and fusion were analyzed by RT-PCR, with expression levels of fission genes (FIS1, DRP1) and fusion genes (MFN1, MFN2, OPA1) compared between busulfan-treated and untreated control groups.

Results: Busulfan treatment did not significantly affect Sertoli cell viability, as indicated by MTT assay results. However, RT-PCR analysis showed increase in the expression of both mitochondrial fission and fusion genes in the busulfan-treated group compared to controls.

Conclusion: This study is the first to examine the effects of busulfan on mitochondrial dynamics-related genes in Sertoli cells. The findings suggest that busulfan does not significantly affect cell viability, but induces changes in mitochondrial dynamics as a compensatory mechanism to maintain cellular homeostasis under stress conditions.

C.O.I. Disclosure:

N. Farhangzad: None. **O.S. Aydos:** None. **T. Ozkan:** None. **M. Tiryaki:** None. **A. Sunguroglu:** None. **K. Aydos:** None.

EP02.012 Detection of Parvovirus B19 and CMV foetal infection by real time PCR - clinico-pathological presentations and outcomes

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Background: Human parvovirus B19 and cytomegalovirus (CMV) are two common viruses that can have significant and devastating effects on a foetus. Both infections may cause nonimmune foetal hydrops and anaemia, as well as abnormal foetal morphology. The objective was to evaluate the prenatal incidence of these infections in suspected cases (with positive serology), their clinical presentation and pregnancy outcome.

Material and methods: The presence of foetal anaemia was assessed by the peak of systolic velocity of the middle cerebral artery (PVS-MCA) Doppler. For foetal diagnosis, real time PCR was performed through amniocentesis or cordocentesis. Six prenatal cases are included in the presentation, one pregnancy was bichorial/biamnial.

Results: In 5 out of 6 cases (83%), we detected foetal infection - 2 positive cases for CMV and 3 positive cases for Parvovirus B19. Clinical presentation of CMV infection, along with foetal anaemia, included echogenic intestines, cardiomegaly, calcifications in white brain matter, large placenta, splenomegaly, ventriculomegaly, abnormal gyration; the pregnancy ended with foetal death. The main clinical presentation in Parvovirus B19 infection was foetal anaemia and foetal effusions (ascites or pleural effusion); in one case there was cardiomegaly and tricuspid regurgitation. Selective foeticide was performed in the twin pregnancy; otherwise, the effusions were resorbed and condition was normalized.

Conclusion: The combination between foetal anaemia and positive serology indicates high probability for foetal viral infection and requires the performance of PCR viral diagnostics in foetal samples. Pregnancy outcome depends on the additional symptoms of the foetus.

C.O.I. Disclosure:

M. Hristova-Savova: None. **P. Chaveeva:** None. **V. Kalev:** None. **T. Vassilev:** None. **A. Shterev:** None. **I. Dimova:** None.

EP02.013 When hearts reveal hidden genes: The role of recurrent cardiac rhabdomyomas in identifying familial Tuberous Sclerosis

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Background: In some families without any positive family history, the genetic condition only becomes apparent when a foetus or child exhibiting related phenotypic features undergoes detailed evaluation. We present a family in which one offspring and one foetus were clinically suspected to have Tuberous Sclerosis (TSC) which led to diagnosis of TSC in husband.

Material and methods: A non-consanguineously married couple came for preconception counselling. Their first female child, passed away at seven months of age from cardiac rhabdomyoma. During their second pregnancy, cardiac rhabdomyoma was detected at 22 weeks of gestation, leading to the family choosing to terminate the pregnancy. Both partners were healthy, without significant family history of epilepsy or intellectual disability. On detailed evaluation suspecting TSC, the male partner exhibited ash leaf macules, angiofibroma's, confetti skin lesions, dental enamel pits, multiple retinal hamartomas, and a retinal achromic patch (two major and one minor diagnostic criteria).

Results: Next generation sequencing revealed a pathogenic variant in TSC2 gene, c.5155G>C (p.Ala1719Pro), (PP3,PM1,PM5,PM2,PP5, 10P-0B = 10 points). The family found it challenging and shocking to accept the reality of a genetic syndrome affecting one of the partners. However, with in-depth genetic counselling and ongoing support, they could understand and accept the diagnosis and opted for prenatal testing next pregnancy.

Conclusion: This case highlights the importance of genetic counselling and testing in families where genetic conditions may be present, even when family history is not initially apparent. It also underscores the value of early diagnosis, surveillance, and prenatal options in managing autosomal dominant disorders with variable expressivity.

C.O.I. Disclosure:

L.D. Goyal: None. **S.K. Goyal:** None. **S. Gupta:** None.

EP02.014 An unbalanced translocation between chromosomes Y and 15 in a male with infertility

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Background: Patients with infertility and repeated pregnancy losses have a higher likelihood of being carriers of balanced chromosome rearrangements; therefore, genetic testing is advised.

Material and methods: Here, we present a couple (a 36-year-old male and a 30-year-old female) with 7-year history of infertility who where referred to genetic consultation. During this period, the couple experienced two miscarriages and had not undergone any assisted fertility treatments. G-banded karyotype of peripheral blood lymphocytes was performed for the couple. C-banding and Fluorescent in situ hybridization (FISH) analysis were done to clarify detected chromosome aberration.

Results: The female's karyotype showed no large structural aberrations (46,XX), while the male's karyotype revealed unclear additional material on the short arm of chromosome 15p11.2. C-banding showed a increased heterochromatin block on chromosome 15p region, as well as heterochromatin on chromosome Yq. Additional FISH analysis with Xq/Yq subtelomeric probes was performed. It showed Yq subtelomeres present on the enlarged 15p and on Yq. The final karyotype was concluded to be 46,XY,der(15)?t(Y;15)(q12;p11.2). A similar derivative chromosome 15 has been reported for pregnant woman and her female fetus [1].

Conclusion: We found an unbalanced translocation between chromosomes Y and 15 in a male with infertility. Increase heterochromatin on Yq is found more often in infertile males. It is thought that it could interfere with homologous pairing but affect on translocated region is even more unclear. However, is highly unlikely, that this rearrangement is the cause of the couples infertility.

C.O.I. Disclosure:

A. Kybartiene: None. **Z. Zemeckiene:** None. **M. Sukys:** None. **J. Peciulaitiene:** None. **D. Medelinskiene:** None. **R. Ugenskiene:** None.

EP02.015 Increased prevalence of *MTHFR* 677TT genotype in spontaneously aborted embryos

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Background: Up to 60% of fetal deaths have no identified etiology. Attempts to determine the cause of fetal death remain important, as this can influence preconception counseling. Methylenetetrahydrofolate reductase (MTHFR, EC 1.5.1.20) is a key enzyme in folate-dependent homocysteine remethylation. Common single nucleotide polymorphisms *MTHFR* 677C/T (rs1801133) and 1298A/C (rs1801131) decrease the activity of enzyme, leading to hyperhomocysteinemia, particularly in folate-deficient states. The aim of our study was to investigate the prevalence of *MTHFR* 677C/T and 1298A/C polymorphism in spontaneously aborted fetuses.

Material and methods: DNA was isolated from 55 fetal tissue samples (FTS) and from 45 peripheral blood samples of children control. Genetic testing of *MTHFR* 677C/T and 1298A/C polymorphism was performed by polymerase chain reaction (PCR-RFLP method) using *HinfI* and *MboII* restriction endonuclease (Thermo Fisher Scientific, USA).

Results: The results showed the higher frequency of heterozygous genotype 677CT in control group - 51.0% compared to 33.0% in the fetal tissue samples. The 677TT genotype was observed more frequently in aborted fetuses than in control group (18.0% vs 4.0%, P = 0.026). As a result it was found a nearly 5-fold increased risk of spontaneous abortion for the fetus with 677TT genotype compared to those with the wild-type genotype 677CC (OR = 4.8, CI: 0,99–23,91). For the *MTHFR* 1298 A/C polymorphism was not found significant difference among examination group.

Conclusion: The present finding of high prevalence of *MTHFR* 677TT genotype in spontaneously aborted embryos indicate its influence on foetal development and emphasize the important role of preconceptional folic acid supplementation.

C.O.I. Disclosure:

L. Chorna: None. **Y. Zahanyach:** None. **O. Kolodiy:** None. **S. Tsybulka:** None.

EP02.016 Routine Genetic Testing in Infertility: A Single-Center Experience (2022–2024)

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Background: Infertility affects 10–15% of couples worldwide, with genetic factors playing a significant role. Routine genetic tests, such as karyotyping, Y-chromosome microdeletion analysis, and thrombophilia-related gene testing, are widely used but often yield limited results. This study evaluates the diagnostic yield of routine genetic tests performed at a single Genetics and Molecular Medicine center from 2022 to 2024 to assess their contribution to infertility diagnostics.

Material and methods: A total of 401 infertility patients underwent routine genetic testing. Karyotyping was performed for all patients to detect chromosomal abnormalities. Y-chromosome microdeletion analysis was conducted for 74 males with azoospermia/oligospermia, *CFTR* sequencing for 27 patients with suspected congenital absence of the *vas deferens* (CABVD). *F2* and *F5* gene testing was carried out for 93 female patients with recurrent pregnancy loss (RPL).

Results: Karyotyping identified 11 chromosomal abnormalities (11/401; 2.74%), including balanced translocations and Klinefelter syndrome. Y-chromosome microdeletion analysis detected 3 AZFc deletions (3/74; 4.05%) among patients with azoospermia or oligospermia, *CFTR* sequencing identified two compound heterozygous genotypes associated with azoospermia (2/27; 7.4%). *F2* and *F5* testing revealed 3 variants (3/93; 3.22%) in RPL patients. Overall, the diagnostic yield of routine genetic testing was low, highlighting the complexity of infertility.

Conclusion: Routine genetic testing provides limited diagnostic yield, particularly for RPL, where thrombophilia screening is discouraged without additional risk factors, per ESHRE guidelines. Expanding genetic testing beyond standard protocols could improve diagnostic accuracy. Large-scale, targeted genetic testing should be integrated into infertility diagnostics to enhance personalized care and optimize patient outcomes.

C.O.I. Disclosure:

K. Aleknavičienė: None. **M. Šukys:** None. **R. Traberg:** None. **I. Pocevičienė:** None. **R. Ugenskienė:** None.

EP02.017 Genomic characterization of partial chromosomal abnormalities in early pregnancy losses

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Background: Pregnancy loss (PL), especially early pregnancy loss (EPL), affects 15% of confirmed pregnancies. Chromosomal abnormalities are found in over half of EPLs, with trisomies being most common, while partial abnormalities, occur in up to 10% of cases. This study focuses on characterization of partial abnormalities previously identified by Quantitative fluorescent polymerase chain reaction (QF-PCR) and multiplex ligation probe amplification (MLPA) analyses.

Material and methods: Using array comparative genomic hybridization (aCGH), we analyzed 20 EPLs with partial abnormalities.

Results: A total of 32 partial chromosomal abnormalities, including 18 deletions and 14 duplications were identified, with an average size of 33.2 Mb. Notably, two abnormalities previously undetected by QF-PCR and MLPA were revealed (deletions in 7q36, and 1p36.32p36.31 regions), emphasizing the necessity of high-resolution genomic tools. Chromosomes 1, 18, and 13 emerged as frequently involved, aligning with previous associations with recurrent pregnancy loss. Recurrent abnormalities were identified in six chromosomal regions, with chromosome 1p36.33-p36.32 exhibiting the highest frequency. Gene Ontology (GO) enrichment analysis of recurrent regions highlighted disruptions in critical biological processes, including molecular binding, enzymatic activity, and cellular development. Many genes in these regions are linked to multisystem syndromes, suggesting their involvement in embryonic development and pregnancy viability.

Conclusion: Our findings underscore the complexity of EPL's genetic landscape, demonstrating that large CNVs may disrupt multiple genes critical for development. Although, subtelomeric MLPA reliably detects telomeric partial chromosomal abnormalities in EPLs, aCGH is essential for detection and precise characterization of all CNVs, thus enhancing diagnostic and counseling strategies in affected couples.

C.O.I. Disclosure:

G. Bozhinovski: None. **M. Terzikj:** None. **K. Kubelka-Sabit:** None. **D. Plaseska-Karanfilska:** None.

EP02.018 Genetic polymorphisms in BMP15 in women with premature ovarian insufficiency: association with laboratory and clinical parameters

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Background: Bone morphogenetic protein 15 (BMP15) is an oocyte-specific protein with crucial for early folliculogenesis and is a modulator of granulosa cell development and differentiation. Understanding the important part BMP15 plays in the development of Premature ovarian insufficiency (POI), we set out to investigate the prevalence of its genetic variations in a cohort of women with POI and correlate them to the patients' clinical-laboratory characteristics.

Material and methods: Direct Sanger sequencing was conducted on the BMP15 gene in 37 patients diagnosed with POI. The identified variants were then compared with data from an internal database consisting of unselected individuals to assess frequency and potential association with POI.

Results: We detected one or two of the following variations in 56.8% of the patients: c.-9C > G, c.308A>G, c.852C>T, and c.538G>A. Heterozygotes for polymorphisms c.-9C > G (40.5%) and c.308A>G (29.7%) were found to be substantially more common in POI patients. We also discovered that in patients with POI, the combined carriership of these two frequent variations is substantially linked to elevated FSH levels and the duration of amenorrhea.

Conclusion: Our research highlights the importance of BMP15 as a candidate gene and the necessity of conducting studies in a range of demographic groups before it can be incorporated into the algorithm for the early diagnosis and management of POI patients.

C.O.I. Disclosure:

I. Oprova: None. **P. Andreeva:** None. **K. Belemezova:** None. **I. Dimova:** None.

EP02.019 Comprehensive clinical and genetic study of a couple with five recurrent biochemical pregnancies and three miscarriages

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Background: Recurrent biochemical pregnancy (BP) is defined as more than two early pregnancy losses that occur before a gestational sac is detected. The etiology remains unclear. Similar to pregnancy loss, the cause of BP may be embryonic (embryonic aneuploidy), maternal (thinned endometrium) or even possibly paternal (sperm abnormalities). However, there is no information on the correlation between biochemical pregnancies and sperm defects.

Aim: We aimed to perform a comprehensive clinical and genetic study in a couple with five repeated biochemical pregnancies and three miscarriages.

Materials and methods: Whole exome sequencing (WES) was performed on DNA samples from both partners and one of the miscarriages; the latter was also subjected to arrayCGH analysis.

Results: There were no pathogenic CNV in the embryonic DNA or in the genetic analysis from WES. The following pathogenic/likely pathogenic variants were detected in the couple: mother - c.244dupT in ACADM (acyl-CoA dehydrogenase deficiency), c.73_86dupCGGCTTCCTGGCGC in BCKDHB (Maple syrup urine disease), c.30dupA in GNRH1 (hypogonadotropic hypogonadism) and c.11804 C > T in DNAH11 (ciliary dyskinesia); father - c.274C>T in FCGR1A (IgG receptor I, phagocytic, familial deficiency), c.1087C>T in ASS1 (citrullinemia), c.150C>A in TNFRSF13B (immunodeficiency, common variable; immunoglobulin A deficiency 2) and VOUS c.5224G>A in LOXHD1 (deafness).

Discussion: In addition to heterozygous recessive mutations, we found two paternal mutations in genes related to immune defence (FCGR1A and TNFRSF13B). Clinical examination revealed persistent urogenital infections in the father despite treatment.

Conclusion: We suggested that immune-related sperm abnormalities could be the cause of recurrent BP and pregnancy loss.

C.O.I. Disclosure:

K. Belemezova: None. **D. Kachakova-Jordanova:** None. **K. Kamenarova:** None. **K. Mihova:** None. **T. Timeva:** None. **R. Kaneva:** None. **I. Dimova:** None.

EP02.020 Whole-genome sequencing reveals two distinct causative variants underlying pregnancy loss and neonatal death in a consanguineous couple

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Background: To investigate the genetic factors contributing to the reproductive challenges faced by a consanguineous couple who experienced multiple pregnancy losses and neonatal deaths due to polymalformative syndromes and to discuss the potential role of preimplantation genetic testing (PGT) in future pregnancies.

Materials and methods: The couple underwent genetic testing following a history of two neonatal deaths caused by severe congenital deformities and one early pregnancy loss. Whole-genome sequencing and subsequent targeted analysis of genes associated with congenital malformations were performed on both parents.

Results: One pathogenic variant in *CC2D2A* (c.4675-1G > C) and one likely pathogenic variant in *PIGN* (c.2237_2238del), present in both parents, were identified. The *CC2D2A* gene is associated with Joubert syndrome, a disorder affecting brain development and resulting in developmental delays, systemic malformations, and neonatal death in severe cases. Genetic defects in *PIGN* are linked to PIGA-related intellectual disability syndromes, which can present with intellectual disabilities and congenital deformities, often leading to early postnatal mortality. Both conditions are inherited in an autosomal recessive manner, significantly increasing the risk of recurrence in consanguineous couples.

Conclusion: Given the couple's genetic profile, their history of neonatal deaths, and the high likelihood of recurrence of genetic conditions in future pregnancies, PGT is strongly recommended. PGT, performed in conjunction with in vitro fertilization, could identify unaffected embryos, thereby reducing the risk of passing on these severe conditions. Genetic counseling is essential to help the couple understand their reproductive options and optimize their chances of achieving a healthy pregnancy.

C.O.I. Disclosure:

S.Y. Yaneva Staykova: None. **M. Atanasoska:** None. **L. Balabanski:** None. **V. Staykov:** None. **S. Raykov:** None. **R. Vazharova:** None.

EP02.021 Whole exome sequencing approaches to deciphering the genetic landscape of miscarriage

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Background: Spontaneous abortion is the most common complication in the first trimester of pregnancy. A variety of the factors can contribute to pregnancy loss, including genetic factors, structural abnormalities, disorders of the coagulation system, endocrine pathologies, and inflammatory processes. Numerical chromosomal abnormalities are the primary cause of miscarriage. However, the cause of up to 50% of spontaneous abortions with an euploid embryo remains unidentified.

Material and methods: The 852 samples of abortive material were collected. All samples were analyzed using standard karyotyping or FISH methods. A group of chorionic samples (n = 73) with a normal karyotype was further studied using a chromosomal microarray analysis (CMA). The euploid chorionic samples and the biological parents of the fetus (trio) were analyzed using exome sequencing. For the analysis of sequencing data a gene panel consisting of 881 genes associated with pregnancy loss was utilized. Furthermore, an analysis of pathogenic and likely pathogenic variants in genes associated with autosomal recessive diseases in the abortive material samples was conducted.

Results: The frequency of chromosomal abnormalities in the group studied was 61%.CMA revealed discrepancies with the results of cytogenetic analysis in three cases: one case exhibited trisomy 14, and two cases presented structural rearrangements. Pathogenic variants were identified in the genes *DHCR7, MIB1, FBN1, FLNB, KCNQ1, NF1*, and *THBD* as potential genetic causes of miscarriage in 8 samples of abortive material.

Conclusion: The results of the study confirm the importance of studying monogenic causes of early fetal death.

C.O.I. Disclosure:

Y.A. Nasykhova: None. **Z.N. Tonyan:** None. **T.E. Lazareva:** None. **N.M. Dvoynova:** None. **M.M. Danilova:** None. **O.V. Malysheva:** None. **A.A. Mikhailova:** None. **A.S. Glotov:** None.

EP02.022 Management of couples requesting preimplantation genetic testing for monogenic disease (PGT-M): from referral to clinical application

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Background: PGT aims to detect genetic abnormalities in in vitro *fertilized* embryos to help couples have 'unaffected' babies. For couples referred for PGT-M with a specific indication, it is essential to offer full genetic evaluation in the context of preconception care, to detect other genetic risks that could be addressed.

Material and methods: The stages from PGT-M referral to clinical application included: 1. Evaluation of medical/reproductive history and recommendation for complementary genetic tests or carrier screening, followed by genetic counselling. 2. Personalized information on PGT, based on the genetic findings, and discussion on the appropriate diagnostic strategy, protocol development requirements (testing relatives or DNA from a previous pregnancy, non-developing embryos and single sperm) and possibilities/limitations of the procedure. 3. Development of PGT protocols with an initial stage of whole genome amplification in blastocyst biopsies, allowing testing of multiple indications, with appropriate techniques for direct/indirect variant detection and chromosomal assessment.

Results: During 2021–2024, following appropriate management, around a quarter of PGT cycles were performed for multiple indications. In total, 178 PGTs were performed in 147 couples. Of these, 135/178 (76%) were performed for one monogenic disease and 43/178 (24%) for more than one indication: 38/43 for monogenic disease and chromosomal abnormalities, 2/43 for hemoglobinopathy and histocompatibility, 2/43 for two and 1/43 for three monogenic diseases.

Conclusion: A thorough investigation of the couple's family and reproductive history, the application of robust PGT strategies for the analysis of multiple indications in a single biopsy constitute the best practice in the implementation of PGT.

C.O.I. Disclosure:

T. Mamas: None. **G. Kakourou:** None. **S. Chatzirvasanis:** None. **K. Kosma:** None. **A. Mitrakos:** None. **N. Selenti:** None. **A. Kampouraki:** None. **I. Sfontouris:** None. **A. Papatheodorou:** None. **E. Mantoudis:** None. **E. Papanikolaou:** None. **E. Nikitos:** None. **F. Makrygiannakis:** None. **S.M. Morou:** None. **G. Kokkali:** None. **K. Chatzimeletiou:** None. **E. Hatzi:** None. **J. Traeger-Synodinos:** None. **C. Sofocleous:** None. **C. Vrettou:** None. **P. Makrythanasis:** None.

EP02.023 First results of two pilot carrier genetic screening projects for monogenic disease risk groups and target population cohort

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Background: As new gene therapies target an increasing number of genetic diseases, implementing universal primary prevention programs for preconception and carrier screening of monogenic diseases is essential.

Material and methods: We provide two genetic screening programs for couples planning pregnancy. The first, focuses on 60 couples with a family history of cystic fibrosis (CF), phenylketonuria (PKU), spinal muscular atrophy (SMA). Testing for variants in CFTR, PAH, SMN1 is conducted using PCR, real-time PCR, MLPA, sequencing. The second program includes 300 couples, screening women for 34 diseases; if a woman is a heterozygous carrier, her male partner is tested for variants in relevant genes. Techniques used include exome sequencing, RT PCR (SMN1), fragment analysis (FMR1), MLPA (DMD, CFTR). Participants receive preventive measures for hereditary diseases through PGT and/or prenatal diagnostics.

Results: As part of targeted screening 27 families with CF, 6 with PKU, 1 with SMA have been studied. The risk of having affected children was ruled out for all families, with natural conception recommended. In 3 families, the carriage of more than one disease was detected. Three families successfully conceived healthy children. Among 290 couples in the second project, carrier status was detected in 12 of the 90 studied families. One family showed a duplication in the DMD wt/dup 38–39 ex(c.(5325 + 1_5326-1)_(5586 + 1_5587-1)dup.

Conclusion: Initial results indicate increased interest in pregnancy planning using genetic technologies among the general population compared to those at risk for monogenic diseases. Collecting data on allele frequencies is vital for monitoring hereditary diseases in the population.

C.O.I. Disclosure:

A. Glotov: None.

EP02.024 Does the inversion carriage is an indication for PGT-SR testing?

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Background: There is no consistent opinion whether different types of inversion cause unbalanced gametes development. In this study we analyzed PGT-SR results of inversion-carriers.

Material and methods: The total number of patients was 28, that was grouped by age: 25–35 years (10 patients, 49 embryos) and 35–42 years (18 patients, 45 embryos). Collected data includes rates of euploidy, aneuploidy, unbalanced translocations and mosaicism. PGT-SR was performed by whole genome amplification followed by NGS.

Results: We got comparable results in different age groups of euploidy rate (55,1% against 54% and 37,8% against 44%), as well

Table 1. Compare PGT-A/PGT-SR data in inversion-carriers and control groups

Groups with chromosomal inversions	Number of patients	Number of embryos	Euploidy rate, %		Aneuploidy rate, %		Segmental event rate, %		Mosaicism rate, %	
			inversion-carriers	control group	inversion-carriers	control group	inversion-carriers	control group	inversion-carriers	control group
25–35 years	10	49	55.1	54	18.37	20	12.24	7	14.29	15
35–42 years	18	45	37.78	44	35.56	37	4.44	4	22.22	12

as percentage of aneuploidy (18,37% against 20% and 35,56% against 37%) over two sample groups. As a control group we used data gotten from over 1000 patients without notable karyotype abnormalities. We found out that the percentage of segmental events was higher in the inversion carriers among younger patients, but mosaicism rate was higher among older patients. There were only 4 samples with segmental translocations involving the breaking point inverted chromosome.

Conclusion: Patients with chromosomal inversions tend to have comparable euploidy and aneuploidy rates and increased percentage of segmental and mosaicism rates.Low rate of embryos with segmental events involving the breaking point of inverted chromosome seems doesn't require necessarily PGT-SR as it occurs in a reciprocal translocation carriers. This implies that inversions have a limited genetic impact in a reproductive potential.

C.O.I. Disclosure:

Y. Rumyantseva: None. **D. Ferdman:** None. **E. Vasilyeva:** None. **R. Bikanov:** None. **N. Isaeva:** None. **V. Zaharova:** None.

EP02.025 Analysis of copy number variations by aCGH in infertile males

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Background: Many genetic changes can underlie male infertility, primarily manifested as sexual dysfunction, oligoasthenospermia, azoospermia, abnormal seminal plasma, and immune infertility. Problems during spermatogenesis are reflected in lower, irregular, or absent sperm production. In cases of idiopathic azoospermia, a genetic cause of infertility is most likely. In our study, genomic CNVs were examined. We expected to detect microdeletions or microduplications on the Y chromosome and autosomal chromosomes, in the regions somehow associated with male infertility.

Material and methods: From a cohort of 400 male patients treated in our infertility clinic, only patients with non-obstructive azoospermia, a normal karyotype or balanced chromosomal changes, without microdeletions on the Y chromosome, without mutations in the CFTR gene, and also without known environmental and other disease conditions that would affect the formation of sperm cells were selected, total 51 patients. Array-CGH was performed.

Results: We identified four pathogenic CNVs and three VUS, together representing 14%. The pathogenic variants included: one duplication on 15q11.1q11.2 and three deletions on Yq11.223q11.3. VUS were detected on autosomes: two on 15q11.1q11.2 and one on 8p11.21p11.1.

Conclusion: Structural abnormalities of the Y chromosome often adversely affect spermatogenesis. We noted that the analysis of CNVs on microarrays could be a complementary method for diagnosing male infertility, especially regarding large deletions on the Y chromosome, which are not covered by the standard analysis of Y microdeletions using the PCR method. Array-CGH could also be the method of choice when the male infertility is associated with microdeletions or microduplications on autosomal chromosomes.

C.O.I. Disclosure:

A. Zagorac: None. **N. Kozar:** None. **M. Reljič:** None. **N. Kokalj Vokač:** None.

EP02.026 Experience with clinical and technical complexities of Preimplantation Genetic Testing for monogenic disease (PGT-M)

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Background: PGT-M is a valuable tool for couples at risk of passing on genetic disorders, but it comes with clinical and technical challenges. This study investigates couples opting for PGT, each facing diverse genetic scenarios.

Material and methods: We reviewed 90 couples counselled for PGT-M between 2023–2024, focusing on 20 couples presenting unique considerations for PGT. These involved conditions with variable expressivity/incomplete penetrance (e.g. carrying variants in *PTPN11, EPHB4, CDKN1C* or CNVs: 22q11.21 microduplication, 1q21.2q22.2 microdeletion), variants associated with mild phenotypes (CF, Hemoglobin H disease, deafness), VUS (*CFTR, ABCA4, MYBPC3*), de novo variants (*RB1, VHL* or 2q11.1q11.2 microdeletion) or lack of relatives (CMT1A, β-thalassemia). Pre- and post-referral clinical/genetic data were analyzed to assess their impact on genetic counselling, protocol development and patient decisions.

Results: Eight couples required additional counseling/investigation for variable expressivity or VUS variants. Four were referred to specialists for information on mild phenotypes/treatment. Nineteen couples decided to proceed with PGT-M. One couple (with VUS) conceived naturally and chose prenatal testing. Seven couples required modified PGT strategies (direct variant testing, single-sperm testing or linkage analysis based on the couples' IVF

embryos). So far, twenty IVF/PGT cycles have been initiated/completed for seventeen couples.

Conclusion: This study highlights the growing complexity of PGT-M referrals. It emphasizes the need for a personalized approach that includes detailed genetic analysis, family history, phenotype assessment and tailored PGT strategies to improve counseling and decision-making. Ethical dilemmas often arise, requiring a careful balance of benefits and emotional impact to help couples make the best reproductive choices.

C.O.I. Disclosure:

G. Kakourou: None. **T. Mamas:** None. **S. Chatzirvasanis:** None. **K. Kosma:** None. **N. Marinakis:** None. **D. Veltra:** None. **A. Kampouraki:** None. **E. Tsorva:** None. **C. Oraiopoulou:** None. **T. Pastroma:** None. **D. Christopikou:** None. **E. Nikitos:** None. **M. Asmarianaki:** None. **S. Mantzouranis:** None. **G. Kokkali:** None. **K. Chatzimeletiou:** None. **M. Tzetis:** None. **J. Traeger-Synodinos:** None. **C. Sofocleous:** None. **C. Vrettou:** None. **P. Makrythanassis:** None.

EP02.027 PGT-M application for tandem genomic duplication leading to autosomal dominant leukodystrophy

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Background: Typical autosomal dominant adult-onset demyelinating leukodystrophy (ADLD) is neurologic disorder characterized by slowly progressive white matter damage, caused by an extra copy of *LMNB1* gene. Patient with inherited 242kb tandem duplication was assigned to PGT-M followed by PGT-A.

Material and methods: Genomic DNA of the proband, his partner and his father were analysed. Whole-genome amplification of ten trophectoderm samples was performed using an Ion Single Seq™ Kit. SeqStudio Genetic Analyzer was used for Sanger sequencing (direct analysis) and fragment analysis (indirect analysis). The PGT-M panel included 11 pairs of self-designed primers. One pair for direct analysis was placed on the duplication junction, thus PCR product could only be detected in case of duplication. Ten polymorphic short tandem repeated markers were amplified using 5'fluorescent-labeled primers to identify disease-associated haplotype. Two markers were defined as uninformative due to the homozygous status in the proband.

Results: In the first IVF cycle, all five embryos inherited an extra copy of *LMNB1* gene. In the second IVF cycle, tree out of five embryos didn't have duplication that was confirmed by both direct and indirect analyses of PGT-M. According to the subsequent PGT-A results, all these three embryos were euploid and recommended for transfer. As a result, the couple gave birth to a child without inherited ADLD.

Conclusion: This study demonstrates the successful application of PGT-M to tandem genomic duplication with known genomic location. Unlike conventional PGT-M cases that target single-nucleotide variants or small deletions, detection of larger structural variations requires an advanced methodological approach.

C.O.I. Disclosure:

E. Vasileva: None. **N. Andreeva:** None. **D. Ferdman:** None. **N. Isaeva:** None. **R. Bikanov:** None.

EP02.028 Real time PCR analysis revealed the correlation between endometrial microbiome and ovarian disfunction

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Background: Several studies have recently defined that the uterine microbiota is important for women's reproductive health. Variations in the microbial community may be associated with hormonal regulation and interfering with reproductive functions. However, there is no consensus on the bacterial core of the endometrium and its role in human reproduction. The primary goal of our research was to identify the characteristics of endometrial microbiota in individuals with diminished or insufficient ovarian function.

Material and methods: A three-year cohort research on 62 patients with their own or donated gametes was conducted. The main objective was to compare the endometrial microbiome of patients with ovarian disfunction (AMA - advanced maternal age; POI - premature ovarian insufficiency; POR - poor ovarian response; DOR - diminished ovarian reserve)($n = 35$) and controls ($n = 27$). It was performed real-time PCR quantification of total bacterial mass, Lactobacillus, facultative and obligate anaerobic microorganisms.

Results: The microbiota composition within the control and study groups showed remarkable similarity with the most predominant genus of lactobacillus spp. with almost equal range (58 % and 49.8%, respectively, NS). Additional subgroup analysis of the patients with ovarian disfunction revealed a significantly reduced percentage of lactobacillus spp in patients with DOR (33.3% vs 51.3%/AMA; 50%/POI; 51.64%/POR,;$p = 0.5$). Subsequently, we conducted Alpha diversity indices of the endometrial microbiota. The patients with POI demonstrate a significantly reduced biodiversity with Shannon Diversity Index(H): 0.1.

Conclusion: The percentage of lactobacillus species in patients with DOR was substantially lower, and biodiversity was considerably reduced in patients with POI even when lactobacillus species levels were normal.

C.O.I. Disclosure:

P. Andreeva: None. **I. Oprova:** None. **M. Dimitrov:** None. **S. Danova:** None. **I. Dimova:** None.

EP02.030 Investigation of genetic etiologies in patients with non-obstructive azoospermia

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Background: Routine genetic evaluation of non-obstructive azoospermia (NOA) includes chromosome and Y chromosome microdeletion (YCM) analysis. Sequencing technologies have also contributed to uncovering genetic causes in male infertility. In this study, we aimed to investigate new genetic causes in NOA patients.

Material and methods: Between November 2022 and June 2024, 31 adult men admitted to the medical genetics outpatient clinic for NOA were included in the study. Patients with secondary causes of azoospermia were not included in the study. All 31 patients first underwent chromosome and YCM analysis. Whole Exome Sequencing (WES) test was performed in 25 patients in whom no cause was found to explain azoospermia.

Results: Chromosomal disorders (47, XXY (3 cases), 45,X/46,X,del(Y)(q11.23) (1 case), 46,X,der(Y) t(Y;?)(q12;p13) (1 case))

were detected in 16.1% of the 31 NOA cases and YCM (AZFc deletion) was detected in 3.2% (1 case). In 16% of 25 NOA patients who underwent WES, a cause that could explain the clinic was found in *MSH4* (2 cases), *STAG3* (1 case) and *NR5A1* (1 case) genes. Novel c.2639_2640del, p.Pro880ArgfsTer14 variation in *MSH4* gene was found homozygous in two unrelated cases from two different families. Novel c.671C>G, p.Ser224Ter variation in *STAG3* gene was homozygous and *NR5A1* gene c.251G>T, p.Arg84Leu variation was heterozygous.

Conclusion: In this study, the success rate of genetic diagnosis in NOA cases with chromosomal analysis, YCM and WES was 32.2% (10/31). The use of WES in NOA cases increases the success of genetic diagnosis and helps infertile couples in the process of pregnancy planning.

C.O.I. Disclosure:

K. Adanur Sağlam: None. **G. Atnallar:** None. **M.A. Ramazanoğlu:** None. **N. Cinkara:** None. **S. Demir:** None. **P. Ata:** None. **I.O. Kazaz:** None. **A. Cebi:** None. **A. Turkyilmaz:** None.

EP02.031 Novel application of whole exome sequencing for optimizing preimplantation genetic testing of monogenic disorders

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Background: Preimplantation genetic testing for monogenic disorders (PGT-M) enables embryo analysis before implantation, but current methods do not provide comprehensive clinically relevant information, including de novo mutations. Whole exome sequencing (WES) allows embryo analysis even without parental genomic data. The recently developed Primary Templated Amplification (PTA) method allows high-quality exome analysis from just a few cells, offering a promising approach for genetic testing. However, the method may introduce artefacts that need to be considered when interpreting the results.

Material and methods: The study used PTA-WGA on small groups of fibroblast cells (4, 10, 16 and 25 cells) to simulate the limited number of cells typical of blastocyst biopsies. PTA-WGA was conducted per BioSkryb Genomics instructions, and exome enrichment was done with Agilent V8 probes. Samples were sequenced in paired-end mode on the DNBSEQ-G400 platform (MGI Tech). Strict criteria for artifact filtering were selected through bioinformatics processing.

Results: In this study, we evaluated WES using PTA-based WGA on small cell groups. PTA amplification provided sufficient DNA for library preparation without compromising data output despite variable cell counts. The sequencing results showed 97.5% coverage of the target region at 10x depth for all samples, meeting ACMG standards. A custom artifact-filtering approach ensured accurate variant detection, with approximately 2–3% of variants filtered out.

Conclusion: The quality metrics of PTA-amplified cell group samples meet the requirements for accurate variant detection in PGT-M. Our filtering approach effectively removes false positives while preserving clinically significant variants, ensuring the data is suitable for further interpretation.

C.O.I. Disclosure:

A. Samitova: None. **V. Belova:** None. **I. Vasiliadis:** None. **Z. Repinskaia:** None. **T. Gorodnicheva:** None. **E. Romanov:** None. **M. Pogosyan:** None. **E. Gaysin:** None. **T. Nazarenko:** None. **D. Rebrikov:** None. **D. Korostin:** None.

EP02.032 Scaling up EmbryoMap PGT-A to 96 samples per run without compromising reliability and data quality

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Background: Originally, EmbryoMap PGT-A workflows was developed for Illumina MiSeq, which provide reliable data but are limited in sample throughput. As the first in the world, we transitioned to the Illumina NextSeq to improve efficiency, data throughput and quality for PGT-A.

Material and methods: Genetic testing was performed using the EmbryoMap PGT-A kit (Vitrolife). NGS was conducted on Illumina MiSeq (48 samples per run) and NextSeq 550 (96 samples per run) systems, following the same protocol for both platforms. Sequencing reads were processed using EmbryoMap software (Vitrolife).

Results: Utilizing the NextSeq 550 for EmbryoMap PGT-A enabled a two-fold increase in laboratory productivity while simultaneously increasing the number of reads per sample and enhancing data quality. The NextSeq 550 produces significantly higher average read counts ($2{,}750{,}000 \pm 130{,}000$) compared to MiSeq ($707{,}000 \pm 31{,}000$), representing a ~ 289% increase. The MiSeq showed an average noise level of 0.19 ± 0.03, while the NextSeq demonstrated a 37% reduction in overall noise, achieving a lower and more stable level - 0.12 ± 0.03. This improvement highlights enhanced data consistency and quality with NextSeq platform. The percentage of failed results decreased from 3.5% on MiSeq to 1% on NextSeq.

Conclusion: This study is the first to report the use of the Illumina NextSeq 550 system for EmbryoMap PGT-A testing, demonstrating its potential in clinical applications. Transitioning from MiSeq to NextSeq enabled a significant increase in sample throughput, scaling from 48 to 96 samples per run, meeting the growing demand for high-throughput PGT-A testing.

C.O.I. Disclosure:

A. Kartuesov: None. **M. Temirgalieva:** None. **I. Shoyusupova:** None. **A. Kullyev:** None. **S. Avdeichik:** None. **Y. Goikhman:** None.

EP02.033 Targeted carrier screening for inherited genetic disease in Romanian couples: a single center study

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Background: Carrier screening aims to identify couples at an increased risk of having an affected child to facilitate informed reproductive decision-making. Current genetic testing technologies have enabled the expansion of carrier screening to multiple conditions or genes, allowing all couples to benefit from this comprehensive test.

Material and methods: This study included 66 couples referred to our center for routine targeted carrier screening. DNA was isolated from peripheral blood or buccal swab. 150 genes were analyzed by sequencing (NGS), TP-PCR technique, and MLPA.

Results: A total of 23.5% (31/132) were found carriers of at least one recessive condition. The average number of variants found in carriers was 1.35, ranging from 0 to 5. Out of 66 couples screened, in 97% ($n = 64$) one partner was a carrier of at least one condition, in 55% ($n = 36$) both partners were carriers of at least one condition and in 9% ($n = 6$) both partners were heterozygous for the same recessive disorder. Of all couples with both partners carriers of the same genetic condition, only 2 had a family history of genetic disorders or congenital anomalies.

Conclusion: Carrier screening has the potential to improve couples' reproductive decisions by informing them about their risk of having children affected by severe genetic conditions. Our data show that rather than offering targeted carrier screening only to high-risk families based on ancestry or family history, this test could be offered to any couple wishing to have children, as a universal preconception test.

C.O.I. Disclosure:

A. Pavel: None. **A. Tutulan-Cunita:** None. **D. Prepelita:** None. **D. Bratu:** None. **R. Truica:** None. **M. Stratan:** None. **I. Focsa:** None. **D. Stambouli:** None.

EP02.034 ReproNAGEN: description of reproductive risk variants identified in the ReproNAGEN cohort in Navarra, Spain

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Background: Genetic carrier screening is essential to prevent transmission of inherited diseases. These tests identify asymptomatic individuals who are carriers of pathogenic variants. We have evaluated in the ReproNAGEN project the increase of carriers as a result of extending the study from the original 3 to 113 genes proposed by the ACMG with the aim of preventing the onset of severe diseases in paediatric age; key for a correct risk assessment and decision making before, during and after pregnancy.

Material and methods: We recruited and performed whole genome sequencing in 74 couples with fertility problems. Firstly, we analysed CFTR, FMR1 and SMN1. Subsequently, we analysed the same cases using the expanded panel of 113 genes recommended by the ACMG, which are associated with autosomal recessive or X-linked recessive inheritance diseases.

Results: Variants associated with reproductive risk were found in 61 individuals when the expanded panel was used, compared to 16 if the analysis was restricted to the original 3 genes. The increase in the number of genes to be studied meant an increase in the detection of carriers from 10.81% to 41.21%. As for the couples, 2 had variants in the same gene, 2.70%, to whom a Preimplantation Genetic Test for Monogenic Diseases (PGT-M) was performed.

Conclusion: The remarkable increase number of carriers highlights the effect that would derived from the implementation of this expanded screening in general population at reproductive age. Such preventive measure will hugely contribute to the health system since it will massively improve early-detection of several disorders.

C.O.I. Disclosure:

S. Ciria Abad: None. **M. Fernández Hernández:** None. **V. García Solaesa:** None. **F. Alvira-Iraizoz:** None. **S. Razquin Sola:** None. **L. Matalonga Borrel:** None. **G. Bullich Vilanova:** None. **I. Paramonov:** None. **M. Miranda Pérez:** None. **D. Cobos Hermosa:** None. **N. Sota Echarri:** None. **A. Martínez Carrasco:** None. **R. Tonda Hernázdez:** None. **Á. Alonso Sánchez:** None. **J. Zabaleta Jurío:** None.

EP02.035 Oligogenic inheritance in congenital hypogonadotropic hypogonadism

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Background: Congenital hypogonadotropic hypogonadism (CHH) is a rare genetic endocrine disorder caused by deficient production, secretion or action of gonadotropin-releasing hormone (GnRH), the master hormone regulating reproduction. The disorder is characterized by absent puberty and infertility, and exhibits clinical and genetic heterogeneity with variable expressivity and incomplete penetrance. To date >40 different causal genes have been identified acting either alone or in combination (oligogenicity).

Material and methods: To assess the extent of oligogenic inheritance in CHH, we studied a cohort of 538 unrelated CHH probands and 601 controls using high-throughput sequencing data. We focused on combinations of rare variants in 41 CHH genes with a MAF < 1% for autosomal recessive and 0.01% for autosomal dominant genes and performed an enrichment analysis for known CHH gene pairs in CHH vs controls.

Results:135 out of 538 probands (25%) exhibited oligogenic events involving 40 CHH genes. A significant enrichment for oligogenic events for several CHH genes (e.g. *DMXL2* and *FGFR1* exhibiting the highest significance with adjusted p-values of 3.38×10^{-10} and $1.84x^{10-7}$, respectively) was found.

Conclusion: Altogether, these data show a statistically higher frequency of oligogenicity (25%) than previously reported and may help differentiate syndromes with overlapping clinical phenotypes.

C.O.I. Disclosure:

I. Habibi: None. **Y. Wang:** None. **Y. Zouaghi:** None. **J. zhai:** None. **A. Ameti:** None. **F. de Azevedo Correa:** None. **T. Todisco:** None. **M. Adamo:** None. **A. Jacobs:** None. **C. Perdices-López:** None. **Z. Kolesińska:** None. **G. Papadakis:** None. **F. Phan-Hug:** None. **S. Pekic:** None. **P. Duarte:** None. **M. Bonomi:** None. **M. Cools:** None. **A. de Paepe:** None. **M. Geffner:** None. **Y. Mamoojee:** None. **L. Persani:** None. **V. Popovic:** None. **R. Quinton:** None. **W. Dhillo:** None. **N. Sato:** None. **A. Boizot:** None. **R. Millar:** None. **F. Ambrin:** None. **M. Ansar:** None. **A. Messina:** None. **N. J Niederlander:** None. **J. Acierno:** None. **F. Santoni:** None. **N. Pitteloud:** None.

EP02.036 Uncovering miRNA Dysregulation in Male Infertility: A Comprehensive Small RNA-Sequencing Analysis

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Background: Male infertility is a multifactorial condition with diverse underlying causes. This study aimed to investigate the small RNA (sRNA) landscape in sperm samples from normozoospermic men and those with various sperm defects to identify microRNAs (miRNAs) associated with these conditions and to uncover their potential roles in sperm quality and function.

Material and methods: Small RNA sequencing was performed on sperm samples from 37 patients, categorized into the following groups: normozoospermic, oligozoospermic, oligoteratozoospermic, asthenoteratozoospermic, and oligoasthenoteratozoospermic. Differential expression analysis was conducted to identify the differentially expressed miRNAs between groups. Target genes of differentially expressed miRNAs were predicted, and Gene Ontology (GO) and KEGG pathway analyses were performed.

Results: A total of approximately 40,000 sRNAs were detected, with significant differences in small RNA expression ($\log_2$fold change≥2, p-value < 0.05) observed across groups. Infertile samples, particularly those with lower sperm counts or mixed pathologies, exhibited increased miRNA dysregulation compared to normozoospermic samples. Among these, teratozoospermic samples displayed the highest levels of dysregulation, distinguishing them as having the most unique miRNA profiles. Pathway analysis linked these dysregulated miRNAs to processes associated with signaling pathways and metabolism. In contrast, normozoospermic samples showed a relatively stable miRNA expression profile.

Conclusion: This study highlights significant differences in miRNA profiles between fertile and infertile groups, with teratozoospermia emerging as the most distinct condition based on miRNA expression patterns. The findings provide valuable insights into miRNA-mediated mechanisms underlying male infertility and identify potential miRNA biomarkers and therapeutic targets for improving diagnostic and treatment strategies.

C.O.I. Disclosure:

M. Kyrgiafini: None. **A. Kaltsas:** None. **A. Chatziparasidou:** None. **Z. Mamuris:** None.

EP02.037 Impact of Ovarian Stimulation Protocols on Gene Expression and Reproductive Outcomes in Assisted Reproductive Technology

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Background: Assisted reproductive technology (ART) outcomes are influenced by ovarian stimulation protocols, which may differentially impact gene expression and reproductive success. This study compared the effects of controlled ovarian hyperstimulation (COH) and modified natural cycle (MNC) protocol on the expression of key folliculogenesis genes (AMH, AMHR2, FSHR, AR) and their associations with oocyte, zygote, and embryo quality, as well as fertilization and embryo transfer success.

Material and methods: A total of 124 cumulus-oocyte complexes were collected from 55 normo-ovulatory women undergoing assisted reproduction with COH or MNC. Gene expression was analyzed using RT-PCR, and oocyte, zygote, and embryo morphology were assessed. Chi-square and multivariate regression tests, were used to determine associations between gene expression levels and reproductive outcomes.

Results: No significant differences in gene expression levels were found between the protocols. Higher AMH expression was associated with successful fertilization ($p = 0.020$), while elevated AMHR2 expression correlated with embryo transfer success ($p = 0.038$). Morphological assessments showed no significant differences in cell quality between protocols. The trend of a higher transfer success for the natural cycle, though not statistically significant, indicated potential protocol effects on the uterine environment.

Conclusion: Both COH and MNC protocols result in comparable oocyte, zygote, and embryo quality. However, AMH and AMHR2 gene expression levels may serve as potential biomarkers for fertilization and embryo transfer success. These findings highlight the complexity of ART outcomes and suggest that incorporating gene expression markers with protocol adjustments may optimize individual ART strategies. Further research with larger cohorts is needed to confirm these findings.

C.O.I. Disclosure:

S. Dević Pavlić: None. **L. Saftić Martinović:** None. **T. Sušanj Šepić:** None. **A. Radojčić Badovinac:** None.

EP02.038 Exome sequencing in idiopathic infertility phenotypes: Novel genetic targets

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Background: Recent studies have identified new genes that play fundamental roles in the human reproduction, as well as genetic variants that are involved in the fertilization or cause different infertility phenotypes as premature ovarian insufficiency (POI) or male infertility. The aim of the study is to identify candidate variants and genes related to idiopathic infertility problems.

Material and methods: This study included 148 patients (50 females and 98 males) with different infertility phenotypes. Among females, the phenotypes included premature ovarian insufficiency (POI, 9.5%), empty follicle syndrome (EFS, 4.7%), and high embryo aneuploidy rate (19.6%). In males, the observed phenotypes were sperm abnormalities (39.2%) and poor fertilization rate (27.0%). WES was performed to investigate genetic variants associated with each phenotype. Controls were included in the POI, high aneuploidy rate and sperm abnormalities groups.

Results: We identified candidate variants in 68 patients, which corresponds to a detection rate of 45.9%. The highest detection rate was achieved in the POI group (100%), followed by the EFS group (71.4%) and males with sperm abnormalities (50%). Most of the genes represent novel candidates that have not been linked to these infertility phenotypes but are implicated in biological processes associated with folliculogenesis, cell division, regulation of gene expression, maintenance of genomic integrity and DNA repair, mRNA/microRNA processing and signal transduction.

Conclusion: We propose new genetic targets related to different infertility problems, and involved in critical processes during male and female gametogenesis, fertilization and embryo development. These data will help to establish new genotype-phenotype correlations in infertility.

C.O.I. Disclosure:

R. Morales: None. **B. Lledo:** None. **J. Ortiz:** None. **F. Lozano:** None. **A. Turienzo:** None. **A. Pay:** None. **A. Bernabeu:** None.

EP02.039 Genetic concordance between the trophectoderm and inner cell mass of day-5 preimplantation embryos using next generation sequencing

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Background: Preimplantation genetic testing for aneuploidies (PGT-A) is used for embryo selection, on a 5–10 cell biopsy from the trophectoderm (TE) which later forms the placenta. Whether this biopsy reflects the inner cell mass (ICM) which develops into the fetus, is still questionable. Additional viability biomarkers were proposed, including mitochondrial DNA (mtDNA) copy number.

Material and methods: Thirty-two donated blastocysts were dissected into TE and ICM and tested with low-pass whole-genome sequencing using the Embgenix PGT-A Core kit (CE-IVD, Takara Bio Inc.). For 14 of these 32 embryos, TE biopsy was previously performed for PGT-A. Ploidy status, mtDNA copy number and the morphology of, the TE and the ICM were evaluated.

Results: 81% of blastocysts showed full ploidy concordance and 19% showed partial concordance between their ICM and TE. Out of the 14 embryos with previous PGT-A result, 86% showed full concordance and 14% partial concordance with the TE ploidy. Non-euploid blastocysts showed a significantly higher but variable mtDNA copy number both in ICM and TE. No significant correlation was found between the mtDNA copy number and the morphology of the whole blastocyst, the TE or the ICM.

Conclusion: These results show that TE ploidy correctly reflects the ICM and a TE biopsy is accurate for embryo status prediction. Also, a high mtDNA copy number in TE or ICM alone may also be indicative of aneuploidy. Our data supports the connection between TE ploidy and embryo ploidy and thus the clinical utility of PGT-A, especially when combined with additional biomarkers.

C.O.I. Disclosure:

N. Salameh: None. **A. Theodosiou:** None. **L. Kousoulidou:** None. **Y. Papaevripidou:** None. **P. Evangelidou:** None. **D. Michaelides:** None. **A. Agathangelou:** None. **S. Fasouliotis:** None. **C. Sismani:** None.

EP02.040 Enhancing quality control in preimplantation genetic testing to prevent misdiagnosis from mosaicism artifacts

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Background: Preimplantation genetic testing for aneuploidies (PGT-A) is crucial for selecting viable embryos in assisted reproduction, but mosaicism misdiagnosis due to artifacts can reduce available embryos for transfer, impacting pregnancy outcomes. The newer EmbryoMap PGT-A software has distinct artifacts that differ from the older BlueFuse software. Laboratories new to EmbryoMap might mistakenly identify these artifacts as true mosaicism or segmental abnormalities.

Material and methods: We analyzed data from 4,500 embryos processed with EmbryoMap PGT-A solution to create a comprehensive catalog of artifacts, which often appear as segmental anomalies like deletions or duplications across various chromosomes. Our methods included frequency analysis and re-biopsy procedures to differentiate genuine mosaicism from artifacts.

Results: More than half of the segmental mosaic anomalies were found to be artifacts through frequency analysis and re-biopsy confirmation. The re-biopsies, conducted on both the inner cell mass (ICM) and the trophectoderm (TE), did not confirm these artifacts, while the control group of embryos with regular aneuploidies consistently confirmed their diagnoses.

Conclusion: This study underscores the importance of rigorous quality control in PGT to avoid misdiagnosis due to mosaicism artifacts. By establishing a catalog based on the analysis of 4,500 embryos, we have demonstrated that a significant portion of what is classified as mosaicism might actually be artifacts, particularly when using newer platforms like EmbryoMap. Our approach of frequency-based cataloging and re-biopsy validation provides a pathway for improving diagnostic accuracy, ensuring that more viable embryos are available for transfer, thus potentially enhancing the success rates of assisted reproduction procedures.

C.O.I. Disclosure:

S. Avdeichik: None. **A. Kullyev:** None. **A. Kartuesov:** None. **Y. Goikhman:** None.

EP02.042 Non-Invasive analysis of culture media as an indicator of embryonic chromosomal status

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Background: Non-invasive preimplantation genetic testing (niPGT) analyzes free DNA in embryo culture media as an alternative to biopsy. However, its reliability and interpretability remain controversial due to inconsistencies in DNA fragmentation

patterns and contamination risks, limiting its clinical application. Previous studies suggest that euploid embryos may also release fragmented or chaotic DNA into the medium, complicating differentiation from aneuploid embryos.

Material and methods: The study includes embryos from IVF patients undergoing preimplantation genetic testing. Paired analysis of embryo biopsies and culture media was performed using next-generation sequencing (NGS). DNA fragmentation patterns and chromosomal status correlations were evaluated to determine the reliability of niPGT.

Results: Preliminary data suggest that euploid embryos can exhibit chaotic DNA fragmentation patterns in culture media, contradicting the assumption that only aneuploid embryos show such profiles. This raises concerns about the current interpretation of niPGT results. Further statistical validation is needed to confirm if specific DNA signatures can reliably distinguish between euploid and aneuploid embryos without direct biopsy.

Conclusion: If confirmed, these findings could reshape embryo selection protocols by reducing reliance on invasive biopsies, ultimately improving IVF outcomes while preserving embryo integrity.

C.O.I. Disclosure:

Á.M. Díaz: A. Employment (full or part-time); Modest; Bioarray. **C. Valdivia:** A. Employment (full or part-time); Modest; Bioarray. **A. Seller:** A. Employment (full or part-time); Modest; Bioarray. **P. Brígido:** A. Employment (full or part-time); Modest; Bioarray. **A. Peñin:** A. Employment (full or part-time); Modest; Bioarray. **L. Alcaraz:** A. Employment (full or part-time); Modest; Bioarray.

EP02.043 Chromosomal abnormalities in reproductive health: data from cytogenetic analysis in a Greek cohort the last dacade

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Background: Chromosomal abnormalities play a significant role in reproductive challenges. Advances in cytogenetic analysis have facilitated the detection of chromosomal aberrations in individuals undergoing cytogenetic evaluation.

Material and methods: Between 2014 and 2024, 2.372 individuals underwent cytogenetic investigation. These were classified based on the reason for referral which included infertility, recurrent miscarriages, IVF and screening using data from the Laboratory of Medical Genetics, Athens University. Retrospective analysis of data allowed characterization and prevalence of findings across the different groups.

Results: Infertility: In females 5% presented mosaicism of sex chromosomes, while 1% reciprocal translocation. In males 6% was diagnosed with Klinefelter syndrome, 1% presented mosaicism of sex chromosomes and 1% reciprocal translocation.Recurrent miscarriages: Males and females presented similar percentages; 1% sex chromosome mosaicism, 2% translocations while males ahead also inversions (1%) and 1% robertsonian translocation. IVF: In females cytogenetic analysis revealed sex chromosome mosaicism (1%) and translocations (2%). Screening: In females 3% presented sex chromosome mosaicism, while 2% structural aberrations. In males 1% was diagnosed with Klinefelter syndrome, while in 2% a structural abnormality was revealed.

Conclusion: Cytogenetic analysis in individuals tested revealed numerical and structural aberrations that are in line with the literature. This study underscores the importance of conventional karyotype as a diagnostic tool in individuals undergoing genetic evaluation during reproduction and enhances clinical management towards improved reproductive outcomes.

C.O.I. Disclosure:

A. Theou: None. **N. Selenti:** None. **K. Kosma:** None. **C. Vrettou:** None. **G. Kakourou:** None. **T. Mamas:** None. **A. Kampouraki:** None. **E. Tsentemidou:** None. **H. Tsarouha:** None. **S. Kitsiou - Tzeli:** None. **H. Fryssira:** None. **A. Kalpini Mavrou:** None. **C. Sofocleous:** None. **P. Makrythanasis:** None.

EP02.044 Study of breast cancer risk in women planning in-vitro fertilisation pregnancy

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Background: According to the data of Ministry of Health of the Sverdlovsk Region, every 30th child in the region is conceived using in vitro fertilisation (IVF). However, studies suggest an increased risk of breast and ovarian cancer in women who undergo hormone stimulation in preparation for IVF. The aim of this study was to investigate the carriage of pathogenic variants in genes associated with the development of breast and ovarian cancer in women planning to become pregnant using IVF.

Material and methods: The study included women from 100 infertile couples with a mean age of 34 [31;37] years. The study was performed using high-throughput sequencing (Miseq, Illumina). The target panel (PARSEK LAB (St. Petersburg, RF) included 63 genes associated with hereditary cancer. The manufacturer's software and international databases were used to interpret the genetic variants.

Results: We identified 4 carriers of pathogenic variants of BRCA1, BRCA2, CHEK2 and NBN genes among 100 women. The duration of infertility for these women ranged from 4 to 14 years, and they all had a history of unsuccessful IVF attempts. Only 1 woman had a family history of cancer.

Conclusion: The presence of a hereditary predisposition to unforeseen side effects of hormonal infertility therapy will allow a more correct medical and genetic counselling in terms of informing the family about the risks of reproductive therapy for the body of a female carrier of BC-related genes. Moreover, the PGT-M technique could avoid the transmission of pathogenic variants to the offspring.

C.O.I. Disclosure:

S.S. Deryabina: None. **O.V. Lagutina:** None. **S.V. Sazonov:** None. **O.P. Kovtun:** None.

EP02.045 To use or not to use the carrier screening test: an Italian monocentric study experience

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Background: Carrier screening provides prospective parents with information about their risk of having a child affected by an autosomal or X-linked recessive genetic condition. If both partners carry the same AR disorder, each pregnancy has a 25% risk of being affected. In XLR conditions, a carrier mother has 50% chance of passing the condition to male offspring. About 1–2% of couples in the general population are at increased risk, often unaware until their child is diagnosed.In this study we explore strengths and limitations of carrier testing in 500 couples enrolled during last two years to elucidate practical utility.

Materials and methods: WES trio analysis was performed on DNA derived from healthy subjects' peripheral blood. Reads were aligned to human genome build GRCh38/hg38 and variant calling, variant annotation and classification were performed with eVAI platform (enGenome).The analysis focuses on 330 genes associated with 600 recessive autosomal or X-linked disorders.

Results: To assess couples' reproductive risk, genetic variants classified as Class V/Class IV according to ACMG and ClinGen classifications were considered. The extimation of reproductive risk is based on the frequency of these variants in the population and targeted genetic testing, thereby supporting informed family planning decisions.

Conclusion: Understanding decision-making factors is crucial, as broader screening panels may increase perceived benefits, while some couples remain hesitant about reproductive options with genetic testing. Moreover, it is essential to monitor long-term effects and consider the ethical impact. Globally, establishing shared criteria for the responsible implementation of genetic screening would be beneficial.

C.O.I. Disclosure:

A. Franzoni: None. **D. Fabbro:** None. **E. Betto:** None. **J. Zucco:** None. **C. Mio:** None. **F. Faletra:** None. **G. Damante:** None.

EP02.046 Exploring shared genetic mechanisms between endometriosis and adenomyosis through whole exome sequence analysis of adenomyosis cases utilizing an endometriosis-associated gene panel

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Background: Adenomyosis and endometriosis are benign conditions characterized by the ectopic localization of endometrial tissue and stroma, which are highly comorbid and thought to share common pathogenesis. We compiled a panel of candidate genes of endometriosis obtained from GWAS (Genome-wide Association Study), NGS (Next Generation Sequencing) and functional studies. Utilizing whole exome sequencing (WES), we aimed to detect rare and/or novel genetic variants in pathologically confirmed sporadic adenomyosis cases.

Material and methods: Adenomyosis was diagnosed via hysterectomy due to abnormal uterine bleeding and dysmenorrhea. Genomic DNA isolated from peripheral blood was subjected to WES on Illumina Novaseq6000, followed by bionformatic analysis on Pairend platform. Protein-altering variants in the gene panel were filtered for MAF < %1. Pathogenicity scores of known variants were determined in accordance with ACMG, ClinVar and human phenotype ontology (HPO), while in silico prediction tools were used in novel variant scoring.

Results: 33 women received adenomyosis diagnosis, while the diagnosis of endometriosis was excluded. WES analysis revealed variants in previously-identified endometriosis genes VPS13B, LAMA5, SYNE1, GREB1 and MIB1 in 10 cases (30%). Moreover, 25 genetic variants associated with irregular menstruation, as one of the shared symptoms among adenomyosis and endometriosis, were detected in 20 out of 33 cases (61%) according to the HPO database.

Conclusion: Our results demonstrate a shared underlying genetic mechanism between endometriosis and adenomyosis, suggesting that their pathogenesis may be inextricably linked. Supporting evidence can be obtained by replication of our findings in independent, larger cohorts from diverse populations; collectively leading to discovery of non-invasive biomarkers.

C.O.I. Disclosure:

S. Aydin: None. **N. Eser MA:** None. **N. Topbas Selcuki:** None. **E. Oral:** None. **F. Tuncer:** None.

EP02.047 Plausible role of SLC12A2 gene expression in menorrhagia, dysmenorrhea, and pain symptoms of adenomyosis

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Background: Adenomyosis is a benign uterus disorder characterized by invagination of ectopic endometrial-like tissue into the myometrium, causing menorrhagia, dysmenorrhea and chronic pelvic pain. The invagination hypothesis proposes that basalis endometrial cells infiltrate a damaged junctional zone of myometrium and form ectopic adenomyotic lesions. Our previous study involving whole-exome sequencing of ten cases identified variations in *SLC12A2* and *SLC12A2*-related genes. We aimed to determine the expressions of *SLC12A2* in formalin-fixed, paraffin-embedded (FFPE) adenomyotic tissues following hysterectomy in these cases.

Material and methods: Ten women, who received adenomyosis diagnosis through histopathological examination along with 1 control case filled out the World Endometriosis Research Foundation-Endometriosis Phenome Harmonisation Project (WERF-EPHect) questionnaire that provided scores for adenomyosis symptoms. RNA was isolated from FFPE adenomyotic tissues, which was utilized in cDNA synthesis. Quantitative real time PCR (qRT-PCR) was performed by SYBR Green PCR Mastermix. Relative quantification of *SLC12A2* expression was calculated by $2^{-\Delta\Delta Ct}$ method, where *ACTB* was the housekeeping gene.

Results: Three cases presented with severe menorrhagia, dysmenorrhea, and chronic pelvic pain that corresponded to two-, three- and four-fold increased expressions of *SLC12A2*, respectively. None of the remaining cases exhibited significant changes in *SLC12A2* expressions.

Conclusion: According to the databases, *SLC12A2* has very low expression levels in the uterine. Therefore, our results might indicate a correlation between *SLC12A2* expression and the severity of adenomyosis symptoms. As this gene has been linked to invasion and pain in disparate conditions, further investigations are needed to prove our preliminary findings for adenomyosis.

C.O.I. Disclosure:
N. Eser MA: None. **S. Aydin:** None. **I. Ozaydin:** None. **N. Topbas Selcuki:** None. **E. Oral:** None. **F. Tuncer:** None.

EP03 Prenatal Genetics

EP03.001 Double dose of epigenetics: can assisted reproductive technologies (ART) and surrogacy have an impact on the child's genomic future?

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Background: The genome undergoes different phases of epigenetic programming during gamete formation and early embryonic development in conjunction with ART treatments. However, it is difficult to separate the effects of ART procedures from the effects of maternal and paternal reproductive disorders. Epidemiological studies in humans have found altered birth weight profiles in ART-conceived singletons compared to spontaneously conceived singletons.This study provides a brief overview of the existing literature on the relationship between epigenetic changes, ART, intrauterine development, surrogacy and the health of the child.

Material and methods: Pubmed and Google Scholar databases for published articles on this topic during 1980–2024 were searched and only English language articles were included.

Results: ART techniques have been associated with imprinting disorders. Manipulation of embryos during freezing and thawing can lead to changes in foetal development through epigenetic modifications. Several studies have been conducted to measure DNA methylation signatures across the genome to analyse the changes in epigenetic programming caused by ART, however not many studies have been conducted to perform genome-wide studies of differential methylation after conception by ART in the case of malnutrition in humans.

Conclusion: The relationship between ART, intrauterine exposure and child development is still controversial. In addition, the effects of epigenetic changes that occur during pregnancy on the health of the surrogate mother and/or the child are not sufficiently known. Future and long-term studies should be conducted to analyse the epigenetic changes in children conceived through ART.

C.O.I. Disclosure:
H. Cobanogullari: None. **P. Savash:** None.

EP03.002 The frequency of cytogenetic abnormalities in patients with normal screening test results

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Introduction and Aim of the Study: Non-invasive screening methods, such as the combined test and free fetal DNA (NIPT), evaluate the likelihood of genetic abnormalities in the fetus, including trisomy 21 (Down syndrome), trisomy 18 (Edwards syndrome), and trisomy 13 (Patau syndrome). Normal result of both the combined test and free fetal DNA, along with a normal ultrasound image, in most cases helps reassure expectant parents. However, some patients, despite these results, choose to undergo invasive diagnostics. The aim of this study is to assess the frequency of cytogenetic abnormalities in patients with normal screening test results.

Methodology: The study included 34 patients diagnosed with fetal growth restriction. All patients underwent laboratory diagnostics, as well as invasive diagnostics—amniocentesis examination. In all patients, ultrasound images showed no other abnormalities. These patients opted for invasive diagnostics due to a complicated obstetric history or a significant fear of genetic defects in the fetus.

Results: Molecular karyotype analysis revealed abnormalities in 26.47% of cases, while cytogenetic testing detected abnormalities in 8.82% fetus. In the remaining patients, the result of the cytogenetic karyotype and molecular karyotype was normal. In two cases, an inversion was detected, and in one of the patients, mosaicism with an additional chromosome of pair 5 was found in 6 out of 30 analyzed cells.

Conclusions: During amniocentesis, it is recommended to perform both molecular karyotyping and cytogenetic testing. It is also worth considering psychological indications for invasive diagnostics, which are increasingly recognized as important.

C.O.I. Disclosure:
K. Cholewa-Nowak: None. **M. Malarska:** None. **M. Zyla:** None. **S. Kubik:** None. **P. Pachniak:** None. **K. Szmyt:** None. **M. Grzesiak:** None.

EP03.003 Aberrant findings from genomic array analysis with CytoScan HD Accel in clinical samples from amniocentesis

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Background: Genomic array can be performed to detect addition or loss of chromosomal segments in prenatal samples obtained by amniocentesis. At the Department of Molecular Diagnostics in Lund, Duo-QF-PCR for detection of aneuploidy and identity control is the first analysis for all prenatal samples. If a normal result is reported, genomic array screening with CytoScan HD Accel is recommended in clinical routine. Here we present the frequency of aberrant results in a clinical setting.

Material and methods: With CytoScan HD Accel the whole genome is investigated with a high-resolution SNP-array to detect addition or loss of chromosomal segments at a resolution of approximately 10 kb for deletions and 30 kb for duplications. Data was collected from all samples received from amniocentesis where genomic array analysis was ordered in our lab data system during 2024.

Results: In 2024, genomic array was ordered for 228 samples from amniocentesis whereas in DNA from 23 of them (10%) aneuploidy was detected by QF-PCR. Therefore, 205 amniocentesis samples were run on genomic array analysis. The indications for the medical doctor to order the analysis were ultrasound findings, known heredity, or increased risk on NIPT (non-invasive prenatal test). Of the 205 samples, in total 7 pathogenic findings were reported (3,4%). Three of the pathogenic copy number aberrations correlated well with the ultrasound findings.

Conclusion: Copy number aberrations detected with genomic array analysis were found in only a small proportion of samples from amniocentesis indicating that a method with higher resolution could be considered.

C.O.I. Disclosure:
C.I. Björk: None.

EP03.004 Validation of ChromoQuant QF-PCR for Rapid Prenatal Aneuploidy Screening in Brazil

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Background: Aneuploidy investigation is crucial in prenatal diagnosis to detect chromosomal abnormalities that impact human development. In Brazil, modern methodologies such as quantitative fluorescent polymerase chain reaction (QF-PCR) have gained traction due to their cost-effectiveness, accuracy, and speed. This study validates ChromoQuant QF-PCRfor genetic diagnostics using chorionic villus sampling (CVS) and amniotic fluid, with Multiplex Ligation-dependent Probe Amplification (MLPA) as a confirmatory method.

Material and methods: We tested 50 samples with abnormal first-trimester ultrasonographic findings using the ChromoQuant QF-PCR kit, targeting chromosomes 21, 13, 18, X, and Y. Doubtful or inconclusive cases were reanalyzed with MLPA to ensure accuracy. PCR amplification products were analyzed with GeneMarker software.

Results: QF-PCR detected trisomy 18 (three cases), partial trisomy 18 (one case), trisomy 13 (three cases), trisomy 21 (six cases), and triploidy (two cases). Chromosomal rearrangements and maternal contamination, and were also identified. All QF-PCR results were consistent with MLPA findings. This method reduces diagnostic ambiguity, alleviates parental anxiety, and accelerates clinical decision-making for high-risk pregnancies.

Conclusion: QF-PCR proved to be an efficient and reliable method for rapid prenatal aneuploidy screening. It is a valuable tool for pregnant women with increased fetal nuchal translucency or abnormal first-trimester ultrasound findings. Possible false-negative results were further investigated using SNP array.

C.O.I. Disclosure:

T. Assis: None. **M.M. Santos:** None. **A. Baranzeli:** None. **B.K. Coelho:** None. **N.M. Silva:** None. **B.H. Dominski:** None. **M. Ocampos:** None.

EP03.005 Invasive prenatal diagnosis in Bizkaia: a 30-year experience

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Background: Since the early 1970s, prenatal cytogenetic diagnosis, specifically the analysis of fetal karyotype obtained after transabdominal amniocentesis, has been recognized as a reliable method for detection of fetal chromosome abnormalities, which are one of the main causes of congenital defects. The objective of our study is to analyze the trends in the number of this invasive procedure and the detection of chromosomal abnormalities over a 30-year single institution experience.

Material and methods: Data of 17,394 invasive prenatal procedures performed between 1993 and 2024 in Bizkaia were descriptively and retrospectively reviewed, with particular emphasis on indications and number and type of abnormal results.

Results: Traditionally, maternal age had been the main referral reason for prenatal testing (51.3%) but during the last years, non-invasive prenatal test (NIPT) has become the most important clinical indication, decreasing the number of invasive procedures performed. Related to karyotype analysis, chromosomal abnormalities were detected in 560 out of the 17,394 (3.22%) cytogenetic studies, being the classical autosomal aneuploidies, Trisomy 21, 18 and 13, the most frequent cases (1.09%). Sex chromosome aneuploidies were found in 72 cases (0.46%) and balanced rearrangements corresponded to 0.67% of the structural abnormalities while unbalanced rearrangements were found in 0.15%.

Conclusion: Our study contributes to establish a standard reference incidence of chromosomal abnormalities in pregnancies in Bizkaia. Our data also confirms the karyotype as a reliable method for detecting complex chromosome abnormalities, providing an important basis for prenatal counseling and future pregnancies.

C.O.I. Disclosure:

I. Huerta: None. **M. Télez:** None. **I. Zubiaur:** None. **C. Pérez:** None. **M. García-Barcina:** None.

EP03.006 Novel likely pathogenic variant in *SHH* gene causing holoprosencephaly

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Background: Sonic Hedgehog Signaling Molecule (*SHH*) gene encodes highly conserved sonic hedgehog protein that plays a crucial role in early embryo development. Several autosomal dominant diseases are associated with *SHH* mutations, including holoprosencephaly 3, microphthalmia/coloboma 5 and single median maxillary central incisor. Additionaly, hypotelorism, microcephaly, cleft lip and/or palate have been described in holoprosencephaly due to *SHH* mutations.

Material and methods: Non-consanguineous couple was referred to genetic counseling at 20th gestational week due to fetal ultrasound anomalies (alobar holoprosencephaly, suspected unilateral microphthalmia). Aside from hypotelorism observed in pregnant woman, she and her two children from the first marriage were healthy. The father and the first common daughter were born with cleft palate, while second common pregnancy was terminated due to microcephaly.

Results: QF PCR for common aneuploidies (Aneufast kit), as well as chromosomal microarray analysis (Agilent SurePrint G3 Human CGH Bundle, 8×60K) revealed a normal male, fetal DNA profile. Clinical exome sequencing (Illumina DNA Prep with Enrichment protocol, TruSight One panel) identified heterozygous variant in *SHH* gene (NM_000193.4:c.469A>T, p.(Lys157Ter)). According to in silico predictions variant causes nonsense-mediated decay mechanism and loss of the functional protein. Following the ACMG/AMP classification system it was classified as likely pathogenic.

Conclusion: This is the first reported case of the c.469A>T variant in *SHH* gene. The prenatal manifestations in this case were consistent with holoprosencephaly 3. Parental testing would be recommendable in order to further investigate possible association between *SHH* variant and other anomalies presented in the family. Our case further expands *SHH* mutational spectrum.

C.O.I. Disclosure:

M. Toljic: None. **A. Stankovic:** None. **N. Karadzov Orlic:** None. **I. Joksic:** None.

EP03.007 Integrating antenatal radiological findings with prenatal whole exome sequencing (WES) to diagnose alazami

syndrome: A case study of a consanguineous Pakistani couple

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Objective: Alazami Syndrome (AS) is a rare autosomal recessive disorder characterized by congenital anomalies. This is the first reported antenatal case from Pakistan in a consanguineous couple. Whole exome sequencing (WES) confirmed the fetal diagnosis of AS.

Case Report: A consanguineous couple presented at 20 weeks and 3 days of gestation for an antenatal scan at a tertiary care hospital. The scan revealed multiple fetal anomalies, including agenesis of the corpus callosum, cerebellar hypoplasia, frontal bossing, ventricular septal defect, mild ventricular hypertrophy, bilateral short humeri, early-onset intrauterine growth restriction, absent cavum septum pellucidum, and bilateral hypoplastic kidneys. Amniocentesis was performed for genetic testing, including karyotyping and WES. Due to the severity of the anomalies, the couple opted for pregnancy termination before receiving genetic results. Karyotyping was normal, but WES identified a *homozygous likely pathogenic variant in LARP7: c.646+3_646+6del (IVS6 + 3+6del), a splice donor variant* affecting RNA splicing. This variant, associated with AS, was previously reported in 2017, submitted to *ClinVar*, and holds a *one-star entry* for being *likely pathogenic*.

Conclusion: This case expands the antenatal phenotype of Alazami Syndrome and emphasizes the importance of molecular diagnosis in consanguineous populations. Without genetic testing, recurrence risk counseling would not have been possible. This highlights the clinical utility of prenatal WES in diagnosing rare genetic disorders and aiding reproductive decision-making.

C.O.I. Disclosure:

Z. Hasan Ali: None. **S. Iqbal:** None. **M. Nasir:** None. **F. Akbar:** None. **S. Kirmani:** None.

EP03.008 Prenatal diagnosis of mosaic trisomy 2: a case report

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Background: Mosaic trisomy 2 is extremely rare chromosomal anomaly in prenatal diagnosis and is associated with multiple system abnormality such as Intrauterine Growth Restriction (IUGR), microcephaly, scoliosis, heart defects, grow and motor delay. To date, only few cases of mosaic trisomy 2 have been reported in the scientific literature.

Material and methods: We describe a 41-year-old pregnant woman, with a negative family history, with high risk of aneuploidy of chromosome 2 in Non-Invasive Prenatal Testing (NIPT) and normal fetal ultrasound. The amniocentesis was performed at 17 weeks of gestation. DNA extracted from cultured amniocytes was used for Array-CGH and UPD2 analysis.

Results: Cytogenetic analysis revealed a mosaic female karyotype with two cell lines: a minority (approximately 34%) aneuploid female, with 47 chromosomes, with trisomy of chromosome 2 (47,XX, + 2); the other prevalent (approximately 66%) normal female (46,XX). Array-CGH analysis revealed a result of arr(2)x2 ~ 3, which indicated that chromosome 2 may be mosaic trisomy. UPD2 analysis allowed us to exclude the presence of uniparental disomy of chromosome 2. The subsequent fetal ultrasaund in second trimester at 20 weeks showed IUGR. It was not possible to discriminate whether it was confined placental mosaicism (CPM) or true costitutional fetal mosaicism since the pregnancy was terminated.

Conclusion: We presented a case with positive NIPT and cytogenetic analysis that confirmed the presence of mosaic trisomy 2. Genetic counseling about fetal mosaicism is a challenge because of unpredictable outcome and it is therefore important combined the genetic results with fetal ultrasound signs.

C.O.I. Disclosure:

R. Lucente: None. **A. Vitelli:** None. **D. Darelli:** None. **A. Orchi:** None. **E. Romanelli:** None. **M. Canestrelli:** None. **E. Orlandi:** None. **F. Picciotto:** None. **E. Salzano:** None. **A. Pantaleo:** None. **A. Novelli:** None. **A. Micalizzi:** None.

EP03.009 When non-invasive prenatal testing fails - a cystic fibrosis case study

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Background: Cystic fibrosis is one of the most common severe conditions caused by loss-of-function variants in the CFTR gene. Both parents of an affected child are carriers of a single CFTR mutation, and the risk of having another affected child who inherits both mutations from the parents is 25%. The non-invasive prenatal testing (NIPT) method is more accurate than other screening tests and carries no risk of miscarriage.

Material and methods: We present a family of an affected child with Cystic fibrosis. The couple's second pregnancy was investigated using the NIPT test due to their previous child having two confirmed mutations in the CFTR gene. Sanger sequencing and MLPA analysis of the amniotic fluid were conducted to detect the point mutation and the large deletion.

Results: The cfDNA analysis suggested the presence of the variant c.38C>T(p.Ser13Phe) of the CFTR gene (of maternal origin) but could not determine whether the fetus had inherited the paternal variant deletion of exons 2–3 due to the technical limitations of the method. Consequently, it was not possible to exclude the possibility that the fetus is compound heterozygous for the maternal and paternal variants in the CFTR gene. Confirmation through invasive prenatal diagnosis established that the fetus was a carrier of the paternal variant but not of the maternal variant.

Conclusion: This case highlights that NIPT may lead to incorrect results in monogenic diseases. Therefore, for accurate pregnancy management, it is essential that a positive cfDNA analysis is confirmed through an invasive prenatal test following amniocentesis or chorionic villus sampling.

C.O.I. Disclosure:

N. Yaneva: None. **G. Petrova:** None. **I. Bradinova:** None. **K. Kercheva:** None. **R. Raynova:** None. **Y. Petrova:** None. **A. Savov:** None.

EP03.010 Prenatal phenotypic expansion of the Schaaf-Yang syndrome: a fetus with congenital heart defects

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Background: Schaaf-Yang syndrome (SYS) is a rare heterogeneous multisystem disorder that shares multiple clinical features (such as developmental delay/intellectual disability, neonatal hypotonia, and behavioral abnormalities) with the genetically related Prader-Willi syndrome. SYS is caused by heterozygous mutation in the *MAGEL2* gene (OMIM # 605283) with high penetrance and phenotipic variability. The 15q11.2 locus, that includes *MAGEL2*, is maternally imprinted, meaning that only the paternally derived allele is expressed while the maternally derived allele is silenced.

Material and methods: We report the case of 35-year-old pregnant woman brought to our attention for ultrasound finding of agenesis of the ductus venosus and aberrant right subclavian artery (ARSA). No family history of congenital malformations. Amniocentesis was performed at 16 weeks of gestation.

Results: Array-CGH and conventional karyotype were normal. Trio NGS in-silico panel, focusing on genes related to the clinical query of congenital heart defects, identified heterozygous nonsense variant c.987G>A (p.Trp329*) in *MAGEL2* gene (NM_019066), segregated from his heterozygous father. The variant can be classified as a likely pathogenic variant (class 4), according to the ACMG guidelines.

Conclusion: To date, only cases with atrial septal defects have been reported in the literature associated with MAGEL2 mutation. The presence of agenesis of the ductus venosus and ARSA identified in fetus has not been reported before. These results expand the clinical phenotypic spectrum of SYS and demonstrates the importance between fetal ultrasound signs and NGS data analysis in prenatal diagnosis.

C.O.I. Disclosure:

A. Micalizzi: None. **V. Pinna:** None. **E. Romanelli:** None. **R. Lucente:** None. **A. Vitelli:** None. **F. Colantoni:** None. **V. Lanari:** None. **M. Di Gregorio:** None. **G. Mastrogiorgio:** None. **A. Novelli:** None.

EP03.011 A rare case of prenatal Stuve-Wiedemann syndrome presenting with asymmetric femoral involvement

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Background/Objectives: Stuve-Wiedemann syndrome (SWS MIM 601559) is rare autosomal recessive bent-bone skeletal dysplasia caused by mutations in LIFR gene. It is characterized by short stature, bilateral bowing of the long bones, dysmorphic features, autonomic dysfunction and respiratory failure. Although SWS is typically a severe and often lethal condition in early infancy, prenatal manifestations have been reported in fewer than 46% of cases. We present a rare case of prenatally diagnosed SWS with asymmetric involvement of femora.

Methods: A 15-year-old Roma woman was referred for genetic counseling at the 33rd week of her first pregnancy due to significant shortening of the long bones. All measured long bones were below the 1st percentile for gestational age, with a noted curvature of the right femur. Amniotic fluid sampling and clinical exome sequencing (CES) using the TruSight One panel (Illumina) were performed. Variants were classified according to the ACMG/AMP classification system.

Results: CES revealed homozygous null variants in LIFR gene, c.756dup (p.(Lys253Ter), NM_001127671.2), classified as pathogenic which is consistent with diagnosis of SWS in proband.

Conclusion: Before introduction of exome sequencing (ES) in prenatal setting, SWS was rarely diagnosed antenatally due to unspecific and late presentation of symptoms. Asymmetric bone bowing should alert suspicion for severe forms of skeletal dysplasias. Given the severity and often lethal nature of SWS, early prenatal detection is crucial for informed counseling and perinatal management. This case confirms utility of ES in prenatal diagnosis of severe childhood conditions and adds to clinical spectrum of prenatal manifestations of SWS.

C.O.I. Disclosure:

I. Joksic: None. **M. Toljic:** None. **A. Stankovic:** None. **N. Karadzov Orlic:** None.

EP03.012 Severe prenatal findings of the *CUL3*related syndrome

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The CUL3 gene is associated with neurodevelopmental disorders (NDD) with or without autism or seizures and follows an autosomal dominant inheritance pattern. We present a case of an inherited CUL3 mutation with prenatal findings.The patient was referred to genetic counseling during her third pregnancy, which showed severe early IUGR and a hypoplastic cerebllum. Her first pregnancy was terminated at 37 weeks due to severe intrauterine growth restriction (IUGR) and a hypoplastic cerebellum. After termination, chromosomal microarray and exome sequencing were performed. Exome sequencing revealed a maternally inherited deletion, encompassing two exons in the SYN1 gene, which did not explain the sonographic findings. Exome reanalysis was performed 3 years after test completion, and revealed a likely pathogenic CUL3variant (c.1549_1552del). The variant was inherited from a healthy father. Exome sequencing of the fetus from the patient's third pregnancy showed the sameCUL3 variant, a finding that led to the termination of pregnancy.This case report expanded the spectrum of sonographic findings, the *CUL3* gene can lead to severe prenatal phenotype. Every case report with a different phenotype allows a better understanding of this gene's role and can help healthcare providers to make informed decisions about care and interventions. This case report also reminds the well known importance of exome reanalysis.

C.O.I. Disclosure:

T. Dery: None.

EP03.013 Comparison of the effectiveness of NIPT and combined test in detecting fetal trisomies

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Introduction and Aim of the Study: Combined test and free fetal DNA tests (NIPT) are non-invasive screening methods that assess the risk of genetic defects in the fetus, such as trisomy 21, 18 and 13. The aim of the study was to compare effectiveness of these tests in detecting trisomies with amniocentesis, which is an invasive method used to confirm the diagnosis.

Methodology: The study included 47 pregnant women in whom the combined test, NIPT and amniocentesis were performed. Patients had amniocentesis performed due to abnormalities in one of the above tests. In all patients, ultrasound images showed no abnormalities Results:In patients who underwent amniocentesis due to a high risk of one of the trisomies in the combined test, despite a low risk in NIPT, the abnormality was found in 63.83%In cases of intermediate risk in the combined test, and an indication of another type of chromosomal abnormality in NIPT, the amniocentesis results in all patients in terms of molecular and classical karyotype came out normal.Patients in whom both the combined test and NIPT a high risk of abnormality obtained an abnormal result in 85.29%. In 15% the combined test indicated the presence of trisomy, NIPT showed a low risk the patient was found to have another chromosomal abnormality.

Conclusions: Both tests are of great prognostic importance and should be performed. However, when suggesting them to patients, attention should be paid to the recommendation to offer them, and they should be reminded of only their statistical significance

C.O.I. Disclosure:

M. Malarska: None. **P. Pachniak:** None. **S. Kubik:** None. **M. Żyła:** None. **K. Cholewa-Nowak:** None. **A. Albinowski:** None. **K. Szmyt:** None. **M. Grzesiak:** None.

EP03.014 Genetic causes of fetal growth restriction

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Introduction and Aim of the Study: Fetal growth restriction (FGR) occurs when a fetus does not achieve its growth potential due to pathological factors, with placental dysfunction being the most common. Globally, FGR is a major contributor to stillbirth, neonatal death, and both short-term and long-term health issues.The aim of this study is to find the genetic causes of FGR Methodology: The study included 56 patients diagnosed with fetal growth restriction. All patients underwent laboratory diagnostics, including infectious factors, as well as invasive diagnostics—amniocentesis examination. In all patients, ultrasound images showed no other abnormalities. Additionally, all patients underwent an ultrasound examination performed by an experienced physician.

Results: In the ultrasound examination, six patients were found to have abnormalities other than just FGR; however, no abnormalities were detected in the cytogenetic analysis. In eight patients, genetic abnormalities in fetal material were identified, which could explain the clinical presentation. Additionally, an infectious cause was confirmed in three patients.

Conclusions: The presented results suggest the need to deepen the diagnosis of fetal growth restriction with WES or NGS panel tests.

C.O.I. Disclosure:

S. Kubik: None. **M. Malarska:** None. **M. Żyła:** None. **P. Pachniak:** None. **K. Cholewa-Nowak:** None. **K. Szmyt:** None. **M. Grzesiak:** None.

EP03.015 POMT2homozygous gene splicing variant: an atypical fetal presentation of Walker-Warburg Syndrome

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Background: Walker-Warburg syndrome is an autosomal recessive disorder characterized by congenital muscular dystrophy, hydrocephalus, cobblestone lissencephaly, and retinal dysplasia. One of the main genes involved is POMT2. We present a 28-year-old female with a history of 2 consecutive pregnancies complicated by hydrocephalus. Both pregnancies after the first trimester combined test evaluation were classified as low risk. Later, a regular ultrasound examination in the second trimester of gestation showed a hydrocephalus in both cases. Family pedigree, maternal and paternal clinical personal history were unremarkable. After intensive counseling, the parents decided to terminate both pregnancies due to the poor fetal prognosis.

Methods and results: A post-mortem chromosomal analysis, only on the second fetus, detected a normal male karyotype, while Trio Next Generation Sequencing analysis identified a never-described POMT2 homozygous splicing variant, c.1332+4A > G inherited from heterozygous carrier parents. The mutation was considered of uncertain clinical significance, by different in silico prediction software. The pathogenic potential of the variant was assessed using synonymous-enabled in-silico predictors. TraP, MaxEnt, SPiP, SpliceAI, and Pangolin tools were further used to verify their impact on the splicing machinery. Large agreement among tools confirms the loss of donor sites in exon 12 and the gain of a new donor site (+4 bp) for c.1332+4A > G leading to a premature predicted stop codon p.Gln528Ter.

Conclusion: Based on bioinformatic prediction, the c.1332+4A > G variant seems to have a deleterious effect on the POMT2 protein. The couple has a recurrence risk of 25% for condition POMT2-related. RNA sequencing is ongoing to identify aberrant splicing and/or aberrant gene expression.

C.O.I. Disclosure:

S. Ottombrino: None. **A. Terracciano:** None. **T. Mazza:** None. **S. Magliulo:** None. **A. Di Meglio:** None. **A. Novelli:** None.

EP03.016 Comparison of chromosome 21-derived miR-99a expressions in amniocyte culture samples of fetuses and plasma samples of pregnants undergoing amniocentesis due to increased risk of Down syndrome

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Background: Down syndrome is a chromosomal disorder that

cause intellectual disability, congenital developmental anomalies and phenotypic changes. Down syndrome phenotype is a complex entity that includes genetic and epigenetic changes. In between epigenetic mechanisms, microRNAs (shortly miRNAs), as involved in many cellular processes are thought to take part. In this study, it is aimed that the comparison of the expression of chromosome 21-derived miR-99a in amniocytes and simultaneously obtained plasma samples of pregnant women who carry increased risk of Down syndrome.

Material and methods: In this study, 92 cases undergoing amniocentesis due to increased risk of Down syndrome are included. miR-99a expression in both tissues are examined via real-time PCR and analyzed by using relative quantification method.

Results: miR-99a expression was observed as increased in amniocyte cultures of fetuses with Down syndrome compared to the fetuses with normal chromosomal structure that undergoing amniocentesis due to various indications. These findings were not statistically significant. In between miR-99a expression levels of amniocyte cultures and maternal plasma, no significant correlation was observed.

Conclusion: In our study, miR-99a expression level was found to be increased in amniocyte cultures of Down syndrome fetuses compared to the amniocyte cultures of the fetuses with normal chromosomal structure. However, this change was not statistically significant. In order to observe exact results of miR-99a expression in amniocyte cultures and maternal plasma samples, further studies with larger sample size are needed.

C.O.I. Disclosure:

I. Kaplan: None. **H. Ilgın Ruhi:** None.

EP03.017 Bartter Syndrome Type V: A Rare Genetic Factor in Prenatal Polyhydramnios

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Background: Bartter syndrome type V is a rare hereditary disorder linked to mutations in the MAGED2 gene, affecting renal electrolyte regulation. This X-linked condition results in imbalances such as hypercalciuria, hyponatremia, and hypokalemia, elevated levels of renin and aldosterone in the fetus. One of the prenatal signs is polyhydramnios, which typically diminishes by weeks 30–33 as amniotic fluid levels stabilize. Despite this transient improvement, postnatal complications, including persistent renal dysfunction and medullary nephrocalcinosis, may arise.

Case report: A 31-year-old woman, expecting second child, presented for a second-trimester check-up. Early screening tests indicated no significant abnormalities, with a low-risk assessment from first-trimester combined screening. However, a later ultrasound detected an amniotic fluid volume nearing the upper limit, leading to referral for further maternal-fetal evaluation. At 21 weeks of gestation, a ultrasound confirmed polyhydramnios, prompting an amniocentesis for genetic assessment. Chromosomal microarray analysis returned a normal male karyotype. Additional maternal metabolic evaluations were conducted due to a history of unexplained hypoglycemia. No conclusive metabolic anomalies were identified. Further genetic testing was conducted using NGS.

Results: The presence of a c.1386+2T > C mutation in the MAGED2 gene was detected, confirming Bartter syndrome type V in both the mother and the fetus.

Conclusion: This case underscores the role of genetic testing in unexplained cases of prenatal polyhydramnios. Early identification of underlying disorders can guide clinical decision-making and postnatal management, ensuring appropriate long-term. The findings highlight the necessity of considering rare syndromes in fetal medicine, especially when conventional diagnostic approaches do not yield clear results.

C.O.I. Disclosure:

P. Pachniak: None. **M. Malarska:** None. **A. Albinowski:** None.

EP03.018 First Case of Regressive Fetal Hydrocephalus Associated with a Pathogenic *SMARCC1* Variant

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Background: *SMARCC1*, encoding a SWI/SNF complex subunit, has been implicated in autosomal dominant congenital hydrocephalus with reduced penetrance. To date, only four fetuses from three unrelated families have been reported prenatally, all resulting in pregnancy termination.

Material and methods: A healthy couple was referred at 20 + 4 weeks of gestation (WG) due to isolated hydrocephalus in JA of a monochorionic diamniotic pregnancy, while JB was unaffected. Ultrasound follow-up until 34 + 4 WG showed complete regression of hydrocephalus, confirmed by fetal MRI. Array-CGH and trio exome sequencing were performed on native amniotic fluid from JA.

Results: Array-CGH was normal. Trio exome sequencing identified a paternally inherited heterozygous nonsense pathogenic variant in *SMARCC1* (NM_003074.4:c.1597G>T, p.(Gly533Ter)), also present in JB. The couple opted to continue the pregnancy.

Conclusion: This is the first reported case of regressive hydrocephalus associated with a pathogenic *SMARCC1* variant in monochorionic twins. This phenotypic variability further complicates genetic counseling.

C.O.I. Disclosure:

A. Bourges: None. **F. Boussion:** None. **C. Battault:** None. **R. Benarbia:** None. **F. Biquard:** None. **C. Bris:** None. **A. Civit:** None. **B. Delorme:** None. **C. Houdayer:** None. **M. Lodin:** None. **D. Loisel:** None. **M. Pacault:** None. **V. Procaccio:** None. **P. Van Bogaert:** None. **A. Guichet:** None. **E. Colin:** None.

EP03.019 Prenatal interpretation of a paternally inherited *BICRA* variant: challenges in genetic diagnosis

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Background: Increased nuchal translucency (NT) often prompts genetic testing. In this case, a 3.0 mm increased NT was detected in a fetus from non-consanguineous parents. Trio exome analysis

revealed a paternally inherited heterozygous *BICRA* nonsense variant (c.3940C>T, p.(Gln1314Ter)) that had not been described in the literature. According to ACMG guidelines, the variant was classified as likely pathogenic (ACMG class 4). However, the father exhibited no signs of a BICRA-related neurodevelopmental disorder.

Material and methods: An episignature study was conducted to identify epigenetic alterations associated with other *BICRA* variants, complemented by RNA sequencing as well as segregation analysis. An autopsy of the fetus was also performed.

Results: Segregation analysis revealed that the variant occurred de novo in the father. RNA sequencing showed no abnormal splicing or non-mediated decay, consistent with the nonsense mutation's position in the last exon. The father's episignature aligned with unaffected controls rather than typical *BICRA* patients. Analysis of a fetal sample was not possible due to the lack of control samples. Fetal autopsy revealed no organ malformations or dysmorphisms.

Conclusion: This case underscores the challenges in prenatal genetic interpretation, especially in assessing parentally inherited variant pathogenicity. Despite following ACMG guidelines, the variant's clinical relevance remains unclear and should be classified as of unknown significance.

C.O.I. Disclosure:

J. De Geyter: None. **T. Koopmann:** None. **S. Barakat:** None. **Y. Shi:** None. **F. Isabel:** None.

EP03.020 Prenatal presentation of *RMRP*-related disorder in two fetuses with lethal skeletal dysplasia

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Background: RNA subunit of mitochondrial RNA processing endoribonuclease is encoded by *RMRP*. Biallelic variants in *RMRP* causes anauxetic dysplasia 1 (AD1), cartilage-hair hypoplasia (CHH) and metaphyseal dysplasia without hypotrichosis (MDWH). Prenatal onset of disease is rarely documented.

Material and methods: We ascertained a non-consangineous european couple who visited for antenatal care. A detailed clinical, radiological, pathological examination followed by quad exome sequencing (ES) and *RMRP* (NR_003051.4) sequencing were performed.

Results: Ultrasonography (P1-28weeks, P2-18weeks) and post-mortem radiographs of both fetuses showed bilateral shortening of the long bones, bowed femora and humeri, platyspondyly, short and narrow ribs with bell-shaped thorax. ES was non-diagnostic. Coverage of genes known for lethal skeletal dysplasia revealed no reads for *RMRP*. Sanger sequencing of *RMRP* identified the variants n.-21_-2dup and n.6C > T in both fetuses. 78 SNVs and 16 del/dup in the mRNA transcript which impair rRNA/mRNA cleavage and 45 variants which impair/reduce the transcription of *RMRP* have been described so far. CHH variants impair both mRNA and rRNA cleavage, AD1 variants affect rRNA cleavage. Previously reported early onset CHH-AD spectrum cases involved variants which either reduce transcription or impair mRNA cleavage. Variants we identified here reduce the transcription (n.21_-2dup) and impair rRNA cleavage (n.6C > T).

Conclusion: Prenatal onset of short and bowed long bones with normal mineralization in the absence of any obvious known skeletal dysplasia, could be the important indicator of *RMRP*-related disorder. Addition of RNA genes to exome capture kits would aid in resolving the diagnosis. Genotype-phenotype correlation contributes to counsel for prognosis and management.

C.O.I. Disclosure:

R. Periyasamy: None. **G. Manegold-Brauer:** None. **T. Menter:** None. **F. Isabel:** None.

EP03.021 The impact of first-tier prenatal screening policies on the detection of triploidy pregnancies

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Background: Non-invasive prenatal testing (NIPT), is a widely used screening method for fetal aneuploidy detection. Within Sweden, some health care regions offer NIPT as first-tier screening while most regions offer combined first trimester screening (cFTS). Due to the decreasing cost of NIPT, more health care regions may change their prenatal screening policies toward abandoning serum-based screening and implement NIPT as their first-tier screening. One limitation of NIPT is that it does not detect triploidy, in which the fetus has three copies of every chromosome. Triploidy accounts for approximately 1% of conceptions and although most triploid fetuses are aborted spontaneously during the first trimester due to complications of the condition, a considerable amount can lead to the birth of a child who will not survive infancy.

Material and methods: All detected triploidy cases between 2020–2024 at the Department of Clinical genetics in Lund, Sweden, and the indication of testing were documented as part of clinical routine.

Results: Overall, 26 cases of triploidy were detected. 14 detected by cFTS. 8 by ultrasound. 3 due to spontaneous abortion. 1 case was an incidental finding.

Conclusion: Most triploidy cases (22/26, 85%) were detected by cFTS or ultrasound. The majority (14/26, 54%) by cFTS. Offering NIPT as a first-tier screening would delay the diagnosis for triploidy pregnancies leading to unnecessary medical risks and mental suffering for the patient. Thus, we advocate for all health care regions in Sweden to offer cFTS as the first-tier screening in pregnancies.

C.O.I. Disclosure:

K. Nilsson: None.

EP03.022 Evidence of utility of rapid prenatal exome sequencing beyond termination of pregnancy: a 2-year cohort from across the North East of England and Yorkshire

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Background: The prenatal exome sequencing (pES) service provides rapid genetic testing for pregnancies with significant fetal anomalies for which a monogenic cause is suspected, and where antenatal diagnosis may alter pregnancy or delivery management. pES tests requested across the North East and Yorkshire GMSA catchment areas are undertaken by the Birmingham team as per national pathways. We reviewed all pES cases between 01/08/2021 and 31/12/2023 to evaluate the impact of prenatal diagnoses on pregnancy management including: termination or continuation of pregnancy, altered delivery plans and neonatal management.

Material and methods: A retrospective analysis of patient records was undertaken in 3 clinical genetics services: Leeds, Sheffield, and Newcastle. Patients were identified through local genomics laboratories. Electronic patient records and paper notes were used to collect data using a predetermined proforma.

Results: 109 pES were completed, identifying 29 diagnoses (pathogenic or likely pathogenic variants; diagnostic yield 26.6%). In cases where a diagnosis was made, the following pregnancy outcomes were documented: 8 terminations, 15 live births, and 4 stillbirths (information was not available for 2 cases). Of the live births, antenatal genetic diagnosis facilitated a postnatal palliation plan for 1 and supported a change in management plan to deliver in tertiary/ quaternary level service for 11.

Conclusion: Information from pES aids decision making beyond continuation or termination of pregnancy. It facilitates prognostic information beyond what could be interpreted from antenatal scans alone. Timely planning of delivery and postnatal management including access to tertiary/quaternary services or palliation is valuable to patients and health services.

C.O.I. Disclosure:

S. Best: None. **A.Z. Imam:** None. **L. Amel Riazat Kesh:** None. **A. Stewart:** None. **R. Sayers:** None. **L. Yates:** None. **P. Wells:** None. **M. McClatchey:** None. **R. Kate:** None. **S. Allen:** None. **K. Fogg:** None. **D. Barnes:** None. **J. Campbell:** None.

EP03.023 Genetic causes of polyhydramnion

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Introduction and Aim of the Study: Polyhydramnios is a common obstetric complication whose causes can include maternal diabetes, chromosomal abnormalities, immune diseases, congenital defects, multiple pregnancies or unknown factors. It is associated with a high risk of complications for mother and child. Effective treatment requires appropriate diagnosis, and preterm labor can be delayed by therapeutic amniocentesis.

Methodology: The study included 88 patients diagnosed with polyhydrosis. In all patients, an amnioreduction procedure was performed at least once, with genetic material secured for analysis. In all of them, microarray and classical cytogenetics were performed. In 36 patients, WES analysis was performed. In addition, each patient had testing for diabetes. In all patients, ultrasound images showed no other abnormalities.

Results: In 4 patients, chromosomal abnormalities were detected that could explain the polyphagia, in 22 patients Alport syndrome was detected in the fetus and in 1 patient Bartter type V syndrome was detected. Diabetes-including gestational and pre-pregnancy diabetes-was detected in 4 patients.

Conclusions: The presented results suggest the need to deepen the diagnosis of polyhydrosis with WES or NGS panel tests for kidney disease and monogenic diabetes as well as other genetic syndromes.

C.O.I. Disclosure:

M. Zyla: None. **M. Malarska:** None. **S. Kubik:** None. **P. Pachniak:** None. **K. Cholewa-Nowak:** None. **K. Szmyt:** None. **M. Grzesiak:** None.

EP03.024 Prenatal diagnosis of congenital heart defects using a 900 candidate gene panel

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Background: Congenital heart defects (CHD) occur in approximately 1% of live births, and are among the most common congenital anomalies. CHD are isolated or combined with other malformations, and are diagnosed during pregnancy using fetal echocardiography. Following genetic counselling fetuses with cardiac abnormalities also undergo prenatal diagnosis using massively parallel sequencing (MPS).

Material and methods: MPS using a 900-candidate gene panel associated with rare diseases, including syndromes linked to heart defects, was performed. DNA was extracted from chorionic villi or amniotic fluid samples/parents' blood, followed by bioinformatic/ clinical evaluation of the detected variants. Parents were tested simultaneously to ensure a short diagnostic turnaround time and clear interpretation of results.

Results: Seven cases of fetuses with prenatally detected heart defects are presented. Two unknown and three previously described de novo pathogenic variants in genes with autosomal dominant inheritance were identified, including missense or nonsense variants in HRAS (NM_005343: c.182A>G), PTPN11 (NM_001330437: c.1507G>A and c.854T>C), and KMT2D (NM_003482: c.15088C>A and c.9292G>T). Two cases showed biallelic pathogenic variants in genes with autosomal recessive inheritance: in EVC (NM_153717: c.1316-7A > G) and LZTR1 (NM_006767: c.401-2_401-1del, c.2219+1G > A). Parents being asymptomatic.

Conclusion: Given genetic findings were of crucial importance for the decision on the further course of the pregnancy. Six of the seven pregnancies were legally terminated based on serious genetic findings, while one child was born with the Kabuki syndrome. Our findings highlight the value of advanced molecular diagnostics in prenatal care and the need for further research on genotype-phenotype correlations to enhance diagnostic precision and counselling.

C.O.I. Disclosure:

P. Cibulkova: None. **V. Burda:** None. **V. Kaduchova:** None. **P. Bednarova:** None. **A. Gregorova:** None. **A. Hladikova:** None. **D. Grecmalova:** None. **J. Schwarzerova:** None. **E. Urbancikova:** None. **V. Skopelidou:** None. **P. Delongova:** None. **J. Skarda:** None. **M. Macek Jr:** None.

EP03.025 Non-invasive prenatal screening for Identification of aneuploidies and CNVs

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Background: Non-invasive prenatal screening (NIPS) is the most sensitive and specific screening test for common chromosomal aneuploidies. This test reduces the number of invasive procedures and patient anxiety. Nevertheless, some factors such as placental mosaicism or vanishing twins can modify NIPS results.

Material and methods: We performed NIPS in 7366 single and 180 multiple gestations using Veriseq NIPT technology (Illumina) to detect common trisomies and sex chromosome aneuploidies (SCAs), rare autosomal trisomies (RATs), as well as copy number variants (CNVs).

Results: We calculated the positive and negative predictive values and the percentage of non-evaluable results. Of the 7546 included cases, there were 0.2% false-positive and 0.07% false-negative cases. Confirmed placental mosaicism, generalized mosaicism and vanishing twins were the main reasons for the false-negative cases. We also examined the relationship between the body mass index and the fetal fraction and the effect of mosaicism of vanishing twins on false-positive and false-negative results.

Conclusion: NIPS has high sensitivity and specificity, enabling the detection of aneuploidies and SCAs, while reducing the number of invasive diagnostic tests. Nevertheless, despite the above potential benefits, notable limitations exist. The incidence of placental mosaicism or the presence of vanishing twins are related to false positives and negative results and women should be informed about the limitations of this technology.

C.O.I. Disclosure:

I. Madrigal: None. **M. Jodar:** None. **C. Badenas:** None.

EP03.026 Prenatal diagnosis of congenital heart disease: molecular analysis of a large cohort

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Background: Congenital heart defects (CHD) represent a group of clinically and genetically heterogeneous cardiac disorders and constitute the most common congenital malformations at birth, with an incidence of 8–10 per 1,000 live births per year. Fetal echographic can detect up to 70% of fetal cardiac anomalies which remain apparently isolated heart defects, while the remaining cases show CHD associated with other fetal malformations.

Material and methods: We evaluated 405 prenatal cases with echographic congenital heart features and the molecular analysis of fetal DNA was performed in Trio analysis by next generation sequencing on the NovaSeq 6000 platform at the Medical Genetics Laboratory of the Bambino Gesù Pediatric Hospital.

Results: NGS analysis identified different variants classified as likely pathogenic (LP) and/or pathogenic (P) in 24% of the analyzed cases. Using a pipeline for CNV detection, we discovered in 9% of our cases the 22q11.21 deletion syndrome also referred to DiGeorge syndrome. Variants of uncertain significance (VUS) were detected in 10% of fetal samples, mostly in different genes associated with isolated forms of CHD and showed variable intrafamilial expressivity. The majority of positive cases (P and/or LP variants) were identified in genes linked to syndromic forms (e.g., Cornelia de Lange syndrome, Noonan syndrome), which are generally de novo and sporadic cases.

Conclusion: Fetal screening and molecular diagnosis of prenatal cases can support the management of CHD during the prenatal development stages by allow an early diagnosis which improved an efficient postnatal care, particularly for the newborn that require immediate surgical interventions.

C.O.I. Disclosure:

D. D'Angelantonio: None. **M. Magliozzi:** None. **P. Bontempo:** None. **D. Ferretti:** None. **S. Ottombrino:** None. **G. Mastrogiorgio:** None. **M. Di Gregorio:** None. **D. Perrino:** None. **V. Pinna:** None. **A. Novelli:** None.

EP03.027 Diagnostic outcomes in clinical exome sequencing for fetal lymphatic anomalies: a retrospective study

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Fetal lymphatic anomalies (fLAs) cause the accumulation of fluid in specific body areas such as behind the fetus's neck (increased nuchal translucency), in abdominal, pleural and pericardial cavities (ascites, pleural and pericardial effusion, respectively) or affect more compartments, leading to nonimmune hydrops fetalis. They can occur as isolated echographic sign or together with other defects (cardiopathies, skeletal and renal anomalies). According to SIGU-SIEOG (Italian Society of Human Genetics and Obstetric, Gynecological Ultrasound, Biophysical Methodologies) recommendations of 2017, invasive prenatal testing is recommended in fetuses with fLAs; when karyotype and chromosomal microarray (CMA) are negative is suggested to perform Rasopathies genes analysis. Between 2022 and 2024, at Bambino Gesù Children Hospital, we collected around 600 fetal samples with fLAs negative for structural anomalies. Trio Clinical Exome Sequencing was performed on NovaSeq 6000, Illumina and analysed with a customized "in silico" panel of genes associated to Rasopathies. In fetuses negative for Rasopathies genes with persisting ultrasound findings the data analysis was expanded, after obtaining informed consent from parents, to whole clinical exome and only pathogenic and likely pathogenic SNVs have been reported. In total 15.2% disease-causing variants were detected, the most localize in Rasopathies genes (58.2%), while 41.8% were identified in genes associated to other syndromic and non-syndromic conditions, such as congenital heart defects, cardiomyopathies, syndromic neurodevelopmental delay, skeletal and ocular abnormalities. To our experience in prenatal testing Clinical Exome Sequencing can improve and accelerate genetic diagnosis revaluating sequencing data based on evolution of ultrasound anomalies, reducing stressful impact for the families.

C.O.I. Disclosure:
E. Lorefice: None. **V. Pinna:** None. **S. Ottombrino:** None. **M.G. Di Gregorio:** None. **V. Lanari:** None. **A. Micalizzi:** None. **F. Lepri:** None. **A. Novelli:** None.

EP03.028 PLoV: a comprehensive database of genetic variants leading to pregnancy loss

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Background: Genetic factors play an important role in such reproductive health problems as spontaneous miscarriage and recurrent pregnancy loss, and the effect of genomic variants is recognized as one of the major causes of pregnancy loss in euploid fetuses. In this work, we aimed to aggregate information about point mutations that have been implicated in pregnancy loss.

Material and methods: We collected information about 536 independent genetic variants observed in 421 cases across 47 studies. The data are stored in an SQL database that includes four main tables: variants, cases, studies and observations. These tables provide detailed descriptive information about each variant and the cases/families in which it was found.

Results: We developed the Pregnancy Loss genetic Variant (PLoV) database that allows users to browse, search, download and upload data on genetic variants linked to pregnancy loss. The resource is freely available at https://plovdb.ott.ru/. Besides browsing, the application enables the user to submit information about new variants and/or suggest changes to the existing data. For all genetic variants we provide the level of confidence based on the ACMG classification, parental and fetal genotypes and phenotypes, gestational age. *PIEZO1*, *COL2A1*, *DYNC2H1*, *FGFR2*, *FOXP3*, *PKHD1*, and *RYR1* genes contain the largest number of high confidence causal variants.

Conclusion: We believe that PLoV could become an important tool to aid interpretation of NGS results in couples affected by pregnancy loss, which could, in turn, increase the effectiveness of early detection of risk alleles and prevention of pregnancy loss.

C.O.I. Disclosure:
E.& Maksiutenko: None. **I.V. Bezdvornykh:** None. **Y.A. Barbitoff:** None. **Y.A. Nasykhova:** None. **A.S. Glotov:** None.

EP03.029 Whole exome sequencing enables the diagnosis of genetic syndromes in fetuses with nonspecific ultrasound abnormalities

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Background: Ultrasound scanning in pregnancy can reveal fetal structural abnormalities from the first trimester onwards. Such findings may indicate an underlying genetic condition but making a definitive diagnosis can be difficult because of unspecific abnormalities, especially in the early pregnancy. Here we present a rare case of a fetus with isolated increased NT (nuchal translucency) and diagnosis of Intellectual Developmental Disorder with hypotonia and behavioral abnormalities (IDDHBA) following prenatal whole exome sequencing (pWES).

Methods: We performed pWES in several hundred fetal cases with abnormal ultrasound findings but normal results for karyotyping. Diagnostic efficiency and its correlation to organ systems involved, phenotypic spectrum, and the clinical impacts of pWES results on pregnancy outcomes were analyzed.

Results: Trio pWES improved the clarification rate of cases with isolated increased NT by 20 %. In our case, isolated increased NT was detected by ultrasound scan in the 12 + 0 week of pregnancy. Trio pWES identified a likely pathogenic heterozygous de novo variant c.611T>A p.(Leu204Gln) in the *CDK8* gene of the fetus. Variants in the *CDK8* gene result in IDDHBA, a neurodevelopmental disorder characterized by onset of hypotonia and variably impaired global developmental delay in infancy.

Conclusion: Pathogenic and likely pathogenic genetic variants detected by pWES are present in a significant proportion of fetuses with increased NT but normal standard karyotype, also when no additional structural fetal anomalies are detected by ultrasound scan. Further studies are needed to confirm these findings and to define a uniform diagnostic algorithm in fetuses with isolated increased NT.

C.O.I. Disclosure:
K. Kornelson: None. **M. Isau:** None. **R. Wimmer:** None. **M. Stumm:** None.

EP03.030 Analysis of residual plasma from prenatal cell-free DNA screening for oncologic somatic variants via circulating-tumor DNA analysis: An assessment of samples at high-risk for maternal cancer

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Background: Maternal cancer can be incidentally detected in prenatal cell-free DNA (cfDNA) screening for fetal aneuploidy, most often associated with multiple structural variants (MSV) characterized by regions of depletion and duplication across the genome. This proof-of-principle study hypothesizes that MSV samples will have oncologic findings via circulating-tumor DNA analysis.

Material and methods: Pregnant patient blood samples were previously tested on the Verifi™ Prenatal Screen via whole genome sequencing. Residual plasma from normal (control cohort) and MSV results was tested with a liquid-based comprehensive genetic profile assay, TruSight Oncology 500 (TSO-500) ctDNA. This is a targeted hybrid-capture based next-generation sequencing assay with median exon coverage ≥1300X, enabling detection of variants present in plasma at low variant allele fractions (VAF) ≥ 1% with high sensitivity and specificity. The panel optimizes coverage of 523 cancer genes by detecting single nucleotide variants, insertions/deletions, rearrangements, and copy number variations.

Results: The control group had 0 somatic variants with VAF ≥ 1%; whereas 5 MSV cases totaled 104. Each MSV case had ≥1 entries in Catalog of Somatic Mutations in Cancer (COSMIC), an oncologic database of somatically acquired mutations. Table 1 demonstrates one case with a cancer gene detected by TSO-500 ctDNA associated with two solid tissue cancers.

Conclusion: Prenatal MSV cases demonstrated statistically significant elevations in somatic variants compared to controls and linked to specific malignancies on TSO-500 ctDNA testing.

This proof-of-principle may drive further exploration of ctDNA as a reflex when MSV is detected.

MSV case example: ctDNA results and associated cancers in COSMIC

GENE	VAF	Nucleotide	COSMIC Primary tissue type	COSMIC Tissue subtype	COSMIC Primary histology
BRCA	0.065	c.5576_5579del	biliary tract	bile duct	carcinoma
			breast	not specified	carcinoma

C.O.I. Disclosure:

S. Hancock: A. Employment (full or part-time); Significant; Illumina, Inc. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Illumina, Inc. **F. Liao:** A. Employment (full or part-time); Significant; Illumina, Inc. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Illumina, Inc. **T. Boomer:** A. Employment (full or part-time); Significant; Illumina, Inc. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Illumina, Inc. **K. Brown:** A. Employment (full or part-time); Significant; Illumina, Inc. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Illumina, Inc. **I. Sangale:** A. Employment (full or part-time); Significant; Illumina, Inc. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Illumina, Inc. **S. Giluso:** A. Employment (full or part-time); Significant; Illumina, Inc. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Illumina, Inc. **E. de Feo:** A. Employment (full or part-time); Significant; Illumina, Inc. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Illumina, Inc. **S. Comer:** A. Employment (full or part-time); Significant; Illumina, Inc. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Illumina, Inc.

EP03.031 Clinical Experience with Genome-Wide Noninvasive Prenatal Testing in a Large Cohort of Twin Pregnancies

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Backgrounds: Noninvasive prenatal testing (NIPT) in twin gestations shows high detection rates and low false-positive rates for trisomy 21, similar to singleton pregnancies. However, data on NIPT performance for other foetal chromosomal anomalies are limited. This study evaluates, in a large cohort of twin pregnancies, the performance and clinical utility of genome-wide NIPT for common foetal chromosomal anomalies (trisomies 13, 18 and 21), Rare Autosomal Aneuploidies (RAAs) and Copy number Variants (CNVs).

Methods: NIPT was conducted using the VeriSeq™ NIPT Solution v2 assay (Illumina) following the Package Insert. Clinical truth was determined via invasive testing, products of conception testing, karyotype at birth, and newborn physical exam. All samples were tested for common trisomies, with 70% of participants opting for genome-wide NIPT for RAAs and CNVs.

Results: Preliminary results from 1200 twin gestations collected over two years showed a 2.1% NIPT screen-positive rate, with 26 high-risk cases: 12 T21, 4 T18, 2 T13, 8 RAAs/CNVs. Clinical follow-up was attempted for all cases, including 1174 negative cases, all of which were true negatives. All T21 cases were confirmed (100% sensitivity, 99.9% specificity and 94.4% Positive Predictive Value), along with 3 T18 and 1 T13. The number of screen-positive cases for additional foetal anomalies is limited, and follow-up is ongoing.

Conclusion: NIPT is reliable screening method in twin pregnancies, demonstrating high performance in T21 with no false positive and false negatives. Further recruitment of twin pregnancies is needed to complete the analysis of performance and clinical utility for additional foetal anomalies.

C.O.I. Disclosure:

L. De Falco: None. **G. Savarese:** None. **R. Sirica:** None. **R. Ruggiero:** None. **M. Ianniello:** None. **N. Petrillo:** None. **A. Mori:** None. **R. Castiello:** None. **A. Di Carlo:** None. **E. Evangelista:** None. **M. Casillo:** None. **L. Circelli:** None. **C. Ramiro:** None. **T. Suero:** None. **R. D'Angelo:** None. **M. Bruno:** None. **F. Del Peschio:** None. **M. Fantuz:** None. **M. Barbato:** None. **S. De Falco:** None. **D. Cino:** None. **V. Feola:** None. **P. Sabbatino:** None. **S. Marcella:** None. **F. Capone:** None. **U. Lazzaro:** None. **A. Fico:** None.

EP04 Sensory Disorders (Eye, Ear, Pain)

EP04.001 A rare *ABCA4* gene variant in a case of retinal dystrophy

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Background: Retinal dystrophies are degenerative retinal diseases characterized with loss of retinal photoreceptor and epithelial cells. Although a huge number genetic abnormalities on various genes were determined in most of them, clinical heterogeneity of retinal impairment creates difficulty on certain diagnosis. Here we present a 25 years old female case of retinal dystrophy with macular atrophy.

Material and methods: Clinical exome analysis was performed by using next generation sequencing method (Illumina Next-Seq2000). Variants were analyzed using Sophia DDM program. Variant information servers dbSNP, ClinVar, Ensembl and ACMG 2015 criteria were used for interpretations.

Results: : On *ABCA4* gene, c.662G>A (p.Gly221Glu) and c.4463G>T (p.Cys1488Phe) missense variants were detected in compound heterozygous state. Although the c.4463G>T change was reported as "pathogenic" variant in ClinVar (ID: 99286), the second c.662G>A variant was not reported in ClinVar. Variant effect programs were not in agreement on predicting the effect of this change on protein function. This variant was observed rarely in population studies (rs377637509, gnomAD: 0.0000018) and there is no report indicating its clinical importance within our knowledge. According to these data, the c.662G>A change was classified as variant of uncertain clinical significance.

Conclusion: Rare missense variants with uncertain clinical importance were frequently encountered in diagnostic area of retinal dystrophies recently. A detailed bioinformatic analysis and clinical surveillance were required to determine these candidate variants significance on retinal abnormalities. To better understand their clinical effect, segregation analysis of the variants in both the patient's family and in appropriate population needs to be performed.

C.O.I. Disclosure:

A. Toylu: None. **M. Dogan:** None. **H. Ilhan:** None. **O. Altiok Clark:** None.

EP04.002 Beyond the surface exploring Wolfram syndrome from genetics to therapy

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Background:. Wolfram syndrome type 1 (MIM# 222300) is an extremely rare autosomal recessive disorder with an incidence of 1 in 770,000 live births. It is characterized by juvenile-onset type I diabetes mellitus (DM), progressive optic atrophy (OA), diabetes insipidus (DI), and deafness (D), often referred to by the acronym DIDMOAD. In addition to these major signs, urinary, neurological, and psychiatric manifestations may also occur. A positive diagnosis requires the presence of type I DM and optic nerve atrophy. WS1 is caused by mutations in the WFS1 gene located on chromosome 4p16, which encodes a transmembrane protein called wolframin.

Material and methods: The authors present the case of a 28-year-old patient diagnosed with type 1 DM at the age of 6 and progressive optic atrophy at the age of 26

Results: Whole Exome Sequencing Plus (WES) revealed that the patient is a compound heterozygote, carrying a heterozygous nonsense variant c.1943G>A, p. (Trp648*) and a heterozygous missense variant WFS1 c.1675G>C, p. (Ala559Pro). WES analysis for the entire family showed that the patient's father is heterozygous for the first mutational variant, the mother is heterozygous for the second variant, and the patient's brother and wife do not carry either of the identified mutational variants

Conclusion: Early diagnosis of Wolfram syndrome is crucial for correct genetic counselling to alleviate suffering and improve the quality of life for these patients.

C.O.I. Disclosure:

C.M. Jurca: None. **S. Jurca:** None. **K. Kozma:** None. **A. Jurca:** None. **D. Bembea:** None. **C. Petchesi:** None. **A. Jurca:** None.

EP04.003 Jalili Syndrome in two adults siblings

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Background: Jalili syndrome is a rare multisystem disorder with the most prominent features consisting of cone-rod dystrophy and amelogenesis imperfecta. Few cases have been reported in the literature. Here we describe two adults brothers with Jalili syndrome examined at our genetic counseling clinic.

Material and methods: We present the case of a 60-year-old male patient with non-specific neurological symptoms (loss of strength) for whom a related molecular study is requested. In this study, only one homozygous molecular variant in the CNNM4 gene (gene related to Jalili syndrome) is reported. The patient is scheduled for a Genetic Counseling consultation to collect phenotypic data and family history.

Results: In the molecular study, the CNNM4 gene variant is characterized (NM_020184): c.731G>T; p.(Cys244Phe) in homozygosity, it is confirmed that the patient presents loss of vision and the absence of multiple teeth from a young age. From a phratry of seven siblings, the patient and his 64-year-old sister have the same clinical phenotype. The molecular study of his sister characterizes the same variant of the CNNM4 gene in homozygosity.

Conclusions: In this case, it is demonstrated that when making a syndromic diagnosis, it is of crucial importance to collect all phenotypic and family data that help interpret the results of molecular studies within a clinical context. This is the first time that a family case of Jalili syndrome has been reported in our community.

C.O.I. Disclosure:

A. Poyatos-Andújar: None. **S. García-Linares:** None. **L. Papay-Ramírez:** None. **F. Castellano-Miñán:** None. **M. Del Aguila-García:** None. **J. Morales-Camino:** None. **M. Martínez-Atienza:** None. **A. Martínez-Cañamero:** None. **S. Pedrinaci-Rodríguez:** None. **G. Alvarez-Corral:** None. **T. De Haro-Muñoz:** None.

EP04.004 Oftalmogenetica - the first Romanian registry of patients with inherited eye diseases

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Background: Inherited eye diseases are considered a critical public health concern since many of them lead to blindness. Because the number of patients affected by this type of illnesses is low, establishing a registry is advantageous for both patients and professionals. "Oftalmogenetica" was launched in September 2023 and it is the first Romanian registry for patients diagnosed with ophthalmogenetic diseases. The objective of this poster is to present the accomplishments of this registry since its inception.

Material and methods: Data from "Oftalmogenetica" were analyzed, emphasizing the number and demographics of patients and the type of their inherited eye disease. Moreover, facilities offered to patients were discussed (courses, workshops, articles).

Results: Since its debut, the registry "Oftalmogenetica" has enrolled over 30 patients with inherited eye diseases from Romania. The registry provides useful information regarding: access to funded treatments in Romania, articles regarding different inherited eye diseases and different courses for patients. Three online courses for patients are offered: “Life with low vision (The first Romanian low vision program)”, “A healthy baby - the importance of genetic diagnosis and preconceptional diagnosis in ophthalmogenetic diseases", “Useful devices for patients with low vision”. Also, an online workshop regarding Leber Hereditary Optic Neuropathy was organized.

Conclusion: Since its inception the registry "Oftalmogenetica" has achieved its objective to create a data base of patients with inherited eye diseases in Romania and offered them support to better understand and manage their condition.

C.O.I. Disclosure:

E. Avram: None.

EP04.005 The first Moroccan patient with Alström syndrome and potential pancreatitis association

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Background: Alström Syndrome (ALMS), an autosomal recessive ciliopathy arising from biallelic pathogenic variations in *ALMS1*, is commonly marked by multisystemic manifestations, including early-onset cone-rod retinal dystrophy leading to blindness, hearing impairment, childhood obesity, type 2 diabetes mellitus, cardiomyopathy, fibrosis, and multiple organ failure. Although the exact function of ALMS1 is not fully understood, studies have revealed its involvement in endosomal and ciliary transport, as well as regulation of the cell cycle. More than 950 cases of Alström patients have been documented worldwide, however, with a notable absence of report within the Moroccan population.

Case presentation: In this context, we present an inaugural case of Moroccan patient that was misdiagnosed for 5 years. An 18-years-old male from a consanguineous union with no significant family history presented for evaluation of a complex medical history. He has congenital obesity, bilateral congenital blindness due to retinitis pigmentosa, hearing impairement, dyslipidemia and a history of diabetes diagnosed at age 10 following ketoacidosis due to severe acute pancreatitis. The diagnosis was initially suspected following extended clinical observation and subsequently verified through genetic analysis, exhibiting a homozygous *ALMS1* pathogenic variant.

Results and Conclusion: A novel frameshifting deletion of *ALMS1* confirms the diagnosis of Alström syndrome despite the complexity of the patient's presentation, expanding the spectrum of genetic variants associated with the syndrome.

C.O.I. Disclosure:

H. Ihlal: None. **M. Ouarhache:** None. **A. Ouskri:** None. **J. Tarabeux:** None. **A. Lebechec:** None. **L. Bouguenouch:** None. **J. Muller:** None.

EP04.006 Identification of novel and known mutations in Iranian families with Leber Congenital Amaurosis

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Background: Leber congenital amaurosis (LCA), one of the most severe inherited retinal dystrophies is responsible for 20% of childhood blindness. LCA can result from pathogenic mutations reported in at least 38 genes participating in the visual cycle. This study reports four homozygous mutations related to LCA in Iranian families.

Material and methods: Whole exome sequencing (WES) was performed in four unrelated patients from consanguineous parents who had at least two affected children. The identified mutations were confirmed by sanger sequencing.

Results: In the first patient a pathogenic known missense variant in RPE65 gene was detected (NM_000329:exon4:c.271C>T:p.R91W). This gene provides an instruction for making RPE65 protein. In second patient a novel homozygous frameshift deletion mutation was found in GUCY2D gene (NM_000180:exon3:c.751delC:p.L251fs) which is classified as "probably pathogenetic" according to American College of Medical Genetics and Genomics guidelines. This gene has an important role in phototransduction or converting light into electrical signals for the brain. Another variant was a known pathogenetic missense mutation in the RDH12 gene with crucial roles in metabolizing retinoids (NM_152443:exon5:c.G226C:p.G76R). Last patient had a novel missense mutation in the KCNJ13 gene (NM_001172417:exon2:c.136T>C:p.F46L). Mutations in this gene are associated with vitreoretinal degeneration. Co-segregation analysis revealed that all affected individuals were homozygous for variants with the heterozygous carrier parents.

Conclusion: This study expands the mutational spectrum of LCA as we report two novel variants and two known pathogenic mutations in related LCA genes which could lead to more knowledge about genetic basis of this disorder.

C.O.I. Disclosure:

P. Nikuei: None. **M. Farazi fard:** None. **S. Tabasi:** None. **K. Alavi:** None. **G. Tabasi:** None. **P. Archangy:** None. **M. Saberi:** None.

EP04.007 Clinical exome (NGS) in Mexican hearing loss patients, diagnostic yield

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Background: Hearing loss is defined as total or partial loss of that function. Sensorineural hearing loss (SNHL) is the most common sensory impairment in humans (1:3000 newborns). Current studies report a diagnosis rate of around 50%.

Material and methods: Eleven patients with hearing loss were studied using a panel of 224 related genes. Sequencing (≥50x) was performed using Illumina technology, aligning to GRCh37. Variants were reported according to HGVS and confirmed with validated methods (invitae). Results were correlated with patients' phenotype and family history.

Results: Of the 11 cases (3 men/8 women), 1 was familial, seven (63.6%) showed a positive result, three cases (27.3%) had variants of uncertain significance (VUS), and one case had a negative result. Pathogenic variants (PV) were identified in the GJB2 gene (3 heterozygotes and one compound heterozygous). Compound heterozygotes were found in USH2A and ADGRV1. Two patients presented inner ear malformation.

Conclusion: The panel of genes related to hearing loss (226 genes) showed acceptable performance, detecting 63.6% of PV. The main gene found was GJB2, however, not in the same proportion as in other populations, and many in heterozygosis. There is no correlation with the degree of hearing loss and PV and its state in heterozygosis or homozygosis, VUS could very likely contribute to SNHL, modifying the phenotype. Segregation studies are necessary to be more precise in the diagnosis and in genetic counseling.

C.O.I. Disclosure:

M. Arenas-Sordo: None. **N.D. Marín-Ríos:** None. **A.E. Jiménez-Romo:** None.

EP04.008 Phenotypic variability of OTX2 mutation in three members in a family

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Background: OTX2 is a homeobox gene that encodes a transcription factor that plays a role in early brain development. Intragenic mutations cause impaired proliferation and differentiation of dopaminergic neuronal cells leading to several different phenotypes: syndromic microphtalmia (#610125); combined pituitary hormone deficiency (#613986) and early onset retinal dystrophy (#610125).

Material and methods: We performed exome sequencing (ES) in three members of one family - two siblings and their mother. Phenotypic evaluation was performed in all.

Results: Variable eye anomalies were detected in the mother and her two children. The first child is a boy with unilateral microphtalmia and short stature due to growth hormone deficiency (GHD). He had small genitalia and undescended testicles that are suggestive for hypogonadism. MRI of the brain showed unilateral microphtalmia, as well as hypoplastic pituitary gland. The girl had bilateral anophtalmia, with hypoplastic pituitary on MRI as well. IGF1 was low, however she was not tested for GHD. Both children have normal psychomotor development without other malformations. Their mother had minor iris coloboma, normal vision and retinal degeneration bilaterally. They all have heterozygote loss of function mutation of the OTX2 gene (c.402dupC; p.Ser135Leufs*2), leading to the premature stop codon and truncated protein.

Conclusion: Heterozygous mutations in OTX2 gene are common cause for eye malformations detected in more than 150 patients. Phenotypic variability is well recognized even within one family, however the reason is not well understood (modifier genes, genomic mosaicism, etc.). Further investigation is needed for elucidating variable clinical presentations.

C.O.I. Disclosure:

E. Sukarova-Angelovska: None. **E. Shuperliska:** None. **S. Veleska:** None. **G. Ilieva:** None. **G. Milanovski:** None. **A. Petlichkovski:** None. **T. Spasovska:** None.

EP04.009 Clinical and genetic heterogeneity in anterior segment anomalies : A case series

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Background: Congenital anterior segment anomalies (CASA) encompass a diverse group of disorders characterized by abnormal development of the cornea, iris, trabecular meshwork, ciliary body, and lens. Approximately 50% of affected individuals develop glaucoma, often early in life. Therefore, genetic testing is essential for improving diagnostic accuracy, management strategies and refining prognoses.

Material and methods: We present the clinical findings of ten patients referred for CASA accompanied by other signs, primarily dysmorphic features and developmental delay. The genetic investigation included either a multi-gene panel or whole exome sequencing

Results: The cohort included ten patients with a median age of 3 years and 6 months. Congenital cataract was the most frequent ophthalmological finding (8/10 patients), followed by aniridia and glaucoma (2/10 each), and coloboma, iris hypoplasia, and ectopia lentis (1/10 each). Consanguinity was present in 5/10 of families. Dysmorphic features were observed in six patients, and associated systemic signs were noted in nine patients, most commonly developmental delay (5/10) and axial hypotonia (3/10). Other associated signs included epilepsy, growth retardation, and hepatomegaly. Molecular diagnosis was established in eight patients. Single nucleotide variants were found in six patients with four affected genes: *SERPINA1* (alpha-1-antitrypsin deficiency), *FBN1* (Marfan syndrome), *OCRL* (Lowe syndrome), *FOXC1* (anterior segment dysgenesis type 3), *GALT* (galactosemia) and two Copy number variants in 15q26.3 and 11p13 encompassing respectively *ALDH1A3 and PAX6*.

Conclusion: Given the overlapping clinical features of CASA, accurate diagnosis and prognostication can be challenging. Therefore, detailed characterization of these anomalies is crucial for optimizing clinical and genetic management.

C.O.I. Disclosure:

C. Mortadha: None. **H. Syrine:** None. **E. Yasmina:** None. **R. Imen:** None. **B. Yosra:** None. **S. Jihene:** None. **J. Houweyda:** None. **B. Lamia:** None.

EP04.010 Expanding spectrum of LRAT-related retinal disease: two spanish children with fundus albipunctatus

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Background/Objectives: Fundus albipunctatus (FAP) and retinitis punctata albescens (RPA) are rare inherited retinal disorders characterized by night blindness (nyctalopia) and distinct retinal findings. This case series aims to describe new mutations and their mechanism of action in patients diagnosed with FAP or RPA to provide appropriate genetic counselling and management strategies.

Methods: We describe a case series of two unrelated female Spanish patients (14 and 16 years old) followed at Sant Joan de Déu Hospital, Barcelona. Both presented with nyctalopia without detectable visual field constriction. Clinical evaluations included visual acuity testing, visual field assessment, wide-field funduscopy, fundus autofluorescence, optical coherence tomography, and full-field electroretinography (ERG). Next-generation sequencing (NGS) was performed using a Clinical Exome Sequencing (CES) virtual panel for genetic analysis.

Results: Both patients were identified as compound heterozygous for the same two genetic variants in the *LRAT* gene, despite being from unrelated families: *LRAT* (NM_004744.4): c.217_218delAT, p.(Met73Aspfs*48) and *LRAT* (NM_004744.4): c.569G>A,p.(Arg190His). In both cases, mutations were found in *trans*-configuration inherited from each parent. The assessment conducted by ERN-EYE experts enabled the classification of the variants as pathogenic. No additional genetic alterations were detected that could explain their condition.

Conclusions: While *LRAT* mutations are predominantly associated with Leber congenital amaurosis (LCA) and early-onset severe retinal dystrophy (EOSRD), our findings suggest that they may also contribute to the development of FAP or RPA phenotypes. This expands the clinical spectrum of LRAT-related retinal diseases and highlights the importance of genetic testing in accurate diagnosis, enabling targeted genetic counselling for affected families.

C.O.I. Disclosure:

G. Escribano Serrano: None. **D. Salinas Chaparro:** None. **A. Baldaquí Baeza:** None. **D. Yubero Siles:** None. **C. Aguilera Roman:** None. **A. Esteve Garcia:** None. **I. Alonso Colmenero:** None. **J. Diaz Cascajosa:** None. **A. Martinez Monseny:** None. **J. Català Mora:** None.

EP04.011 Identification of novel structural variant using exome sequencing in combination with genome-wide SNP genotyping

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Background: Leber congenital amaurosis (LCA) accounts for 5% of the inherited retinal dystrophies (IRDs) with worldwide prevalence ranging 1:30,000 to 1:80,000. Here we present the genetic analysis of family affected with autosomal recessive LCA using combination of genome-wide SNP genotyping and whole exome sequencing (WES).

Material and methods: Genome-wide SNP genotyping, performed on seven members of a family, followed by WES of a proband. Expression analysis of the target gene was performed in blood samples obtained from patients and normal individuals.

Results: Homozygosity mapping of genome wide genotyping data identified homozygous regions on chromosome 2 (8.31Mb) and chromosome 14 (28.5Mb) which were shared among 3 affected members. WES of the proband could not reveal any potential pathogenic variant. But re-analysis of the mapped homozygous regions identified three consecutive SNPs with no genotype calls in affected individuals. The probability of deletion was further evaluated by analyzing BAM file of the proband which showed absence of reads in the region spanning these missing SNPs. A novel 81.8kb homozygous deletion (chr14:88855277-88937126) was confirmed by chromosomal walking between the intact flanking SNPs (rs7161612 and rs2274736) on both sides. This deletion spans *SPATA7* gene which is already known for LCA. Blood cells-based expression analysis of *SPATA7* confirmed the absence of *SPATA7* mRNA in the affected individuals.

Conclusion: The capturing of structural variants (SVs) using short-read NGS data is difficult. Third-generation sequencing is considered the most appropriate technique for SV identification, yet multiple approaches in combination with short-read NGS can help in the identification of SVs.

C.O.I. Disclosure:

R. Basharat: None. **S. Sajid:** None. **E. Ullah:** None. **J. Ahmed Zai:** None. **M. Ansar:** None.

EP04.012 Identification of possible novel genes and variants in exome-negative Iranian families with non-syndromic hearing loss through reanalysis

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Background: Hearing loss (HL) is a prevalent sensorineural disorder that significantly affects an individual's quality of life. The most frequent genetic form of HL is autosomal recessive non-syndromic HL (ARNSHL). In Iran, congenital HL is the second most common genetic disorder, mainly because of the high frequency of consanguineous marriages, and its diagnosis is complicated by its significant genetic diversity.

Material and methods: We conducted a systematic exome sequencing (ES) data reanalysis 6–9 years after the initial analysis of five consanguineous Iranian HL families that revealed negative results previously using *GJB2* screening, an OtoSCOPE panel (V5 and V6), and ES analysis. After clinician-led reanalysis of the ES data and comprehensive bioinformatics analysis, segregation analysis of potential candidate variants was performed by Sanger sequencing using an ABI 3500 sequencer.

Results: Five variants were identified, including two novel variants in known HL-related genes (*OTOA* and *TBC1D24*) and three variants in new candidate genes (*ANKRD24*, *DBX2*, and *ARHGAP22*). These variants exhibited co-segregation within the family pedigrees, indicating their possible association with HL.

Conclusion: This study highlights the importance of periodic reanalysis of ES data to increase the overall diagnostic yield and uncover missed genetic causes of HL.

C.O.I. Disclosure:

F. Zare Ashrafi: None. **R. Rezvani Rezvandeh:** None. **N. Kazemi:** None. **Z. Bolghan Abadi:** None. **K. Jalalvand:** None. **K. Kahrizi:** None. **H. Najmabadi:** None. **M. Mohseni:** None.

EP04.013 Diagnostic and therapeutic strategies in mitochondrial eye disease

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Background: Mitochondrial disorders are a wide spectrum of conditions which are estimated to affect 1:5000 individuals. There is a paradigm shift in the therapeutic landscape for mitochondrial disorders which highlights the need for early, accurate molecular diagnosis. Mitochondrial disorders are a highly heterogenous group of syndromes with up to 50% of individuals including a clinical ophthalmic phenotype. Key ophthalmic features most commonly include external ophthalmoplegia, retinal disorders and optic neuropathies along with neurological features. Given a relatively high initial presentation to ophthalmology it is paramount that key factors from an ophthalmic perspective are carefully considered.

Material and methods: A scoping literature review was performed with a focus on clinical features, diagnostic pathways, and therapeutic strategies in mitochondrial eye disease.

Results: The unique inheritance, variable severity and non-specific clinical features result in mitochondrial cause often under-considered at initial ophthalmology presentation. Key guidelines exist if patients present to genetics clinic with what is assumed to be a mitochondrial disease. A systematic approach is needed to comprehensively investigate and identify a underlying mitochondrial eye disease which may also have systemic features that could be life threatening. There have been significant advancements towards developing therapy which further demonstrates the importance early diagnosis for emerging treatments.

Conclusion: We aim to highlight key considerations that need to be undertaking when patients present with a potential mitochondrial eye disorder. In particular we aim to highlight the need for application of standardised guidelines as part of the diagnostic process.

C.O.I. Disclosure:

G.A. Borchert: None. **M.F. Baxter:** None.

EP04.014 Association of OPRM1 rs1799971 and COMT rs4680 polymorphisms with clinical phenotype among women with fibromyalgia

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Background: Fibromyalgia (FM) is a complex syndrome characterized by chronic musculoskeletal pain, fatigue and cognitive problems. Catechol-O-methyltransferase (COMT) and the μ-opioid receptor (OPRM1) have been associated with chronic pain sensitivity. Although its exact causes are still not fully understood, genetic factors, such as *OPRM1* and *COMT* polymorphisms could be potential contributors to this syndrome phenotype.

Methods: A observational cross-sectional study was conducted in a cohort of 446 women, 311 fibromyalgia patients and 135 healthy women. We recovered validated questionnaires about quality of life, symptoms and pain. DNA was extracted from collected blood samples, and genotyping for the *OPRM1* rs1799971 and *COMT* rs4680 polymorphisms was performed using TaqMan assays.

Results: We only found a marginal difference in the genotype distribution of *COMT* rs4680 between groups ($p = 0{,}053$). Being the allele A more prevalent in patients. On the other hand, we observed several statistically significant associations between *COMT* rs4680 and fatigue ($p = 0{,}027$), restful sleep ($p = 0{,}009$), Symptom Severity Scale (SSS) ($p = 0{,}007$), sleep quality ($p = 0{,}033$), stress ($p = 0{,}009$) and Borg scale ($p = 0{,}011$). In the case of *OPRM1* we only found an association with sleep quality ($p = 0{,}049$) and a marginal association with visual analogic scale for pain (VAS) ($p = 0{,}080$).

Conclusions: A wide spectrum of symptoms, including severe fatigue, impaired physical function, and cognitive difficulties were found in fibromyalgia patients. Instead of finding differences in the genotypes distribution between FM and healthy women we observed associations with symptoms or clinical characteristics more prevalent in the group of patients, especially for *COMT* rs4680.

C.O.I. Disclosure:

I. Erenas: None. **S. Estepa:** None. **C. Chicharro:** None. **J. Gómez:** None. **A. Fernández-Araque:** None. **Z. Verde:** None.

EP04.016 Molecular characterization of a large case series of Italian hearing-impaired patients attending to the Audiology service of AULSS3 Serenissima

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Background: Congenital or early-onset sensorineural hearing loss (SNHL) is one of the most common hereditary disorders. All known Mendelian inheritance-patterns, involving approximately 150 genes, have been identified. Approximately 50% of pediatric SNHL-cases have an identifiable causative genotype. This study aims to enhance the understanding of the molecular basis of SNHL.

Material and methods: Using the CE-IVD kit Oto-GeneSGKit® (Sistemas Genomicos, Spain), we generated a library covering exonic and adjacent intronic regions of 115 genes, along with the microRNA miR-96, previously associated with SNHL. The library was sequenced on Illumina-MiSeq (Illumina, USA). Variant prioritization was performed using CE-IVD e-Vai software (enGenome, Italy). Since February 2023, 113 probands with bilateral childhood-onset SNHL (64 females, aged 1 to 50 years) have been analyzed, 33 of whom had a family history of SNHL. The DFNB1 locus had been analyzed earlier.

Results: A molecular diagnosis was achieved in 57 (50,4%) probands: 34 had a dominant inheritance-pattern (including 5 with 'de novo' variants in *MITF*, *GATA3*, *MYO7A*, and *ACTG1* genes), and 23 had a recessive inheritance-pattern. In the dominant-model, the most frequently mutated genes were *MYO7A* (5/34, 14.7%) and *WFS1* (4/34, 11.8%), while in the recessive-model, *MYO15A* (4/23, 17.4%) and *MYO7A* (4/23, 17.4%) were most common. Causative variants were identified in 32 different genes, and 39 probands had a causative genotype not previously reported in the literature. Of the 33 probands with familial SNHL, 12 received a molecular diagnosis.

Conclusions: These findings contribute to a deeper understanding of the genetic and allelic heterogeneity underlying the SNHL-phenotype.

C.O.I. Disclosure:

D. Degiorgio: None. **E. Greco:** None. **M. Beggio:** None. **L. Bevilacqua:** None. **G. Favretto:** None. **A. Tuniz:** None. **E. Cama:** None. **C. Gondiu:** None. **E. Natale:** None. **P. Scimemi:** None. **R. Santarelli:** None. **M. Favarato:** None.

EP04.017 Evaluation of the impact of variants in the *SLC26A4* gene found in patients with hearing loss on splicing by minigene assay

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Background: Pathogenic variants of the *SLC26A4* gene are one of the most significant causes of inherited hearing loss. At least 25% of all known pathogenic *SLC26A4* variants, located in both intronic and exonic regions, are thought to result in abnormal splicing, although their pathogenicity is often inferred solely on in silico predictions. Analysis of *SLC26A4* in patients with hearing loss (the Republics of Tyva and Altai, Southern Siberia, Russia) revealed several novel variants, as well as variants of uncertain significance. We aimed to analyze the functional consequences of these *SLC26A4* variants on splicing.

Material and methods: A minigene assay was used to evaluate the impact of the *SLC26A4* variants on splicing.

Results: Six *SLC26A4* variants were analyzed in silico using several predictive tools designed to assess their potential effects on splicing, followed by minigene assay. We experimentally confirmed predicted deleterious effect of rare variant c.2034+1G > A, which leads to aberrant splicing. However, the loss of the acceptor site and exon 16 skipping, predicted for intronic variant c.1708-18T > A were detected only in a small fraction of transcripts. For three intronic variants (c.1545-168A > G, c.1708-125T > C, c.1804-31C > T) located outside canonical splice sites, and exonic variant c.1545T>G located at nucleotide position 1 of exon 14, equivocal predictive scores were obtained, however, the minigene assay showed no effect on splicing for these variants.

Conclusion: Obtained data underscore the importance of an integrated approach combining in silico predictions with functional in vitro analysis for the accurate interpretation of genetic variants and reliable molecular genetic diagnosis.

C.O.I. Disclosure:
V. Danilchenko: None. **E. Panina:** None. **M. Zytsar:** None. **K. Orishchenko:** None. **O. Posukh:** None.

EP04.018 Molecular characterization of a large cohort of Saudi Arabian patients with hereditary hearing loss using next generation sequencing

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Background: Hearing loss is one of the most common sensory disorders in humans with both genetic and environmental etiologies. Genetic causes of hearing loss are extremely heterogeneous; more than 100 genomic loci for hearing loss have been mapped so far. Molecular diagnosis is of great significance in revealing the molecular pathogenesis and aiding the clinical diagnosis of this disease. Our study aims to comprehensively delineate the genetic basis of hearing loss in the individuals of Saudi Arabian origin.

Material and methods: The identification of the causative gene in affected families with hearing loss is difficult due to extreme genetic heterogeneity and lack of phenotypic variability. Consanguineous families are a powerful resource for genetic linkage studies/homozygosity mapping for recessively inherited hearing impairment. Homozygosity mapping, linkage analysis and Whole Exome sequencing were conducted.

Results: Using the combined approaches, so far mutations in 32 different deafness genes have been identified in 300 familial/sporadic cases, including novel variants in known HL genes and novel genes.

Conclusion: Using these innovative molecular approaches, we were able to document the most common forms of hereditary hearing loss, their incidence and distribution in the Saudi population. The overall results of this study are highly suggestive that the underlying molecular basis of hearing loss in Saudi Arabia is very genetically heterogeneous. The benefit of this study will hopefully provide the foundation for knowledge and awareness through screening of carrier status and genetic counselling, thereby having a major impact upon early intervention for and prevention of hereditary hearing loss.

C.O.I. Disclosure:
K. Ramzan: None.

EP04.019 Isolated vs. syndromic structural and retinal eye disorders: experience at Children's Clinical University Hospital (CCUH) since 2022

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Background: Genetic eye diseases include a diverse group of inherited disorders affecting vision, presenting as either isolated or syndromic phenotypes. Accurate diagnosis often requires advanced molecular techniques, with whole exome sequencing (WES) emerging as a crucial tool for identifying genetic causes.

Material and methods: Since 2022, 46 individuals (28 paediatric and 18 adult patients) with structural or retinal/optic nerve disorders have been referred for genetic testing at CCUH, Riga, where WES was performed.

Results: For structural disorders (n = 19), the diagnostic yield was higher in cases with a systemic phenotype (n = 11), or broader phenotypic presentation compared to isolated cases (n = 8): 54% versus 25%. Among patients with systemic or multiorgan involvement, three (27%) had additional genetic findings. Most structural eye phenotypes were associated with autosomal dominant variants (88%).For retinal and optic nerve disorders (n = 27), most positive cases (n = 13, 48%) had an isolated phenotype (n = 20). Three patients had additional genetic findings, confirming multiple diagnoses. The isolated phenotype was primarily caused by X-linked or autosomal recessive mechanisms. Only one patient had a positive finding in multi-panel screening (n = 7), but the identified variant explained only the eye phenotype.

In both groups, the diagnostic yield was significantly lower in females compared to males (Chi-squared test, p = 0.0045).

Conclusion: Structural and retinal/optic nerve disorders demonstrated similar diagnostic yields at CCUH (~43%). Structural eye phenotypes were more frequently associated with syndromic findings, while isolated gene panel analysis was more beneficial for retinal and optic nerve diseases.

C.O.I. Disclosure:
L. Freimane: None. **L. Bārdiņa:** None. **E. Berga-Švitina:** None. **D. Enkure:** None. **I. Nartiša:** None. **I. Mičule:** None. **M. Masinska:** None. **I. Grīnfelde:** None. **G. Tauriņa:** None. **D. Ločmele:** None. **D. Rots:** None.

EP04.020 Crosstalk of GJB2 and mt-DNA in congenital hearing loss: a path less travelled

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Background: Sensorineural hearing loss (SNHL) is a common genetic condition, often influenced by mitochondrial dysfunction. Since cochlear cells have high energy demands, mitochondrial defects can exacerbate hearing loss caused by GJB2 mutations. Patients with GJB2 mutations may be more vulnerable to additional stressors, such as D-loop variations, which can further impair cochlear function and accelerate disease progression. Our study aimed to assess mitochondrial D-loop variations in patients with GJB2 mutations to better understand their potential role in worsening hearing impairment.

Material and methods: Blood samples were obtained from 18 patients for genomic DNA extraction, followed by mt DNA based D-loop variations. This methodological approach facilitates the investigation into the association between mitochondrial D-loop variations and hearing loss in individuals carrying GJB2 gene mutations.

Results: In our study, we identified four GJB2 mutations in 23 patients and analyzed mitochondrial D-loop variations in GJB2-positive cases. Two common mutations, 263 (A > G) and 310–315 (CCCCC), were found in 94% of samples. The 303–309 (ccccccc>cccccccc) mutation appeared in 53%, while 152 (t > c) and 489 (t > c) were present in 35% and 29%, respectively. Additionally, 18 other distinct D-loop mutations were detected.

Conclusion: The mitochondrial D-loop can influence the severity and progression of hearing loss in patients with GJB2 gene mutations by contributing to mitochondrial dysfunction, which in turn affects the energy-dependent processes crucial for cochlear function. Further research into the interactions between mitochondrial mutations and GJB2 mutations can provide deeper insights into the mechanisms of hearing loss and potential therapeutic targets.

C.O.I. Disclosure:
M. Kavidayal: None. **M. Kavidayal:** None.

EP04.021 Implementation of a new Ocular Genetics Clinic: A 5-year clinical audit

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Background: Ocular genetics is a rapidly evolving field where genetic diagnoses have high utility. We describe a multidisciplinary Australian Ocular Genetics Clinic (OGC) provided jointly by the Royal Melbourne Hospital and Royal Victorian Eye and Ear Hospital.

Material and methods: We present data from a complete audit of the OGC from its inception in December 2018 to December 2023, where mutation detection/diagnostic yield is reported and compared across multiple phenotypes.

Results: We observed a high rate of diagnostic testing, along with comparable or more favourable diagnostic yields reported in internationally cohorts. Of 535 patients seen through the OGC, 319 diagnostic tests amounted to 59.63% uptake rate of diagnostic testing during the 5-year period. The overall rate of a clinically actionable result (likely pathogenic or pathogenic variant) was 66.67%. This facilitated a further 82 predictive and segregation tests for at-risk family members. Molecular confirmation of a diagnosis was more likely with young age of clinical diagnosis (under 20 years) ($p < 0.022$) and when there was a family history of disease ($p < 0.026$).

Conclusion: We attribute the high yield of diagnostic variants to expert phenotyping by the ophthalmologists. These data support a model of care expanding mainstreaming of genomics into ophthalmology to keep up with increasing demand for ocular genetic diagnostics. Enhanced provision of genomic confirmation of a diagnosis ends the diagnostic odyssey, presents opportunities for predictive testing and reproductive options, and may provide the entry point into targeted therapies and novel clinical trials.

C.O.I. Disclosure:

L. Tan: None. **J. Schulz:** None. **L. McCall:** None. **T. O'Brien:** None. **A. Huq:** None. **M. Cleghorn:** None. **J.B. Ruddle:** None. **T.L. Edwards:** None. **A.W. Hewitt:** None. **L.S. Kearns:** None. **P. James:** None. **I. Winship:** None.

EP04.022 Improved variant detection using long-read sequencing and optical mapping: illustration in STRC-related hearing loss

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Background: Biallelic loss-of-function variants in *STRC* contribute to mild-moderate hearing loss. Here, we report a case with mild hearing loss. Exome sequencing and MLPA analysis revealed *STRC* biallelic inactivation due to a nonsense variant and a *CKMT1B-STRC* deletion. Analysis of the self-reported normal-hearing parents revealed inconsistent mendelian inheritance. Indeed, the mother was a heterozygous carrier of a *CKTM1B-STRC-CATSPER2* deletion, and the father shared the same genotype as his daughter. He was later found to also have moderate hearing loss.

Results: To address these discrepancies, we used long-read sequencing and optical genome mapping. We demonstrated that the father, in fact, carried a *CKMT1B-STRC-CATSPER2* deletion in trans with the *STRC* nonsense variant and a tandem duplication of *CATSPER2-CKMT1A*. The proband inherited this latter haplotype, together with the maternal *CKMT1B-STRC-CATSPER2* deletion. Following this characterization, we estimated at 50% the risk of having another child with mild to moderate hearing loss and at 12.5% the risk of having a boy with Deafness-Infertility syndrome.

Conclusions: Combining these two technologies allowed us to fully elucidate the complex structural rearrangements at the *STRC* locus and provide appropriate genetic counselling. Moreover, this methodology has the potential to be extended to other complex genomic regions of clinical significance

C.O.I. Disclosure:

M. Guipponi: None. **S. Laurent:** None. **A. Vannier:** None. **C. Gehrig:** None. **M. Abramowicz:** None. **A. Paoloni-Giacobino:** None. **H. CaoVan:** None.

EP04.023 Genetic and phenotypic characterization of inherited retinal diseases in an italian cohort

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Background: Inherited retinal diseases (IRDs) are the main cause of legal blindness in Europe. IRDs are both clinically and genetically heterogeneous encompassing more than 20 different phenotypes and 350 known genes. Even though molecular genetic diagnosis is evolving, Next-generation sequencing (NGS) gene panels have proven highly effective for diagnosing IRDs.

Aim: To describe clinical and genetic findings on a cohort of patients undergoing targeted NGS panel for suspect IRD.

Material and methods: A cohort of 534 patients with suspect IRD was analyzed by a 296-gene NGS panel in a single Center. Cases were retrospectively deemed as solved, unsolved, partial or potentially solved according to their clinical and genetic data.

Results: We achieved a mean diagnostic rate of 61.6%. The highest detection rate was found for Stargardt disease (75%), followed by Usher syndrome (73%) and Retinitis pigmentosa (65%). A total of 82 genes and 332 pathogenic variants were identified, including 139 novel variants. Non-syndromic RP and Stargardt disease were the most common phenotypes in the cohort of isolated IRDs (56% and 9% of solved cases, respectively). Usher syndrome was the prevalent syndromic phenotype (13%). The three most frequently involved genes were *USH2A* (16.7%), *ABCA4* (9.1%), and *RPGR* (6%). Forty-two of disease-causing genes (51%) were identified in just one proband thus confirming the high genetic heterogeneity of IRDs.

Conclusion: This study highlights the relevance of gene panel testing as well as the importance of a multidisciplinary interaction for an accurate diagnosis, counselling and treatment of IRDs.

C.O.I. Disclosure:

A. Peruzzi: None. **R. Modugno:** None. **M. Tavolato:** None. **F. Parmeggiani:** None. **F. Boaretto:** None. **C. Bertolin:** None. **C. Rigon:** None. **L. Salviati:** None. **E. Di Iorio:** None.

EP04.024 Genetic diagnosis of inherited hearing loss through the application of targeted genomic strategies

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Background: Hearing loss (HL) is a common sensory disorder, with about half of all cases having a genetic etiology. Inherited hearing loss exhibits high clinical and genetic heterogeneity, making genetic diagnosis challenging. For this reason, massive sequencing techniques have represented a major advancement in this field.

Material and methods: This study focuses on the genetic analysis of hearing loss using two targeted strategies: a gene panel (59 genes) for 286 cases and a clinical exome (209 genes) for 75 patients. The aim of the study is to evaluate the efficacy of both analysis strategies for their future implementation in clinical practice.

Results: Using the gene panel, the genetic cause was identified in 100 patients (35%), whereas the clinical exome resolved 30 cases (40%). Among cases resolved by the gene panel, 66 had autosomal recessive (AR) inheritance, 26% associated with *GJB2*, and 29 had autosomal dominant (AD) inheritance, with *MYO6* the most frequent. Two patients had X-linked recessive (XLR) inheritance (*POU3F4*), two had mitochondrial inheritance, and one had X-linked dominant inheritance. Among 30 cases resolved with the clinical exome, pathogenic variants were identified in 19 patients with AR inheritance, with *GJB2* again the most frequent. Additionally, ten cases had AD inheritance, and one had XLR inheritance.

Conclusion: The implementation of clinical exome sequencing in the genetic diagnosis of HL has improved the resolution rate compared to the gene panel. However, some cases remain unsolved due to the heterogeneity of both the patient cohort and the disorder itself.

C.O.I. Disclosure:

A. Pedro-Ibor: None. **A. Berzal-Serrano:** None. **L. Cavallé:** None. **C. De Paula:** None. **J. Millán:** None. **E. Aller:** None. **G. García-García:** None.

EP04.025 Spatial transcriptomics in human cornea

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Background: Keratoconus (KTCN) is a corneal disease in which histopathological changes can be observed in all five corneal layers: outermost - epithelium, Bowman's Layer, stroma, Descemet's Membrane and innermost - endothelium. To investigate the location of differentially expressed genes (DEGs) with the preservation of histological information, spatial transcriptomics (ST) was used in KTCN and non-KTCN corneas.

Material and methods: The O.C.T.-embedded corneas, derived from 6 KTCN patients after penetrating keratoplasty, and 5 unaffected corneas derived from Tissue Bank were evaluated. Two/three consecutive cryosections per cornea were placed within a single Capture Area of the Spatial Gene Expression Slide (10xGenomics). The spatial gene expression analysis and sequencing libraries were prepared according to the manufacturer's protocol and subjected to NGS. The raw reads were processed using the spaceranger_count pipeline. Then multiple Visium libraries were aggregated and analyzed using Loupe Browser (version 8.0.0).

Results: We implemented the ST on corneal cryosections. The barcodes were assigned to the KTCN or control corneas. The UMAP plot showed the division consistent with the separation into KTCN and control corneas, with an emerging additional cluster, identified as a tissue fragment adjacent to the cornea, present only in controls. Then, excluding the additional cluster, we identified the DEGs, of which 3 were recognized as the elements of the epithelial-mesenchymal hallmark, previously noted as elevated in KTCN corneal epithelium analysis.

Conclusion: The application of ST reflects the real histology of the human cornea and provides information regarding the location of DEGs.

C.O.I. Disclosure:

A. Wysocka: None. **K. Jaskiewicz-Rajewicz:** None. **J. Wozniak:** None. **R. Płoski:** None. **M. Rydzanicz:** None. **J.P. Szaflik:** None. **M. Udziela:** None. **M. Gajecka:** None.

EP04.026 Improving the diagnostic yield of inherited retinal dystrophies through WES reanalysis, WGS and functional validation

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Background: Inherited retinal dystrophies (IRDs) are a leading cause of blindness worldwide, characterized by extensive clinical and genetic heterogeneity. Despite advances in next-generation sequencing (NGS), approximately 40% of IRD cases remain undiagnosed due to limitations in whole exome sequencing (WES), such as incomplete gene coverage and difficulty detecting non-coding and structural variants. This study aimed to improve diagnostic yield by integrating WES reanalysis, whole genome sequencing (WGS), and functional validation.

Methods: A cohort of 677 IRD patients, including 602 index cases and 75 familial cases, underwent genetic analysis. Initial testing involved WES, followed by reanalysis with updated bioinformatics pipelines and expanded gene panels. WGS was performed on unresolved cases, prioritizing those with suspected deep-intronic or structural variants. Variants were validated using Sanger sequencing, digital PCR, and functional assays. Diagnostic yield was calculated before and after reanalysis and WGS.

Results: Initial WES analysis yielded a diagnostic rate of 62.99% (376/597 cases). The integration of WES reanalysis, WGS, and variant reassessment increased the diagnostic yield to 69.18% (413/597 cases), a 9.84% improvement. Key findings included the identification of novel deep-intronic variants in *ABCA4* and structural variants, along with the reclassification of variants of uncertain significance (VUS) through functional assays.

Conclusions: This study demonstrates that combining WES reanalysis, WGS, and functional validation significantly improves the diagnostic yield for IRDs. These findings contribute to a deeper understanding of novel genetic variants and mechanisms, improving genetic counseling and access to emerging therapies. Comprehensive genomic approaches and multidisciplinary discussions are essential to addressing IRD diagnostic challenges.

C.O.I. Disclosure:

A. Esteve Garcia: None. **A. Padró-Miquel:** None. **J. Català-Mora:** None. **C. Sau:** None. **Z. Corradi:** None. **F. Cremers:** None. **P. Barberán-Martínez:** None. **J. Salvador:** None. **G. García-García:** None. **D. Yubero-Siles:** None. **E. Cobos:** None. **C. Aguilera:** None.

EP04.027 Jalili Syndrome, the Rare retinopathy with Amelogenesis Imperfecta, Clinical Insights, novel variant and genetic Perspectives in five patients

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Background: Jalili syndrome is a rare progressive retinal-ciliopathy disorder displayed with cod-rod dystrophy and amelogenesis imperfecta (AI) caused by CNNM4 gene. The onset of visual impairment starts in infancy or early childhood. We report on four Egyptian patients affected by Jalili syndrome, highlighting the clinical presentation and implications of the disorder. Additionally, we expand the molecular characterization of the *CNNM4* gene by reporting a novel pathogenic variant, contributing to understanding its role in this rare condition.

Material and methods: Five patients (P1-P5) from three unrelated families who complained of eye problems and amelogenesis imperfecta were included in the study. The patients were subjected to detailed clinical and dental examinations. WES was used to identify the causative gene molecularly.

Results: Exome sequencing identified three different variants in *CNNM4* gene of Jalili syndrome, one novel homozygous missense variant in Exon 2 c.1423G> A (p.Val475Met), homozygous missense variant c.599C>A p.S200Y(P2) and a homozygous deletion of the entire exon 1 of the gene c.1-?_1403þ?del (P3-P5). P2 showed hypomagnesemia and tachycardia.

Conclusion: This study focuses on Jalili syndrome, highlighting and expanding its clinical and genetic phenotypes. Hypomagnesemia and cardiac manifestations highlight comprehensive management and monitoring across multiple organ systems. Moreover, identifying a novel CNNM4 mutation enriches the genomic landscape of the syndrome, helping to guide future genetic testing and counseling efforts. Finally, this research emphasizes the essential role of specialists, particularly dental geneticists, in unveiling the puzzle of rare disorders.

C.O.I. Disclosure:

N.F. Hassib: None. **H. El-Sayed:** None. **I.S. Sayed:** None. **M.S. Abdel-Hamid:** None.

EP05 Internal Organs and Endocrinology (Lung, Kidney, Liver, Gastrointestinal)

EP05.001 Urogenital anomalies in a girl with 15q11.2 deletion

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Background: Prader-Willi syndrome (PWS) is caused by a genetic deletion or imprinting defect in the 15q11-q13 chromosomal region. The clinical presentation include neurodevelopmental delay in the first year of life, after that there is morbid obesity, accompanied by hypogonadism and cognitive delay. Rare represented anomalies in children with PWS are urogenital anomalies.Case presentation:We present an obese girl, who was referred to our clinic due to significant hair loss, later linked to trichobezoar formation in her stomach after operation. According to the medical history the child was born small for gestational age (SGA) and she had developmental delays in her first year. Also she had recurrent urinary tract infection due to hydronephrosis and mega ureter. During puberty, the patient gained substantial weight and developed metabolic syndrome with associated type 2 diabetes. At the time of the examination she has delayed puberty with little breast development and absent menarche. The hormonal profiles were normal, and further investigations were performed and revealed the presence of genital malformations, including a bicorn ate uterus and a double vagina, with one side being blind. Than we made a detailed array comparative genomic hybridization (aCGH) analysis that proved deletion on chromosome 15 (15q11.2q13.1) spanning 5,356 kbp, containing 19 OMIM genes.Discussion and Conclusion:The primary aim of this case report study was to correlate the relationship between genetic deletions, such as the 15q11.2q13.1 deletion, and the development of urogenital malformations in PWS patients. It is not sufficiently studied which gene in the deletion causes the genitourinary anomalies.

C.O.I. Disclosure:

E. Shuperliska: None. **E. Sukarova-Angelovska:** None. **S. Veleska:** None. **G. Ilieva:** None. **M. Krstevska-Konstantinova:** None.

EP05.003 Analysis of markers of *Helicobacter pylori* resistance to clarithromycin and levofloxacin in primary patients in Moscow according to PCR diagnostics

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Background: The growing antibiotic resistance of Helicobacter pylori is a serious problem. H. pylori resistance to clarithromycin and levofloxacin remains high in most countries, so choosing an effective *H. pylori* eradication regimen is crucial. Currently, bismuth quadrotherapy continues to achieve high rates of eradication. The need to diagnose antibiotic resistance is extremely important. *H.pylori* before starting therapy, which avoids the use of ineffective antibiotics. PCR diagnostics for the presence of molecular markers of antibiotic resistance is very promising from the point of view of economic efficiency, accuracy and timing of the study.

Material and methods: molecular markers of resistance to clarithromycin and levofloxacin were studied in biopsy samples of the gastric mucosa of 223 patients who had not previously taken antibacterial drugs. The study was carried out by RT-PCR using a kit (GastART LLC, Russia) on a CFX-96 thermal cycler (BIO-RAD, USA).

Results: Markers of resistance to clarithromycin were found in 51 (23%) samples and to levofloxacin in 45 (20%). Double resistance was observed in 38 (17%) samples.

Conclusion: We have demonstrated the level of resistance of *Helicobacter pylori* DNA to clarithromycin in Moscow exceeding the 15% threshold. According to the VI Maastricht Consensus, in regions with clarithromycin resistance levels >15%, bismuth quadrotherapy or bismuth-free quadrotherapy is recommended. PCR diagnostics of molecular resistance can be used in diagnostic practice before starting H. pylori eradication therapy.

C.O.I. Disclosure:

V.V. Serebryakov: None. **V.V. Polyakova:** None. **A.A. Golodushkin:** None. **D.S. Bordin:** None. **K.A. Nikolskaya:** None. **M.V. Chebotareva:** None. **N.A. Neyasova:** None. **N.A. Bodunova:** None. **L. Tsapkova:** None.

EP05.004 Short Telomere Syndromes andmulti-systemfailure

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Background: The telomere-related pulmonary fibrosis and/or bone marrow failure syndrome-3 (PFBMFT3) is caused by heterozygous mutation in the RTEL1 gene under umberla of Short Telomere Syndromes (STS) are accelerated aging syndromes caused by inheritable gene mutations resulting in decreased telomere lengths. As a consequence, organs systems with increased cell turnover. Due to diverse clinical presentations, STS pose a diagnostic challenge, with bone marrow failure and idiopathic pulmonary fibrosis being frequent manifestations. Telomere lengths can be assessed in peripheral blood granulocytes and lymphocytes using a sensitive technique called flow-FISH, while mutational analysis can be performed using next generation sequencing assays. Idiopathic pulmonary fibrosis (IPF) is the most frequent pulmonary manifestation seen in patients with STS (70%).

Material and methods: case report

Results: Herein, we report a 36-year-old female her presentation started 3-years ago, cough, dyspnea, O2 dependent diagnosis with idiopathic pulmonary fibrosis. HRCT-chest showed diffuse lung disease with severe ILD. liver failure Imaging showed hepatic cirrhosis and portal hypertension. Persistent bone marrow failure. Significant for feature of accelerated aging including history of Premature graying of the hair started at age 15-year. Family history of pulmonary fibrosis and Leukemia in her brother, liver cirrhosis in her mother and maternal aunt. A clinical genome were done for her, which reported a compound heterozygous variant in RTEL1 gene, c.707G>C p.(Arg236Thr) and c.749A>G p.(Asp250Gly). Segregation analysis of the family member aproved the pathogencity.

Conclusion: Telomerase mutations should be consider in patients with idiopathic pulmonary fibrosis and pancytopenia.

C.O.I. Disclosure:

A.M. Alatawi: None. **M. AlMannai:** None.

EP05.005 Study of genetic heterogeneity of genetic variants of the *CFTR* gene in the Russian population

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Background: Cystic fibrosis is a genetic hereditary disease characterized by a wide range of clinical manifestations and genetic variants. **Objective**: to study the genetic heterogeneity of allelic variants of the *CFTR* gene in the Russian population according to the 2023 Registry.

Material and methods: The 2023 Registry included data from 4058 patients. The overall cumulative frequency of identified alleles was 93.4%.

Results: A total of 275 pathogenic variants were identified, 148 of them repeatedly. The most common variants were: F508del (51.4%), CFTRdele2.3 (6.1%), E92K (3.71%), 1677delTA (2.51%), 3849+10kbC->T (2.24%), 2143delT (1.99%), 2184insA (1.93%), W1282X (1.73%), L138ins (1.71%), N1303K (1.62%). 26 variants are absent from international databases. 46 variants were identified with a frequency greater than 0.10%. "Severe" genotypes dominate among both children and adults, but their frequency is 80.0% in those under 18 years of age and 66.0% after 18 years of age. "Mild" genotype was detected in 19.5% of patients under 12 years of age and in 59.6% of patients over 36 years of age. Distribution by classes was as follows: I - 110 variants, II - 7, III - 5, IV - 11, V - 13, VI - 1, VII - 17, unknown class in 108 variants. Of the 4058 patients, F508del/F508del was found in 28.0%, F508del / not F508del in 46.8%, and not F508del / not F508del in 25.2%.

Conclusion: Allelic frequencies of genetic variants of the *CFTR* gene in the Russian Federation were determined.

C.O.I. Disclosure:

Y. Melyanovskaya: None. **E. Kondratyeva:** None. **A. Voronkova:** None. **E. Amelina:** None. **S. Krasovsky:** None. **N. Kashirskaya:** None. **O. Shchagina:** None. **A. Stepanova:** None. **M. Starinova:** None. **R. Zinchenko:** None. **S. Kutsev:** None.

EP05.006 Fanconi-Bickel syndrome with variable phenotypes: A case series highlighting a successful pregnancy outcome

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Background: Glycogen storage disorder type XI, also known as Fanconi-Bickel syndrome, is an autosomal recessive disorder caused by mutations in the *SLC2A2* gene on chromosome 3q26.2.

Material and methods: We report five patients from India, including three members from the same family who exhibit considerable intrafamilial and interfamilial variability.

Results: In the first family, one sibling succumbed to the illness at 12 years of age, the other two are now thriving at 16 and 27 years. The eldest, now 27 years old, is married and has had a normal pregnancy outcome. Exome sequencing identified a known homozygous pathogenic variant c.1093C>T (p.Arg365Ter) in exon 9 of the *SLC2A2* gene. Interestingly, our second patient a 2 year old male harboring the same variant presented to us with delayed attainment of motor milestones and abdominal distension. The last patient in this series is a 5 year old female who presented to us with complaints of failure to thrive and recurrent bone fracture, whole exome sequencing identified a homozygous missense variant c.1330T>C in Exon 10 of the *SLC2A2* gene that results in the amino acid substitution (p.Trp444Arg).

Conclusion: This series highlights the intrafamilial and interfamilial variability and includes one of the few documented successful pregnancy outcomes in females with this disorder.

C.O.I. Disclosure:

K. Mandal: None. **P. Kafley:** None. **V.M. Srinivasan:** None. **C. Ravi:** None. **P. Motwani:** None.

EP05.008 Deleterious bioinformatic prediction of a splicing variant in the DUOX2 gene: a case of congenital hypothyroidism with TSH resistance

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Background: Resistance to thyrotropin (RTSH) is a condition with reduced sensitivity to thyrotropin (TSH). Mutations of the DUOX2 gene have recently been reported to cause RTSH. The DUOX2 gene encodes the Dual Oxidase 2 protein involved in the generation of hydrogen peroxide required for thyroid hormone synthesis. DUOX2 gene mutations cause variable phenotypes with transient hypothyroidism, especially in individuals with a monoallelic pathogenic variant. Here we report a case of a child with a positive neonatal screening for congenital hypothyroidism, but subsequent investigations ruled out any pathological conditions or therapeutic needs. At the age of 2 years, hormonal tests revealed elevated levels of TSH and Free triiodothyronine.

Material and methods: Clinical exome sequencing of the proband and his parents was performed on genomic DNA using the NovaSeq6000 platform (Illumina).

Results: Genetic analysis revealed a splicing heterozygous mutation c.160+5G > A in intron 3 of DUOX2 gene. The variant, inherited from the apparently healthy mother, has not been reported in literature and, to date, it has been classified as a Variant of Uncertain Significance. The general pathogenic effect of the variant was assessed using in-silico predictors which suggest a loss of the intron 3–4 donor site with two potential consequences: 1) intron retention between exons 3–4 and the consequent introduction of a stop codon (TAG); 2) activation of alternative contiguous donor sites.

Conclusion: Based on bionformatic prediction, the c.160+5G > A variant seems to have a deleterious effect on DUOX2 protein. RNA sequencing is ongoing to identify aberrant splicing and/or aberrant gene expression.

C.O.I. Disclosure:

R. Ruta: None. **T. Mazza:** None. **F. Carlomagno:** None. **M. Mucciolo:** None. **A. Giovannetti:** None. **L. Vincenzi:** None. **S. Petrocchi:** None. **A. Isidori:** None. **A. Novelli:** None.

EP05.009 Genetic diagnosis of kidney diseases in the neonatal and early childhood period

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Background: Renal abnormalities are the most common congenital malformations in newborns. They can affect the proper function of the kidneys or urinary tract anomalies. Renal tract malformations are also frequent in patients with microdeletion of or microduplication syndrome (MMS). MMS can lead to a wide range of symptoms depending on the genes involved. Symptoms can vary and may include neurodevelopmental disorders and numerous birth defects. Therefore, the establishment of identifying the genetic cause of the disorder as early as possible is crucial for proper treatment and monitoring surveillance of the patients.

Here we present a newborn with renal abnormalities diagnosed at birth. No clinical diagnosis could be established based on his clinical presentation.

Material and methods: Genetic testing by whole exome sequencing (WES) was performed.

Results: Analysis revealed an approximately 1.3 Mb deletion in size at chromosome 17q12.

Conclusion: 17q12 deletion syndrome results from a recurrent 1.4 Mb heterozygous deletion on the long arm of chromosome 17 with an estimated incidence of 1 in 1,000,000 newborns. Multicystic kidneys and other structural and functional kidney anomalies occur in 85% to 90% of patients with 17q12 deletion, maturity-onset diabetes of the young type 5 (MODY5) in approximately 40%, and mild to severe developmental delay or learning disability in approximately 50% of patients with 17q12 deletion syndrome. Without genetic testing, the syndrome remains unrecognizable undiagnosed due to its broad and nonspecific clinical presentation. The clinical presentation of the newborn and his follow-up will be presented, as well as the course of genetic diagnostics.

C.O.I. Disclosure:

D. Krgović: None. **I. Trutin:** None. **T. Nikuševa Martić:** None.

EP05.010 Case report of a pediatric patient with acute kidney injury and tubular proteinuria

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Background: Acute kidney injury with tubular proteinuria is commonly attributed to interstitial nephritis and acute tubular necrosis. Clinical findings consist of malaise, nausea, vomiting, high blood pressure and dizziness. Laboratory tests show elevated markers of renal function and tubular damage in urinalysis, such as low specific gravity, proteinuria and glycosuria. Main causes are medicines, infections and genetic diseases.

Material and methods: Our patient presented febrile and vomiting. His test results revealed high inflammation markers and deranged renal function with eGFR = 51ml/1,73m2/min. He also had persistent tubular proteinuria with no response to steroid treatment. Assessing patient's morphologic features and clinical findings, such as obesity, bilateral cataracts, large nose and lips, short fingers and toes and delayed speech development from medical history, Whole Exome Sequencing was performed.

Results: Genetic variant c.2060T>G in OCRL was found, which leads to amino acid replacement p.(Leu687Arg) and has not been described in literature and databases. It is characterized as Variant of Unknown Significance (VUS), based on ACMG Guidelines and it correlates to patient's phenotype. There was an additional finding of 16p11.2 recurrent deletion which is characterized by motor speech and language disorder, motor coordination difficulties, psychiatric conditions and autistic features.

Conclusion: Our patient has normal renal function and receiving Angiotensin Converting Enzyme (ACE) Inhibitors. Proteinuria remains 1,4gr/24hrs. Persistent tubular proteinuria not responding to ACE inhibitors is consistent with LOWE syndrome. Since the genetic variant is characterized as VUS, close monitoring of the patient is required.

C.O.I. Disclosure:

V. Christodoulaki: None. **K. Kosma:** None. **N. Stergiou:** None. **C. Chronis:** None. **O. Filippou:** None. **N. Marinakis:** None. **F. Tilemis:** None. **A. Kampouraki:** None. **V. Karava:** None. **E. Botsa:** None. **J. Traeger- Synodinos:** None. **C. Kanaka-Gantenbein:** None. **P. Makrythanasis:** None.

EP05.011 The role of oxytocin and vasopressin receptors in inflammatory bowel disease

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Background: The oxytocin receptor (OTR) and vasopressin receptor (VR) in intestinal tissue play opposing roles in the stress response by regulating gastrointestinal physiology, including epithelial permeability and inflammation. As enteric OTR initiates a signaling pathway to suppress inflammation, V1bR is assumed to have a proinflammatory effect. To assess the influence of OTR and V1bR on the pathogenesis of inflammatory bowel disease (IBD), the expression of proinflammatory factors (TNF-α, IL-1β) was also analyzed in this study.

Material and methods: Intestinal tissue samples were obtained from 16 patients with irritable bowel syndrome (IBS) and 39 pediatric IBD patients at the University Children's Clinic "Tirsova". Performing qPCR analysis, the relative gene expression of OTR, V1bR, TNF-α and IL-1β markers was assessed using the $2^{-\Delta\Delta Cq}$ method.

Results: A trend of increased expression in IBD compared to IBS patients was observed for the V1bR (1.4 vs. 1.1), TNF-α (1.6 vs. 1.1), and IL-1β (1.4 vs. 1.1) genes. Contrary, there was a trend of decreased expression of the OTR gene in IBD compared to IBS patients (0.7 vs. 1.1). In terms of disease activity, the IBD score was predominantly moderate.

Conclusion: Our results suggest that increased expression of V1bR in intestinal tissue in response to stressors may lead to impairment of the epithelial barrier, leading to inflammation through increased expression of proinflammatory markers such as TNF-α and IL-1β. Due to opposing effects of OTR and V1bR on stress-induced inflammation, our data of decreased expression of enteric OTR may indicate higher disease activity in the gastrointestinal tract.

C.O.I. Disclosure:

B. Popovic: None. **B. Stevanovic:** None. **I. Milovanovic:** None.

EP05.012 Association of germline variants in the ZFX gene with primary hyperparathyroidism

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Background: Somatic variants in the *ZFX* gene have been found in human sporadic parathyroid adenomas. This gene encodes a transcriptional factor recently described as a transcriptional activator in multiple types of human tumours.

Material and methods: We present the clinical and molecular characterization of three patients diagnosed with primary hyperparathyroidism (PHPT) who have germline variants in the *ZFX* gene. For Next-Generation Sequencing, a targeted panel for a selection of genes identified as potentially associated with hyperparathyroidism and/or hypercalcemia was designed. Samples were sequenced using the Ion GeneStudio S5 System.

Results: The first patient had a pathogenic missense variant (c.2321A>G; p.(Tyr774Cys)) in the heterozygous state. This patient exhibited PHPT along with ear, nose and forehead abnormalities. Additionally, she presented other characteristics seen in patients with pathogenic variants in the *ZFX* gene, such as hearing loss and multiple cutaneous nevi. The second and third patients had a missense variant (c.1606C>T; p.(Arg536Cys)) of uncertain significance and a likely benign in-frame insertion (c.452_460dup; p.(Gly151_Val153dup)), respectively, both in the heterozygous state. These patients did not have hearing loss, cutaneous melanocytic nevi, bone or facial deformities, long fingers, scoliosis, or developmental abnormalities.

Conclusion: *ZFX* may be one of the genes to be analysed in women affected by PHPT with suspected genetic inheritance, especially if they have other features such as facial deformities, hearing loss, and cutaneous melanocytic nevi.

C.O.I. Disclosure:

A.C. Aranaga: None. **P. González:** None. **S. Gómez:** None. **L. Madariaga:** None. **N. Valdés:** None. **L. Castaño:** None. **A. Garcia:** None.

EP05.013 Bardet-Biedl Syndrome: a rare cause of end-stage Kidney Disease

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Background: Bardet-Biedl syndrome (BBS) is a rare autosomal recessive disorder. Diagnosis can be established with specific symptom criteria, confirmed by genetic testing. Early detection and monitoring are crucial due to the high risk of serious organ damage in affected children. We report a case of Bardet-Biedl syndrome with End-stage kidney disease at younger age managed on hemodialysis.

Case presentation: A 18-year old female born to consanguineous parents, with a history of psychomotor and reduced visual acuity. She was admitted for severe kindey failure with GFR of 5 mL/min. On clinical examination, the patient was obesity, polydactyly, a dysmorphic facial appearance. The fundoscopy showed a pigmentary retinopathy and pigmentary dystrophy in both eyes.Our patient was diagnosed with end-stage kidney disease due to bardet biedel syndrome based on the presence of six major criteria. Management consisted of hemodialysis with treatment of complications of Chronic Kidney Disease (CKD)

Discussion: BBS is a multisystem disorder classified as a non-motile ciliopathy due to defects in cellular organelles (cilia), which explains its diverse clinical manifestations. The frequency of CKD secondary to bardet-biedl syndrome is variable. The management of CKD in BBS does not differ from that due to any other cause and all three modalities of long-term renal replacement therapy

Conclusion: Early diagnosis and multidisciplinary care are crucial to managing complications and improving patient outcomes. Further research is needed to explore the genetic factors behind its complex manifestations.

C.O.I. Disclosure:

N. Serbati: None. **M. Bouchoual:** None. **K. Dadi:** None. **S. Anibar:** None. **M. Jabrane:** None. **M. Arrayhani:** None.

EP05.014 Results of *UGT1A1* gene sequencing by Sanger in individuals with phenotypic Gilbert syndrome

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Background: The variant rs3064744, the detection of which is the most common method of DNA diagnostics Gilbert's syndrome, has incomplete penetrance and variable expression. The aim of our work is to search for mutations that can explain the causes of unconjugated hyperbilirubinemia in patients who are not homozygous carriers of the variant rs3064744.

Material and methods: The group of patients with unconjugated hyperbilirubinemia, with no other causes except for the genetic ones was 24 people. Genotyping was carried out for rs3064744 *UGT1A1* gene using a polymerase chain reaction. Direct automatic sequencing was carried out by Sanger for exons and for the part of the promoter of the *UGT1A1* gene.

Results: Distribution of rs3064744 genotypes in the group: 5 people - genotype 7TA/7TA, 5 people -genotype 6TA/6TA, 12 people - genotype 6TA/7TA, 1 person - genotype 5TA/7TA, 1 person - genotype 6TA/8TA. Single nucleotide variants of uncertain clinical significance rs3755319 (in 21 people) and rs28899472 (in three people with the 7TA/7TA genotype rs3064744) were identified in the promoter of the *UGT1A1* gene, rs2125984650 in the first exon of the *UGT1A1* gene (in one person with the 5TA/7TA genotype rs3064744). In two individuals with genotypes 6TA/7TA rs3064744, gene variants were identified that are pathogenic or probably pathogenic for the Gilbert's syndrome according to some sources (rs4148323, rs1273237448).

Conclusion: Direct automatic Sanger sequencing has identified new variants in the *UGT1A1* gene that can explain the cause of unconjugated bilirubinemia in patients who are not homozygous carriers of the variant rs3064744.

C.O.I. Disclosure:

Y.V. Ivanova: None. **A.A. Ivanova:** None. **N.E. Apartseva:** None. **A.P. Kashirina:** None. **E.G. Nemcova:** None. **S.A. Kurilovich:** None. **M.V. Kruchinina:** None. **V.N. Maksimov:** None.

EP05.015 Autosomal recessive polycystic kidney disease associated with a homozygous variant in the *CYS1* gene: the second case reported in the literature

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Background: The *CYS1* gene mutations in mice have been associated with autosomal recessive polycystic kidney disease (ARPKD) but in humans, to our knowledge, it has been reported in only one case in the literature.

Material and methods: During our outpatient practice, a patient with renal cysts and glaucoma was admitted to genetic diseases evaluation center. We performed renal diseases next-generation sequencing (NGS) gene panel testing for his diagnostic work-up. Sequencing was performed using the Illumina Nextseq 550 platform (San Diego, CA, USA) and SOPHIA Clinical Exome Solution V3 (Boston, USA) kit was used to analyze single nucleotide variants (SNVs) and copy number variants (CNVs). Variant annotations were conducted according to the GRCh37-hg19 reference genome. Data analysis was performed using the Sophia DDM-V4 (Boston, USA) platform.

Results: A 20-year-old male patient was referred to our clinic for bilateral polycystic kidney disease. Renal cysts were first detected when he was 10 days old. The patient had no perinatal respiratory distress and no significant liver involvement. At 1.5 years of age, he underwent bilateral glaucoma surgery. The parents were first cousins, but no similar history was reported in the patient's siblings. Clinical exome sequencing (CES) revealed a homozygous *CYS1* (NM_001037160) c.192dup (p.Asp65Argfs60) variant and this variant is segregated.

Conclusion: In this study, we report the second homozygous *CYS1*-associated ARPKD case in the literature. Functional studies may further support the relationship between *CYS1* and ARPKD. To establish a stronger genotype-phenotype correlation, functional studies are necessary to prove molecular pathogenesis.

C.O.I. Disclosure:

O. Hanoğlu: None. **E. Arslan:** None. **A. Ersoy Karayumak:** None. **S. Olgun:** None. **N. Çiçek:** None. **B. Demirelli:** None. **&. Gökce:** None. **P. Ata:** None.

EP05.016 Detecting *PKD2* variants in Lithuanian patients with cystic kidney

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Background: *PKD2* gene pathogenic variants are the second most common cause of autosomal dominant polycystic kidney disease (ADPKD), the most common phenotype of cystic kidney diseases. The ADPKD variant database lists more than 190 pathogenic/likely pathogenic variants in *PKD2*, while more than 20 remain variants of uncertain significance (VUS). This study aimed to identify and classify genetic variations within the *PKD2* gene among patients exhibiting multiple renal cysts.

Material and methods: 94 unrelated patients with multiple renal cysts were included in the study. The study group comprised 51 females and 43 males; 85 adults and 9 children. Patients were tested using a kidney-focused next-generation sequencing panel of 498 genes, including the main polycystic kidney disease genes *PKD1*, *PKD2*, *PKHD1*, *ALG5*, *DZIP1L*, *DNAJB11*, *GANAB*. The pathogenicity of detected variants was assessed using the American College of Medical Genetics and Genomics guidelines.

Results: Overall, phenotype-related variants were detected in 11 genes for 76 patients (80.9%). Eleven different *PKD2* gene variants were identified in 13 patients, of which 4 had not been described in the literature. After evaluating the pathogenicity of the variants, 9 were classified as pathogenic/likely pathogenic, and 2 as VUS. All *PKD2*-patients were clinically diagnosed with ADPKD; however, family history was negative for 2 of them.

Conclusion: A genetic diagnosis was established for the majority of patients in the study, with *PKD2* identified as the second causative gene. The majority of *PKD2* variants identified in the study were unique to the families.

C.O.I. Disclosure:

D. Brazdziunaite: None. **G. Mazur:** None. **A. Utkus:** None.

EP05.017 Novel *OTX2* mutation in two siblings with combined pituitary hormone deficiency (CPHD) provides further phenotype delineation

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Background: Mutations in the transcription factor OTX2 can lead to a broad spectrum of ocular and endocrine abnormalities due to its critical role in early embrionic development, particularly in eye and pituitary gland formation. Endocrine manifestations primarily involve combined pituitary hormone deficiency (CPHD), affecting growth, metabolism and reproduction. Description of new mutations will provide insight into molecular mechanism and phenotype.

Material and methods: Two siblings aged 13 and 11 presented with symptoms of hormone deficiency including short stature, obesity and hypogonadism. Their parents and brother were healthy. MRI identified pituitary stalk interruption syndrome (PSIS) as the cause of CPHD as they had absent pituitary stalk, anterior pituitary aplasia/hypoplasia and ectopic neurohypophysis. Targeted WES was performed to find variants in common in CPHD-associated genes.

Results: Both siblings were heterozygous for the c.808T>G p.(Phe270Val) mutation in *OTX2* (NM_172337.3), which locates in the C-terminal domain, between 2 tandem motifs that activate transcription factors such as HES1, involved in pituitary gland development. This is consistent with the genotype-phenotype gradient described for this gene, with the first portion being associated to ocular defects and the latter to endocrine features. The father was also heterozygous although he only had short stature and few body hair, according to the incomplete penetrance reported for *OTX2* mutations.

Conclusion: This mutation locates in a more terminal position than the *OTX2* missense mutations published so far. The fact that it causes CPHD supports that terminal mutations lead to endocrine phenotypes. Moreover, incomplete penetrance should be considered when offering genetic couselling.

C.O.I. Disclosure:

E. Borràs Angosto: None. **C. Sánchez Garré:** None. **B. Mañé Valverde:** None. **M. Gamundi Rodríguez:** None.

EP05.018 Cantu Syndrome with rare endocrine manifestations

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Background: Cantu syndrome (CS) (MIM #239850) is a rare genetic disorder caused by heterozygous gain of function alterations in *ABCC9* and *KCNJ8* genes. Clinical presentation of CS typically includes congenital hypertrichosis, facial dysmorphism and cardiovascular anomalies. Endocrine abnormalities may be implicated, but rarely constitute representing symptoms. In this study, we report a girl with growth hormone deficiency, primary amenorrhea, facial dysmorphism, hypertrichosis and edema with molecular genetic findings.

Methods: An 18-year-old female presented with primary amenorrhea, mild hypertrichosis and non-pitting edema. Prenatal polyhydramnios was noted, and she was born at term via cesarean section, weighing 3800 grams, with severe hypertrichosis at birth. She had history of surgically corrected patent ductus arteriosus (PDA) and growth hormone deficiency. CS was considered in differential diagnosis based on her clinical history and exome sequencing (ES) was performed.

Results: A heterozygous pathogenic variant c.3461G>A/p.(Arg1154Gln) in *ABCC9* gene (NM_005691.3) was identified through ES analysis. Segregation analysis confirmed that the variant occurred de novo in the proband.

Conclusion: Although CS is a well-defined syndrome, its rarity and diverse clinical presentation make diagnosis challenging. Our case exhibits milder spectrum of hypertrichosis and subtly coarse facial features, which likely contributed to relatively late diagnosis at age of 18. Growth hormone deficiency and hypogonadotropic hypogonadism indicative of multiple pituitary hormone deficiencies (MPHD), were observed, highlighting the importance of considering CS in the differential diagnosis of MPHD.

Conflict of Interest: None declared

C.O.I. Disclosure:

M.C. Elmas: None. **G. Memiş:** None. **M. Yücesoy:** None. **V. Karaman:** None. **A. Aslanger:** None. **A. Bayrak:** None. **Z.O. Uyguner:** None.

EP05.019 Genetic profile of ciliopathies with renal involvement in Bulgarian patients

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Background: Ciliopathies are a group of more than 35 genetic diseases resulting from molecular defects of the cilia. They affect various organs and systems, and are often considered to be parts of a clinical spectrum. At least 100 genes are involved in the pathogenesis of these disorders, with the number expected to increase thanks to the widespread use of next generation sequencing (NGS). Many ciliopathies are characterized with renal cystic disease, which is a common cause of chronic kidney disease.

Material and methods: Whole exome sequencing using NovaSeq 6 000 (Illumina) was used for mutation screening of patients with renal diseases. An extended panel including 773 genes, previously associated with kidney pathologies, was used for the analysis. The pathogenicity of relevant variants was evaluated based on the ACMG criteria.

Results: Pathogenic or likely pathogenic variants in ciliary genes, many of them novel, were identified in 16 unrelated patients, the majority of them children. As expected, more than half of the affected individuals carried mutations in the *PKD1/PKD2/PKHD1* genes, responsible for autosomal-dominant and recessive polycystic kidney disease. Less frequent were pathogenic variants in genes for other proteins involved in cilia function and formation, like those of the BBSome (*BBS7* and *BBS9*); basal bodies (*MKS1*) and transition zone (*CPLANE1*).

Conclusion: Application of NGS with extended gene panel for mutation screening revealed highly variable molecular basis of ciliopathies involving the kidneys in Bulgarian patients. The identification of pathogenic variants led to re-evaluation or amendment of the initial diagnosis in a number of cases.

C.O.I. Disclosure:

O. Beltcheva: None. **K. Kamenarova:** None. **K. Mihova:** None. **M. Georgiev:** None. **D. Kachakova:** None. **D. Kostova:** None. **G. Zlatanova:** None. **G. Mihneva:** None. **I. Dimova:** None. **R. Kaneva:** None.

EP05.020 Functional analysis of splicing variants in patients with NBAS associated phenotypes

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Background: Pathogenic variants in the *NBAS* gene are associated with multisystemic clinical symptoms including infantile liver failure syndrome 2, short stature, optic nerve atrophy, Pelger-Huet anomaly and frequently observed immunodeficiency. Among all described pathogenic variants in *NBAS*, splicing-affecting variants are the least studied. The identification of such variants and the determination of their molecular mechanisms are essential for establishing genotype-phenotype correlations and developing prognostic insights.

Material and methods:WES or WGS was performed in patients. The SpliceAI tool was used to predict splicing variants. Total RNA was extracted from patient fibroblast samples. The identification of splicing alterations and allelic imbalance, caused by the identified variants, was carried out using deep NGS sequencing of RT-PCR products.

Results: In three patients, novel variants in *NBAS* were identified in a compound heterozygous state with known pathogenic variants. To assess the impact of these variants on splicing, we conducted RNA analysis. The analysis revealed: c.954G>T caused the skipping of exon 11 and truncation of the protein; c.3932-9A > G led to partial skipping and partial elongation of exon 34, both events cause protein truncation; c.2019-6A > G resulted in the skipping of exon 19 and retention of intron 18, causing protein truncation and NMD-mediated degradation. The clinical presentation varied among all three patients: episodes of liver failure during respiratory infections and undifferentiated connective tissue dysplasia; primary immunodeficiency; and spastic paraplegia with optic nerve atrophy, respectively.

Conclusion: The obtained data contribute to the understanding of the molecular mechanisms underlying diseases associated with pathogenic splicing variants in the *NBAS* gene.

C.O.I. Disclosure:

D. Akimova: None. **N. Semenova:** None. **A. Murtazina:** None. **A. Marakhonov:** None. **S. Ionova:** None. **T. Milovidova:** None. **V. Tabakov:** None. **A. Filatova:** None. **M. Skoblov:** None.

EP05.021 Primary Hyperoxaluria Type 1: First Reported Case with the p.Gly103Arg Variant in Homozygosity

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Background: Primary hyperoxaluria type 1 (PH1) is an autosomal recessive disorder caused by variants in the AGXT gene, leading to alanine:glyoxylate aminotransferase deficiency. This results in oxalate accumulation in the kidneys and other tissues, causing nephrolithiasis, nephrocalcinosis, and eventually chronic kidney disease. With an estimated prevalence of 1 in 120,000 live births, it is a rare disease. Herein, we report a novel homozygous variant in the AGXT gene causing PH1

Material and methods: A 9-year-old male patient with prenatal findings of right renal pyelectasis, oligohydramnios, and parental consanguinity. Postnatal evaluation confirmed renal pyelectasis and recurrent nephrolithiasis, requiring eight surgical interventions and management of recurrent urinary tract infections. Under pediatric nephrology care, he was referred to clinical genetics for etiological evaluation. Current treatment includes potassium citrate, pyridoxine, and enalapril. Physical examination showed no abnormalities. Laboratory studies revealed hyperoxaluria, microalbuminuria, proteinuria, cystinuria, and calcium oxalate/phosphate-positive calculi (cystine-negative). Molecular testing with an NGS nephrolithiasis panel and parental variant evaluation was recommended.

Results: Molecular analysis identified the homozygous c.307G>A p.Gly103Arg variant in the AGXT gene (2q37.3), with both parents confirmed as carriers. This variant fulfills pathogenicity criteria in Varsome, ClinVar, LOVD, and HGMD. In silico analysis indicates protein damage across multiple predictors. The affected amino acid resides in a highly conserved region, supporting pathogenicity

Conclusion: A pathogenic variant was identified, representing the second reported case of this variant in Colombia and the first in homozygosity worldwide. This case emphasizes the importance of interdisciplinary management and genetic counseling.

C.O.I. Disclosure:
M.F. Salamanca: None. **S. Pico:** None. **G. Mantilla:** None. **G. Contreras:** None.

EP05.022 A national experience of a novel publicly funded nephrogenetic sequencing program

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Background: Nephrogenetics has enhanced our understanding of the genetic architecture of kidney diseases, with potential to transform diagnosis, treatment, and prevention. However, accessibility and costs hinder its widespread implementation. The prevalence of chronic kidney disease (CKD) in Israel is notably high, influenced by consanguinity and bottleneck populations. We describe a national experience of a publicly funded exome sequencing program for individuals with suspected genetic kidney diseases.

Methods: Trio exome sequencing was conducted for CKD patients (onset <21) meeting the following criteria: CKD stage 5; kidney transplantation; dialysis treatment; steroid resistant nephrotic syndrome; primary kidney disease with major anomaly/ intellectual disability (ID); CKD with family history.

Results: During 2023–2024, forty individuals (0–29 years), across Israel met eligibility criteria. Twenty-nine probands (72.5%) completed sequencing, with a positive diagnostic yield of 38%, variants of unknown significance were detected in 21%, and an incidental finding in 3%. Steroid resistant nephrotic syndrome was the most common indication (n = 13), with negative results for all sequenced cases (n = 10). Among individuals with primary kidney disease with major anomaly/ ID (n = 11), diagnostic yield was 33% (3/10). Six diagnoses were attributed to dominant conditions (*HNF1*B associated, n = 3); four to recessive conditions (*NPHP1* deletion, n = 3); mitochondrial inheritance (n = 1).

Conclusions: Our diagnostic yield of 38%, is lower in comparison to prior reports (40–70%) possibly attributed to selection bias of prior negative testing and extended accessibility. Reassessment of steroid resistant nephrotic syndrome as an indication is warranted. Furthermore, 27.5% of cases did not complete sequencing, demanding improvements in the referral to sequencing process.

C.O.I. Disclosure:
A. Singer: None. **J. Grinshpun-Cohen:** None. **I. Maya:** None. **L. Sagi-Dain:** None. **A. Vivante:** None. **O. Chorin:** None.

EP05.023 Phenotype/genotype correlations of 24 patients with Alport Syndrome gene variations

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Background: Alport Syndrome is a heterogeneous disease resulting from variations in genes encoding alpha-3, alpha-4 and alpha-5 chains in the structure of type IV collagen. Type IV collagen is found in the kidney, cochlea and eye basement membranes and its structural abnormalities can cause dysfunctions in these organs. This study aimed to evaluate the clinical and laboratory findings of individuals with variations of COL4A3, COL4A4, COL4A5, and to evaluate genotype/phenotype correlations.

Material and methods:24 patients who were referred to the medical genetics outpatient clinic between 2021 and 2024 and who were examined using next-generation sequencing analysis and found to have disease-causing variants in AS-associated genes were included.

Results:17 different variants were detected in 24 patients: 6 in COL4A3, 10 in COL4A4, and 8 in COL4A5 gene. 4 different variants seen in 6 different patients were novel variations. Homozygous variations were detected in 2 cases, hemizygous were detected in 4 cases, and heterozygous variations were detected in rest of the patients. Of the detected variations, 17 were missense, 4 were splice site, 2 were nonsense, and 1 was frameshift variation. All missense variants affected the glycine residue in the alpha helix structure of collagen.

Conclusion: Early diagnosis and initiation of treatment are important to slow down the deterioration in renal function and provide genetic counseling. In addition, audiological and ocular evaluation should be performed at the time of diagnosis and during follow-up in all diagnosed patients. Reporting the novel variants detected will contribute to the expansion of genotype/ phenotype correlation.

C.O.I. Disclosure:
O. Demir: None. **E. Bahat ozdogan:** None. **A. Cebi:** None. **A. Turkyilmaz:** None.

EP05.024 Phenotypes and mutational spectrum in the GNAS complex locus

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Background: Disorders associated with the GNAS complex locus present a broad phenotypic spectrum, including pseudohypoparathyroidism types 1a, 1b, 1c (PHP1a, PHP1b, PHP1c), pseudopseudohypoparathyroidism (PPHP), progressive osseous heteroplasia (POH), osteoma cutis (OC), and fibrous dysplasia of bone (FD). PHP1a, PHP1b, and PHP1c result from reduced or absent Gsα expression/function from the maternal GNAS allele, caused by an inactivating GNAS variant or multiple differentially methylated regions (DMRs) abnormalities. PPHP, POH, OC, and FD arise from paternal GNAS allele inactivation. We describe nine probands with different GNAS locus alterations, illustrating their diverse phenotypic and mutational spectrum.

Material and methods: - Methylation-Specific MLPA (MS-MLPA)_ME031 *GNAS* probemix- Sanger sequencing - Microsatellite analysis for chromosome 20 (D20S100, D20S109, D20S120, D20S175)

Results:- PHP1a [ORPHA:79443]. Two female patients with Albright Hereditary Osteodystrophy (AHO): 9-year-old girl with TSH resistance, brachydactyly and subcutaneous calcifications and 32-year-old woman with hypopituitarism, childhood-onset seizures and bone dysplasia- PHP1b [ORPHA:94089]: two familial PHP1b cases due to *STX16* exon 5–7 deletions, affecting *GNAS* expression (loss of methylation in exon A/B) (12-year-old and 45-year-old) and two sporadic PHP1b cases involving DMRs (LOM:NESPas, XLαs, XA/B; GOM:NESP55) (21-year-old and 60-year-old)- PPHP [ORPHA:79445]: one 3-year-old boy with macrocephaly, shortening of the 4th-5th metacarpals/tarsals and brachydactyly- POH [ORPHA:2762]: one 8-year-old boy referred for calcifications in the heel, suspected osteoma cutis- FD [ORPHA:249]: one 78-year-old woman with multiple lytic lesions suggestive of polyostotic fibrous dysplasia

Conclusion: *GNAS* mutations generate multiple syndromes depending on their nature and epigenetic context. Given their heterogeneous and progressive course, multidisciplinary follow-up from childhood to adulthood is crucial.

C.O.I. Disclosure:

M. Martínez-Romero: None. **A. Serrano Antón:** None. **M. Sánchez-Soler:** None. **M. Ballesta-Martínez:** None. **V. López-González:** None. **A. Vera-Carbonell:** None. **C. Dolores-Sánchez:** None. **M. Álvarez Martín:** None. **P. Carbonell-Meseguer:** None.

EP05.025 Identification of a pathogenic founder variant in the WFS1 gene that causes Wolfram syndrome in the Druze population

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Background: Wolfram syndrome (WS) is an autosomal recessive neurodegenerative disorder caused by pathogenic variants in the WFS1 gene. It is characterized by central diabetes insipidus, juvenile-onset diabetes mellitus (DM), optic atrophy (OA), and deafness. The natural history of WS is variable, even within the same family and with the same variant.

Material and methods: Five patients belonging to three core families were enrolled. Clinical, biochemical, and genetic data were retrieved retrospectively from their medical files.

Results: All five patients carried the same previously reported homozygous WFS1 pathogenic variant: c.2649del, p.Phe884fs. In all patients, the first presentation was DM at a mean age of 5.2 years (range 4–7), diagnosed initially as type 1 DM with negative anti-pancreatic autoantibodies, and all were treated with insulin by either pump or multiple injections. All five patients had OA that appeared at a mean age of 12.3 years (range 4–30). Three had hearing loss and neurological involvement, and none had diabetes insipidus. One patient was treated with a glucagon-like peptide 1 receptor agonist with a good response.

Conclusion: This is the first report of a founder pathogenic variant in the WFS1 gene in the Druze population in Israel. Our findings imply that molecular analysis is warranted in children presenting with DM and negative pancreatic antibodies. The identified variant should be considered for genetic testing in individuals of Druze ancestry diagnosed with young-onset non-autoimmune diabetes. Early diagnosis of WS is important for therapeutic approaches, especially since novel medications are becoming available.

C.O.I. Disclosure:

M. Abutbul: None. **I. Halabi:** None. **L. Sagi-Dain:** None. **K. Iana:** None. **T. Yardena:** None.

EP05.026 *TRPV6* VARIANTS SHOULD BE TESTED IN NON-ALCOHOLIC CHRONIC PANCREATITIS

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Background: Genetic factors play a pivotal role in the pathogenesis of non-alcoholic chronic pancreatitis (NACP). The transient receptor potential vanilloid 6 (TRPV6) channel, a calcium-selective ion channel critical for epithelial calcium transport, has emerged as a promising candidate in recent studies. We aimed to assess the impact of rare functionally deficient TRPV6 variants in NACP susceptibility.

Materials and methods: Systematic search was conducted in PubMed, Embase, and Web of Science on January 3, 2025, with a prospectively registered protocol (CRD42023438777). Conference abstracts and preprints were excluded. Utilizing a random-effect model with REML estimation, odds ratios (OR) with 95% confidence intervals (CI) were computed. Rare functionally deficient TRPV6 variants were collectively pooled and compared to cases without mutations affecting TRPV6 function. Risk of bias was assessed using the Joanna Briggs Institute's tool.

Results: Altogether, 307 records were screened from which 3 case-controls were included in the quantitative synthesis and one additional cohort study in the qualitative synthesis. Based on data from French, German, Polish and Japanese case-control studies, functionally deficient *TRPV6* variants were associated with a 35-fold increased odds of developing NACP compared to individuals without these variants (OR = 35.32, CI 20.72 to 60.22, across 4 cohorts, 2236 cases and 2748 controls). The quality of the included studies was sufficient, but additional data on the study population would minimize the risk of bias.

Conclusion: Our meta-analysis highlights the significant association of functionally deficient *TRPV6* variants with NACP. These results warrant the inclusion of *TRPV6* in genetic screening panels for NACP.

C.O.I. Disclosure:

K. Ocskay: None. **M.F. Juhász:** None. **P. Mátrai:** None. **M. Sahin-Tóth:** None. **A. Párniczky:** None.

EP05.027 Reduced *TMEM100* expression in newborns with lethal lung developmental disorders

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Background: Lethal lung developmental disorders (LLDDs) involving alveolar capillary dysplasia with misalignment of pulmonary veins (ACDMPV), congenital alveolar dysplasia (CAD), acinar dysplasia (AcDys), and primary pulmonary hypoplasia (PH) are rare diseases manifested by acute respiratory failure refractory to treatment. In most patients with ACDMPV, SNVs or CNVs encompassing *FOXF1* are identified, while abnormalities including *TBX4* or *FGF10*, are detected in newborns with AcDys, CAD, and PH. Recent studies indicated reduced *TMEM100* expression in the lungs of LLDD patients with *FOXF1*, *TBX4*, or *FGF10* deficiency.

Material and methods: Using genome sequencing and histopathological evaluation, we analyzed lung-derived material from one newborn (LLDD014) with clinical manifestation indicating LLDD. In addition, qPCR analysis was performed in this and three previously described LLDD patients compared to age-matched control lungs.

Results: Histopathological and genomic evaluation for patient LLDD014 revealed ACDMPV due to a heterozygous c.852_856del (p.Tyr284*) variant in *FOXF1*. Expression analysis performed in this and previously recognized patients with PH (LLDD009: c.121G>T, *TBX4*), ACDMPV (LLDD011: c.445C>T, *FOXF1*), and AcDys (LLDD013: ~2.2 Mb CNV deletion at 17q23.1q23.2) showed a statistically significant ($P < 0.005$) decrease of lung *TMEM100* expression in all studied patients (LLDD009:49.2%, LLDD011:58.5%, LLDD013:54.8%, LLDD014:44.5%).

Conclusion: Both lung histopathological evaluation and genetic testing may be useful in diagnosing newborns with a history of acute respiratory failure shortly after birth. Identifying novel genetic variants is necessary to understand the etiology of LLDD. Our study suggests that a reduced level of *TMEM100* may be valuable in diagnosing LLDD.

C.O.I. Disclosure:

K. Bzdega: None. **G.H. Deutsch:** None. **M. Rydzanicz:** None. **W. Błaż:** None. **E. Rafińska-Ważny:** None. **A.P. Terpin:** None. **D. Klepach:** None. **V. Zakharova:** None. **R. Płoski:** None. **T. Szczapa:** None. **J.A. Karolak:** None.

EP05.029 *FOCAD*-Associated Neonatal Liver Cirrhosis. A Novel Pathogenic Variant and Previously Undescribed Clinical Feature

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Background: Liver cirrhosis is a severe condition affecting both adults and children, with neonatal liver cirrhosis (NLC) being particularly rare and often of genetic origin. Pathogenic variants in *FOCAD* have been associated with NLC in a small number of families. We identified a patient with a novel homozygous pathogenic *FOCAD* variant, expanding the genetic spectrum of this disorder. Additionally, we report a previously undescribed clinical feature—dyslipidemia.

Material and methods: Whole-exome sequencing (WES) identified a FOCAD variant, confirmed by Sanger sequencing. RNA analysis was performed to assess its functional impact.

Results: The proband, a male infant, presented with hepatomegaly, progressive liver dysfunction, failure to thrive, and persistent gastrointestinal symptoms. WES revealed a homozygous c.1455+1G > T splice site variant in *FOCAD*, classified as pathogenic based on ACMG criteria. Compared to previously reported cases, our patient exhibited an additional clinical feature —dyslipidemia—further refining the phenotypic spectrum of *FOCAD*-associated NLC. Due to disease progression, liver transplantation was performed at 25 months. One year post-transplant, the patient showed normalization of liver function tests, resolution of coagulopathy, and appropriate weight gain, though hematological abnormalities persisted.

Conclusion: We identified a new homozygous pathogenic *FOCAD* variant associated with NLC. This case highlights the genetic basis of pediatric liver diseases, where monogenic causes should be considered alongside metabolic and cholestatic disorders. One-year post-transplant follow-up confirms the effectiveness of liver transplantation while underscoring the need for ongoing monitoring. WES is crucial for early diagnosis, as targeted gene panels may not include all relevant genes.

C.O.I. Disclosure:

O. Shatokhina: None. **E. Nuzhnaya:** None. **V.V. Zabnenkova:** None. **A. Surkov:** None. **A. Arakelyan:** None. **E. Bessonov:** None. **A. Filin:** None. **A. Metelin:** None. **O. Ryzhkova:** None. **N. Semenova:** None.

EP05.030 Genetic variant analysis in patients referred for pancreatitis: insights from a single-center cohort

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Background: Chronic pancreatitis is a multifactorial disease often preceded by recurrent acute pancreatitis. Both genetic and environmental factors significantly contribute to disease severity, complications, clinical course, and treatment response. Early identification of genetic risk factors enables timely intervention to prevent progression to chronic pancreatitis and associated complications. Failure to identify the underlying cause or treatable etiology increases the risk of recurrent acute pancreatitis and ultimately chronic pancreatitis.

Material and methods: A retrospective evaluation was conducted on patients referred to the Department of Medical Genetics, Ege University, with unexplained recurrent or chronic pancreatitis, who underwent clinical exome panel testing over the past two years. The primary focus was on the mutation spectrum and associated clinical findings.

Results: Genetic variants classified as pathogenic (P), likely pathogenic (LP), or variants of uncertain significance (VUS) according to ACMG criteria were analyzed in 31 adult patients. Clinically relevant variants were not found in 10 patients (32.3%). In the remaining patients (21/31, 67.7%), variants were identified in the following genes: hereditary pancreatitis-related genes, including *CFTR*, *PRSS1*, *SPINK1*, and *CTRC*; among patients with suspected lipemic pancreatitis (15/31, 48.4%), variants in *APOB*,

LDLR, *APOE*, and *APOA5*; additionally, variants in *HFE*, *ATP7B*, and several other genes were also identified.

Conclusion: This study underscores the significance of genetic testing in patients with unexplained recurrent or chronic pancreatitis. Early detection of pathogenic variants can guide clinical management to prevent disease progression. Further investigation is required to optimize diagnostic and therapeutic approaches based on genetic findings.

C.O.I. Disclosure:

I. Oflaz: None. **A. Durmaz:** None. **N. Oruc:** None. **I. Yildirim Simsir:** None. **H. Akin:** None. **A. Aykut:** None.

EP06 Skeletal, Connective Tissue, Ectodermal and Skin Disorders

EP06.001 Genetic factors predisposing to atopic dermatitis - genes related to the skin barrier

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Atopic dermatitis is characterized by a persistent course and a complex genetic and pathophysiological basis. The development of this allergic inflammatory disease is mainly influenced by factors including predisposing genes, innate susceptibility, environmental and infectious agents, impaired structure and function of the skin barrier and immune response, abnormal colonization of the skin by microorganisms. The genes that affect the function of the epidermal barrier and have been best studied in the context of AD include *FLG* (filaggrin) and *SPINK5/LEKTI* (serine protease inhibitor kazal type 5 / lympho-epithelial kazal-type-related inhibitor). Filaggrin is responsible for binding cells in the stratum corneum of the epidermis, and mutations in its gene lead to excessive water loss and skin dryness. In turn, SPINK5 polymorphisms may result in deficiencies in the LEKTI inhibitor, leading to the activation of pro-inflammatory responses, damage to the skin barrier, and worsening of disease symptoms. The proper maintenance of the skin barrier is also influenced by kallikrein (*KLK7*) and *STAT6* (signal transducer and activator of transcription 6). Mutations in *KLK7* can lead to severe exfoliation of the epidermis, while mutations in *STAT6* can contribute both to skin barrier damage and the intensification of inflammatory conditions due to increased IgE production.

C.O.I. Disclosure:

T. Nowak: None.

EP06.002 Genetic Insights into Isolated Dental Agenesis: A Whole Exome Sequencing Study on an Italian Family

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Background: Isolated dental agenesis (DA) is one of the most common congenital dental anomalies, characterized by the absence of one or more teeth. It is a heterogeneous condition with a strong genetic component, but the underlying genetic basis remains unclear in many cases. This study aims at exploring the genetic determinants of DA in an Italian family with multiple affected members.

Material and methods: We investigated a family in which the proband, her father, her paternal grandfather and two of his brothers (one female and one male) present with non-syndromic DA. Whole exome sequencing (WES) was performed on the proband, her father, and her unaffected mother. The analysis focused on identifying rare, likely pathogenic variants inherited from the father that could explain the observed phenotype. Variants are being filtered based on their allele frequency, predicted pathogenicity, and known or putative roles in dental development.

Results: Preliminary data analysis aims at identifying candidate variants shared between the affected father and daughter but absent in the unaffected mother. Further segregation analysis within the family will be performed to assess the potential pathogenic role of identified variants. The analysis is currently ongoing.

Conclusion: This study underscores the importance of WES in investigating the genetic basis of DA. The identification of novel candidate genes may contribute to a better understanding of the molecular mechanisms underlying tooth development and could improve genetic diagnosis in patients with non-syndromic DA.

C.O.I. Disclosure:

F. Bogliardi: None. **C. Modafferi:** None. **E. Tabolacci:** None. **P. Chiurazzi:** None. **P. Concolino:** None. **C. Grippaudo:** None.

EP06.003 BIU*X2X2*

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This retrospective observational based study was designed to examine pre-existing data the mostcommon genodermatosis seen in Dhofar region.It is a 2-years study, with a sample size of 18 patients in Dhofar who attended the Genetic disorder clinicand dermatology clinic at SQH. The sample group include individuals who are clinical and molecularlydiagnosed with Genodermatosis. The data compiled directly from clinical records and moleculardiagnostic findings documented in Excel sheet.Identifying the phenotype/genotype of the most common Genodermatosis. The data encompassingclinical presentations, genetic findings, and demographic information were reviewed and analyzed.Other information like patient's age, sex, region, and genetic mutations associated with the conditionwere also reviewed. The main objectives of the study is to identify common Genodermatosis syndromein Dhofar region. In addition to assess, the molecular variants linked to those syndromes and theirgeographical distribution.To our knowledge, no previous records of the phenotype /genotype of the common genodermatosis inDhofar region. This registry, though might not fully representing the region, but will be the first pillar tobuild on further detailed and comprehensive study and researcher in the future.

C.O.I. Disclosure:

M.M. Al Awadi: None.

EP06.004 De novo variants in RPL13 and FN1 genes: clinical evaluation and genotype-phenotype of spondylometaphyseal dysplasia with neurodevelopmental delay

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BACKGROUND: We report a 2 y boy of non-consanguineous parents without familiar history of intelectual diability; the father had Perthes disease and hip displasia. He was delivery at term with alength of 47 cm.

Physical Examination: At 4 years old his weight was 9.640kg (−2.42 SD), lenght 78 cm (−3.86 SD) and head circunference was 46.5 cm (- 2.39 SD). The examination showed prominent forehead, low anterior hairline, bilateral epicanthal folds big eyes, sparse eyebrows, a flat nasal bridge, large nose with bulbuos tip,short and flat filtrum, short neck,his musculoskeletal features included short upper and lower extremities with wrist joint laxity and brachidactyly.Clinodactyly of 5th finger. Signs of hyperactivity.

Investigations and Results: Neurometabolical studies, EEG and brain MRI were normal. Array: 14q11.2(19474772_23614930)x1, 22q11.21(20641693_21505417)x1 Deletion in 14q11.2 of 4.14 Mb classified as pathological, the doses of 323 genes are altered, 52 of them with OMIM annotation. It is worth mentioning the CHD8 gene (OMIM*610528), deletions of this gene can cause ASD(AUTS-18 ≠ 615032). Deletion of 853 Kb in 22q11.21 altered 40 genes, 12 of them with OMIM annotation. Whole exome sequencing showed: A heterozygosis variant in the RPL13 gene (OMIM#113703) classified as a pathogenic variant has been identified according to the recommendations of the ACMG. It is related to autosomal dominant spondyloepimetaphyseal dysplasia, Isidor-Toutain type (OMIM #618728). The variant found in the patient is not found in the general population databases consulted.

CONCLUSION: Here we emphasize the importance of all genetics testing using Human Phenotype Ontology to ensure early diagnosis.

C.O.I. Disclosure:

T. Carrion: None. **D. de Sotto-Esteban:** None. **E. Moratalla:** None. **V. Asensio:** None.

EP06.005 Terminal osseous dysplasia with pigmentary defects: a family case study with *FLNA* gene variant

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Background/Objectives: Terminal osseous dysplasia with pigmentary defects (TODPD, OMIM#300244) is one of the X-linked filaminopathies and is lethal in males. First clinically described by Zhang et al., and Sun et al. reported a single recurrent variant in the *FLNA* gene in 2010 (c.5217G>A;p.Val1724_Thr1739del). It's characterized by pigmentary abnormalities in the skin, skeletal abnormalities, recurrent digital fibromas in childhood, and multiple frenulum clinics. X-inactivation may play a role in the expression of the disease. Distinctive cutaneous features are pivotal in distinguishing it from other diseases in the otopalatodigital spectrum. There are approximately 30 cases with genetic diagnosis in the literature. Here, we presented a daughter (proband) and her mother with TODPD.

Methods: Proband (3y, female) and her mother (27y) were referred due to finger-toe anomalies, contractures, and cysts on the fingers. The proband's anthropometric measurements were normal. There was a broad forehead, ptosis, a bulbous nose, thick lips, brownish pigmentary spots around the zygomatic arch, and multiple frenulum. The fingers were short, with limited movement and contractures. There were fibromas on the dorsum of the fingers. The metacarpal and metatarsal bones were disorganized on the radiographs. Her mother also had similar findings, although they were milder.

Results: Sanger sequencing analysis revealed a pathogenic heterozygous recurrent variant in the *FLNA* gene for the proband and her mother (NM_001110556.2,c.5217G>A, p.Thr1739=). The proband's karyotype analysis was normal.

Conclusion: TODPD is a rare syndrome within filaminopathies, with few familial cases. This report highlights the TODPD's clinical features and molecular mechanism of the *FLNA* gene.

C.O.I. Disclosure:

G. Karamik: None. **T. Kalkan:** None.

EP06.006 Case of prenatal diagnostics of Apert syndrome in a young Ukrainian family with primary infertility and miscarriage

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Background: Apert syndrome is a rare autosomal genetic disease part of craniosynostosis group. Frequency of this syndrome has not been studied in Ukraine. The objective of this research is diagnostics of molecular changes and clinical signs in prenatally diagnosed Apert syndrome.

Material and methods: syndromological, ultrasonography, molecular genetic (Ukraine).

Results: Spouses age: woman - 26 years old, man - 30 years old; primary infertility in past medical history within the last 3 years. I and II pregnancies - missed miscarriage in 8,9 week of pregnancy respectively. Dermoid ovarian cyst in past gynaecologic history. No history of hereditary diseases. According to the results of the combined prenatal screening of I trimester the pregnant woman was not at risk of chromosomal pathology. During ultrasonography in 21 week symmetric ventriculomegaly, ventricular septal defect, bilateral syndactyly of 2–5 fingers, thickening of the proximal phalanges of the lower extremities and deviation of the I fingers, acrocephaly, hypertelorism, bulging orbits, nasus selliformis, flat face, retrognathia, low-set auricles were diagnosed. Parents decided to terminate pregnancy considering poor prognosis for the child's future health. Macroscopic examination revealed a typical aspect of coronary craniosynostosis and bilateral syndactyly of both hands and feet. Genetic examination of placental villi revealedmutation of *FGFR2* gene (c.755C>G(p.Ser252Trp)), which allowed to verify the diagnosis by molecular genetic methods.

Conclusion: in families with infertility, habitual miscarriage there is a high risk of birth of children with genetic pathology. Apert syndrome also occurs in young families. Detailed clinical assessment is of primary importance for patients' classification.

C.O.I. Disclosure:

M. Bondarenko: None. **A. Dutchak:** None. **R. Kozoviy:** None. **N. Kitsera:** None.

EP06.007 Case of rare form of Harlequin-type ichthyosis in newborn

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Background: over 50 nosological entities of genetic ichthyosis have been described, Harlequin-type ichthyosis is the most severe of them. It occurs in newborns with frequency 1:300000. Objective: to analyze a clinical case of Harlequin-type ichthyosis.

Material and methods: ichthyosis, syndromological analysis, ultrasonography.

Results: The child was born at 36 weeks of gestation with a weight of 2850 grams, a length of 44 cm. The course of pregnancy was complicated by signs of the threat of miscarriage. The family denies any influence of environmental and household factors. According to clinical-genealogical analysis, the wife has two healthy children born in her first marriage, and the husband - one healthy child. In the second marriage, this is their first common child. The couple denies consanguinity. The combined first-trimester screening indicated no increased risk for common chromosomal abnormalities. Ultrasound screenings of the 1st and 2nd trimesters did not reveal any abnormalities in fetal development. At 28 weeks of gestation, ultrasound diagnostics confirmed changes: brachycephalic skull, edematous "fan-shaped" eyelids, diminished nasal bones, a shortened chest. Finger phalanges were shortened, curved, deformed. Syndromological analysis of the newborn revealed the following diagnoses: hyperkeratosis of the skin; deep, moist, erythematous cracks affecting the entire body. Eclabium, ectropion, flattening of the nasal bone, underdeveloped auricles, semi-flexed limb positioning, and shortened fingers were observed. Despite treatment, the child died at the age of 2 days due to respiratory distress syndrome.

Conclusion: The delayed phenotypic expression complicates timely prenatal diagnosis, requiring a multidisciplinary approach to prevent complications and manage associated disorders.

C.O.I. Disclosure:

A. Dutchak: None. **U. Petryshchuk:** None. **O. Kornutii:** None.

EP06.008 A Novel Variant in the *SULT2B1* Gene Linked to Congenital Ichthyosis: A Family Study

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Background: Autosomal Recessive Congenital Ichthyosis Type 14 (OMIM # 617571) is a rare genetic disorder characterized by disrupted skin barrier function. This condition arises from mutations in the SULT2B1 gene located on chromosome 19q13. The SULT2B1 gene encodes an enzyme crucial for skin barrier maintenance, specifically the sulfation process. Mutations in SULT2B1 disrupt this process, leading to increased water loss and the characteristic scaly skin of ichthyosis.

Material and methods: Genomic DNA extracted from a peripheral blood leukocyte sample of the proband was subjected to clinical exome sequencing (CES) using the Genomize Seq platform for bioinformatics analyses. Segregation analysis of the identified variant was subsequently performed in the proband's parents and four siblings via Sanger sequencing.

Results: Clinical exome sequencing identified a homozygous LP variant, c.448C>T (p.Arg150Trp), in the SULT2B1 gene of a patient with the disease. Segregation analysis confirmed parental carrier status and identified a homozygous affected sibling. Other siblings were unaffected and did not carry the variant. This finding, supported by four lines of evidence (PM2, PP1, PP4), classifies the variant as likely pathogenic.

Conclusion: This study presents a family with ichthyosis, a rare genodermatosis. Genetic testing identified a novel variant, expanding our understanding of the diverse genetic landscape of ichthyosis. Family segregation analysis confirmed the variant's pathogenicity, emphasizing the importance of genetic testing in diagnosing monogenic disorders and informing genetic counseling for reproductive planning.

C.O.I. Disclosure:

A. Islamova: None. **A. Aydin Gumus:** None. **L. Yilmaz Celik:** None.

EP06.009 A Case Report of Acromesomelic Dysplasia in a 10-Year-Old Male Patient

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Background: Acromesomelic dysplasia, type Maroteaux (AMDM), is a rare autosomal recessive skeletal dysplasia characterized by severe shortening of the middle (mesomelic) and distal (acromelic) segments of the limbs. It is caused by mutations in the *NPR2* gene, which encodes natriuretic peptide receptor B, a key regulator of endochondral bone growth. Affected individuals typically present with disproportionate short stature, limb deformities, and characteristic radiographic findings. Early diagnosis is crucial for appropriate clinical management and genetic counseling.

Materials and methods: We report the case of a 10-year 4-month-old male patient who presented with growth retardation and limb shortening. Clinical evaluation, radiographic imaging, and genetic analysis were performed. Targeted sequencing of the *NPR2* gene was conducted to identify pathogenic variants. Growth parameters, skeletal abnormalities, and family history were also assessed.

Results: The patient exhibited disproportionate short stature with prominent shortening of the forearms, lower legs, hands, and feet. Radiographic examination revealed mesomelic and acromelic shortening with metaphyseal irregularities, characteristic of AMDM. Genetic analysis confirmed a homozygous/pathogenic variant in the *NPR2* gene consistent with the diagnosis. Family history suggested a consanguineous relationship between parents, reinforcing the autosomal recessive inheritance pattern.

Conclusion: This case highlights the clinical and genetic features of AMDM due to an *NPR2* mutation. Early recognition and molecular diagnosis are essential for accurate prognosis, clinical management, and genetic counseling. Although no curative treatment is available, growth hormone therapy and supportive interventions may improve quality of life in affected individuals. Further research is needed to explore potential therapeutic approaches targeting the NPR2 signaling pathway.

C.O.I. Disclosure:

D. Karaer: None. **T. Durak:** None. **K. Karaer:** None.

EP06.010 New case of hipomelanosis Ito with normal intelligence

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Hypomelanosis of Ito (HI) is a rare neurocutaneous disorder characterized by linear and swirled hypopigmentation along Blaschko's lines, often associated with neural, ophthalmic, and orthopedic abnormalities. We present the case of a 6-year-old boy with vitiligo-like hypopigmentation, developmental milestones achieved with mild delays, recurrent respiratory infections, and dysmorphic features such as hypertelorism, wide forehead, and low-set eyebrows. Initial blood karyotyping and microarray analysis did not reveal abnormalities. However, analysis of skin fibroblasts demonstrated mosaic tetrasomy of chromosome 13q, confirming the diagnosis of HI.A review of literature indicates that cases of mosaic tetrasomy 13q frequently present with features consistent with HI, including neurological and pigmentary abnormalities. These findings emphasize the diagnostic significance of detecting chromosomal aberrations in different tissues, particularly fibroblasts in cutaneous disorders. This case highlights

the importance of analyzing tissue-specific samples in diagnosing mosaic disorders.

C.O.I. Disclosure:

E. Preizner-Rzucidlo: None. **H. Zurek:** None.

EP06.012 A rare case of a mosaic Turner syndrome with marker chromosome and complex molecular karyotype

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Background: Turner syndrome (TS)[1] is a genetic disorder due to monosomy or structural aberrations of the X chromosome, leading to a wide range of phenotypic manifestations. A marker chromosome[2] is an abnormally small, derivative chromosome, which conventional cytogenetic techniques can not characterize.

Material and methods: We describe a case of a 5-year-old patient referred for a medical genetics consultation due to dysmorphic features and impaired growth. Clinical evaluation, karyotyping, chromosomal microarray analysis (CMA) and FISH were performed.

Results: The patient was born preterm. She had metopic synostosis at birth, which was surgically corrected. Physical examination revealed low weight (0.1%), height (0.0%), small head circumference (0.0%), broad chest, disproportionately short lower extremities, and orbital and nipple hypertelorism. Karyotyping revealed a marker chromosome. CMA showed the following molecular karyotype (arr[GRCh38]):

Deletions	Microdeletions
Xp22.33p11.22(249588_54774109)x1	Xq12q13.1(65846495_69829338)x1
Xp11.22p11.1(54775794_65920053) x1 ~ 2	Xq13.1q13.2(69829589_73077702) x1 ~ 2
Xq13.2q22.2(74026302_103328938)x1	Xq13.2(73048443_73536244)x1
Xq22.3q28(108126513_156029899)x1	Xq13.2(73523565_74051074)x1 ~ 2
	Xq22.2q22.3(103328938_105645270) x1 ~ 2
	Xq22.3(105813035_106051182)x1 ~ 2
	Xq22.3(106047812_106484565)x1
	Xq22.3(106458060_108120926)x1 ~ 2

The diagnosis of TS was made. FISH analysis using chromosome X alpha satellite[3] probe (region - Xp11.1-Xq11.1) revealed the presence of marker chromosomes in 60.5% of cells, and monosomy of X chromosome in the remaining cells (mos 46,X,+mar/45,X). The presence of CNVs from different regions only in half of the cells means that these regions are preserved in the marker chromosome, therefore it was probably formed by chromothripsis[4] when shattered fragments are assembled into the new chromosome. XIST gene[5] is preserved on the marker.

Conclusion: Patients with TS can have complex molecular karyotypes, which can only be detected utilizing modern genetic tools. This case also highlights the possible association of craniosynostosis with TS.

C.O.I. Disclosure:

N. Dzoyan: None. **A. Aywaz:** None. **T. Hovhannisyan:** None. **N. Kostandyan:** None. **T. Sarkisian:** None.

EP06.013 Three Cases Diagnosed with Roberts Syndrome-Clinical and Molecular Evaluation

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Background: Roberts syndrome is a rare autosomal recessive developmental disorder characterized by prenatal growth retardation, symmetric mesomelic limb deformities, microcephaly, and distinct dysmorphic facial features. The condition results from biallelic pathogenic variants in the *ESCO2* gene, which encodes an acetyltransferase involved in regulating sister chromatid cohesion.

Material and methods: Genomic DNA was isolated from peripheral blood samples, and the exons and exon-intron boundaries of the ESCO2 gene were analyzed using Sanger sequencing.

Results: Three patients were referred to our genetics clinic for their clinical and dysmorphic features. A 17-year-old girl presented with short stature, mesomelic shortening of the upper extremities, thumb hypoplasia in the left hand and dysmorphic facial findings. Dysmorphic features included long face, broad forehead, hypertelorism, down-slanting palpebral fissures and hypoplastic ala nasi. Sanger sequencing of the *ESCO2* gene identified a homozygous splice-site variant, c.1131+1G > A, previously classified as pathogenic in the ClinVar database. The other two siblings were 6 and 4 year-old female patients. Both had similar clinical findings including severe growth retardation, developmental delay, extremity defects and dysmorphic features. The first sister had bilateral cataract. The other sibling had corneal dystrophy, esotropia and nystagmus. Sanger sequencing revealed a homozygous nonsense *ESCO2* gene variant, c.679G>T (p.Glu227*), in both of the siblings. This was a novel variant which was predicted as likely pathogenic according to ACMG guidelines.

Conclusion: This study highlights the clinical findings of three patients with *ESCO2*-related Roberts syndrome, including a previously reported pathogenic variant and a novel nonsense mutation.

C.O.I. Disclosure:

C. Yilmaz Uzman: None. **S. Gürsoy:** None. **D. Ayyıldız Emecen:** None. **F. Hazan:** None.

EP06.014 X-linked hypophosphatemia: analysis of the c.2051T>G (p.Phe684Cys) variant and literature review

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Background: X-linked dominant hypophosphatemic rickets (XLHRD) (OMIM #307800) is characterized by inadequate bone and teeth mineralization due to hypophosphatemia and hypocalcemia, caused by variants in the PHEX gene. These variants inactivate Phosphate-Regulating Endopeptidase (PHEX) protein and increase Fibroblast Growth Factor 23 (FGF-23). With an estimated prevalence of 3.9:100,000 live births, patients present with isolated hypophosphatemia, bone deformities, and renal failure. Hereby, we report a patient with a novel variant in the PHEX gene.

Material and methods: A 28-year-old woman presented with childhood-onset growth alterations, genu varum, and rickets. Her mother showed a similar phenotype, and her brother died of multiple malformations. Laboratory studies revealed

hypophosphatemia, phosphaturia, elevated parathyroid hormone, and bone alkaline phosphatase. She exhibited grade 3 obesity, walking difficulties, hip and lower limb pain, and bilateral mild hearing loss. A next-generation sequencing (NGS) panel for hypophosphatemic rickets was performed for molecular diagnosis.

Results: Genetic testing identified the variant c.2051T>G (p.Phe684Cys) in the PHEX gene. While classified as probably pathogenic in Varsome, ClinVar, and LOVD, the variant is considered pathogenic given the patient's clinical presentation and family history.

Conclusion: This variant has been previously reported in a patient with elevated FGF23. Complementing variant information in databases is crucial for guiding interdisciplinary management with monoclonal antibody therapies.

C.O.I. Disclosure:

S.G. Pico Muñoz: None. **M.F. Salamanca:** None. **G.A. Contreras:** None. **G. Mantilla:** None.

EP06.015 McCune-Albright syndrome diagnosed in adulthood: a case report

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Background: McCune-Albright syndrome (MAS) is a rare disorder that affects the bones, skin, and several hormone-producing (endocrine) tissues. Althoug it is genetic disorder caused by GNAS gene mutations, diagnosis is complicated as mutations are somatic and difficult to establish.

Material and methods: We present a MAS case with difficult clinical diagnosis - from symptoms to final diagnosis about 12 years.

Results: From 30 years of age patient had 14–16 major and minor bone fractures. At 40 y.o. he had surgeries of hepatic adenoma, lipomas of the neck and arm, myxofibrosarcoma of the upper arm. The patient was tested for Li Fraumeni syndrome using NGS panel. The test was negative. After several tests (CT, PET CT, bone scintigraphy) widespread and progressively advancing fibrocystic dysplasia was discovered, as well as multiple lesions in the femurs, tibias, humeri, with a high risk of fractures. At 42 y.o. MAS diagnosis was pursued due to fibrotic dysplasia finding. Laboratory testing for hormonal changes was performed: IGF-1 and GH values were slightly increased. On the physical examination pigmentation signs were noted on the upper back. MAS diagnosis was confirmed and treatment with zoledronic acid was started

Conclusion: MAS is a rare disorder and without clear laboratory tests it relies on clinical experience to recognise disorder.We do not perform GNAS gene testing from affected tissues as negative test does not rule diagnosis. Myxofibrosarcoma might be consequence of fibrotic dysplasia transformation which is rare event.

C.O.I. Disclosure:

L. Kucinskas: None. **A. Gintilaite:** None. **R. Matukaitiene:** None. **M. Sukys:** None. **R. Ugenskiene:** None.

EP06.016 Clinical and genetic characteristics of a patient with type III osteogenesis imperfecta caused by a novel pathogenic deletion in the last splicing site of the *COL1A1* gene

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Background/Objectives: The diversity of phenotypes in *COL1A1* gene variants arises from various mechanisms of pathogenicity. Understanding the impact of splicing variants is crucial as they can alter mRNA and protein structure through distinct molecular events. This study aims to delineate the molecular and clinical characteristics of a patient with Osteogenesis Imperfecta (OI) Type III due to a novel pathogenic deletion in the last acceptor splicing site of the *COL1A1* gene.

Material and methods: Data from an 8-year-old patient were examined extensively. Diagnostic methods included clinical assessment, radiography, whole-genome sequencing. Minigene splicing reporter was used to assess the impact of the COL1A1 variant of RNA splicing.

Results: This study details clinical and radiological features of an OI Type III patient with a de novo deletion at the last acceptor site, c.4249-15_4252del in the 51st intron of *COL1A1*. Functional analysis using minigene system revealed that the c.4249-15_4252del variant leads to 2 independent events: intron 50 retention p.(Ser1417Valfs*46) and exon 51 shortening by 105 nucleotides p.(Ser1417_1451del). Fetal ultrasounds at 20 and 26 weeks revealed initial signs of the disease, including bone shortening, bending, and fractures. Main clinical features encompassed multiple fractures, bone and spine deformities, short stature (−4.2 SD), bluish sclerae, imperfect dentinogenesis, and impaired ambulation. By age eight, the patient had experienced approximately 60 fractures.

Conclusion: The variant c.4249-15_4252del, located in the C-terminal domain, disrupts triple-helix association explaining the severe clinical progression of OI. Our clinical-genetic study revealed new data that allows to clarify genotype-phenotype correlations.

C.O.I. Disclosure:

E.S. Merkuryeva: None. **I.V. Viakhireva:** None. **V.M. Kenis:** None. **O.P. Ryzhkova:** None. **M.Y. Skoblov:** None. **T.V. Markova:** None.

EP06.017 An investigation of autophagy and related genes in psoriasis

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Background: Psoriasis is a chronic, non-contagious, painful, disfiguring and disabling disease. The pathogenic mechanism of psoriasis is not fully understood. The initiation and execution of autophagy, which is essential for cellular homeostasis, is controlled by Atg gene products. It is not known how one of this gene family, Atg16l1, mechanistically contributes to skin biology or psoriasis pathogenesis. The aim of this study was to investigate ways that can regulate the risk of psoriasis by examining the Atg16l1 gene and a total of 11 related genes as a result of the scans.

Material and methods: Tissue biopsies of 30 adults consisting of 10 newly diagnosed psoriasis patients, 10 treated psoriasis patients and 10 healthy individuals as a control group were used.

RT-PCR was performed on the RNAs obtained from these tissues. Immunohistochemical analysis was also performed for protein levels.

Results: Except for Nod2, all mRNA expressions decrease in newly diagnosed patients compared to controls. It was determined that the mRNA expressions of only two genes (Atg5 and Wıpı2) were not statistically significant compared to the control. The products of four statistically significant genes were examined by IHC method. It was determined that the highest cell proliferation was in the patient group diagnosed with Psoriasis.

Conclusion: As a result, according to the immunohistochemical analysis applied to the It was observed that these protein expressions were highest in the control group. When autophagy is inhibited, it causes keratinocytes to proliferate. This suggests that autophagy may have a role in the pathogenesis of psoriasis.

C.O.I. Disclosure:

F. Çetİn arslantürk: None. **T. Kiliçaslan ayna:** None. **F.S. Çam:** None.

EP06.018 Novel *ABCA12* missense variant is the likely cause of lamellar ichthyosis

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Background: Autosomal recessively inherited ichthyosis is a heterogeneous group of skin cornification disorders that result in defective keratinocyte differentiation and abnormal epidermal barrier formation. Biallelic loss of function variants in *ABCA12* cause harlequin ichthyosis, whereas other *ABCA12* genotypes are associated with congenital ichthyosiform erythroderma or lamellar ichthyosis. We report on a girl with lamellar ichthyosis with palmoplantar keratoderma born of consanguineous parents.

Material and methods: DNA from a blood sample was extracted and analysed by exome sequencing and virtually focused on genes related to ichthyosis. The coding and flanking intronic regions (±10 bp) were sequenced at a mean sequencing coverage of 121X.

Results: Homozygosity for the variant *ABCA12* (NM_173076.3): c.[6347C > T];[6347C > T], (p.(Ser2116Phe) was detected. The variant has not formerly been associated with disease (HGMDv2024.4, ClinVar) and is seldomly seen in the frequency database gnomAD 4.1 (0.0002%). The amino acid is highly conserved, and prediction tools (REVEL, AlphaMissense) predict the variant to be deleterious. ABCA12-staining revealed deficient ABCA12 protein expression in the patient's granular layer compared with normal skin tissue.

Conclusion: In order to confirm that the detected variant in *ABCA12* is the likely cause of ichthyosis in this patient, we are currently confirming homozygosity for the variant in the patient's affected cousin of consanguineous parents and confirming that the patient's healthy brothers do not carry the variant in a homozygous state.

C.O.I. Disclosure:

L. Graversen: None. **M. Sommerlund:** None. **H. Vinter:** None. **N. Brix:** None. **K. Rønholt:** None. **U.B. Jensen:** None. **J. Blechingberg:** None.

EP06.019 Heterogeneity of the type II collagen disorders caused by missense variants in the *COL2A1* gene: three cases from our clinical practice

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Background: Type II collagen disorders comprise a phenotypic spectrum caused by defects in type II collagen. This protein forms the cartilage and eye structures and plays a role in endochondral ossification.

Material and methods: Clinical and radiological evaluations were performed. For one patient, clinical exome sequencing (CES), which covers 4800 genes, was performed using the Illumina TruSight One panel. A targeted next-generation sequencing (NGS) panel for skeletal dysplasia (Blueprint Genetics) was performed for the other two patients.

Results: The first case was a boy whose dysmorphic features, cleft palate, short thighs and trunk were observed at birth. Postnatally, he developed respiratory insufficiency, leading to tracheostomy. The skeletal dysplasia gene panel identified a heterozygous variant *COL2A1*, c.1781G>A (p.Gly594Glu), classified as pathogenic. He died at the age of 5 months. The second case was a boy whose shorter femurs were seen prenatally. At birth, dysmorphic features, cleft soft palate, short neck, trunk, and III. right-hand finger and disproportionally short femurs were observed. X-ray showed bilaterally underdeveloped femoral and tibial epiphyseal ossification centers. CES identified a heterozygous variant *COL2A1* c.3329G>A (p.Gly1110Asp), classified as likely pathogenic. He underwent surgical cleft palate repair at the age of 11 months. The third case was a 29-year-old, short-statured male professional athlete with signs of congenital spondyloepiphyseal dysplasia. The skeletal dysplasia gene panel identified a heterozygous variant *COL2A1*, c.2974A>G (p.Arg992Gly), classified as likely pathogenic.

Conclusion: These cases reaffirm that missense variants in the *COL2A1* gene are associated with a broad phenotypic spectrum, from perinatally lethal to milder forms.

C.O.I. Disclosure:

I. Tečer: None. **A. Šimek:** None. **K. Gotovac Jerčić:** None. **S. Huljev Frković:** None.

EP06.020 POLYMORPHISMTAQIOF THE VDR GENE IN CHILDREN WITH GROWTH HORMONE DEFICIENCY

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Background: Vitamin D, which is a powerful regulator of bone and calcium homeostasis, as well as immunomodulation, cell differentiation and replication in various target tissues.

Material and methods: A genetic study was carried out in 42 children with growth hormore deficienty who were treated at the State Institution "V.P. Komisarenko Institute of Endocrinology and Metabolism of the National Academy of Medical Sciences of Ukraine". The average age of the children included in the study was 11,28 ± 3,06 years. There were taken 47 children for the control group.

Results: To determine polymorphic variants of TaqI (rs731236), modified protocols of the VDR gene, that predict involving the use of oligonucleotide primers in the PCR.The presence of a

heterozygous TC genotype increases the risk of developing GHD (OR = 4,07, 95% CI 1,61-10,29; p = 0,01), and the presence of a homozygous TT genotype is protective (OR = 0,23, 95% CI 0,09–0,57; p = 0.01).When analyzing alleles in patients with GHD, the fol-lowing data were obtained: the carrier of the C allele of the polymorphic locus TaqI (rs731236) of the VDR gene is associated with the risk of GH deficiency (OR = 2,52, 95% CI 1,30–4,86; p = 0.01).The main allele in the control group is T (pT = 0,7872), as well as in the group of GHD patients (pT = 0,5952).

Conclusion: The presence of a heterozygous TC genotype increases the risk of developing. The carriage of the C allele of the polymorphic locus TaqI (rs731236) of the VDR gene is associated with the risk of GHD.

C.O.I. Disclosure:

M. Ryznychuk: None.

EP06.021 The application of whole exome sequencing in children's dermatology

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Background: Whole-exome sequencing (WES) is an effective and rapid method for diagnosis genodermatoses [1]. The prevalence of these diseases is estimated at 1:250 to 1:1,000,000 [2]. Many of these conditions, such as xeroderma pigmentosum and neurofibromatosis, may have a poor prognosis due to an increased risk of tumor development or oncological predisposition [3]. Therefore, early and accurate diagnosis is essential for personalized strategy.

Material and methods: WES was performed on pediatric patients with suspected genodermatoses after a physician`s examination.

Results: The study included 19 patients, with a mean age of 9.15 years ± 3.76 (10 males and 9 females). All patients underwent dermatological consultation and deep phenotyping. This approach facilitated the identification of children who most urgently required genetic testing. The clinical diagnosis and WES findings were concordant in 9 cases (47.37%). A confirmed inherited disorder was established in 12 cases, while no carriers of pathogenic variants were identified. The *SPINK5*(NM_006846.4):c.1048C>T (p.Arg350Ter) variant was confirmed in 2 patients with Netherton syndrome. Two mutations in *NIPAL4* were detected in patients with congenital ichthyosis. One female patient had risk alleles for psoriasis (rs34367357, rs2066964). Eight previously unreported mutations were identified. In total, 18 variants were detected, including 11 pathogenic, 2 likely pathogenic, and 3 variants of uncertain significance.

Conclusion: WES with deep phenotyping is a highly effective diagnostic strategy in pediatric dermatology.

References: 1. Zhu X. et al., 2023. https://doi.org/10.1186/s12920-023-01628-2. 2. Süßmuth, Kira et al., 2020. https://doi.org/10.1111/ddg.14049. 3. Aşkın, Ö., et al., 2020. https://doi.org/10.1016/j.clindermatol.2020.03.009.

C.O.I. Disclosure:

O.P. Parshina: None. **A.A. Buianova:** None. **E.R. Gumennaia:** None. **M.A. Sidorchuk:** None. **A.S. Botkina:** None. **A.F. Samitova:** None. **A.A. Kuznetsova:** None. **A.O. Shmitko:** None. **V.A. Belova:** None. **J.S. Lashkova:** None. **D.O. Korostin:** None.

EP06.022 Palmoplantar keratoderma caused by a novel variant in *KLF4*, indicating Lys409 as a hotspot?

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Background: Palmoplantar keratodermas (PPKs) is a group of disorders caracterized by hyperkeratotic thickening of the skin of the palms and soles. To this date 63 causal genes are known. Here we present a patient with PPK harbouring a novel variant in *KLF4*, a gene just recently shown to be associated with inherited PPK.

Material and methods: Whole genome sequencing of DNA from whole blood from the patient was done. Sequencing data of all exon and intron sequences of 63 genes associated to PPK were analysed. Testing of the parents of the patient was done by Sanger sequencing.

Results: The variant c.1227G>T (p.(Lys409Asn)) in exon 4 of *KLF4* in heterozygous form was detected. The same variant has not been reported before, but another variant in the same amino acid was recently reported as pathogenic for PPK. After testing the healthy parents of the patient, the variant was confirmed to be de novo in the patient. Functional studies of the effect of the variant is currently awaiting.

Conclusion: We have identified a novel de novo variant in *KLF4* as likely causative for PPK. This is the third pathogenic variant associated with PPK reported in *KLF4*, and the second in Lys409 indicating this amino acid as a hot-spot for KLF4 function. This further strengthens the evidence of *KLF4* as an important player in inherited PPK.

C.O.I. Disclosure:

J. Blechingberg: None. **K. Malovitski:** None. **E. Sprecher:** None. **M. Sommerlund:** None. **L. Graversen:** None.

EP06.023 Novel pathogenic variants in hereditary multiple exostoses

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Background: Hereditary multiple exostoses (HMEs) or multiple osteochondromas represent a rare and severe skeletal disorder, characterized by multiple benign cartilage-capped bony outgrowths, usually in the epiphyseal and metaphyseal regions of long bones, scapulae, vertebrae, pelvis, cranium. Exostoses apply pressure on adjacent tissues, including nerves or blood vessels, with multiple consequences; the most severe complication is their malignant transformation (3.9% of cases). HME incidence is unknown, as probably a significant proportion of asymptomatic patients remain undiagnosed. Germline or somatic alterations in *EXT1* and *EXT2* genes are found in most of the patients.

Material and methods: 4 patients were referred for genetic investigations following the clinical diagnosis of HMEs. In addition of exostoses, one of them presented with limitations in limb movements. Sequencing of DNA isolated from peripheral blood or buccal cells was performed on Thermo Fisher Ion S5 and Illumina platforms.

Results: Three of the patients carried germline pathogenic or likely pathogenic variants in *EXT1* gene: c.552G>A, c.893G>T, c.963-2A > C, while the remaining patient presented c.461_462del in *EXT2*.

Conclusion: While the number of patients is very small, 3/4 of them carried pathogenic variants in *EXT1*, and only 1/4 in *EXT2*, in agreement with the incidence in the literature. Similarly, in our group, the highest frequency of causative variants in *EXT1* is represented by indels; in our study, 2 missense and one splicesite variants were detected; indels in *EXT2* are the most common variant type for this gene. Two of these variants (c.893G>T, c.963-2A > C) are novel causative variants in HMEs.

C.O.I. Disclosure:

A.C. Tutulan-Cunita: None. **A.G. Pavel:** None. **R.A. Truica:** None. **F.M. Nedelea:** None. **V. Plaiasu:** None. **D. Prepelita:** None. **M. Stratan:** None. **D. Stambouli:** None. **I.O. Focsa:** None.

EP06.024 Synergistic Impact of RUNX2 and MSX1 Mutations on Craniofacial and Dental Development: A Rare Case Report

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Background/Objectives: The RUNX2 and MSX1 genes are critical for craniofacial development, including tooth formation. We report a rare case of compound mutations in these genes to elucidate their genetic impact on skeletal and dental development. A 38-year-old male presented with maxillary hypoplasia, frontal suture synostosis or bone dysplasia, and oligodontia, seeking surgical orthodontic treatment.

Methods: Saliva samples were collected from the patient and family members for genomic DNA extraction. Exome sequencing was performed to investigate the genetic cause. Wild-type and mutant vectors were constructed based on the identified mutations and functionally analyzed using a Dual-Luciferase Reporter Assay.

Results: Exome sequencing identified a novel nonsense variant in *MSX1* gene and a novel missense variant in *RUNX2* gene. Luciferase assays demonstrated that the transcriptional activity of the RUNX2 variant was functionally null.

Conclusion: The patient exhibited craniofacial anomalies and severe tooth agenesis, emphasizing the critical roles of RUNX2 and MSX1 in orofacial development. This case illustrates the synergistic impact of mutations in these genes on skeletal and dental development, providing insights into the genetic etiology of combined skeletal dysplasia and tooth agenesis. Comprehensive genetic and functional analyses are essential for understanding rare hereditary conditions.

C.O.I. Disclosure:

J. Machida: None. **M. Ando:** None. **Y. Sano:** None. **H. Miyachi:** None. **A. Ota:** None. **M. Goto:** None. **Y. Tokita:** None.

EP06.025 A Rare Cause of Disproportionate Short Stature:PAPSS2-related Brachyolmia

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Background: Brachyolmia is an autosomal recessive skeletal dysplasia characterized by short trunk-short stature, platyspondyly, and short lower limbs. Loss-of-function biallelic variants in the PAPSS2 gene associated with Brachyolmia Type 4 (BCYM4/MIM#612847) and may lead to androgen excess by disrupting dehydroepiandrosterone sulfate (DHEAS) synthesis from dehydroepiandrosterone (DHEA). This report presents cases of PAPSS2-related Brachyolmia admitted due to short stature.

Material and methods: The clinical, molecular, and radiological findings of seven individuals with PAPSS2-related Brachyolmia were comprehensively reviewed to elucidate the diagnostic approach. Exome sequencing (ES) was performed in the first three cases, while Sanger sequencing of the PAPSS2 gene was performed in the remaining cases based on elevated DHEA and low DHEAS levels.

Results: All seven cases were from four unrelated families, with consanguineous parents. Clinical examination revealed short stature, short trunk, short lower limbs, increased lumbar lordosis, and brachydactyly. Radiological findings, including squared-off platyspondyly and close-set pedicles, along with early-onset osteoarthritis symptoms in the second decade, were noted. All detected variants were in a homozygous state. The pathogenic variants c.372del, c.1078del, and c.27+3A > C have been previously reported in cases from Türkiye. Additionally, a novel homozygous pathogenic variant, c.1038G>A was identified.

Conclusion: Our study expanded the mutation spectrum of Brachyolmia in cases from Türkiye. Radiological findings such as platyspondyly and overfaced pedicles, combined with low DHEAS levels and the onset of early-onset arthropathy symptoms, may serve as important diagnostic clues for *PAPSS2*-related Brachyolmia.

C.O.I. Disclosure:

G. Koc: None. **E. Karakilic Ozturan:** None. **V. Karaman:** None. **C. Gulec:** None. **T. Kandemir:** None. **F. Bas:** None. **F. Darendeliler:** None. **A. Aslanger:** None. **Z.O. Uyguner:** None.

EP06.026 FKBP14-Related Kyphoscoliotic Ehlers-Danlos Syndrome (FKBP14-kEDS) or Type 2: A New Ehlers-Danlos Phenotype

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Background: Kyphoscoliotic Ehlers-Danlos Syndrome type 2 (EDSKS2) is an autosomal recessive disorder caused by mutations in the *FKBP14* gene (7p14.3), which encodes the FKBP22 protein. This protein interacts with type III and VI collagens. The syndrome is characterized by congenital hypotonia and muscle weakness (typically improving during childhood), progressive scoliosis, joint hypermobility, hyperelastic skin, delayed gross motor development, myopathy, and hearing loss. Here, we report the first case in our region of an adolescent with a homozygous FKBP14 mutation.

Material and methods: A 12-year-old adolescent from was referred for cardiological evaluation. He presented delayed psychomotor development, bilateral congenital hip dislocation, syndromic neuromuscular scoliosis, joint hyperlaxity. He was diagnosed with Desbuquois Dysplasia (DBQD) but it does not

include hearing loss or vascular involvement, which are present in this case. Cardiological, neurological, otolaryngological, ophthalmological, and orthopedic studies were conducted; however, no conclusive diagnosis was reached. Therefore, a genetic study was conducted using Sanger sequencing. Parental testing was also carried out.

Results: A variant in homozygosity for the FKBP14 gene was found, c.362dupC, p.Glu122fs. This mutation results in a cytosine duplication at position 362, leading to a frameshift mutation and it is listed in ClinVar (ID: 279809) as pathogenic and probably pathogenic. This variant found is previously reported in the literature as a recurrent cause of Ehlers-Danlos syndrome and it is found at low frequencies in population databases.

Conclusion: Genetic analysis enables an accurate diagnosis, leading to a more precise multidisciplinary management treatment.

C.O.I. Disclosure:

S. Franco Freire: None. **M.C. González:** None. **M. García De Burgos:** None. **L. Alcaraz Mas:** None. **R. Yahyaoui Macías:** None.

EP06.027 *WNT10A Homozygous Mutation in Odontoonychodermal Dysplasia: A Comprehensive Case Report*

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Background: Odontoonychodermal dysplasia (OODD) is a rare autosomal recessive ectodermal dysplasia syndrome caused by mutations in the ***WNT10A*** gene. It primarily affects ectodermal-derived structures, leading to severe hypodontia or anodontia, nail dystrophy, and dermatological abnormalities such as palmoplantar hyperkeratosis and hyperhidrosis. The ***WNT10A*** gene plays a crucial role in the Wnt signaling pathway, which regulates the development of teeth, nails, and skin.

Material and methods: The patient underwent a comprehensive clinical examination, including dermatological, dental, and oncological assessments. Genetic analysis was performed using whole-exome sequencing (WES) to identify pathogenic variants. DNA was extracted from peripheral blood, and sequencing data were analyzed using bioinformatics pipelines for variant calling, annotation, and interpretation. The identified *WNT10A* mutation (c.490C>T, p.Arg164*) was confirmed via Sanger sequencing.

Results: The patient exhibited congenital hypodontia, progressing to complete anodontia by age 18, along with severe nail dystrophy, palmoplantar hyperkeratosis, and hyperpigmentation. Dermatological findings included multiple actinic keratoses and a history of basal cell carcinoma. WES identified a homozygous nonsense mutation in *WNT10A* (c.490C>T, p.Arg164*), confirming the diagnosis of OODD. Family history revealed parental consanguinity, but no affected relatives.

Conclusion: This case underscores the phenotypic heterogeneity of WNT10A-related ectodermal dysplasias and highlights the importance of genetic testing for diagnosis. While no curative treatment exists, early recognition enables personalized interventions, including dental prosthetics, dermatological management, and cancer surveillance. Future research into WNT10A pathways may offer targeted therapeutic opportunities for OODD.

C.O.I. Disclosure:

A. Subasioglu: None. **R. Gasimli:** None.

EP06.028 whole-exome sequencing identified a novel mutation of the SMARCA4 gene causing coffin-siris syndrome 4

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Background: Coffin-Siris syndrome 4 (CSS4) is a rare autosomal dominant disorder associated with mutations in the SMARCA4 gene, a key component of the SWI/SNF chromatin remodeling complex. CSS4 is characterized by intellectual disability, coarse facial features, microcephaly, hypoplastic nails, and skeletal abnormalities. Here, we report a novel SMARCA4 mutation in a patient presenting with CSS4-related phenotypic features, identified through whole-exome sequencing (WES).

Material and methods: A 15-month-old female patient presented with global developmental delay, microcephaly, hypotonia, feeding difficulties, and dysmorphic facial features. WES was performed, and variant classification was conducted based on ACMG guidelines. In silico pathogenicity prediction tools (REVEL, SIFT, PROVEAN) were used to assess the functional impact. Segregation analysis was conducted using parental samples.

Results: WES identified a heterozygous missense mutation in the SMARCA4 gene (c.2815T>G, p.Phe939Val). This variant was absent in population databases (gnomAD, ExAC) and was not detected in the parents, indicating a de novo origin. Computational modeling predicted a damaging effect on SMARCA4 protein function, supporting its pathogenicity. Given the patient's clinical presentation and genotype-phenotype correlation, the variant was classified as likely pathogenic.

Conclusion: This study reports a novel SMARCA4 mutation associated with CSS4, expanding the genotypic spectrum of the disorder. These findings reinforce the diagnostic value of WES in rare genetic syndromes and emphasize the need for further functional studies to understand SMARCA4-related disease mechanisms and therapeutic implications.

C.O.I. Disclosure:

B. Saglam Ada: None. **&.M. Tekin:** None. **E. Kılıç:** None.

EP06.029 Intrafamilial variability in Type II Collagenopathies

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Background: Type II collagen is the primary collagen synthesized by chondrocytes. This same type of collagen is also found in the vitreous. *COL2A1* gene variants are associated with autosomal dominant type II collagenopathies, presenting a wide range of clinical manifestations, from congenital spondyloepiphyseal dysplasia, Stickler syndrome type I, predominantly ocular non-syndromic Stickler syndrome, rhegmatogenous retinal detachment, osteoarthritis with mild chondrodysplasia, avascular necrosis of the femoral head, among others.

Materials and methods: A male newborn was admitted to intensive care from birth due to respiratory failure caused by Pierre Robin sequence. Clinical studies were performed, including karyotype and CGH Array. Molecular biology NGS was carried out on genes associated with Pierre Robin sequence and craniofacial abnormalities. All these studies were performed on his family.

Results: The patient presented retrognathia, mandibular distractors, and a cleft palate. A peak acoustic test showed moderate acoustic impairment. The phenotype was consistent with Stickler syndrome type I. Karyotype analysis revealed a pericentric inversion of chromosome 12: inv(12)(q21.3q24.31). The CGH array 180k detected a 1.9 Mb duplication at cytoband

10q11.22, identified as a variant uncertain clinical significance (VUS). NGS for Pierre Robin sequence identified a VUS variant, c.1527+6G > A in the *COL2A1* gene in heterozygosity. It was inherited paternally, having his father, his uncle and his paternal grandfather the same variant, presenting a heterogeneous phenotype.

Conclusion: considerable inter- and intrafamilial variability in the clinical expression of type II collagenopathies is described. It is challenging to predict the severity of the phenotype based solely on the *COL2A1* mutation genotype.

C.O.I. Disclosure:

J. Porta Pelayo: None. **S. Franco Freire:** None. **M.C. González:** None. **M. Benito López:** None. **F. Mérida de la Torre:** None. **R. Yahyaoui Macías:** None.

EP06.030 Identification of Recurrent Mutations in Xeroderma Pigmentosum Genes in Morocco: A Molecular Genetic Study

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Background: Xeroderma pigmentosum (XP) is a rare autosomal recessive disorder characterized by photosensitivity, early-onset skin cancers, and ocular lesions. The underlying genetic defects are assigned to seven complementation groups—XP-A to XP-G—and one variant form (XPV). While XPV affects the translesional DNA polymerase η, the genes involved in the seven complementation groups are all part of the nucleotide excision repair (NER) pathway.

Material and methods: This study included 13 Moroccan XP patients. Comprehensive clinical evaluations and pedigree analyses were performed. DNA was extracted from peripheral blood samples, and targeted sequencing of exons 9 and 6 of the XPC and XPA genes, respectively, was conducted using Sanger sequencing. Identified variants were compared with reference sequences and existing variants databases.

Results: Among the 13 patients, 9 (70%) were consanguineous. The North African founder variant p.Val548Alafs*25 in the XPC gene was detected in 10 patients, while the p.Arg228 founder variant in the XPA gene was identified in one patient. These findings emphasize the predominance of XPC gene variants in the Moroccan population.

Conclusion: This study highlights the genetic heterogeneity of XP and the importance of identifying recurrent mutations in specific populations. These findings are crucial for the development of targeted diagnostic tools, genetic counseling, and early interventions.

C.O.I. Disclosure:

L. Soufian: None. **M. El Qabli:** None. **E. Salman:** None. **M. Mansouri:** None. **F. Bouzid:** None. **K. Dafir:** None. **H. Akallakh:** None. **O. Hocar:** None. **S. Amal:** None. **N. Aboussair:** None.

EP06.031 The spectrum of genetic causes of common hereditary ichthyoses in the Russian Federation

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Background: Ichthyoses (I) present heterogeneous group of genodermatoses, characterized by general skin dryness and exfoliation disorder. Autosomal dominant vulgar (IV) and X-linked recessive (XRI) ichthyoses refer to common ichthyoses and the most frequent monogenic genodermatoses. IV occurs in 1:250 ÷ 1000 individuals, X-linked in 1:2000 ÷ 5000 boys. The cause of IV is heterozygous or biallelic variants in the *FLG* gene. The variants p.R501* and c.2282del4 are the most frequent. The cause of XRI is hemizygous variants or deletions of the *STS* gene. Deletion of approximately 2 Mb encompassing the *STS* gene is the most common cause of XRI. The common I genetic causes spectrum in Russia has not been studied before.

Material and methods: 100 non-related families with common I were included into the study. Clinical examination, PDAF analysis to search for *STS* gene deletions and Sanger sequencing to search for *FLG* variants were performed.

Results: Frequent *FLG* variants p.R501*and c.2282del4 were found in 31/100 (31%) of the probands, while the other rarer *FLG* variants in 4/100 (4%). Xp22.31 region deletions captured the *STS* gene was found in 32/100 (32%). In 34 families, the genetic cause was not determined.

Conclusion: In most probands (67/100, 67%), the diagnosis of I was confirmed, contribution of the genes *FLG* and *STS* was equal and made 35 and 32%. The clinical manifestations of IV and XRI may overlap. In 11/100, (11%) molecular diagnosis changed the referring diagnosis of IV to XRI. In boys with I genetic analysis should be started with the *STS* gene deletion search.

C.O.I. Disclosure:

T. Vasilyeva: None. **O. Lenina:** None. **S. Pankratova:** None. **A. Marakhonov:** None. **R. Zinchenko:** None.

EP06.032 A novel heterozygous variant in the*ALDH18A1*gene in a Russian patient with referral diagnosis of heritable connective tissue disorder

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Background/Objectives: Heritable disorders of connective tissue (HDCT) are a group of highly diverse and overlapping conditions for which differential diagnosis is difficult, yet crucial for the correct stratification of cardiac and surgical risks. We report a case of suspected HDCT where whole exome sequencing (WES) helped establish the definitive diagnosis and correctly manage the patient.

Material and methods: A 53-year-old female with referral diagnosis of HDCT underwent genetic counselling, tandem mass spectrometry assay for acylcarnitines and amino acids (MS/MS), and genetic testing by WES.

Results: The proband presented with soft skin, atrophic scars, easy bruising, skeletal abnormalities, high palate, dental crowding, gingival recession. We observed limited extension of her upper extremity joints and hypermobility of other joints, muscular wasting in the proximal extremities. Medical history included mitral valve prolapse and umbilical hernia. The results of MS/MS analysis were normal. WES identified a novel heterozygous c.1160A>G (p.Glu387Gly) variant in the *ALDH18A1* gene linked to several forms of cutis laxa and spastic paraparesis/paraplegia through amino acid synthesis impairment. The genotype-phenotype correlations for *ALDH18A1* are understudied; in our proband, additional examinations revealed cataract and gastro-esophageal reflux supporting the final diagnosis of autosomal dominant (AD) *ALDH18A1*-spastic paraplegia. Subsequently, the

patient was referred to neurologist and symptomatic therapy was promptly initiated.

Conclusion: This case of AD *ALDH18A1*-spastic paraplegia with atypically prominent HDCT signs expands the known phenotypic spectrum of *ALDH18A1*-related disorders, sheds additional light to the HDCT heterogeneity in Russian patients and underscores the significance of genetic analysis for precise diagnosis of suspected HDCT cases.

C.O.I. Disclosure:

E. Baranovskaya: None. **A. Bukaeva:** None. **V. Rumyantseva:** None. **A. Ershova:** None. **A. Meshkov:** None.

EP06.033 First report of posterior reversible encephalopathy syndrome (PRES) in a patient with *CA2*-related osteopetrosis

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Abstract: Osteopetrosis, autosomal recessive 3 (OP-AR3), also known as Carbonic anhydrase II (CA2) deficiency, is characterized by impaired osteoclast-mediated bone resorption and renal tubular acidosis (RTA) caused by biallelic *CA2* gene pathogenic variants. Neurological manifestations in OP-AR3 are limited to cranial nerve entrapments, metabolic disturbances and developmental delay. Posterior reversible encephalopathy syndrome (PRES) is generally associated with hypertension, renal dysfunction, or autoimmune diseases, but it has never been reported in context with *CA2* deficiency. Case: We report a 10-year-old girl diagnosed with RTA and mild neurodevelopmental delay, who had been followed with stable renal function, well-managed electrolyte levels and blood pressure within normal ranges. Despite the absence of electrolyte decompensation or renal deterioration, she experienced an episode of acute clinical deterioration characterized by neurological symptoms in the absence of infection. Neuroimaging confirmed PRES, and additional findings of basal ganglia calcifications led to genetic evaluation. Subsequent skeletal imaging with osteosclerosis and homozygote *CA2*(NM_000067.3):c.82C>T, p.Gln28* pathogenic variant confirmed OP-AR3.

Results: This case represents the first reported occurrence of PRES in a patient with OP-AR3. While PRES is typically associated with hypertension, autoimmunity or electrolyte imbalances, its presence in this context is unprecedented. The coexistence of basal ganglia calcifications raises further questions regarding underlying neurovascular dysfunction in OP-AR3.

Conclusion: This case highlights a novel association between OP-AR3 and PRES. While the precise mechanism remains unclear, potential vascular contributions warrant further investigation. In patients with genetic disorders, atypical neurological presentations should be considered, even when conventional risk factors appear well-managed or stable.

C.O.I. Disclosure:

E. susam: None.

EP06.034 Unravelling the Rare_First Polish Cases of Classical-like EDS Linked to TNXB Variants

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Background: Ehlers-Danlos syndrome (EDS) is a group of hereditary connective tissue disorders caused by pathogenic variants in genes that encode structural proteins critical for connective tissue integrity, including various types of collagen, proteins involved in collagen metabolism and molecules that participate in maintaining connective tissue stability. Classical-like EDS (clEDS) is an extremely rare autosomal recessive subtype associated with homozygous TNXB mutations, with fewer than 100 cases reported worldwide. It is characterised by hyperextensible skin, generalised joint hypermobility, and easy bruising. This study reports the first diagnosed Polish cases, underlining the significance of genetic diagnostics in clEDS identification.

Material and methods: The study included three patients fulfilling the Ehlers-Danlos Society criteria for clEDS and their immediate family members. Genomic DNA was extracted from peripheral blood samples. Next-generation sequencing (NGS) of connective tissue-related genes was performed, followed by Sanger sequencing for variant confirmation. MLPA was conducted to detect possible copy number variations. Family members were examined to confirm carrier status and validate the autosomal recessive inheritance pattern.

Results: Genetic analysis identified homozygous or compound heterozygous TNXB variants in all three patients. Segregation analysis confirmed the origin of these variants from the parents, supporting an autosomal recessive inheritance model. MLPA detected no large deletions or duplications.

Conclusion: This study presents the first Polish cases of clEDS, demonstrating the importance of NGS, Sanger sequencing, and MLPA in achieving precise molecular diagnosis. Early genetic screening is essential for recognising this rare disorder, allowing timely diagnosis and improved clinical management.

C.O.I. Disclosure:

A. Junkiert-Czarnecka: None. **M. Pilarska-Deltow:** None. **O. Haus:** None.

EP06.035 Presentation of a series of clinical cases of acromesomelic dysplasia, Maroteaux type, with a novel homozygous variant in the *NPR2* gene

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Background: Acromesomelic dysplasia Maroteaux type (AMDM, MIM: 602875) is a rare autosomal recessive skeletal disorder affecting both the axial and appendicular skeleton, with an estimated prevalence of 1 in 1,000,000 births. The condition is caused by a loss-of-function mutation in the *NPR2* gene, which encodes the natriuretic peptide receptor B (NPR-B) in the chondrocyte layers of the growth plate.

Material and methods: We studied 12 pediatric patients from 10 unrelated families in the Republic of Tyva, all diagnosed with AMDM. Our investigation focused on the c.125_126insTGGCG (p.Trp42CysfsTer12) mutation in *NPR2*. Additionally, we performed newborn screening using 1,000 dried blood spots to estimate the mutation's frequency in the general population.

Results: Two families, initially diagnosed with an unspecified skeletal dysplasia resembling achondroplasia, tested negative for achondroplasia mutations. However, whole exome sequencing identified a homozygous five-nucleotide insertion in exon 1 of *NPR2* (c.125_126insTGGCG), resulting in a frameshift and a premature stop codon (p.Trp42CysfsTer12), confirming AMDM. Given the families' origin in Tyva, we hypothesized that this allele might be common in the Tuvan population. Newborn screening revealed a carrier frequency of 0.00876 (95% CI: 0.0045–0.0130), or

1 in 114 (95% CI: 1 in 222-1 in 77), indicating a high carrier rate. The clinical and radiological features of AMDM in 12 Tyvan patients revealed both intra-familial and inter-familial phenotypic variability, as well as age-related disease progression.

Conclusion: The genetic heterogeneity and phenotypic diversity of skeletal dysplasias complicate diagnosis. Differential diagnosis and mass parallel sequencing are critical for accurate diagnosis and management.

C.O.I. Disclosure:

T.V. Markova: None. **V.M. Kenis:** None. **O.S. Omzar:** None. **A.O. Dulush:** None. **T.S. Nagornova:** None. **E.Y. Zakharova:** None.

EP06.036 Unveiling the phenotypic spectrum of spondyloepimetaphyseal dysplasia, Isidor-Toutain type associated with novel variants in the RPL13 gene

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Background: Spondyloepimetaphyseal dysplasia Isidor-Toutain type (SEMD-IST) is a rare skeletal dysplasia caused by heterozygous variants in the *RPL13* gene. To date, only a few cases of SEMD-IST have been reported in the literature. This study aims to expand the molecular and clinical understanding of SEMD-IST associated with novel variants in RPL13 gene.

Material and methods: Three unrelated pediatric patients underwent clinical and radiological examinations. Trio WGS was conducted for two families, and WES for one patient. RNA extracted from a patient's blood sample was analyzed using RT-PCR, followed by Sanger sequencing.

Results: Three heterozygous variants were identified in the *RPL13* gene: two novel variants, c.477+5G > C and c.539_541del (p.Ala180_Ser181delinsGly), and one previously reported variant, c.477+1G > C. RNA analysis demonstrated that c.477+5G > C leads to an extension of exon 5 by 54 nucleotides, resulting in an 18-amino acid insertion. The patients' phenotypes with splice variants were mild, characterized by normal growth, genu varum deformity, and progressive Blount-like changes in the tibia that developed after the onset of walking. Over time, gait abnormalities and lower limb pain due to Perthes-like fragmentation of the femoral epiphyses and bilateral coxa vara appeared. In contrast, the c.539_541del variant was associated with a classic severe phenotype, characterized by disproportionate short stature (−3.75 SD), progressive scoliosis, and genu valgum that developed by age 9.

Conclusion: This study expands the clinical spectrum of SEMD-IST, demonstrating phenotypic variability from mild to severe. It was shown that the novel variant c.477+5G > C causes an 18 amino acids insertion consistent with other 5 intron splice variants previously reported.

C.O.I. Disclosure:

D. Gorodilova: None. **V. Kenis:** None. **D. Akimova:** None. **M. Bulakh:** None. **O. Shatokhina:** None. **A. Orlova:** None. **M. Skoblov:** None. **T. Markova:** None.

EP06.037 Association between telomere length and phenotype of frailty and sarcopenia in older adults

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Background: Telomere length (TL) shortening has been identified as a marker of aging, but evidence of its association with sarcopenia and frailty (S&F) is inconclusive. The aim of this study was to identify the association between TL and phenotype of S&F in older adults.

Material and methods: We examined 197 seniors (aged 82.2 ± 7.6 years) that were classified as non-S&F controls (75 community-dwelling adults, aged 80.2 ± 7.5), and 121 S&F patients (aged 85.3 ± 6.7) according to Fried's criteria and diagnostic criteria of the European Working Group on Sarcopenia in Older People. Phenotypic data were collected via questionnaires, scales (Physical Activity Scale for Elderly (PASE), etc.), and testing of anthropometric-physiological characteristics. Leucocyte relative TL was determined by qRT-PCR on DNA extracted from blood samples and quantified as the telomere/single-copy gene (T/S) ratio.

Results: The average relative TL was 0.408 T/S units for all participants, and TL was shorter in S&F than in non-S&F (0.345 vs. 0.469; $p = 0.002$). A significant interaction was found between age, physical activity and TL: with each year the TL shortening and PASE score decreases ($\beta = -6.52$, 95% CI [-10.56, −2.49], $p = 0.0018$). In the S&F group significant association was found between TL and lower muscle oxygenation ($\beta = -2.27$, 95% CI [-2.27, −0.19], $p = 0.02$).

Conclusion: Our findings indicate a significant correlation between shorter telomeres and greater manifestations of S&F, suggesting that TL may serve as a biomarker for these age-related conditions. A deeper understanding of this association could inform interventions aimed at improving the quality of life in the aging population.

C.O.I. Disclosure:

L. Jurkūnaitė: None. **V. Ginevičienė:** None. **E. Pranckevičienė:** None. **A. Urnikytė:** None. **R. Dadelienė:** None. **J. Kilaitė:** None. **I.E. Jamontaitė:** None. **A. Mastavičiūtė:** None. **I.I. Ahmetov:** None. **V. Alekna:** None.

EP06.038 A rare mechanism of Marfan syndrome: insertion of an Alu element causing a frameshift in FBN1

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Background: Marfan syndrome is a systemic connective tissue disorder usually caused by FBN1 variants. Major features include tall stature, ectopia lentis and thoracic aortic aneurysm. Missense variants are most frequently encountered, followed by frameshift, nonsense and splice-site variants. We present a 10-year-old boy

with typical features of Marfan syndrome in whom primary FBN1 analysis indicated an uncertain structural rearrangement, later confirmed to arise from an Alu element insertion.

Material and methods: Detailed clinical evaluation and records review were completed by clinical geneticists. Cardiac phenotyping undertaken by a paediatric cardiologist included serial measurement of aortic diameter by transthoracic echocardiography, normalized for body surface area. Initial FBN1 Sanger sequencing followed by next-generation sequencing with a bioinformatic analysis pipeline including the mobile element insertion (MEI) detection tools, Mobster and Scramble, was undertaken to characterise a rare variant.

Results: The patient has tall, slim stature, myopia, bilateral lens dislocation, pectus excavatum, severe bilateral planovalgus feet and aortic root dilatation, requiring treatment with Losartan. A de novo heterozygous, likely pathogenic variant, c. 5653_5654ins311 (p.Asp1885Glyfs*76), in FBN1 was characterised as insertion of an Alu retrotransposon element between c.5653 and c.5654. This frameshift variant is predicted to result in functional haploinsufficiency of FBN1. Karyotype and array CGH were normal, excluding larger rearrangements.

Conclusion: Alu retrotransposition forms the basis of Marfan syndrome in this patient. This unusual pathogenic mechanism, only reported once before to our knowledge, enriches the FBN1 mutation spectrum in Marfan syndrome, and adds to the conditions caused by Alu retrotransposition.

C.O.I. Disclosure:

A.N. Kavasoglu: None. **A. Churchill:** None. **A. Kelly:** None. **J. Shortland:** None. **C. Armstrong:** None. **J. Turner:** None. **T. Scott-Adams:** None. **M. Gable:** None. **E. Watson:** None. **S. Smithson:** None.

EP06.039 Analysis of alternative splicing events in lumbar disc degeneration using the RuDDS cohort

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Background: Lumbar disc degeneration (LDD) is a risk factor of back pain - one of the major causes of disability worldwide. LDD is associated with alterations in gene expression profile, which has been characterized quantitatively. No analysis of differential transcript usage (DTU) has been performed previously, despite the evidence of alternative splicing role in spine degeneration. Here we aimed to explore alternative splicing events in LDD.

Material and methods: We analyzed transcriptomes from lumbar discs of 19 conditionally healthy controls (Pfirrmann grades<4) and 50 LDD cases (Pfirrmann grades≥4) from the RuDDS cohort using strand-specific paired-end RNA-sequencing on the DNBSEQ-G400 platform. Transcript abundances quantification was conducted by employing quasi-mapping approach implemented in salmon, followed by DTU analysis using the DRIMSeq package to model transcript usage changes and identify transcripts with significant differences between conditionally healthy and degenerated discs. Transcript- and gene-level results were aggregated and adjusted for multiple testing by Holm-Shaffer procedure as implemented in the stageR package, controlling the overall false discovery rate.

Results: A total of 109 transcripts from 89 genes demonstrated a significant change in their usage between the two conditions analyzed. Twenty genes showed a clear isoform switch. Among the identified genes were *FNDC3B*, which participates in regulation of osteoblast differentiation and associated with bone disease and height, *PHACTR2* also related to height and bone density, and *CSNK1E*, which is an experimental drug target for various disorders including bone disease.

Conclusion: LDD is accompanied by alternative splicing events. DTU may contribute to LDD by affecting osteoblast differentiation.

C.O.I. Disclosure:

D.E. Maslov: None. **A.A. Berdnikova:** None. **Y.A. Tsepilov:** None. **E.E. Elgaeva:** None.

EP06.040 Genome-wide methylation signature of relative telomere length in a cohort of Lithuanian elderly sarcopenia and frailty patients

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Background: Unhealthy lifestyle affects telomere shortening rate and in turn pace of aging and disease risk. We analyzed the relative telomere length (TL) and genome-wide methylation in a cohort of Lithuanian elderly with sarcopenia and frailty.

Material and methods: Methylation of a total of 62 subjects (43 females aged 85.5 ± 6.1 and 19 males aged 82.9 ± 8.8) diagnosed by both: sarcopenia and frailty were profiled by Illumina Infinium EPICv2 arrays. Leucocyte relative telomere length (TL) was determined by real-time qPCR on DNA extracted from blood samples and quantified as the telomere/single-copy gene (T/S) ratio. Phenotypic data were collected via the geriatric assessment tools and anthropometric and physiological testing. Quality control and filtering of the raw methylation data was performed using R packages 'sesame' (v1.24.0) and 'DMRcate' (v3.2.1). Gene-associated traits were collected from NHGRI-EBI GWAS registry.

Results: No association was found between DNA methylation levels and age or gender. The TL was associated with 49 differentially methylated CpG probes at 95% confidence level (FDR < 0.05) covering 7 genetic loci overlapping *FPGT, LRRIQ3, TNFSF9, ZDHHC14, LINC00240, MPL* and *SH3RF3* genes and regulatory regions. These genes conjointly associated with patients phenotypes: smoking, alcohol consumption, educational attainment, depressive symptoms, BMI, body fat mass, bone mineral density.

Conclusion: We found significant association between the TL and methylation levels in genes associated with lifestyle traits potentially linked to the risk of developing sarcopenia and frailty in Lithuanian elderly.

Funding: This project has received funding from the Research Council of Lithuania (LMTLT), agreement No S-MIP-22-36.

C.O.I. Disclosure:

E. Pranckeviciene: None. **V. Gineviciene:** None. **A. Urnikyte:** None. **L. Jurkunaite:** None. **G. Anikeviciute:** None. **R. Dadeliene:** None. **J. Kilaite:** None. **I. Jamontaite:** None. **A. Mastaviciute:** None. **V. Alekna:** None. **I. Ahmetov:** None.

EP06.041 Collagen genetic variations contribute to large clinical spectrum of Bulgarian patients

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Background: There are 29 distinct types of collagens playing essential role in maintaining the integrity of various tissues and having other important functions. Mutations in collagens can cause wide spectrum of diseases. We explored the diversity of genetic variations in collagens and patients' phenotypes referred to our lab.

Material and methods: 80 patients were referred to Genome Diagnostic Laboratory for genetic diagnosis through clinical/whole exome sequencing. Pathogenic/likely pathogenic variants and/or variants with unknown significance (VUS) in various collagen genes were found, according to ClinVar/ACMG.

Results: The most abundant group of patients (55%) with collagen variants had renal diseases (Alport syndrome, hematuria, nephritis, nephrotic syndrome, chronic renal failure). We found pathogenic variants (PVs) most frequently in *COL4A5*, *COL4A4* and *COL4A3*.The second most common group, (23.75%) was with connective tissue and skeletal syndromes (such as Marfan syndrome, osteogenesis imperfecta) with variants in 9 different collagen genes found, mostly in *COL1A1*. Seven patients with Usher syndrome, retinitis pigmentosa and cataract had PVs in *COL17A1*, *COL5A2* and VUS in 3 other collagens.Totally 9 cases with myopathy, muscular dystrophy, mental retardation with ataxia, epilepsy and other neurological diagnosis carried PVs in *COL4A2*, *COL11A1* and VUS in 4 types of collagens.Two prenatal diagnoses were performed due to ultrasound observed micrognathia and variants in COL2A1 and COL11A2 were found.

Conclusion: Collagen mutations were more frequently found in patients with renal and skeletal and connective tissue diseases in our study. Our experience adds further to collagens genetic variations and their important role in pathophysiology of diverse disorders.

C.O.I. Disclosure:

D. Kachakova-Yordanova: None. **K. Kamenarova:** None. **K. Mihova:** None. **M. Georgiev:** None. **O. Beltcheva:** None. **N. Ivanova:** None. **G. Zlatanova:** None. **M. Dikova:** None. **N. Veleva:** None. **I. Tournev:** None. **I. Litvinenko:** None. **V. Bojinova:** None. **D. Avdjieva-Tzavella:** None. **V. Gergelcheva:** None. **I. Dimova:** None. **R. Kaneva:** None.

EP06.042 A novel variant in*COL1A2*gene with autosomal recessive inheritance

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Background: The COL2A1 gene encodes the alpha-1 chain of type II collagen, a key structural component of cartilage and the extracellular matrix. Variants in COL2A1 cause a spectrum of skeletal, orofacial, and ophthalmic phenotypes, ranging from lethal conditions like achondrogenesis type II to milder forms such as early-onset osteoarthritis. Recent studies have identified autosomal recessive COL2A1 mutations associated with skeletal dysplasia.

Methods/Objectives: A male infant, born at 39 weeks via urgent cesarean section for fetal deceleration, was diagnosed with skeletal dysplasia following antenatal ultrasound findings of short upper and lower limbs. His parents were first-degree cousins, with a history of sibling death due to similar skeletal dysplasia. Physical examination revealed dysmorphic features including short neck, retrognathia, small chest, rhizomelic limbs, and redundant skin folds. Skeletal survey confirmed abnormalities.

Results: Molecular testing identified a homozygous missense COL2A1 variant (c.2590G>A, p.Gly864Ser), confirmed through familial segregation analysis. Both parents, heterozygous for the variant, exhibited only mild short stature. Radiographic findings included limb shortening, irregular metaphyses, delayed epiphyseal ossification, short ribs, and abnormal vertebral bodies.

Conclusion: While COL2A1 disorders are typically autosomal dominant, this case supports an autosomal recessive inheritance pattern with incomplete dominance, as homozygous individuals present more severe phenotypes than heterozygous carriers. Further studies are needed to clarify genotype-phenotype correlations and improve clinical management for affected individuals.

C.O.I. Disclosure:

S.H. Alyami: None. **M. Almannai:** None.

EP06.043 Clinical and molecular features of osteogenesis imperfecta: a single centre experience

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Background: Osteogenesis imperfecta (OI) is a collagen related disorder. The genetic etiology in most cases (80–85%) is due to defects in collagen structure and processing, while defects in bone mineralization, collagen modification, and collagen folding and cross-linking can also cause OI. In this study, we aimed to investigate the clinical and molecular findings of a group of OI patients.

Material and methods: The molecular etiology was investigated using exome sequencing and Sanger sequencing. The demographic and clinical findings of patients were reviewed, and genotype phenotype correlations were analyzed.

Results: A total of 110 patients with OI were evaluated, and the genetic etiology was identified in 70 (63.6%). The pathogenic and likely pathogenic variants in the COL1A1 and COL1A2 genes accounted for the majority (59%) of cases, while pathogenic variants in autosomal recessive (AR) OI related genes were present in 41%. Various novel and previously reported variants were detected in COL1A1, COL1A2, FKBP10, SERPINF1, P3H1, IFIH1, CRTAP, PLOD2, BMP1, and TMEM38B genes. Skeletal deformities and fractures in the neonatal period were significantly more common in patients with AR OI related gene mutations ($p < 0.05$). Consanguinity was present in 59% of patients with a confirmed genetic etiology.

Conclusion: OI is genetically heterogeneous, with new gene mutations continuously being identified. Autosomal recessive OI related genes may be more frequently observed in populations with high rates of consanguinity.

C.O.I. Disclosure:

P.O. Simsek-Kiper: None. **T. Dasar:** None. **G. Urel-Demir:** None. **G. Imren:** None. **B. Karaosmanoglu:** None. **Z. Taskiran:** None. **Y. Alanay:** None. **A. Ozon:** None. **N. Gonc:** None. **A. Alikasifoglu:** None. **G. Utine:** None. **K. Boduroglu:** None.

EP06.045 Unraveling the role of a synonymous *SEC24D* variant in aberrant splicing in Cole-Carpenter Syndrome 2

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Background: SEC24D is a key component of the Coat Protein Complex II, primarily involved in the selective sorting and transport of cargo proteins. This function is particularly crucial for the secretion of structural extracellular matrix proteins, including collagens. Biallelic pathogenic variations in the *SEC24D* gene have been associated with Cole-Carpenter Syndrome 2. This study aims to investigate the impact of a synonymous *SEC24D* variant on gene expression.

Material and methods: Genomic DNA was extracted from peripheral blood sample and whole-exome sequencing was performed in the patient. For functional analysis, library preparation was conducted using the TruSeq Stranded mRNA kit and sequenced on the NovaSeqX platform. The data analyzed with Genomics&More platform developed by Phitech.

Results: A 12-year-old male patient was referred to our clinic during the neonatal period due to rhizomelic short stature. At birth, his height was 38 cm (−4.8SDS), and head circumference 33 cm (−0,64SDS). Radiographic imaging revealed signs of previous fractures and deformities in the long bones. Considering the clinical presentation of the patient and the prominent dysmorphic features observed during follow-up, Cole-Carpenter syndrome was considered the primary diagnosis. Whole-exome sequencing identified a homozygous synonymous variant in the *SEC24D* gene (c.2361C>T; p.Asn787=). RNA sequencing, performed to assess the functional impact of this rare synonymous variant not reported in any population database, revealed reduced *SEC24D* gene expression and exon 17 skipping due to splicing disruption.

Conclusion: We identified a novel synonymous *SEC24D* variant in a patient with Cole-Carpenter syndrome 2 and demonstrated its functional impact through RNA sequencing.

C.O.I. Disclosure:

S. Süncak: None. **B. Tüysüz:** None. **R. Yıldırım:** None. **S. Gürsoy:** None. **D. Uludağ Alkaya:** None. **A. Abacı:** None. **E. Özzeybek:** None. **U. Özbek:** None. **Ö. Giray Bozkaya:** None.

EP06.046 From Tendon to Bone: Molecular Profiling of Enthesis Regions

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Background: The enthesis is the specialized tissue at which tendons or ligaments attach to bone, transitioning through distinct morphological zones to accommodate mechanical stress and enable efficient force transmission. Fibrocartilaginous entheses have four distinct zones: tendon, unmineralized fibrocartilage (UFC), mineralized fibrocartilage (MFC), and bone. Enthesis development involves forming both tendon and bone elements, regulated by muscle contraction and Hedgehog signaling. Gli1+ cells contribute to fibrocartilage formation and postnatal mineralization. Although the enthesis shares similarities with growth plate development, it maintains a semi-mineralized state without complete endochondral ossification. Yet, mechanisms establishing postnatal zonation, particularly the unmineralized-to-mineralized transition, and factors preventing full fibrocartilage-to-bone transformation, remain elusive.

Material and methods: Using scRNA-seq, we investigated the dynamics of postnatal Achilles enthesis zonation dynamics. Data analysis employed a specialized pipeline tailored to our research.

Results: In-silico annotation against PanglaoDB identified seven skeletal muscle-related cell populations. We used established gene sets to score each cell's tenogenic and chondrogenic signatures, finding three tenogenic clusters expressing extracellular matrix genes and two chondrocyte clusters expressing mineralization and osteogenic genes. Additionally, we identified a UFC cluster with high tendon and cartilage scores, and an MFC cluster. Single-molecule FISH confirmed the clusters spatially. Interestingly, we observed a transient mineralization spike followed by chondrogenic program resurgence, suggesting a mechanism preventing complete osteogenesis.Gene module analysis identified three key modules governing transcriptional mechanisms, including Runx1, which was previously suggested to have a role in directing chondrocyte-to-osteoblast commitment.

Conclusions: Indeed, limb-mesenchyme Cre-mediated Runx1 knockout showed delayed mineralization and reduced MFC size.

C.O.I. Disclosure:

R. Vinestock: None. **T. Olender:** None. **E. Zelzer:** None.

EP06.047 Expanding the genotypic and phenotypic spectrum of P3H1 related osteogenesis imperfecta

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Background: Osteogenesis Imperfecta (OI) is a genetically heterogeneous disorder with a clinical spectrum ranging from mild, involving few fractures and minimal deformity, to severe, characterized by multiple perinatal fractures. Bi-allelic *P3H1* (OMIM*610339) variants are associated with a severe recessive phenotype. This study reports three cases with phenotypic variability and two novel variants, aiming to expand the phenotypic and genotypic spectrum of *P3H1*-related OI.

Material and methods:A multigene OI panel was performed for genetic analysis. Segregation was analyzed in the families.

Results: Patient 1, a one-year-old girl, presented with multiple fractures and deformities in the neonatal period. A novel homozygous 58 bp exonic deletion, c.2112_2169del (p.Glu708AlafsTer21), was identified in exon 15 of *P3H1*. Her parents were heterozygous carriers. Patient 2, a nine-month-old boy with perinatal fractures, carried compound heterozygous variants: a novel splice site variant, c.1171-2_1171-1delinsC, and a missense variant, c.1224G>C (p.Lys408Arg), previously reported as a VUS in a homozygous state. Segregation confirmed compound heterozygosity. Patient 3, an 11-year-old boy, exhibited a milder phenotype, including a history of few fractures, slightly short stature (SDS:-2.06), and osteoporosis. He was homozygous for the c.446T>G (p.Leu149Arg) variant, reported multiple times in Turkish OI patients. His older brother, sharing the same variant, had severe deformities due to multiple fractures.

Conclusion: This study expands the *P3H1* genotypic spectrum with two novel variants and proposes reclassifying c.1224G>C as likely pathogenic, as it now meets PM3 criteria. It highlights phenotypic variability in patients with the same variant and suggests milder phenotypes may be seen in patients with c.446T>G.

C.O.I. Disclosure:

E. Kindis: None. **E. Avci Durmusalioglu:** None. **E. Eviz:** None.

EP06.048 Non-coding genome in Nail-Patella Syndrome

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Limb malformations are paradigmatic of altered gene regulation in human disease. Nail-Patella Syndrome (NPS) is a rare condition characterized mainly by skeletal defects, glomerulonephritis and glaucoma, with variable expressivity. NPS is caused by the haploinsufficiency or loss-of-function of *LMX1B*, which encodes a transcription factor involved in limb dorsalization, in the renal glomerular filtration barrier and the anterior segment of the eye. The dorsal expression of *LMX1B* in the developing limbs is under the control of *LMX1B* autoregulatory modules (LARMs), which are non-coding regions with a limb-specific enhancer activity. While approximately 95% of NPS individuals carry heterozygous pathogenic variants in the coding regions of *LMX1B*, non-coding alterations explain the diagnosis deadlocks in the remaining cases. NPS due to monoallelic or biallelic alteration in the limb-specific enhancers manifests as isolated skeletal defects of autosomal dominant or recessive mode of inheritance. Here we report on four families affected with NPS due to regulatory anomalies and review the molecular mechanisms involved, beyond the coding genome, including alteration of regulatory sequences or disruption of enhancer-promoter interaction. This work also illustrates the importance of genomic diagnosis for precision medicine and genetic counselling in rare diseases.

C.O.I. Disclosure:

P. Brunelle: None. **A. Jourdain:** None. **S. Audebert-Bellanger:** None. **S. Bouquillon:** None. **V. Cormier-Daire:** None. **D. Lacombe:** None. **A. Molin:** None. **A. Desdoits:** None. **R. Touraine:** None. **J. Van Gils:** None. **F. Escande:** None. **F. Petit:** None.

EP06.049 Efficacy of growth hormone therapy in children with NPR2 and ACAN gene variants: a comparative study

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Background: NPR2 and ACAN gene variants are well recognised causes of idiopathic short stature (ISS). Both genes play a crucial role in growth plate regulation. The study aimed to evaluate and compare the efficacy of growth hormone (GH) therapy in children with NPR2 and ACAN variants.

Material and methods: Next-generation sequencing was performed in ISS children to identify causal NPR2 and ACAN variants. Eligible children met national guidelines for treatment with GH. The main outcome measure was change (Δ) in height SD score (HtSDS). Comparison was made between:• Treated and untreated NPR2/ACAN children• Prepubertal vs. pubertal children receiving GH• Overall NPR2 vs. ACAN patient outcomes.

Results: A total of 16 NPR2 and 17 ACAN children were enrolled. 8/16 NPR2 (6 males, mean age 9.5 ± 3.8 years) and 10/17 ACAN (5 males, mean age 8.2 ± 3.2 years) children received GH (treatment duration: NPR2 3.2 ± 1.7 years, ACAN 5.3 ± 2.2 years). In the untreated subgroups, the mean Δ in HtSDS was +0.07 (NPR2) and −0.23 (ACAN). Children who started treatment at the prepubertal stage had greater height gains (Δ HtSDS; +1.01 in NPR2 and +1.35 in ACAN) than those who started in puberty (Δ HtSDS; +0.37 in NPR2 and + 0.3 in ACAN).Overall, mean height gain in treated children was:• NPR2: + 0.8 ± 0.4 SDS• ACAN + 0.9 ± 0.8 SDS

Conclusion: GH therapy significantly improved linear growth in both NPR2 and ACAN groups compared to the untreated children; with a more pronounced effect in ACAN children. Early initiation of GH was particularly effective in both groups.

C.O.I. Disclosure:

L. Stavber: None. **K. Dovč:** None. **M. Debeljak:** None. **J. Šuput Omladič:** None. **T. Battelino:** None. **P. Kotnik:** None.

EP06.050 Novel RPL13 variant in a 15-year-old male with Spondyloepimetaphyseal dysplasia (SEMD): expanding the genotypic spectrum

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Background: RPL13-related spondyloepimetaphyseal dysplasia

(SEMD-RPL13) is a recently identified skeletal dysplasia characterized by early postnatal growth failure, severe short stature and *genu varum*. Although some patients exhibit incomplete penetrance and are asymptomatic, the majority share these hallmark features. We report a case of a 15-year-old male, affected by SEMD-RPL13 with a novel variant in *RPL13* gene.

Material and methods: Whole exome sequencing (WES) (Illumina) was performed for variant identification. Variant pathogenicity was assessed with in silico tools, population databases, and literature analysis. Sanger sequencing was performed for segregation analysis.

Results: The patient presented with dolichocephaly, slight facial asymmetry, short stature (−5.0 SD), scoliosis, knee pain after exercises, restricted movements, and no evidence of growth hormone deficiency. Radiographic imaging identified a 1.5 cm leg length discrepancy with a shorter left femur. The femoral heads flattened and dipped downward, both tibial axes exhibited *varus* deformities. WES revealed a novel likely pathogenic heterozygous missense variant NM_000977.4(RPL13):c.533C>A (NP_000968.2:p.(Ala178Asp)), which was not present in the mother (the father is deceased). This variant is located in a key domain, encoding the C-terminal end of the eL13 protein, responsible for ribosome assembly.

Conclusion: To date, three splice-site, one frameshift and six missense variants related to SEMD-RPL13 phenotype have been reported. Notably, all known variants cluster in a specific region, suggesting it's importance for eL13 function in skeletal tissues, which is critical for understanding the underlying mechanisms of associated ribosomopathies. This case further expands the genotypic spectrum of SEMD-RPL13 and supports previously reported phenotypic findings.

C.O.I. Disclosure:

E. Narmontienė: None. **G. Žukauskaitė:** None. **A. Matulevičienė:** None.

EP06.051 Extremly rare type of loeys-dietz syndrome with a homozygous*TGFB3*mutation: case reports of two siblings with severe clinical presentation

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Background: Loeys-Dietz Syndrome Type 5 (LDS5) is a rare autosomal dominant connective tissue disorder caused by pathogenic variants in the *TGFB3* gene. It is characterized by aortic aneurysms, craniofacial dysmorphism, skeletal abnormalities, and systemic involvement. While heterozygous mutations are the primary cause, homozygous mutations are exceptionally rare and have not been extensively documented. We report two siblings, a 3-year-old female and a 10-year-old male, born to consanguineous parents, who both presented with unusual clinical signs including multiple joint dislocations, skeletal involvement, extreme skin laxity in addition to the classical features of LDS5.

Material and methods: Whole exome sequencing (WES) was performed on the Illumina platform using the Qlseq Human Exome Kit, with variant classification based on ACMG 2015 criteria.

Results: Whole exome sequencing revealed a homozygous mutation (c.973C>T, p.Arg325) in the *TGFB3* gene in both siblings, consistent with LDS5. The siblings exhibited a significantly severe phenotype of LDS5, along with novel clinical signs. Interestingly, the heterozygous parents also displayed mild clinical features of LDS5. To date, only a limited number of patients with homozygous mutations have been described in the literature. This report expands the known clinical and genetic spectrum of LDS5 and highlights the importance of WES in atypical cases.

Conclusion: In countries with a high rate of consanguineous marriages, such as Turkey, very rare homozygous forms of autosomal dominant diseases can be observed, potentially leading to previously unrecognized clinical phenotypes.

C.O.I. Disclosure:

N. Kan Menku: None. **T. Sismanlar Eyuboglu:** None. **M. Sari:** None. **G. Gozen:** None. **A. Tana Aslan:** None. **F. Ezgu:** None.

EP06.052 A Novel *TEK* Pathogenic Variant in a Patient with Venous Malformations and Recurrent Pregnancy Loss: A Possible New Phenotypic Association

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The *TEK* gene encodes the TIE2 receptor tyrosine kinase, which plays a crucial role in vascular development and endothelial cell function. Germline *TEK* variants are known to cause autosomal dominant venous malformations, but their role in recurrent pregnancy loss has not been previously documented.

A 47-year-old woman with multiple VMs presented for genetic evaluation. She first underwent surgery for an unrelated reason, but during intubation, significant bleeding occurred due to a VM in her larynx. MRI revealed extensive VMs affecting her larynx, lips, arms, fingers, face, feet, and the orbital region. She also had a history of multiple unexplained pregnancy losses. In this patient with multiple venous malformations, a *TEK* variant known to cause these types of manifestations (c.2752C>T, p.Arg918Cys) was identified.

TIE2 signaling is critical for vascular stability, angiogenesis, and endothelial integrity. Disruptions in this pathway can lead to abnormal vessel formation and increased vascular permeability. In pregnancy, proper vascular remodeling is essential for placental function, and impaired angiogenesis could contribute to recurrent miscarriage. This case suggests a possible novel phenotype associated with TEK mutations, expanding the known clinical spectrum of *TEK*-related disease. Further studies are needed to investigate the role of *TEK* in reproductive health and pregnancy outcomes. This case underscores the importance of considering *TEK* in patients with unexplained pregnancy loss, particularly in the presence of vascular anomalies.

C.O.I. Disclosure:

B. Ergin: None. **A.M. Elitok:** None. **G. Kökcü:** None. **T. Tuncalı:** None.

EP06.053 Value of genetic testing in differential diagnostics of syndromic aortic diseases in postpartum period

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Background: Epidemiological data on the prevalence of acute aortic dissection in different populations are limited. The incidence of aortic dissertation ranges from 0.2% to 0.8% of cases. In women with hereditary aortopathies, pregnancy is associated with an increased likelihood of acute aortic events, with the highest risk of dissection occurring from the 3rd trimester to 3 months postpartum.

Material and methods: Genetic testing for probands was performed by whole exome sequencing (WES) with NextSeq 550Dx instrument (Illumina platform). Candidate variants validation and cascade familial screening were performed by capillary Sanger sequencing. Pathogenicity assessment was performed according to current ClinGen recommendations.

Results: Two women, both aged of 20, were diagnosed with postpartum aortic aneurism after their first pregnancies. No aortic pathology was detected at the routine checkup during pregnancy in both probands. First proband died suddenly in 48 hours after cesarian section due to acute aortic dissection (two ruptures in descending thoracic aorta, and abdominal aorta). Vascular type of Ehlers-Danlos syndrome was confirmed postmortem by detection of heterozygous nonsense mutation c.3496C>T (p.Arg1166*) in the COL3A1 gene. Second proband delivered healthy male twins by cesarian section. After childbirth, she noticed the progressive shortness of breath, but did not require investigation. In 1 year after delivery giant thoracic aneurism was detected. Genetic testing revealed a Likely Pathogenic variant c.1460G>A (p.Arg487Gln) in the TGFBR1 gene described in Loyes-Ditz syndrome.

Conclusion: Patients with syndromic aortic diseases require lifelong careful monitoring. Genetic testing allow diagnosis specification to provide gene-specific follow-up and recommendation, and cascade familial screening.

C.O.I. Disclosure:

E.V. Zaklyazminskaya: None. **M.A. Sadekova:** None. **I.O. Islanov:** None. **V.V. Aminov:** None. **R.V. Deev:** None. **L.M. Mikhaleva:** None.

EP07 Cardiovascular Disorders

EP07.001 Genetic Background and Clinical Phenotype in an Italian Cohort with Inherited Arrhythmia Syndromes and Arrhythmogenic Cardiomyopathy (ACM): A Whole-Exome Sequencing Study

Alessandra Ranaldi[1], *Sara Cannito*[1], *Francesco Santoro*[2], *Ilaria Ragnatela*[2], *Rosa Santacroce*[1], *Alessandra Margaglione*[2], *Giovanna D'andrea*[1], *Natale Brunetti*[2], *Maurizio Margaglione*[1], *Maria d'Apolito*[1]

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Background: Inherited arrhythmia syndromes include several different diseases, as well as long QT syndrome (LQTS), Brugada syndrome (BrS), catecholaminergic polymorphic ventricular tachycardia (CPVT), short QT syndrome (SQTS), Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C). Most arrhythmia syndromes areinherited in an autosomal dominant manner and genetic studies are suggested. The purpose of this study was to report the spectrum of genetic variations and clinical phenotype in an Italian cohort with confirmed Inherited arrhythmia syndromes using the whole exomesequencing (WES).

Material and methods: 61 patients with confirmed Inherited arrhythmia syndromes were included in this study. Genomic DNA samples were extracted from peripheral blood and conducted for WES. The variants were annotated using BaseSpace Variant Interpreter Annotation Engine 3.15.0.0 (Illumina). Reported variants were investigated using ClinVar and a literature review and classified according to the criteria of the ACMG.

Results: We evaluated NGS data of 61 patients. In terms of clinical diagnosis BrS was the most common disorder in NGS group. Among the 61 NGS patients, 7 had pathogenic or likely pathogenic mutations, 25 had uncertain significance, and 29 patients with clinical diagnosis BrS had no mutation detected.

Conclusion: Our understanding of inherited arrhythmia syndromes continues to advance. The era of next- generation sequencing has advanced rapidly given new genetic evidence including pathogenicity,background genetic noise, and increased detection of variants of uncertain significance. Although, NGS study has some limitations in obtaining the full genetic data of probands, large-scale gene sequencing can ably and promptly be applied in real clinical practices, especially in inherited fatal arrhythmia syndromes.

C.O.I. Disclosure:

A. Ranaldi: None. **S. Cannito:** None. **F. Santoro:** None. **I. Ragnatela:** None. **R. Santacroce:** None. **A. Margaglione:** None. **G. D'andrea:** None. **N. Brunetti:** None. **M. Margaglione:** None. **M. d'Apolito:** None.

EP07.002 Genetic risk of increased left atrial volume in hypertensive patients with atrial fibrillation in Uzbek population

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Aim. Assess the role of polymorphism of the PITX2 gene, polymorphism of the CAV1 gene, and intergenic polymorphism in the risk of increased indexed left atrial volume (ILAV) in hypertensive (HTN) patients with atrial fibrillation (AF) in Uzbek population.

Materials and methods: The study included 89 patients with AF. The control group ($n = 48$) consisted of HTN patients without AF. The diagnosis of AF was based on ECG results and/or Holter ECG data. Genotyping for the presence of allele variants of the studied polymorphisms was performed using the RFLP-PCR method.

Results: The risk of increased ILAV was associated with the presence of C allele of rs6817105 polymorphism of PITX2 gene ($p = 0.001$),the TC genotype ($p = 0.0004$), and the CC genotype ($p = 0.0004$). It was associated with the presence of the C allele of the rs3807990 polymorphic marker of the CAV1 gene ($p = 0.01$) and the C allele of the intergenic rs11264280 polymorphism ($p = 0.04$).

Conclusion: The genetic risk of increased ILAV in HTN patients with AF in Uzbek population was associated with the presence of C allele, TC, and CC genotypes of rs6817105 of the PITX2 gene; the C allele of the rs3807990 polymorphic marker of the CAV1 gene; and the C allele of the intergenic rs11264280 polymorphism.

C.O.I. Disclosure:

K. Yusupova: None. **G. Abdullaeva:** None. **G. Radjabova:** None. **D. Zakirova:** None. **M. Pulatova:** None. **Z. Mashkurova:** None. **N. Ibrohimov:** None. **A. Abdullaev:** None.

EP07.003 Genetic determinants of clinical and morphofunctional changes in cardiac parameters in patients of the Uzbek population with arterial hypertension complicated by atrial fibrillation

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Background: Arterial hypertension (AH) complicated by atrial fibrillation (AF) is a common cause of adverse cardiovascular outcomes. Aim of the current research study was to illustrate the associations of rs3807990 and rs11773845 polymorphisms with LA remodeling parameters and interactions of genes.

Material and methods: Study included 200 patients with AH and associated AF. A PCR method was used for genotyping. Echocardiography was carried out to determine LA index (LAI) and LA volume index (LAVI). Genotypic associations for the risk of LA remodeling were measured by means of logistic regression analysis; gene-gene interactions were also determined. A risk of LA fibrosis was considered above the sample mean of LAI.

Results: Comparing the two mentioned genotypes, patients with rs3807990 CC showed a higher LAI of 6.34 ± 6.39 and LAVI of 89.13 ± 19.45 compared to TT (LAI: 5.35 ± 0.78, LAVI: 65.60 ± 22.91). The LAI was increased in the rs11773845 AC genotype at 5.51 ± 0.69, without significant difference over the CC ($P = 0.960$). Gene interaction suggested an increased risk for fibrosis, especially in the combination of rs3807990 CC with rs11773845 AC, but there was no significant value.

Conclusion: The presence of the CC genotype of rs3807990 predisposes to LA remodeling with unfavorable changes in size and volume, reflecting the tendency to fibrosis. Less marked but still remaining relevant is the effect of the AC genotype of rs11773845. Re-evaluation studies are required for this finding. These results underline significance of genetic testing for personalized management in hypertension and AF.

C.O.I. Disclosure:

Z.T. Mashkurova: None. **G. Abdullaeva:** None. **B. Rozibaev:** None. **N. Sodikova:** None. **A. Norboyev:** None. **G. Radjabova:** None.

EP07.004 Oligogenic contributors in neonatal hypertrophic cardiomyopathy: a familial analysis

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Background: Neonatal hypertrophic cardiomyopathy (HCM) is a rare condition, characterized by rapid myocardial hypertrophy and severe clinical outcomes. While sarcomeric gene variants are the primary cause of HCM, non-sarcomeric gene variants and genetic modifiers have also been associated with congenital heart disease and cardiomyopathy. This study aimed to explore the genetic basis of neonatal HCM in a family with a history of cardiac disease, focusing on the oligogenic contribution of non-sarcomeric variants.

Material and methods: Clinical data and blood samples were utilized for whole genome sequencing (WGS) and bioinformatic analyses. Maternally inherited variants were analyzed, as the proband's mother had also been diagnosed with HCM at birth. Likewise, candidate variants potentially associated with the cardiac phenotype were identified in the maternal grandfather and aunt, both of whom have cardiac disease.

Results: Compound heterozygous *MYO19* variants were identified in the neonate. Maternally inherited missense variants were found in *SURF1* and *ETFDH*, both linked to mitochondrial function. Additional candidate variants potentially contributing to the cardiac phenotype were also identified in the extended maternal family.

Conclusion: This study is the first to link the *MYO19* gene to HCM, highlighting the potential role of oligogenic inheritance in its etiology. Causative variants in other mitochondria-related genes, including *MYO19*, *SURF1*, and *ETFDH*, were identified, with further investigation of family members supporting the pathogenesis of HCM. These findings provide valuable insights into the genetic basis of pediatric HCM.

C.O.I. Disclosure:

H. Cho: None. **J. Kim:** None. **D. Shin:** None. **O. Kim:** None. **J. Jang:** None. **Y. Chang:** None. **B. Kim:** None. **H. Park:** None. **M. Park:** None.

EP07.005 Genetic foundations of dilated cardiomyopathy with ventricular tachycardia in Kazakhstani patients

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Background: Dilated cardiomyopathy is a heart disease characterized by structural and functional changes in the heart muscle. The study of the epidemiology, pathogenesis and prognosis of the disease has been significantly improved by genetic research. The aim of our study was to assess the spectrum of mutations and genotype-phenotype correlation of patients with dilated cardiomyopathy with ventricular tachycardia (DCM VT)

Material and methods: The patients with DCM VT ($n = 32$) were recruited to the study at the UMC Heart center, Astana. The control group was randomized by age, gender, and ethnicity ($n = 60$). Whole exome sequencing was performed following the Illumina DNA Prep with Exome 2.0 Enrichment on NovaSeq6000. Data analysis was carried out using ClinVar, 1000 Genome, PolyPhen2, ExAC, MutationTaster, and others.

Results: In total, 124 genetic variants were identified in the group DCM VT, among which, by frequency of occurrence, the majority of mutations were identified in genes TTN (43.4%), LAMA2 (36.8%), MYBPC3 (.2%), MYH6 (28.1%), KCNQ1 (15.6%), GAA (15.6. Pathogenic (LMNA, TTN, DSG2, KCNQ1 and MYH7) and likely pathogenic variants (DES, CAV3, MYBPC3), according ACMG was detected in 6 patients. Including one patient with two pathogenic (MYH7:F244L, KCNQ1:c.477+1G > A) and two likely pathogenic (DES:R406Q,CAV3:V39L) variants.Compared to the group of patients DCM VT in the control group we found 23 genetic variants: uncertain significance (13%), likely benign (17%), and benign (70%).

Conclusion: In our research most frequently, genetic variants were identified in genes encoding sarcomere (65,7%), ion flux

(19,8%). Pathogenic mutations were prevalent in genes encoding sarcomere.

C.O.I. Disclosure:

S. Rakhimova: None. **Z. Abilova:** None. **A. Akhmetova:** None. **A. Molkenov:** None. **M. Bekbossynova:** None. **U. Kozhamkulov:** None. **A. Akilzhanova:** None.

EP07.006 Two novel likely pathogenic *TTN* variants identified in Bulgarian patients with dilated cardiomyopathy

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Background: Dilated cardiomyopathy (DCM) is the third most common cause of heart failure and the most frequent indication for heart transplantation. Recent studies show that nearly 60% of cases are inherited. The aim of the study was to identify disease-causing genetic variants related to the pathogenesis of DCM in a group of Bulgarian patients using next-generation whole exome sequencing (WES).

Material and methods: Ten unrelated DCM patients were included in this study. Following informed consent, DNA was isolated and WES was conducted, using Illumina Exome Panel and NovaSeq6000 (Illumina). Bioinformatic analysis was performed using Dragen (Illumina) pipeline and VarSeq ver.2.2.1 (Golden Helix). Classification of the variants was according to ACMG criteria.

Results: In two sporadic DCM patients (males, 50 and 39 years old, respectively) novel likely pathogenic variants in the *TTN* gene were detected. Both nonsense variants, c.103017T>G (p. Tyr34339-Ter) in exon 3 and c.12712G>T (p. Glu4238Ter) in exon 48 were not reported in the ClinVar or population databases. Truncating mutations in *TTN* are the most common cause of DCM. Additional 26 VUS in other DCM related genes were found in 9 of the patients.

Conclusion: According to the literature pathogenic truncating variants in the largest gene *TTN* contribute to 25% of the familial and 18% of sporadic DCM cases (Herman et al 2012). In our study they represent 20% of the analyzed samples. The identification of pathogenic variants using WES can help to provide definitive diagnosis for DCM patients, enabling better clinical management, family screening, and genetic counselling.

C.O.I. Disclosure:

K. Vitanova: None. **R. Tzveova:** None. **K. Mihova:** None. **M. Shumkova:** None. **T. Yaneva-Sirakova:** None. **K. Kamenarova:** None. **M. Georgiev:** None. **I. Dimova:** None. **R. Kaneva:** None.

EP07.007 Exploring *RBFOX2* haploinsufficiency : A New Genetic Link to Hypoplastic Left Heart Syndrome

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Hypoplastic left heart syndrome is a congenital malformative syndrome that affects the development of the left heart and is associated with defects in the mitral valve, the aortic valve and/or the ascending aorta. This condition is characterized by significant morbidity and mortality. Currently, several molecular hypotheses are being studied, including involvement of *RBFOX2* as a candidate gene. The role of *RBFOX2* in left heart hypoplasia was hypothesized from mouse models carrying *RBFOX2* loss-of-function variants.

This report presents a cohort of 19 individuals from 12 different families who have loss-of-function variants or heterozygous deletions of *RBFOX2* gene and have congenital heart disease, mainly hypoplastic left heart. A phenotypic spectrum can be observed within a single family, ranging from hypoplasia of the left ventricle to isolated coarctation of the aorta, with non-compaction of the left ventricle. All the deletions or loss-of-function variants described in the various databases were collected.

The objective of this work is to define the phenotype associated with haploinsufficiency of *RBFOX2* gene and to establish genotype/phenotype correlations by studying an initial international cohort of 19 individuals. These findings may enhance genetic diagnostic precision for congenital heart diseases and can guide genetic counselling and prenatal diagnosis.

C.O.I. Disclosure:

C. Sauvestre: None. **A. Bouchatal-berrahou:** None. **C. Beneteau:** None. **V. Michaud:** None. **P. Blanc:** None. **M. Fradin:** None. **P. Bouvagnet:** None. **W.K. Chung:** None. **J. Marcadier:** None. **M. Thomas:** None. **H. Karstensen:** None. **L.C. Ades:** None. **A. Born:** None. **K. Metcalfe:** None. **S. Xiong:** None. **C. Rooryck-thambo:** None.

EP07.008 Polymorphic variant rs2681472 in *ATP2B1* gene show possitive association with susceptibility to coronary artery disease in Bulgarian males

*Reni Stoyova Tzveova[1], Galya Naydenova[2], Teodora Yaneva-Sirakova[3], Silvia Vandeva[4], Petar Atanasov[5], **Diana Pendicheva – Duhlenska**[6], Vanyo Mitev[7], Radka Kaneva[7]*

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of Pharmacology, Faculty of Pharmacy, Medical University – Pleven, Pleven, Bulgaria, [7]Molecular Medicine Center, Department of Medical Chemistry and Biochemistry, Medical Faculty, Medical University – Sofia, Sofia, Bulgaria.

Background: The *ATP2B1* gene encodes isoform 1 of the plasma membrane calcium ATPase, which exports bivalent calcium ions from eukaryotic cells against very high concentration gradients and plays an essential role in intracellular calcium homeostasis. The objective of this research was to investigate the association between rs2681472(*ATP2B1*) and the risk of coronary artery disease(CAD) and myocardial infarction(MI) in the Bulgarians.

Material and methods: A total of 817 subjects were included in the study. Of them, 323 were patients with CAD (199 with MI and 124 without MI), and 494 were population-based controls. Genomic DNA was extracted from venous blood samples. The rs2681472 (*ATP2B1*) was genotyped by TaqManSNP Genotyping Assay. Non-adjusted χ^2-based analysis was applied for evaluation of genotype and allele association with CAD and MI by PLINK 1.07.

Results: The rs2681472 (allele G) was found to be associated with an increased risk of CAD (OR1.40,CI95:1.09–1.82,p = 0.01) and MI (OR1.48,CI 95:0.10–1.99,p = 0.009). In the subgroup analysis by sex, this trend persisted only in men with CAD (OR1.60,CI95:1.14–2.24,p = 0.01), but not in women (OR1.09,CI95:0.71–1.65,p = 0.75). In the group of patients with MI, similar results were observed, as carrying a polymorphic G allele was associated with an increased risk of the disease in males (OR1.87,CI95:1.29–2.72,p = 0.001).

Conclusions: We found a positive sex-dependent association of the rs2681472 allele G with an increased risk of CAD and MI in the male subgroup. The contribution of *ATP2B1* to the risk of developing CAD/MI still remains unclear, especially in this sex-dependent manner, and it hasto be clarified by further genetic and functional analyses.

C.O.I. Disclosure:

R.S. Tzveova: None. **G. Naydenova:** None. **T. Yaneva-Sirakova:** None. **S. Vandeva:** None. **P. Atanasov:** None. **D. Pendicheva – Duhlenska:** None. **V. Mitev:** None. **R. Kaneva:** None.

EP07.009 Single center experience with arrhythmogenic right ventricular cardiomyopathy genetic testing

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Background: Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a life threating rare genetic disorder. During ARVC development, myocytes are replaced with fatty and connective tissue, that leads to ventricular tachycardia or fibrillations and consecutively may lead to cardiac arrest. 50 percent clinically diagnosed ARVC cases are proved to be due to genetic causes. Without genetic testing, about 10 percent cases can have delayed diagnoses. We review our experience with ARVC genetic testing.

Material and methods: Data from pediatric and adult patients referred for suspected genetic cardiac disorders were collected between 2019 and 2024, and genetic testing was performed at The Hospital of Lithuanian University of Health Sciences. We gathered referral diagnoses and selected patients diagnosed with ARVC.

Results: 751 patients were tested, 467 - due to suspected hereditary cardiomyopathy, of whom 10 because of suspected ARVC (average age 35, min 12, max 63; 9 males, 1 female). 2 patients fulfilled clinical diagnostic criteria and genetic testing revealed *PKP2* (NC_000012.11:g.(?_32945215)_(33051265_?)del; whole gene deletion, previously published) and *DSG2* (NM_001943.5:c.1109dup) gene pathogenic variants. 8 cases fulfilled one major ARVC criteria or several minor (5 cases because or right ventricular dyskinesia and dilation, 3 cases - various arrhythmias). In those cases no pathogenic variant or variants of unknown clinical significance were detected.

Conclusion: Our small sample study shows no additional value of genetic testing in ARVC cases when not all clinical criteria are met. Also, we expected more ARCV cases, thus it shows a need of better cardiac patients screening.

C.O.I. Disclosure:

M. Šukys: None. **E. Ereminiene:** None. **I. Nasvytiene:** None. **R. Jonikas:** None. **K. Aleknaviciene:** None. **D. Cereskevicius:** None. **S. Zekiene:** None. **R. Ugenskiene:** None.

EP07.010 Gene expression of endothelial marker VEGFR2 in peripheral blood of patients with type 2 diabetes mellitus

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Background: Studies of circulating endothelial cells and endothelial progenitor cells provide valuable information about pathological events and response to treatment in different conditions with vascular damage, which is the most common complication of diabetes.

Aim: We aimed to analyze in peripheral blood cells the expression of VEGFR2, which is a specific marker for endothelial lineage, in patients with type 2 diabetes mellitus (T2DM).

Materials and methods: Blood samples were collected from healthy controls (with normal glucose tolerance) and diabetic patients, divided according to the duration of diabetes. VEGFR2 expression was examined by real-time PCR using the relative comparison method between patients and controls.

Results: We detected mean relative levels of 1.77 for the expression of this marker in patients compared to controls, without statistically significant difference. In T2DM subgroups with different diseases duration, we found mean VEGFR2 expression levels, respectively, 1.09 in newly diagnosed diabetes, 2.36 in patients with < 5 years of duration and 2.80 in patients with > 5 years of diabetes. Comparison of VEGFR2 expression according to the presence of polyneuropathy showed a statistically significant decrease in expression ($p < 0.05$) in patients with polyneuropathy - 0.33 vs. 2.18 in patients without polyneuropathy.

Conclusion: Endothelial progenitor cells may be an optimal candidate for the treatment of diabetic neuropathy, as they possess paracrine properties with angiogenic and neurotrophic effects. Data from studies of cells from endothelial lineage will open intriguing perspectives in redefining their role in T2DM.

C.O.I. Disclosure:

P. Gateva: None. **I. Bogdanov:** None. **I. Mihaleva:** None. **I. Dimova:** None.

EP07.011 Lipoprotein glomerulopathy (LPG) with APOE Osaka/Kurashiki pathogenic variant in first reported Argentinian patient

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Background: Lipoprotein glomerulopathy (LPG) is a rare autosomal dominant disease caused by mutations in APOE gene which encodes apolipoprotein E. LPG mainly affects Asian individuals, with occasional cases described in Americans and Europeans. Our aim is to report first Argentinian patient with LPG disease and APOE-Osaka/Kurashiki pathogenic variant.

Material and methods: A 49-year-old man sought genetic assessment for renal failure and resistant hypertension. He have several paternal relatives with chronic kidney disease (CKD), dialysis and transplants backgrounds. Patient denied Asian ancestry. Laboratorial tests showed BUN 120 mg/dL, creatinine clearance 40 mL/min/1.73m^2, total cholesterol 397 mg/dL, LDL 249 mg/dL, triglycerides 606 mg/dL, albumin 3.1 g/dL, proteinuria 3.6 g/24 h, apolipoprotein E serum level 72 mg/dL and negative screening for secondary causes of glomerulopathy. His kidney biopsy was consistent with advanced chronic nephropathy secondary to LPG. Genomic DNA was extracted from blood sample and was analyzed by whole exome sequencing (WES) using NovaSeq X.

Results: Genetic analysis revealed APOE pathogenic variant c.527G>C, p.Arg176Pro in heterozygosis, known as the Osaka/Kurashiki mutation and positioned nearby the LDL receptor binding site.

Conclusion: LPG usually manifests in adulthood and predominantly affects males. Hyperlipoproteinemia type III, high levels of plasma apolipoproteins, proteinuria and microhematuria are common findings. Nephrotic syndrome, resistant hypertension and CKD are often observed. According to actual literature this is the first report of Osaka/Kurashiki variant in Argentina. Kidney transplantation and immunosuppressants are not recommended because the underlying pathophysiological mechanisms are abnormal lipoprotein components in blood instead kidney. Cosegregation variant studies in offsprings was required.

C.O.I. Disclosure:

J.M. Ramirez: None. **R. Sardi:** None. **P. Corral:** None. **E.I. Ramirez:** None. **L. Graziani:** None. **M.A. Tenuta:** None. **N.F. Renna:** None.

EP07.012 Investigation of the Genetic Etiology in Cases Presenting with Cardiovascular Disease Phenotype

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Background: Inherited heart diseases encompass a group of phenotypes, including familial cardiomyopathies, arrhythmia syndromes, hypercholesterolemia, aortopathies, and congenital heart diseases. Determining the molecular etiology contributes to planning the clinical management of the disease and identifying at-risk family members. This study aims to investigate the genetic etiology in cases of inherited heart disease.

Material and methods: Study included 46 index cases and 21 family members with cardiac findings to the Medical Genetics Clinic between 2021 and 2024. The index cases were analyzed using Whole Exome Sequencing (WES).

Results: The index cases were divided into two groups: i) cases presenting with arrhythmia, cardiomyopathy, hypercholesterolemia, and aortopathy (38 cases) ii) cases presenting with congenital heart disease (8 cases). First group, genetic diagnosis rates in subgroups were as follows: cardiomyopathies 64% (14/22), arrhythmias 33% (3/9), hypercholesterolemia 100% (4/4), and complex cardiac findings 33% (1/3). Second group, consisting of 8 patients with congenital heart disease, pathogenic/probably pathogenic variants were detected in 4 patients (50%). Segregation analysis were performed for 2 of the 4 patients in this first group, and the variants were found to be de novo.

Conclusion: Literature indicates genetic diagnosis rates in inherited heart diseases can reach up to 40%. Our study, this rate was assessed as 55%, varying among subgroups. Identifying the molecular etiology in patients presenting with cardiac findings is crucial for clarifying the diagnosis, improving clinical management, and providing genetic counseling by identifying at-risk family members.

C.O.I. Disclosure:

A. Cebi: None. **T. Apuhan:** None. **M. Sahin:** None. **N. Senturk:** None. **A. Turkyilmaz:** None.

EP07.013 Investigation of a mutation in *MYH7* causing hypertrophic cardiomyopathy and restrictive cardiomyopathy by whole-exome sequencing

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Background: Hypertrophic cardiomyopathy (HCM) and restrictive cardiomyopathy (RCM) share a similar genetic background, primarily caused by variants in sarcomeric genes. Most patients with RCM and HCM harbor mutations in sarcomeric protein genes, which can lead to alterations in sarcomere structure and ultimately result in heart failure. *MYH7* is one of the sarcomereic genes identified as causative for both HCM and RCM.

Material and methods: Following clinical analysis, cardiac magnetic resonance imaging, and pathology, whole exome sequencing (WES) was performed on a twenty-two-year-old proband diagnosed with HCM and RCM. Variant confirmation and segregation analysis of available family members were conducted using PCR and Sanger sequencing. The *MYH7* gene was also examined for potential causative variants and their previously reported conditions.

Results: A pathogenic homozygous variant, *MYH7* (NM_000257), c.G1750A, p.G584S, was identified in the proband. This variant is likely the primary cause of HCM in this particular family. Confirmation was achieved through Sanger sequencing, and the variant segregated among other affected family members.

Conclusion: HCM and RCM are significant contributors to heart failure. Genetic testing, such as WES, can identify asymptomatic family members at risk. Furthermore, cardiac evaluations appear beneficial for these patients before the onset of heart-related issues.

C.O.I. Disclosure:

M. Pourirahim: None. **M. maleki:** None. **S. Kalaeinia:** None.

EP07.014 Hereditary Hemorrhagic Telangiectasia type 1: New pathogenic variant in the ENG gene

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Background: Hereditary hemorrhagic telangiectasia type 1 (HHT1, OMIM #187300) is an autosomal dominant vascular disorder caused by pathogenic variants in the ENG gene, which encodes endoglin, a co-receptor of the transforming growth factor-beta

(TGF-β) superfamily. This protein, composed of three domains (orphan, pellucid zone, cytosolic), is involved in angiogenesis and platelet adhesion. Affected individuals present arteriovenous malformations in mucocutaneous areas, and soft organs.

Material and methods: We report a 58-year-old female patient with a paternal family history of epistaxis, early-onset stroke, and pulmonary hemorrhage. The patient has hypothyroidism, spontaneous epistaxis since childhood, telangiectases (lips, fingertips), and chronic iron-deficiency anemia for over 30 years. In 2023, she was diagnosed with angiodysplasia in the stomach, duodenum, ileum, and colon. A next-generation sequencing (NGS) panel for HHT was performed.

Results: NGS analysis identified the ENG variant c.531del (p.Ser178Hisfs*44), which is classified as likely pathogenic in the Varsome database (ACMG: PVS1 and PM2) but has not been described in the literature. However, it meets the PM1 pathogenicity criterion as it is located in the critical "orphan" functional domain that enables angiogenesis pathways. The early truncation of endoglin likely explains the patient's recurrent mucocutaneous bleeding and gastrointestinal angiodysplasia. The addition of the PM1 criterion, along with the correlation to clinical manifestations and family history, supports reclassifying this variant as pathogenic.

Conclusion: It is important to suspect HHT in the presence of telangiectases, recurrent epistaxis, and angiodysplasia in the digestive tract for timely diagnosis and treatment.

C.O.I. Disclosure:

L.A. Trujillo-Moreno: None. **L.A. Melo-Porras:** None. **G.A. Contreras-García:** None. **C.I. Vargas-Castellanos:** None.

EP07.015 A Complex Cardiovascular Phenotype Caused by an 8p23.1 Deletion Affecting GATA4 and a RBM20 Splicing Variant: Evidence for a Digenic Disease Model

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Background: Next-generation sequencing (NGS) has revolutionized the identification of digenic and oligogenic contributions to complex phenotypes, where a single-gene mutation might not fully explain the clinical presentation. This approach enables a more comprehensive understanding of genetic interactions underlying cardiovascular disorders.

Material and methods: The patient, a 30-year-old female, presented with a complex cardiovascular phenotype, including atrial septal defect, pulmonary valve stenosis, arrhythmia, and heart failure with reduced ejection fraction. Her family history was positive for sudden cardiac death due to dilated cardiomyopathy.

Results: A pathogenic 1.8 Mb deletion in the 8p23.1 region, covering 32 genes, including GATA4, was identified. This deletion was followed by an intact segment and a 1.5 Mb duplication, consistent with an inversion-deletion-duplication aberration. Additionally, a novel, potentially pathogenic missense variant c.2550G>C p.(Glu850Asp) in exon 9 of RBM20 was detected. This variation is likely to disrupt the donor splice site following exon 9, potentially activating a cryptic splice site. Loss of function is a recognized pathogenic mechanism in this context.

Conclusion: This case underscores the utility of next-generation sequencing in uncovering complex genetic factors in cardiovascular phenotypes. The 8p23.1 deletion involving GATA4, associated with monogenic congenital heart anomalies, and the RBM20 variant, associated with dilated cardiomyopathy type 1DD, emphasize the importance of considering digenic or oligogenic causes in complex cases. These findings stress the need for comprehensive genetic analysis, aiding in more precise diagnosis and management strategies.

C.O.I. Disclosure:

L. Kepczynski: None. **T. Kaluzewski:** None. **U. Wysocka:** None. **Z. Krepsztul-Baczewska:** None. **A. Kucinska:** None. **A. Bielecka-Dabrowa:** None. **A. Gach:** None.

EP07.016 Importance of CNV identification in FH patients: analysis of a cohort of 395 patients studied by NGS panels

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Background: Mutations in LDLR are the most common cause of familial hypercholesterolemia (FH). Copy number variants (CNVs) represent for ~10% of mutations in this gene. This high prevalence of CNVs is mainly related to the abundance of Alu elements in LDLR. Most of known CNV breakpoints are located where the Alu elements are most concentrated. To highlight, loss-of-function variants in LDLR, such as CNV deletions, are often associated with a more severe phenotype and milder response to conventional drugs. NGS has proven to be a powerful tool to accurately identify CNVs. This study aims to assess the prevalence of CNVs in a cohort of patients with a mutation in LDLR, and to perform a descriptive analysis of them.

Material and methods: 395 unrelated patients with a causal variant in the LDLR gene identified by NGS. CNVs were confirmed by an alternative methodology.

Results: 34 patients carried a CNV (8.6%): 73.53% heterozygous deletions spanning several exons, 20.59% heterozygous deletions of a single exon, 2.94% homozygous deletions of several exons and 2.94% duplications. 17.65% of CNVs involved deletion of exons 4–6, 11.8% deletions of promoter and exon 1 and 8.82% deletion of exons 16–18. Most CNVs-breakpoints were located in introns where Alu elements are most concentrated.

Conclusion: The prevalence of CNVs was 8.6%. Most of them were deletions and their breakpoints match with the location of the Alu sequences. The identification of CNVs is essential in the diagnosis of FH as it considerably increases the diagnostic yield and avoids false negative diagnoses.

C.O.I. Disclosure:

P. Vélez Viéitez: None. **D. Cabrera Argaña:** A. Employment (full or part-time); Modest; Health in Code.

EP07.017 The Effect of Vitamin D on Global DNA Methylation in Adipose Tissue of Metabolic Syndrome-Induced Rats

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Background: Metabolic syndrome (MetS) is a serious health condition characterized by a combination of abdominal obesity, hypertension, hyperglycemia, insulin resistance, elevated serum triglycerides, and reduced HDL cholesterol levels, which is rapidly increasing worldwide. The increasing global prevalence of MetS cannot be explained by environmental or genetic factors alone, raising the need to investigate the possible roles of epigenetic factors. Vitamin D levels have been associated with both MetS and

DNA methylation. This study aims to investigate the effects of vitamin D on global DNA methylation in adipose tissue of MetS induced rats and to obtain new insights into the epigenetic basis of MetS.

Methods: Adipose tissue was collected from 19 male Sprague Dawley rats aged 26–28 weeks with MetS caused by a high-fat, high-fructose diet. Control groups were fed standard chow. Following week 15, both groups were given vitamin D supplements for 12 weeks. Global 5-mC levels were quantified by ELISA using equal amounts of DNA including MetS and control groups, as well as vitamin D-treated MetS and untreated control groups.

Results: The median global 5-mC levels were similar both in MetS (median ± SE = 0.98 ± 0.18;n = 4) and controls (median ± SE = 1.05 ± 0.25; n = 6) and vitamin D-treated MetS (median ± SE = 0.98 ± 0.18; n = 4) and untreated control (median ± SE = 1.32 ± 0.15; n = 5) groups.

Conclusion: Vitamin D treatment did not significantly alter global DNA methylation levels in adipose tissue of MetS-induced rats. Further research is needed to explore potential gene-specific or tissue-specific epigenetic effects of vitamin D in metabolic disorders.

C.O.I. Disclosure:

F. Geyik: None. **G. Guven:** None. **H. Akbas:** None. **O. Karadeniz:** None. **D. Temizkan:** None. **E. Komurcu-Bayrak:** None.

EP07.018 Interplay between genetic and lifestyle factors in modulating LDL-C levels and atherosclerotic cardiovascular risk

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Background: Low-density lipoprotein cholesterol (LDL-C), a key contributor to cardiovascular diseases (CVD), increases mortality risk. While lowering LDL-C reduces risk, its optimal lower limit remains debated. In particular, numerous observational studies have reported that low LDL-C levels may be a risk factor for hemorrhagic stroke. This study aimed to analyze the association between genetically determined LDL-C (GLDL-C) and cardiovascular diseases.

Methods: This analysis was conducted using the Korean Cancer Prevention Study-II (KCPS-II). Using the genetic results from the KCPS-II biobank, a GWAS was performed to identify genetic variations associated with LDL-C. Environmentally determined LDL-C (ELDL-C) was calculated by subtracting GLDL-C from the measured LDL-C (MLDL-C).These levels were divided into quintiles, and their associations with CVD were analyzed

Results: In the final analysis of 136,263 participants, MLDL-C levels were associated with confounding factors, while GLDL-C was independent of these factors. Even after excluding participants with a history of CVD or dyslipidemia treatment at baseline, GLDL-C showed a linear association with the risk of CVD, without exhibiting J-shaped or U-shaped patterns. Similarly, no J-shaped or U-shaped patterns were observed in the analysis of GLDL-C in relation to hemorrhagic stroke. Notably, even in high genetic risk groups, the risk of CVD was reduced when environmentally determined ELDL-C was low.

Conclusion: This study demonstrates that lower LDL-C levels are linearly associated with a reduced CVD risk. Furthermore, low LDL-C was not a risk factor for hemorrhagic stroke. These findings suggest that individuals with genetically high LDL-C can lower their cardiovascular risk through lifestyle modifications.

C.O.I. Disclosure:

K. Jung: None. **Y. Yang:** None.

EP07.019 Functional characterisation of splicing effects of rare MYBPC3 gene intronic variants

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Background: Rare pathogenic variants affecting the *MYBPC3* gene are the most common genetic causes of cardiomyopathy. The pathogenic mechanism is strongly related to truncating *MYBPC3* gene variants, where deep intronic variants account for a significant proportion of solved cases (up to 9%), requiring functional analysis at the RNA level. The *MYBPC3* gene is predominantly expressed in cardiac muscle cells; however, RNA can be successfully extracted from peripheral white blood cells.

Material and methods: Patient-specific Sanger sequencing of cDNA samples was performed to characterize the possible splicing defects of rare intronic *MYBPC3* gene variants (c.2068-9T > A, c.2148+25A > C, c.2309-3C > G, and c.2738-147A > G (*MYBPC3*, NM_000256.3). These intronic variants were detected using a virtual panel of 389 cardiomyopathy-related genes (SOPHiA Genetics, Switzerland).

Results: The aberrant splicing of *MYBPC3* pre-mRNA was proven to be a consequence of variants c.2068-9T > A (out-of-frame insertion of 7 bases leading to the creation of a premature termination codon) and c.2309-3C > G (complete degradation of aberrant transcripts by nonsense-mediated mRNA decay), whereas the deeper intronic variants c.2148+25A > C and c.2738-147A > G are likely rare but benign polymorphisms.

Conclusion: Intronic DNA variants of the *MYBPC3* gene are important causes of cardiomyopathy. We proved the diagnostic utility of custom, patient-specific, RNA analysis from the leukocyte transcriptome in our patients for the characterisation of splicing effects due to rare intronic variants in the *MYBPC3* gene.

C.O.I. Disclosure:

M. Wayhelová: None. **P. Peldová:** None. **M. Havelka:** None. **V. Zoubková:** None. **A. Krebsová:** None. **M. Macek:** None.

EP07.020 Copy Number Variants in Cardiac Channelopathies: Still a Missed Part in Routine Arrhythmic Diagnostics

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Background: Inherited cardiac channelopathies are major causes of sudden cardiac death (SCD) in young people. Genetic testing is focused on the identification of single-nucleotide variants (SNVs) by Next-Generation Sequencing(NGS). However, genetically elusive cases can carry copy number variants(CNVs), which need

specific detection tools. We underlie the utility of identifying CNVs by investigating the literature data and internally analyzing cohorts with CNVs in KCNQ1, KCNH2, SCN5A, and RYR2.

Material and methods: This study is divided into two major parts: literature data collection of patients described with channelopathy genes CNVs, and the same category of patients from our internal cohort.The patients with inconclusive NGS testing were analyzed to investigate CNVs in cardiac genes through Multiplex Ligation-dependent Probe Amplification (MLPA) kits.

Results: The literature data include 119 individuals with CNV in one of the analyzed genes. Our cohort included 485 MLPA tests for patients without pathogenic or likely pathogenic variants in NGS analysis for cardiac and arrhythmic genes. We identified microrearrangements in 21 individuals (4.3%). Young patients with CNVs in KCNQ1 and KCNH2 show a Long QT(LQT) phenotype >480ms. CNVs in SCN5A were represented by the Brugada pattern, with major cardiac events mainly in males. RYR2-exon3 deletion showed a broader phenotype, including left ventricular non-compaction(LVNC) and catecholaminergic polymorphic ventricular tachycardia(CPVT).

Conclusion: The detection of CNVs can be of greater yield in two groups: familial channelopathies and patients with suspected Jervell and Lange-Nielsen syndrome or CPVT. The limited number of reported individuals makes it mandatory for multicentric studies to give future conclusive results.

C.O.I. Disclosure:

M. Gnazzo: None. **G. Parlapiano:** None. **F. Di Lorenzo:** None. **D. Perrino:** None. **S. Genovese:** None. **V. Lanari:** None. **D. Righi:** None. **F. Calì:** None. **M.S. Silvetti:** None. **E. Falcone:** None. **A. Bauleo:** None. **F. Drago:** None. **A. Novelli:** None. **A. Baban:** None.

EP07.021 The genotype and phenotype relationships for *MYBPC3* in hypertrophic cardiomyopathy

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Background: Most hypertrophic cardiomyopathy (HCM) related variants are detected in the *MYBPC3*. There is a lack of genotype-phenotype correlation data based on the variant's location within the gene. Our study analyzes molecular and phenotypical characteristics of *MYBPC3*-related HCM.

Material and methods: We analyzed clinical and genetic data from adult HCM patients treated at the Vilnius University Hospital Santaros Klinikos between 2005 and 2024. Probands and relatives with HCM and a single pathogenic/likely pathogenic variant (P/LPv) in the *MYBPC3* were included for the evaluation.

Results: The study involved 55 individuals with HCM and *MYBPC3* P/LPv, 55% males. The median age at HCM diagnosis was 42 ± 15 years. During a median follow-up of 4.1 [1.7–9.5] years, 2 patients (3.6%) deceased. 44 individuals had P/LPv in Ig-like C2 type domains, 9 - in fibronectin type III domains, 2 - in interdomain region. P/LPv in fibronectin type III domains was associated with thicker left ventricular wall (maximal thickness) on transthoracic echocardiography (OR = 1.23; 95% CI: 1.02–1.52, p = 0.036) compared to P/LPv in the Ig-like C2 type domains. *MYBPC3* c.3697C>T P/LPv was associated with higher HCM risk score of sudden cardiac death (OR = 2.39, 95% CI: 1.23–5.43, p = 0.019), less with brain natriuretic peptide (BNP) (OR = 0.99, 95% CI: 0.97–0.99, p = 0.03) and late gadolinium enhancement at the right ventricular insertion point on cardiac magnetic resonance imaging (OR = 0.14, 95% CI: 0.01–0.75, p = 0.04).

Conclusion: The patients with *MYBPC3* P/LPv in the fibronectin type III domains and c.3697C>T P/LPv exhibit distinct phenotypic features compared to carriers of other P/LPv in this gene.

C.O.I. Disclosure:

D. Zebrauskiene: None. **E. Sadauskiene:** None. **R. Puronaite:** None. **R. Masiuliene:** None. **J. Barysiene:** None. **A. Jakaitiene:** None. **E. Preiksaitiene:** None.

EP07.022 Genetic architecture of hereditary cardiomyopathy, first results of the Czech national database

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Background: Inherited cardiomyopathy (CMP) is an important cause of heart failure in patients <50 years. Genetic stratification may lead to individualized therapy and primary prevention in CMP relatives. We assessed the genetic background of CMP in a representative Czech population.

Material and methods: The Cardiogenetics Working Group of the Czech Society of Medical Genetics & Genomics (kardiogenetika.cz; slg.cz) initiated a shared pseudoanonymised nationwide CMP DNA variant database comprising 4217 patients (2775 males/1442 females). Six accredited molecular genetic laboratories performed CMP testing using next-generation sequencing, ranging from various candidate gene panels to exome sequencing, with 99 genes analyzed in all cases.

Results: We tested the following CMP: hypertrophic- (HCM-2069), dilated- (DCM-1572), arrhythmogenic- (ACM-333), restrictive- (RCM-33) and uncategorized cardiomyopathies (210) were collected. ACMG.net class 4(LP) and 5(P) variants were identified in 1230/4217 (29 %) of all CMP cases. The most frequently identified genes harboring P/LP DNA variants comprised *MYBPC3* (321/1230; 26 %), *TTN* (266/1230; 22 %), *MYH7* (172/1230; 14 %), and *PKP2* (58/1230; 5 %). The *MYBPC3* gene has several frequent DNA variants (85 index patients with NM_000256.3(MYBPC3):c.3697C>T (p.Gln1233Ter), 17 index patients with NM_000256.3(MYBPC3):c.821+1G > A, 14 index patients with NM_000256.3(MYBPC3):c.3490+1G > T) differing from the current W. European datasets suggest a mutation hot spot or even a founder effect in the Czech population.

Conclusion: Hereby, we present the results of the nationwide Czech CMP variant database with P/LP variants observed in approximately 1/3 of all analyzed cases. Interlaboratory coordination led to a standardized diagnostic approach and a high diagnostic yield.

C.O.I. Disclosure:

M. Adamová: None. **E. Kutílková:** None. **A. Krebsová:** None. **M. Macek:** None.

EP07.023 Descriptive analysis of a cohort of patients with familial hypercholesterolemia: diagnostic utility of the polygenic risk score

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Background: Familial hypercholesterolemia (FH) has a complex

etiology. The genetic yield for monogenic FH varies widely depending on the clinical criteria used (20%-80%), and it has been observed that up to 80% of patients with a negative monogenic result may have a polygenic etiology. Even in patients with causal variants, an elevated polygenic risk score (PRS) of hypercholesterolemia contributes to elevated c-LDL. Monogenic FH has a higher cardiovascular risk (CVR) and a less favorable response to treatment than polygenic hypercholesterolemia. Polygenic hypercholesterolemia is associated with a higher CVR than non-genetic hypercholesterolemia and has a non-Mendelian inheritance pattern. Distinguishing between these entities is clinically difficult, which underlines the importance of genetic diagnosis.The aim of this study is to analyse the usefulness of NGS sequencing for the diagnosis of monogenic and polygenic hypercholesterolemia and to evaluate the usefulness of PRS of hypercholesterolemia (12 SNPs).

Material and methods:134 apparently unrelated patients with clinically suspected FH were analyzed by NGS. PRS of polygenic hypercholesterolemia was calculated.

Results:12.6% had identifiable mutations and 87.4% did not. Among patients not carrying causal variants, 92% had high PRS, suggesting a polygenic etiology. A significant proportion of patients with causal variants also had a high PRS.

Conclusion:A high PRS could explain the phenotype in a large proportion of patients without identified causal variants. The PRS could also explain the phenotypic variability observed in patients with monogenic FH. Knowing the genetic etiology of FH has implications for risk stratification and treatment, as well as for family management.

C.O.I. Disclosure:

D. Cabrera Argaña: A. Employment (full or part-time); Modest; Health in Code. **P. Vélez Viéitez:** A. Employment (full or part-time); Modest; Health in Code.

EP07.024 Aspirin reverses the inhibitory effect of soluble fms-like tyrosine kinase-1 on trophoblast invasiveness and ciliogenesis through Sonic Hedgehog signaling in preeclampsia

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Background: Soluble Flt-1(sFlt-1) is upregulated in preeclamptic patients and is a predictive biomarker for preeclampsia risk and progression. Aspirin is an effective agent to prevent preeclampsia and has been shown to suppress sFlt-1 production in trophoblasts; however, the underlying mechanism is not fully understood.

Material and methods: We used two trophoblastic cells (HTR-8/SVneo and JAR) to investigate the effects of sFlt-1, the underlying pathways on trophoblast biology, and cilia formation in sFlt-1-pretreated, FLT-1-knockdown cells in a hypoxic chamber (0.1% O2). The plasma and placenta sFlt-1 expression and cilia formation were also evaluated in a preeclampsia mouse model.

Results: sFlt-1 production was upregulated under hypoxia in two trophoblastic cells (HTR-8/SVneo and JAR). sFlt-1 was shown to inhibit trophoblast invasion and migration under hypoxia, and this inhibitory effect occurred through Sonic Hedgehog (SHH) signaling pathway. Ciliary number and length of trophoblasts were inhibited by sFlt-1 treatment and were enhanced after FLT-1 knockdown. Aspirin was revealed to reverse the sFlt-1-mediated inhibitory effect on trophoblast invasion/migration, ciliogenesis and SHH signaling. Moreover, plasma and placental sFlt-1 level were upregulated in the preeclampsia mouse model, and the primary cilia formation was impaired in the mouse placenta. Aspirin-fed mice were presented with a reduced sFlt-1 expression and the placental ciliogenesis of preeclamptic mouse model was improved as well.

Conclusion: Soluble Flt-1 impairs trophoblast motility and ciliogenesis through Sonic Hedgehog signaling under hypoxia. Aspirin exerts its suppressive effect on sFlt-1 upregulation and sFlt-1-mediated cell function and signaling in vitro and in vivo.

C.O.I. Disclosure:

M. Su: None.

EP07.026 Arrhythmia Risk in Ogden Syndrome

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Background: Ogden syndrome is a X-linked condition that is reported in just over 100 individuals. The *NAA10* gene encodes for enzyme N-alpha-acetyltransferase 10 (NAA10), which is part of NatA, a major N-terminal acetyltransferase complex involved in N-terminal acetylation. Genetic changes residing within the N-terminal acetyltransferase domain are known to be associated with arrhythmias. The reported arrhythmia phenotypes include supraventricular tachycardia, ventricular arrhythmias and sudden cardiac arrest at an early age. We highlight an ultrarare cardiogenetic case that we longitudinally follow at our hospital.

Material and methods: Exome sequencing revealed a de novo pathogenic variant (c.100C>T, p.H34Y) in the *NAA10* gene. This genetic change resides in the NAA15 and acetyltransferase domains.

Results: 3-year-old boy diagnosed with Ogden syndrome initially presented with global developmental delays, lacrimal fistula, macroglossia, oropharyngeal dysphagia, gastroesophageal reflux, and an elevated alpha fetoprotein level that eventually normalized. He remains asymptomatic from a cardiac perspective. Last electrocardiogram showed anterior T wave inversion with normal QTc interval. Holter monitors have been normal with no significant ectopy or arrhythmia and no evidence of conduction disease. Echocardiograms show bicuspid aortic valve with normal valve function and no evidence of cardiomyopathy.

Conclusion: Despite the severe clinical features that can be associated with Ogden syndrome, management guidelines are not available for providers given the dearth of research and the rarity of this condition. This necessitates the importance to better identify individuals at higher risk for sudden cardiac arrest and better establish longitudinal guidelines for primary cardiac prevention strategies in those living with Ogden syndrome.

C.O.I. Disclosure:

A. Nouraee: None. **J.P. Ramos:** None. **L. Desai:** None. **B. Chalazan:** None.

EP07.027 Spectrum of genetic findings in patients operated for hypertrophic cardiomyopathy

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Background: To analyze the spectrum of causative genetic variants in patients operated for hypertrophic cardiomyopathy (HCM).

Material and methods: Myectomy was performed on 330 patients diagnosed with HCM. All patients were symptomatic, and

half of them had heart failure Class III-IV (NYHA). Genetic testing was performed for 138 (41%) of them. NGS sequencing was performed on the Illumina NextSeq 550Dx platform with the SureSelect_allExonsV7 kit or TrueSightCardio sequencing kits. Pathogenicity of the rare variants was assessed in accordance with current ClinGen recommendations.

Results: We identified 94 causative variants in 76 probands: 42 of them were belong to the IV-V class of pathogenicity and were detected in 40 probands, 52 VUS were detected in 40 cases. Most of mutations were found in the *MYBPC3* (n = 18) and *MYH7* (n = 17). 63 of causative variants were found in the genes encoding thin filaments and 31 in non-sarcomeric genes (*ALPK3, APOB, CREB3L3 CSRP3, DSP, ELAC2, ELN, FBN1, FBN2, FHOD3, FKTN, FLNC, HCN4, LAMA2, LAMA4, LDLR, MYO6, NRAP, RBM20, RPL3L, RYR1, SCN5A, TRPM4)*. Syndromic forms were identified in 7 probands; 7 patients with biallelic form. Double-hit mutations were found in 2 patients: one had 2 variants in *MYH7* and other one had 2 VUS in *MYBPC3*.

Conclusion: According to the study results, genetic variants were found in 55%. The number of patients without any findings is 45% (62 cases). The majority of pathogenic and likely pathogenic variants are found in genes encoding sarcomeric proteins or in genes responsible for the development of syndromic forms.

C.O.I. Disclosure:

M.A. Sadekova: None. **M.S. Balashova:** None. **I.O. Islanov:** None. **A.P. Motreva:** None. **S.L. Dzemeshkevich:** None. **E.V. Zaklyazminskaya:** None.

EP07.028 Clinical and genetic spectrum of RASopathies in left ventricular hypertrophy cohort

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Background: RASopathies are a heterogeneous group of diseases caused by genetic alteration of the RAS-MAPK pathway. RASopathies have multisystemic symptoms including short stature, facial dysmorphism, and a wide spectrum of cardiac involvement. We aim to evaluate the ratio and spectrum of RASopathies in symptomatic patients with LVH.

Material and methods: We performed genetic counseling and NGS testing on 330 symptomatic probands who required surgical correction of cardiac hypertrophy. Median follow up period was 3 years (1–8). Whole exome sequencing was performed on the Illumina NextSeq550Dx (Exome or TruSightCardio sequencing kits). Pathogenicity was assessed in accordance with current recommendations.

Results: We found 19 rare variants in the genes encoding RAS-MAPK proteins in 18 probands aged 1–68 years. Fifteen probads (4.5%) had RASopathy diagnosis confirmed by the P/LP variants in the genes *PTPN11, RIT1, LZTR1, MRAS*, and *HRAS*. Six out of 15 probands had an entering diagnosis of Noonan syndrome, and 9 of them were initially diagnosed with HCM. Two probands with *RIT1* mutations had macrosomic phenotype rather than growth deficit. Four probands died during the follow-up period, and 1 was transplanted. Three LVH probands carried rare VUS variants the role of which in LVH should be further elucidated.

Conclusion: Despite well-recognized phenotypes, many patients with RASopathies are underdiagnosed. Phenotypic spectrum of RIT1 mutations can be expanded with macrosomy. When presenting with LVH, patients with RASopathies have an earlier age of symptoms, operations, and higher mortality compared with non-syndromic HCM probands.

Additional information: study was supported by research projects FURG-2024-0004, FURG-2023-0009

C.O.I. Disclosure:

M. Balashova: None. **M. Sadekova:** None. **A. Motreva:** None. **N. Kotlukova:** None. **S. Dzemeshkevich:** None. **L. Dzik:** None. **E. Zaklyazminskaya:** None.

EP07.029 Pathogenic variants with a founder effects in the underrepresented Dagestani cohort of patients with cardiomyopathies

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Background: North Caucasian Highland Region contains approximately 110 ethnic groups belonging to Caucasians and largely underrepresented in global genetic databases. Representatives of over 30 indigenous nationalities live in the Republic of Dagestan over an area 50270 square kilometers, and characterized by high inbreeding rate. We found clinically significant variants enriched in Dagestani patients with cardiomyopathies.

Methods: Genetic study was performed on DNA samples from 80 arrhythmogenic cardiomyopathy (ARVC) probands, and 300 probands with hypertrophic cardiomyopathy (HCM). Genotyping was performed using various NGS approaches with following Sanger sequencing. Potential role of revealed genetic variants was evaluated according to ACMG criteria. Evaluation of the minor alleles frequencies was carried out in DNA of healthy volunteers (130 of Slavic origin, 223 of Dagestani origin).

Results: Homozygous mutation p.Ser194Leu in *DSG2* gene was found in two Dagestani probands with ARVC. High prevalence of p.Ala57Asp mutation in *MYL3* gene was found in Dagestani probands with HCM. Variant was identified in 11 probands (6 homozygous and 5 heterozygous carriers) (21% in Dagestani probands), and was absent in patients of other origin. Mutation FV Leiden was found in 20% of all Dagestani probands, in the group of Slavic origin it presents in less than 4% alleles.

Conclusion: Rare recessive forms of ARVC and HCM, and inherited thrombophilia are enriched in Dagestani region. It might be explained by founder effect and high rate of consanguinity. More detailed studies are needed to characterize genetic diversity of North Caucasian Highland Region. **This study was supported by Research projects FURG-2023-0009, FURG-2024-0004**.

C.O.I. Disclosure:

A. Shestak: None. **M. Sadekova:** None. **G. Radzhabova:** None. **O. Blagova:** None. **Y. Lutokhina:** None. **A. Motreva:** None. **S. Dzemeshkevich:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Research project FURG-2024-0004. **E. Zaklyazminskaya:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Research project FURG-2023-0009.

EP07.030 Investigation of the relationship between variants in *SCN5A* E-box motifs and phenotype in the 100KGP Brugada syndrome cohort

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Background: Brugada syndrome (BrS) is an inherited rare cardiac disease characterised by an increased predisposition to life-threatening arrythmias and sudden cardiac death (SCD). The variable expressivity in BrS with no clear understanding of what predisposes who to severe symptoms complicates its management. Though links between the circadian clock and BrS exist, there is no understanding of how variants in circadian regulatory regions contribute to BrS, either in its development or by modification of its expressivity. Analysis of variants in clock and clock-controlled genes (CCGs) found the enrichment of a two-variant haplotype (OR = 2.77, p-value = <2.2E-16; OR = 2.11, p-value = 6.23E-14) in the CCG *SCN5A* in the 100,000 Genome Project (100kGP) BrS cohort. This study aimed to find possible phenotype associations with the haplotype.

Material and methods: Genotype-phenotype associations were investigated using the 100kGP BrS data in R. Phenotype enrichment was analysed by Fisher's exact tests and cluster analysis using the random forest machine learning algorithm.

Results: Phenotype analysis showed an enrichment of the phenotypes, 'Episodes of ventricular tachycardia' (OR = 5.73, p-value = 0.013), 'Prolonged QTc interval' (OR = 6.21, p-value = 0.019), and 'Right bundle block' (OR = 6.89, p-value = 0.018) in BrS patients with the two-variant *SCN5A* haplotype in homozygous form. From the cluster analysis, the phenotypes- syncope, ventricular tachycardia, ventricular fibrillation, and SCD were most associated with the homozygous *SCN5A* haplotype.

Conclusion: Findings point out an association between the homozygous *SCN5A* haplotype and adverse BrS phenotypes such as ventricular tachycardia. This implies that the *SCN5A* haplotype may be a modifier of BrS disease severity.

C.O.I. Disclosure:

M. Ampiah: None. **T. Hearn:** None.

EP07.031 Genetic variants associated with clonal haematopoiesis in Kazakhstani atherosclerotic cohort

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Background: Clonal hematopoiesis of indeterminate potential (CHIP) is a common risk factor for atherosclerosis (AS). We aimed to identify the contribution of certain CHIP-related mutations to atherosclerosis in the Kazakhstani cohort.

Materials and methods: We analysed whole-genome sequencing data to identify the presence of CHIP in 380 AS and 300 controls (the Kazakh Whole Genome project) study. The absence of hematologic malignancy was confirmed before study enrollment by hematologic data. Genetic variants in genes, associated with the most prevalent somatic clonal hematopoiesis-related mutations were assessed. CHIP was considered at VAF ≥ 1%. Variants below the minor allele threshold and not present in COSMIC were considered variants of uncertain significance (VUS) and were excluded from this analysis.

Results: Overall, we found 27 patients carriers of genetic variants exhibited a minor allele frequency of ≤0.1% in the Exome Aggregation Consortium Project in *DNMT3A*, *TET2*, *JAK2*, *TP53*, *PPM1D*, *ASXL1* genes, which comprise 4,5%. Mutations were commonly observed in *DNMT3A* (n = 1), *TET2* (n = 10), *JAK2* (n = 5), *ASXL1* (n = 9) and *PPM1D* (n = 1), *TP53* (n = 1). CHIP carriers with these mutations had increased coronary-artery calcification, a marker of coronary atherosclerosis burden.

Conclusion: Our study identifies CHIP in 4,5% (27) cases of AS carriers of genetic variants in clonal hematopoiesis-related genes. Our findings support the association between CHIP and atherosclerosis, but it is necessary to expand our cohort and analysis to establish more evidence to elucidate the risk-enhancing role of CHIP in coronary artery disease and AS.

C.O.I. Disclosure:

T. Shakhmarova: None. **Z. Mirmanova:** None. **M. Zhalbinova:** None. **A. Chamoieva:** None. **N. Satvaldina:** None. **S. Rakhimova:** None. **U. Kairov:** None. **A. Daniyarov:** None. **M. Bekbossynova:** None. **A. Akilzhanova:** None.

EP07.032 Genetic basis of nonischemic cardiomyopathies associated with ventricular tachycardias in Kazakhstani patients

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Background: Advances in the field of human genetics have led to an accumulating understanding of the genetic basis of distinct nonischemic cardiomyopathies associated with ventricular tachycardias (VTs) and sudden cardiac death. This study aimed to elucidate the mutational spectrum in Kazakhstani patients with VTs.

Material and methods: Using a customized, targeted panel of 96 known cardiac disease genes, associated with cardiomyopathy and arrhythmia we screened 92 patients, diagnosed with VT and dilated cardiomyopathy (VT DCM) or idiopathic VT (iVT).

Results: Targeted sequencing and stepwise filtering of the annotated variants identified 307 unique variants in 74 genes totaling up to 456 variants for the overall study group. Variants included one in/del variant, four splice-site variants, and 451 single-nucleotide variants (SNV) within the coding exonic regions. Seven (0.15%) of the SNVs were unique stop-gain variants, three in the TTN gene. 168 HGMD mutations (61 unique) were observed in 37 genes. According to ACMG, variants were classified as nine pathogenic (KCNJ2 R218Q and TTN R5338X in iVT patients, KCNQ1 c.477+1G > A, LMNA Q353X, MYH7 F244L, TTN L17465X and W21011X, DSG2 c.2334+1G > A, GAA W746C in VT DCM patients), 11 likely pathogenic, 97 variants with uncertain significance and rest as benign were observed.

Conclusion: Individuals presenting with VT either secondary to DCM or of idiopathic etiology carry multiple rare mutations and potentially pathogenic sequence variants in cardiac risk genes in a similar pattern and at a comparable frequency. Further studies are needed to identify more mutations causing VT in a larger cohort of patients.

C.O.I. Disclosure:

G. Akylzhanova: None. **Z. Abilova:** None. **T. Shakhmarova:** None. **Z. Mirmanova:** None. **A. Chamoieva:** None. **S. Rakhimova:** None. **A. Chinybayeva:** None. **A. Abdrakhmanov:** None. **M. Bekbossynova:** None. **A. Akilzhanova:** None.

EP07.033 IDENTIFICATION OF BACTERIA BY 16S METABARCODING IN PATIENTS WITH INFECTIVE ENDOCARDITIS

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Background: Infective endocarditis (IE) is an infectious disease caused by the hematogenous dissemination of bacteria into heart valves. Clinical presentation of IE is variable and making its diagnosis challenging. This study was aimed to identify bacterial agents in the native heart valves obtained from IE patients characterized by negative and positive blood cultures.

Material and methods: 10 native heart valves obtained from IE patients undergoing heart valve replacements were analyzed. Bacterial invasion in the heart valves was studied by Gram staining of histological sections. Immunohistochemical analysis of pan-leukocyte marker CD45, platelet marker CD41, and neutrophil myeloperoxidase was performed. The taxonomic diversity of the bacteria was analyzed using 16S rRNA metabarcoding.

Results: Resulting to routine blood culture, the IE etiology was established in only for cases; *Streptococcus gordonii* and *Enterococcus faecalis* were identified. The detected bacteria were localized within microthrombi on the atrial surface at the leaflet's free edge or on the ventricular surface at the leaflet's base. Resulting to Gram staining, Gram-positive cocci and Gram-negative bacilli formed mixed colonies in the IE-affected native heart valves. Resulting to 16S rRNA metabarcoding, we detected the following bacteria: *Pseudomonas* (70% of the studied heart valves), *Roseateles* (60%), *Acinetobacter* (40%), *Sphingomonas* (40%), *Enterococcus* (30%), *Reyranella* (20%), *Sphingobium* (20%), *Streptococcus* (20%), *Agrobacterium* (20%), *Ralstonia* (10%), and *Bacillus* (10%). Polymicrobial contamination was detected in 90% of cases; in one sample, only *Enterococcus* spp. was identified.

Conclusion: The obtained results show the importance of 16S rRNA metabarcoding of heart valves removed due to IE in clinical practice.

C.O.I. Disclosure:

A. Sinitskaya: None. **A. Kostyunin:** None. **M. Asanov:** None. **M. Khutornaya:** None. **A. Poddubnyak:** None. **A. Tupikin:** None. **M. Kabilov:** None.

EP07.034 Genetic profiling in young adult patients with cardiac conduction disorders using Nanopore sequencing

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Background: Cardiac conduction disorder (CCD) is an inherited heart condition that manifests either as a primary electrical disorder or in association with structural heart abnormalities. Our goal is to study and compare nanopore sequencing data obtained from patients with Atrioventricular block and Sinus node weakness under the age of 44 years.

Material and methods: Genetic testing was carried out in 14 patients using nanopore sequencing on a PromethION 48 instrument (Oxford Nanopore). FastQC, Guppy v6.0.1, and MultiQC programs were used to assess the quality of the bioinformatics analysis. Alignment of the obtained reads was performed against the reference human genome (hg38) using the minimap2 v2.24 program, along with the Annovar and ClinVar tools to assess the pathogenicity of variants.

Results: Nanopore sequencing revealed mutations in 8 patients. The most common mutations were SNVs and frameshift variants. No structural variants were found. Mutations were identified in the following genes: APOA2, PYGL, LRPPRC, and SCN5A. Further data analysis and validation of the sequencing results are ongoing.

Conclusion: The use of genetic screening and identification of the causative mutation is critical for risk determination and family counseling. Therefore, it is essential to clarify the potential significance of Oxford Nanopore Technology in clinical practice.

C.O.I. Disclosure:

D. Samatkyzy: None. **S. Rakhimova:** None. **A. Akilzhanova:** None.

EP07.035 Genetic insights into ventricular tachycardia: a Next-Generation Sequencing Study

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Background: Ventricular tachycardia (VT) is one of the most dangerous arrhythmias, requiring timely diagnosis and effective treatment. Modern sequencing technologies make it possible to identify new mutations. Identifying genetic mutations opens up the possibility of a personalized approach to patient treatment. To identify rare and common variations in candidate genes associated with cardiomyopathy and cardiac rhythm disorders using next-generation sequencing (NGS) technology.

Material and methods: This study included 95 patients with various clinical phenotypes of cardiomyopathy, including dilated cardiomyopathy (DCM), idiopathic VT, and patients with ischemic heart disease (IHD) accompanied by ventricular rhythm disturbances. A common characteristic among these three groups was the occurrence of severe VT episodes in all patients. Among them, 64 out of 95 had sporadic forms of the disease, while 26 had familial forms of cardiomyopathy.

Results: Genetic analysis identified 173 mutations previously associated with cardiomyopathies and arrhythmias as documented in the Human Gene Mutation Database (HGMD). Each patient had an average of ≥1.6 mutations, regardless of their initial clinical diagnosis.In addition, 215 unique genetic variants were detected, many of which were predicted to have high pathogenic potential.

Conclusion: Our results show that most patients had multiple genetic mutations and novel variants, regardless of their initial clinical diagnosis.The use of NGS allows the simultaneous detection of multiple pathogenic mutations in key genes associated with cardiac arrhythmias. This approach significantly improves diagnostic accuracy and promotes the development of personalized medicine in cardiology.

C.O.I. Disclosure:

Z. Abilova: None. **A. Akhmetova:** None. **U. Kairov:** None. **S. Rakhimova:** None. **U. Kozhamkulov:** None. **M. Bekbosynova:** None. **A. Akilzhanova:** None.

EP07.036 Congenital Fibrinogen Deficiencies, not so rare

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Background: Congenital fibrinogen deficiencies (CFDs), traditionally considered rare monogenic disorders are now thought to be more prevalent and genetically complex. Estimates using data from gnomAD v2.0, (approximately 140,000 individuals) showed that up to 1/100 individuals could carry deleterious variants in one of the three fibrinogen-encoding genes[1]. Here, we update these estimates using data from gnomAD v4.1.0 (over 800,000 individuals).

Material and methods: Our filtering pipeline was designed to include only high-confidence predicted deleterious variants in the fibrinogen-encoding genes *FGB, FGA*, and *FGG*. We calculated the cumulative allele frequency (cAF) and estimated the prevalences of CFD genotypes using the Hardy-Weinberg equilibrium.

Results: Focusing on the three major isoforms, we identified 798 variants classified as potentially pathogenic (cAF = 0.007515). This equated to recessively-inherited CFDs (homozygous genotypes) in approximately 29/million individuals and dominantly-inherited CFDs (heterozygous genotypes) in approximately 15,000/million (1.5%). Interestingly, the Broad Institute's GeniE online tool for prevalence estimates generated even higher numbers than our in-house approach (109/million and 28,000/million for homozygous and heterozygous genotypes respectively).

Conclusion: These increased prevalence estimates using gnomAD v4 data confirm that CFDs are more common than previously thought and reflect the inclusion of broader datasets, capturing a greater range of rare variants and homozygous cases. However, we believe that GeniE overestimates prevalences, at least for CFDs, by including variants that in our experience, should be considered benign. Our findings provide insights into the genetic landscape of fibrinogen disorders and highlight the necessity of integrating multiple genetic resources and expert-driven criteria for accurate prevalence estimation.

C.O.I. Disclosure:

A. Couzens: None. **M. Neerman-Arbez:** None.

EP07.037 7q11.23 Microduplication Syndrome: Cardiac profile and screening outcome of a cohort of individuals in the North East, United Kingdom

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Background: 7q11.23 Microduplication (dup7q11.23) syndrome is a rare autosomal dominant disorder due to a recurring duplication of the Williams-Beuren Syndrome critical region. Aortic dilatation is a primary cardiac finding and can occur in about half of these individuals. This study analyses the cardiac profile and screening outcome of a cohort of individuals with 7q duplication syndrome.

Material and methods: Forty three individuals confirmed to have 7q duplication syndrome over the last ten years were included retrospectively from the cytogenetic database at Northern Genetics Service, UK. Cardiac screening was done by Consultant Cardiologists. Individuals with no digital records were excluded from data analysis.

Results: Roughly two thirds of the screened were males and in the paediatric age group. The most prevalent copy number variant was the 1.4Mb duplication seen in twenty three individuals. Half of them had a family history of the same duplication. 37% had a familial inheritance. Half of them had diagnostic echocardiographs and roughly a third had follow up scans. Eight individuals (36%) had normal echo findings. Five (23%) had aortic dilatation (Z > 2) at diagnosis and/or follow up. Average cardiac follow up frequency was 1.65 years (range: 1–4 years).

Conclusion: Average cardiac screening rates closely followed recommended international annual screening frequency. The United Kingdom does not have national guidelines for the cardiac screening of these individuals. The findings of this study further reinforce the critical need of clear recommendations for cardiac follow up and health professional awareness of these individuals at risk of progressive aortic dilatation.

C.O.I. Disclosure:

H. Hewavitharana: None. **R. Richardson:** None. **S. Haigh:** None.

EP07.038 Genetic Etiology of Congenital Heart Disease: A WES-Based Study

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Background: Congenital heart disease (CHD) is a genetically based disorder that affects approximately 1% of live births worldwide. Whole exome sequencing (WES) studies help identify genetic variants that may correlate with the clinical presentation of CHD

Material and methods: DNA was isolated from peripheral blood of 4 index cases and their family members. WES was performed on the index cases. Variants identified as pathogenic/likely pathogenic or variants of uncertain significance were evaluated.

Results: Variants were detected in 4 CHD cases with extracardiac anomalies whose microarray-based comparative genomic hybridization analysis (aCGH) was normal: Case 1 (6 months,male), heterozygous *MED13L* c.3613C>T, case 2 (5 months,female), heterozygous *NOTCH1* nonsense c.3319C>T and *TBX1* missense c.1347C>G; case 3 (neonatal,male), homozygous *DCAF6* nonsense c.649C>T; case 4 (prenatal,male) heterozygous *GATA4* frameshift c.680del and *SMAD2* nonsense c.559del. The *MED13L* and *GATA4* variants were confirmed by Sanger sequencing and segregation analysis revealed that they were de novo, classified as "likely pathogenic" by ACMG, and were consistent with the clinical phenotype.Validation and segregation analysis for other variants are ongoing.

Conclusion: The study suggests that identifying causal genetic variants in the etiology of CHD could help predict disease prognosis and provide personalized treatments. In the prenatal period, genetic counseling can be provided to families, and prenatal and preimplantation diagnosis options can be offered in high-risk pregnancies. Studies reveal rare mutations in multiple genes in cases of CHDs may contribute to their development due to their synergistic nature, underscoring the need for further research to address the genetic complexity of this common and genetically heterogeneous disease.

C.O.I. Disclosure:

H. Sentürk: None. **T. Kalayci:** None. **A. Aslanger:** None. **M. Erdogdu:** None. **T. Sarac Sivrikoz:** None. **B. Karaman:** None. **E. Komurcu-Bayrak:** None.

EP08 Metabolic and Mitochondrial Disorders

EP08.001 Celiac disease

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Background: Celiac disease (CeD) is triggered by gluten consumption in genetically predisposed individuals. Common symptoms in children include vomiting, nausea, chronic diarrhea, constipation, weight loss, and delayed puberty. This case involves an 8-year-old boy with slightly delayed physical growth who presented with diarrhea, vomiting, and weight loss. His medical history includes hyperthyroidism, rheumatoid arthritis, proptosis, and retinitis. His parents are first cousins; they reported a similar case in another boy who died young.

Material and methods: Whole exome sequencing (WES) was used to selectively capture the exons of protein-coding genes, along with several critical genomic regions. Next-generation sequencing was performed, yielding approximately 100 million reads on an Illumina sequencer. Sanger sequencing confirmed the WES report in both the parents and the proband.

Results: The WES results revealed a c.G25T:p.G9X mutation in the IL2RA gene in proband (with a high CADD score of 27.4). Sanger sequencing confirmed that both parents were heterozygous carriers of this mutation while the child was homozygous.

Conclusion: These findings indicate that this mutation follows an autosomal recessive inheritance pattern. This pattern, in combination with the clinical symptoms and the family pedigree, supports the hypothesis that this case suffers from CeD. This couple and their family members are advised to pursue genetic counseling to ensure effective prenatal diagnosis and informed decision-making regarding the likelihood of inheriting similar conditions.

C.O.I. Disclosure:

S. Dashti Rahmatabadi: None.

EP08.002 Whole exome sequencing reveals a homoplasmic pathogenic variant in the *MT-RNR1* gene in a case of infantile hypotonia

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Background/Objectives: The *MT-RNR1* mitochondrial DNA (mtDNA) gene encodes 12S ribosomal RNA. Pathogenic variants in *MT-RNR1* have been associated predominantly with nonsyndromic hearing loss and complex IV deficiency. These rare genetic conditions may present with multiple manifestations such as myopathy, hypotonia, encephalomyopathy, lactic acidosis, and hypertrophic cardiomyopathy. We present a case of an infant with hypotonia where whole exome sequencing (WES) reveals a *MT-RNR1* pathogenic variant possibly related to the phenotype.

Methods: A 3-month-old, full term, Albanian male was referred to pediatrics hospital unit for evaluation of decreased muscle tone and feeding difficulties. Growth parameters were within normal range and there were no signs of acute distress. The infant was unable to lift his head and had considerable head lag while pulling to a sitting position. Extended biochemical investigation showed lactic acidemia and high lactate-to-pyruvate ratio in both blood and CSF. Heart and CNS ultrasound were normal. WES (Twist Bioscience) was applied on DNA extracted from peripheral blood. Bioinformatics analyses were conducted by Franklin® (Genoox) pipelines.

Results: A virtual gene panel, based on Human Phenotype Ontology gene-disease associations including mtDNA genes, was applied. Variant interpretation revealed a homoplasmic *MT-RNR1* pathogenic variant, GRCh38: chrM-1555 A > G, revealing a genetic background of the patient's condition.

Conclusions: Primary mitochondrial diseases encompass an impressive range of inherited energy deficiency disorders. The WES we applied readily detects mtDNA along with nuclear DNA variants. Incorporating mtDNA sequencing within the same WES workflow increases the diagnostic yield and is beneficial for patients with hypotonia and failure to thrive.

C.O.I. Disclosure:

G. Christopoulou: None. **S. Bournazos:** None. **A. Oikonomaki:** None. **A. Vagena:** None. **M. Satra:** None. **R. Poulaki:** None. **E. Papadopoulou:** None. **P. Constantoulakis:** None.

EP08.003 New phenylalanine hydroxylase gene mutation spectrum in hyperphenylalaninemia newborns obtained in the first 2 years of Russian expanded neonatal screening

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Background: Since 2023, the Russian Federation has implemented an expanded neonatal screening program, incorporating molecular genetic diagnostics for phenylketonuria (PKU)/ hyperphenylalaninemia (HFA) in newborns with elevated phenylalanine levels.

Material and methods: Frequent variant analysis by MLPA system, panel NGS sequencing (genes *PAH* and *PTS, QDPR, GCH1, PCBD1, SPR, DNAJC12*), repeated tandem mass spectrometry (TMS) for unrelated Russian patients referred were performed.

Results: Over a two-year period in the Russian Federation, 2,498,706 newborns were recorded. Expanded neonatal screening was performed for 2,430,554 newborns (coverage 97,3%). Among them, 1,293 newborns were identified as at-risk for GFA based on tandem mass spectrometry results, followed by confirmatory molecular genetic testing. In 520 newborns (1040 chromosomes), 2 or more pathogenic variants of the PAH gene were detected. The pathogenic variant p.Arg408Trp represents 33,7% of phenylketonuria (PKU) alleles and is the most prevalent mutation among Russian patients. The second most frequent PAH pathogenic variant in Russia is p.Ala403Val, accounting for 4,5% of alleles, followed by p.Ala300Ser, which constitutes 4,3% of the observed variants. Two variants in other genes responsible for the development of hyperphenylalaninemia were also identified in 6 patients. According to the new data, the frequency of phenylketonuria in the Russian Federation was 1:4692 people, in contrast to the previous estimated frequency of 1:7000.

Conclusion: As a result of the study, a change in the spectrum of mutations was observed: a significant change in the frequencies of occurrence of known mutations compared to previous data was recorded.

C.O.I. Disclosure:

E. Lotnik: None. **A. Chukhrova:** None. **N. Ryadninskaya:** None. **V. Kadnikova:** None. **E. Zakharova:** None. **G. Baydakova:** None. **S. Voronin:** None. **A. Polyakov:** None. **O. Shchagina:** None.

EP08.004 Allele dosage in addition to NGS was essential to identify a rare inborn error of metabolism in a case of

hypoglycemia during a viral infection

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Background: Ketotic hypoglycemia is common in school age children as a consequence of a decreased oral glucose intake due to gastrointestinal illness, especially with vomiting and prolonged fasting.

Material and methods: A Georgian 10-year-old female was admitted to the Intensive Care Unit of our hospital for lethargy and vomiting during a H1N1 febrile infection. She presented hypoglycemia with acute lactic acidosis. Plasma acilcarnitine and amino acids were normal. Urinary lactic and keto acids were abnormal. We suspected a gluconeogenesis defect. High coverage NGS targeted-resequencing of *FBP1* and *PC* genes using Illumina Nextera DNA Prep with Enrichment kit (Illumina). CoNVaDING (Johansson 2016, Hum Mutat 37:457-464) for calling copy number variations. MLPA by Probemix P255-B1 (MRC Holland).

Results: There were no pathogenetic single nucleotide variants in the genes sequenced. A second-level analysis of the NGS data revealed two gross gene rearrangements in *FBP1*. MLPA confirmed an exon 1 deletion and a duplication of exons 2 and 3 in the *FBP1* gene. The exon 1 deletion was previously reported as pathogenetic. The exon 2-3 duplication represents a new pathogenetic variant. The patient's parents were not available for genetic testing and further patient samples were not available for additional functional studies.

Conclusion: When ketotic hypoglycemia occurs with lactic acidosis during a viral infection, even in the absence of other typical alterations of FBP1 deficiency (i.e. elevated transaminases, triglycerides, glycerol, alanine and uric acid), we recommend FBP1 gene sequencing. Allele dosage proved to be crucial in achieving a molecular diagnosis for our patient.

C.O.I. Disclosure:

L. Ferri: None. **N. Campagna:** None. **M. Rinaldi:** None. **M. Daniotti:** None. **E. Procopio:** None. **F. Marin:** None. **G. la Marca:** None. **R. Guerrini:** None. **A. Morrone:** None.

EP08.005 A 40-year diagnostic delay in Adult Refsum Disease: A case study highlighting the importance of genetic testing and early intervention in genetic eye disease

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Background: Adult Refsum disease (ARD) is a rare metabolic disorder characterised by the accumulation of diet-derived phytanic acid in adipose and nervous tissues. This build-up results from an enzymatic deficiency in peroxisomes. The condition, associated with biallelic pathogenic variants in PHYH or PEX7 genes, often presents with syndromic retinitis pigmentosa (RP) and systemic manifestations.

Methods: A 53-year-old female, clinically diagnosed with RP at age 11, underwent panel-based genetic testing as a part of the VENTURE study (Victoria, Australia).

Results: Genetic testing identified homozygous PHYH c.823C>T (p.Arg275Trp) variants, pathogenic for ARD. A comprehensive clinical review identified anosmia, paraesthesia, hearing loss and skeletal anomalies, correlating with a Refsum disease diagnosis. Despite surgery for joint abnormalities, and care for other ARD features, the individual was not diagnosed with Refsum disease until 40 years after their initial RP diagnosis. This prolonged diagnostic odyssey resulted from a fragmented healthcare system and limited recognition of associated syndromic symptoms. Since their diagnosis, the patient has adhered to a low-phytanic acid diet, guided by Global Defeat Adult Refsum Everywhere Foundation recommendations, to manage phytanic acid levels and prevent complications.

Conclusion: This case highlights the critical role of genetic testing in accurately diagnosing rare conditions like ARD, enabling targeted interventions such as dietary modifications to mitigate disease progression and reduce the risk of cardiac death. Eye care providers must consider syndromic RP and collaborate with multidisciplinary teams to prevent diagnostic delays. Increased awareness of ARD, genetic testing, early diagnosis, and diet adherence has potential to improve outcomes.

C.O.I. Disclosure:

P. Truong: None. **H.G. Mack:** None. **A.B. Metha:** None. **N. Deen:** None. **A. Britten-Jones:** None. **L.N. Ayton:** None.

EP08.006 SGLT-2 management of neutropenia in GSD1b and G6PC3 deficiency, a Canadian experience

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G6PC3 deficiency is one of the most common monogenic causes of congenital neutropenia, compromising up to 25% of cases in certain ethnicities. SGLT-2 inhibitors have emerged in recent years as a safe and effective off-label use for the congenital neutropenia of both Glycogen Storage Disorder (GSD) 1b and G6PC3 deficiency. The established mechanism is a decrease in renal tubular reabsorption of 1,5-anhydroglycitol (1,5-AG), leading to an intracellular reduction of its metabolite 1,5-AG phosphate that antagonizes glucose metabolism in neutrophils. The existing standard of care is Granulocyte Colony Stimulating Factor (G-CSF), but regular intravenous infusions are required and there is an increased risk for bone marrow malignancies. There remains no clinical consensus on optimal choice of SGLT-2 inhibitor, or on appropriate dosing and monitoring regimens. Additional case series of treated patients would be beneficial in providing further evidence to guide the generation of evidence-based recommendations. Here, we describe two infants treated with Empagliflozin (an SGLT-2 inhibitor) in Calgary, Canada for neutropenia secondary to GSD1b and G6PC3 deficiency. One individual was diagnosed with GSD1b at four weeks of age and initiation of Empagliflozin led to an initial adverse effect of generalized urticaria, which was successfully treated as an outpatient. The second individual was diagnosed at 11 months of age with G6PC3 deficiency on trio exome sequencing, completed in the context of failure to thrive, congenital heart disease and self-resolving pancreatic insufficiency. Pre-initiation of Empagliflozin, 1,5-AG plasma levels were drawn to monitor response to treatment.

C.O.I. Disclosure:

S. Bradbrook: None. **M. Steele:** None. **M. Thomas:** None. **M. Lines:** None.

EP08.007 The *SMPD1* gene mutational spectrum of 49 patients with Niemann-Pick disease in Russia

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Background: Pathogenic variants in the SMPD1 gene are associated with Niemann-Pick disease type A/B (NPD A/B). The availability of enzyme replacement therapy, whose success depends on the disease type, highlights the importance of studying the genetic heterogeneity of NPD A/B.

Material and methods: We present molecular genetic data from 49 patients diagnosed with NPD A/B who underwent counseling at the RCMG between 2009 and 2024. The patients were genotyped using NGS or Sanger sequencing.

Results: In cohort, comprising 45 unrelated families, we identified 40 unique genetic variants in *SMPD1*, including 13 previously undescribed. All patients were categorized based on the clinical forms of NPD A/B: the infantile neurovisceral form (type A), the chronic visceral form (type B), and an intermediate form (type A/B). Among patients with type A, the most common variants were c.742G>A (31.8%), c.1159del (25%). In type B, the predominant variants were c.1493G>A (12.5%), c.1805G>A (12.5%). In type A/B, the most frequent variant was c.1177T>G (26.7%). Additionally, we identified eight previously undescribed variants associated with type B, accounting for 10.2% of alleles in all patients in our cohort. We also found two novel variants associated with type A (15%) and three new variants associated with the A/B form (4%).

Conclusion: This study expands our understanding of the mutation spectrum of the *SMPD1*. Knowledge of the mutation spectrum and the presence of common variants may facilitate the development of screening systems for identifying patients at the preclinical stage and aid in predicting the course and severity of clinical symptoms.

C.O.I. Disclosure:

A. Vasilenko: None. **E. Gusarova:** None. **N. Shatokhina:** None. **S. Mikhaylova:** None. **A. Lavrova:** None. **V. Zarubina:** None. **V. Lobanova:** None. **G. Movsisyan:** None. **E. Zakharova:** None.

EP08.008 Mitochondrial DNA depletion syndromes - case series of late onset presentation in 3 adults from southern India

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Background: The term 'Mitochondrial DNA (mtDNA) depletion syndromes' (MDS) refers autosomal recessive disorders that are characterized by a severe reduction in mtDNA content due to variants in nuclear genes that are needed for mtDNA synthesis or replication. These disorders usually present in infancy or early childhood as encephalopathy or hepatocerebral dysfunction.

Material and methods: We report 3 adult patients from a tertiary care centre in southern India. All were of Tamil ethnicity. Patient1 (19 years) and patient 2 (25 years) presented in early adulthood with progressive muscle wasting, peripheral neuropathy and recurrent pain abdomen. Patient 3 presented at 25 years of age with progressive weakness and wasting of the limbs and poor appetite. MRI showed extensive leukodystrophy in all three patients.

Results: Clinical exome sequencing showed a novel pathogenic homozygous nonsense variant in TYMP gene, c.454G>T (p.Gly152-Ter) in patient 1 while patient 2 had a earlier reported homozygous variant (c.866A>C, p.Glu289Ala).Hence these two patients were diagnosed to have Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) syndrome. The third patient had homozygous missense variant in exon 5 of MPV17 gene (c.293C>T, p.Pro98Leu). This variant has been reported previously to cause Navajo neurohepatopathy / Axonal Charcot-Marie-Tooth neuropathy type 2EE.

Conclusion: We report 3 patients who presented in early adulthood with severe peripheral neuropathy, abdominal symptoms and asymptomatic leukodystrophy. Two patients were diagnosed to have (MNGIE) syndrome while the 3rd patient was diagnosed to have Navajo neurohepatopathy / Axonal Charcot-Marie-Tooth neuropathy type 2EE. This report highlights the relatively late onset manifestations of (mtDNA) depletion syndromes.

C.O.I. Disclosure:

D. Amalnath: None.

EP08.009 Unraveling atypical lysosomal storage disease: molecular insights from adult siblings with hypertrophic cardiomyopathy and retinal dystrophy

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Background: Two adult siblings, diagnosed with hypertrophic obstructive cardiomyopathy, complicated by a ventricular aneurysm, advanced heart failure, ventricular arrhytmias and retinal dystrophy were referred for molecular genotyping. Both patients did not exhibit a neurocognitive phenotype.

Material and methods: We performed massive parallel sequencing on both patients and a healthy brother complemented by a review of family and medical history. Additionally, urinary glycosaminoglycans (GAG) levels were measured.

Results: Before referral, this family had been evaluated by both a neurologist and a cardiologist. Due to therapy-resistant ventricular arrhythmias, both patients ultimately required heart transplants. Exome sequencing analysis established a diagnosis of lysosomal storage disease (LSD) for both affected siblings, identifying bi-allelic variants in the *SGSH* gene responsible for Sanfilippo III A (MPS III A). Elevated urinary GAG levels further corroborated this molecular diagnosis.

Conclusion: The adult-onset presentation of LSD in this family was primarily characterized by hypertrophic cardiomyopathy and retinal dystrophy, leading to an extended diagnostic challenge. While the phenotypic spectrum of many LSDs, including MPS III A,

is broader than previously recognized, a non-classic and attenuated phenotype can significantly obscure diagnosis. Exome sequencing proved to be a crucial clinical tool in diagnosing rare diseases providing essential insights into the genetic basis of atypical phenotypes, as illustrated in this case.

C.O.I. Disclosure:

S. Seneca: None. **A. Gheldof:** None. **M. Vanderheyden:** None. **G. Cypers:** None.

EP08.010 Clinical phenotype of two individuals with multiple mitochondrial dysfunctions syndrome 2 caused by *BOLA3* p.Arg46Ter homozygous pathogenic variants

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Background/Objectives: Multiple mitochondrial dysfunctions syndrome 2 (MMDS2) is a rare autosomal recessive neurodegenerative condition caused by biallelic pathogenic variants in *BOLA3*, a gene which plays a vital role in the biogenesis of mitochondrial [4Fe-4S] proteins. We report the clinical phenotype of two patients with *BOLA3*:c.136C>T (p.Arg46*) homozygous pathogenic variants.

Methods: In this retrospective case series, we identified two individuals with *BOLA3* pathogenic variants by whole exome sequencing and analyzed their clinical phenotype, electroencephalogram (EEG) and brain imaging findings.

Results: Both patients presented with infantile onset epilepsy, hypotonia, global developmental delay with neuroregression, lactic acidosis, hyperglycinemia and hypertrophic cardiomyopathy. MRI brain of patient 1 showed hypomyelination with vacuolation in bilateral posterior limbs of the internal capsule whereas MRI brain of patient 2 showed T2 white matter hyperintensities with restricted diffusion. Electroencephalograms did not capture any seizure activity in both patients. EEG of patient 2 showed slow background, suggestive of encephalopathy. Patient 1 is showing clinical improvement, and is currently being treated with co-enzyme Q10, thiamine, L-carnitine, alpha lipoic acid, vigabatrin and levetiracetam. Patient 2 passed away from severe cardiomyopathy with multiple complications at 5 months of life.

Conclusion: The severe clinical phenotype with infantile onset of symptoms of these two individuals is in keeping with the previously reported patients with *BOLA3* p.Arg46* mutations. However, their different clinical course indicates the possibility of genetic modifiers in this disease.

C.O.I. Disclosure:

M. Chan: None. **J. Lee:** None. **S. Chow:** None. **M. Haniffa:** None. **L. Ngu:** None.

EP08.011 Rare variants in the *LEPR* gene in three patients with severe early-onset obesity and hyperphagia identified in the Russian Federation

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Background: Congenital leptin deficiency is a rare cause of early-onset obesity. We report novel LEPR variants identified through molecular diagnosis in Russian patients with severe hyperphagia and morbid obesity.

Methods: Whole exome sequencing was performed on genomic DNA from affected individuals. Bioinformatic analysis prioritized variants in leptin-melanocortin pathway genes. Sanger sequencing was performed for variants validation.

Results: Two rare pathogenic LEPR variants introducing premature termination codon were identified. The first variant (NM_002303.6:c.133_136dup:p.(Tyr46Ter)) was identified in a homozygous state in two patients with obesity onset at 1 month and 1 year. The third patient with onset of obesity in the first year of life was identified as a compound heterozygote for the c.133_136dup variant and a novel variant (NM_002303.6:c.2590_2591del:p.(His864ProfsTer24); two variant loci in the proband were in the trans position. LEPR encodes the leptin receptor, a critical protein of leptin-melanocortin signaling required for proopiomelanocortin neuron activation and appetite regulation. Both variants are predicted to result in truncated, nonfunctional receptors, consistent with autosomal recessive leptin receptor deficiency.

Conclusion: This study makes a significant contribution to validating the genetic basis of childhood obesity, focusing on the role of rare LEPR gene variants. By validating the role of LEPR variants in childhood obesity, this research paves the way for more precise diagnostic and therapeutic strategies, ultimately improving outcomes for affected individuals.

C.O.I. Disclosure:

A. Nikolaeva: None. **A. Tiulpakov:** None. **Y. Tikhonovich:** None. **O. Ryzhkova:** None. **V. Sigin:** None.

EP08.012 Flow cytometry-based alpha-galactosidase activity of human PBMC and T Cells

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Background: Fabry disease, the most common lysosomal storage disorder, due to deficient or reduced activity of alpha-galactosidase (AGAL) is underdiagnosed worldwide. In our previous work, we have established a single-cell flow cytometry assay suitable for measuring AGAL activity in Jurkat T cell line. The aim of our study was to optimize the developed assay using primary human cells.

Material and methods: Peripherial mononuclear cells were isolated from healthy controls and negatively sorted with flow cytometry. Peripheral mononuclear and T cells were incubated at different fluorescently labelled globotriaosyl-ceramide (Fl-Gb3) with or without 1-deoxygalactonojirimycin and assessed the AGAL activity.

Results: For primary human T cells and monocytes 5.66 nmol/mLFl-Gb3 was required for the detection of AGAL activity. After 6 hours of incubation the detectable mean fluorescence intensity in monocytes was significantly higher compared to T cells (43924 ± 4560 vs. 2129 ± 169 MFI).

Conclusion: Compared to the Jurkat T cell line AGAL assay, a higher concentration of Fl-Gb3 was needed to assess AGAL activity. Moreover, in human PBMCs the detection of AGAL activity was 20-fold higher than in T cells.

C.O.I. Disclosure:

L. Li: None. **N. Fekete:** None. **C. Bödör:** None. **H. Vágó:** None. **É. Pállinger:** None. **Á. Dr. Kovács:** None.

EP08.013 a novel mechanism between diabetes mellitus complications and procalcitonin gene expression

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Background:

Material and methods:

Results:

Conclusion: Procintroduction: The PCT gene, referred to as Calc-1, is located on chromosome 11p15.4 and was sequenced in 1989. The promoter has sites for basal transcription factors but more interestingly, also has sites for Nuclear factor κβ (NFκβ) and activator protein −1 (AP-1), factors induced under inflammatory conditions. Diabetes Mellitus is associated with oxidative stress and elevation of advanced glycation end products (AGEs). AGEs are produced by a non-enzymatic, maillard reaction between reducing sugars and either proteins or lipids. AGEs interacts the receptor for advanced glycation end products (RAGE) and RAGE activation is caused by elevation of transcriptional factors NFκβ and AP-1. These factors induce procalcitonin gene expression. The aim of this study was to determine whether or not procalcitonin is a specific marker in patients with diabetic complications.

Results: Twenty diabetic foot patients were studied along with age and sex matched normal 20 non-diabetic subjects. Blood samples were taken for measurements of procalcitonin. Serum procalcitonin levels were analized with kryptor analizator. Kryptor-PCT is a kit designed for kryptor automated immunofluorescent assay of procalcitonin in serum. Procalcitonin levels were elevated in diabetic foot patients when compared with normal non-diabetic subjects. There was a statistically significant difference in serum procalcitonin of diabetic foot patients versus normal subjects ($P < 0.01$).

Conclusions: Our study revealed a raise in serum procalcitonin in patients with diabetic foot complications. Diabetic complications associated with increased circulating concentrations of procalcitonin may be a mechanism which explains many of the clinical and biochemical features of diabetic complications.

C.O.I. Disclosure:

M. Soylemez: None.

EP08.014 The c.508-26C > G *GALT* gene variant is a deep intronic variant associated positive newborn screening for galactosemia

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Background: Galactose-1-phospate uridylyltransferase (GALT) is the third enzyme of the Leloir pathway responsible for galactose catabolism. Mutations in the *GALT* gene are associated with classic Galactosemia.

Material and methods: A female who tested positive for galactosemia at Newborn Screening and was deficient in GALT enzyme activity (1.8 U/g Hb; normal >4.4) resulted heterozygous for a single pathogenetic *GALT* variant inherited from her father. GALT activity was normal in the patient's father.High coverage NGS targeted-resequencing of *GALT*, *GALK1* and *GALE* genes using Illumina Nextera DNA Prep with Enrichment kit (Illumina). Sanger sequencing of *GALM* gene. CoNVaDING (Johansson 2016, Hum Mutat 37:457–464) for calling copy number variations.

Results: The patient resulted compound heterozygous for two *GALT* variants. A paternally inherited pathogenetic variant c.855G>T p.(Lys285Asn) and a rare novel deep intronic variant of unknown significance c.508-26C > G inherited from her mother. *GALT* transcript analysis from whole blood mRNA showed an unexpected extra-amplicon involving *GALT* exons 5 and 6 in the proband and her mother, but not in the father or in controls. Sequencing analysis confirmed the presence of abnormal 67 bp between exons 5 and 6. The inserted bases were part of *GALT* intron 5 and led to a frameshift with premature stop codon p.(Ile170Glyfs25*).

Conclusion: The c.508-26C > G *GALT* variant is a novel deep intronic pathogenetic variant which is associated with elevated blood galactose at newborn screening. Further analyses will be needed to assess the clinical impact of this new *GALT* variant on our patient's phenotype and dietary regimen.

C.O.I. Disclosure:

M. Rinaldi: None. **L. Ferri:** None. **F. Tubili:** None. **G. la Marca:** None. **E. Procopio:** None. **F. Feo:** None. **S. Falliano:** None. **R. Guerrini:** None. **A. Morrone:** None.

EP08.015 Metabolic and Genetic Profiles of Moldovan Patients with Suspected Mitochondrial Diseases

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Background: Mitochondrial diseases (MD) represent a diverse group of inherited disorders primarily characterized by dysfunctions in oxidative phosphorylation, leading to impaired cellular energy production, and manifesting through a wide range of clinical phenotypes affecting multiple organ systems.

Material and methods: A total of 100 patients presenting with clinical features indicative of MD were analyzed for 8 common pathogenic mitochondrial DNA (mtDNA) point mutations using quantitative PCR high-resolution melting (qPCR-HRM) analysis, followed by Sanger sequencing. In addition, metabolic evaluations included the measurement of plasma amino acids, acylcarnitine profiles, and urinary organic acids.

Results: Patients were selected using the Nijmegen MD scoring system, with 8 classified as possible MD, 79 as probable MD, and 13 as definite MD. Elevated serum lactate was observed in all patients with definite MD and the majority of those with probable MD. Increased alanine levels were identified in 17 patients, while abnormalities in urinary organic acid profiles and acylcarnitine profiles were detected in 11 and 7 patients, respectively. Using the qPCR-HRM method, pathogenic mtDNA mutations were identified in 8 patients, including m.3243A > G (3 cases), m.8993T > G (2 cases), m.3460A > G (2 cases), and m.11778G > A (1 case). Further mitochondrial genome analysis through Sanger sequencing revealed pathogenic or potentially pathogenic mutations linked to the clinical phenotype in 28 patients.

Conclusion: Among the 100 patients assessed for clinically suspected mitochondrial disease, a total of 36 individuals were found to carry pathogenic or potentially pathogenic variants that correlated with their observed clinical manifestations, highlighting the genetic underpinnings of their condition.

C.O.I. Disclosure:

D. Secu: Other; Modest; Ministry of Research, Innovation and Digitalization, CNCS-UEFISCDI, project PN-IV-P8-8.3-ROMD-2023-0249, PNCDI-IV (Romania). **D. Blanita:** None. **N. Usurelu:** None. **C. Deleanu:** None. **A. Nicolescu:** None. **V. Sacara:** None.

EP08.016 Congenital generalized lipodystrophy type 2: importance of early intervention in a rare disease

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Background: Congenital generalized lipodystrophy type 2 (CGL2) (OMIM #269700) is a rare disease (1/1,000,000 births) caused by pathogenic variants in the BSCL2 gene, which encodes seipin, an endoplasmic reticulum protein. Diagnosis is based on clinical features, including acromegalic facies, lipoatrophy, hypertriglyceridemia, hepatomegaly, and insulin resistance, and is confirmed through molecular testing. This study aims to present a case of CGL2 while emphasizing the importance of early dietary and pharmacological treatment in preventing rapid disease progression.

Material and methods: A 6-year-old male was referred to Medical Genetics at 1 year of age due to low weight, generalized lipoatrophy, triangular facies, large hands and feet, and neurodevelopmental delay. Studies revealed nephromegaly, splenomegaly, hepatomegaly, hepatic steatosis, hypercholesterolemia, hyperinsulinemia, hypertriglyceridemia, and elevated liver enzymes. A clinical diagnosis of congenital generalized lipodystrophy was established, and an NGS panel was requested for confirmation.

Results: The c.517dup p.Thr173Asnfs*5 variant in the BSCL2 gene was identified and classified as pathogenic according to the ACMG criteria PVS1, PP5, and PM2, confirming CGL2. A low-lipid diet was initiated at 2 years of age following the clinical diagnosis. Subsequently, metreleptin therapy was introduced at 3 years of age upon molecular confirmation. Continuous metabolic monitoring showed improvement following treatment (Table 1).

Conclusion: This case underscores the importance of early recognition of clinical features in rare diseases, enabling accurate diagnosis, prompt treatment, and interdisciplinary follow-up to prevent complications that reduce the life expectancy of these patients.

Table 1. Metabolic values.

	Normal value	1 year	2 years	3 years	4 years	5 years	6 years
Glycated Hemoglobin (%)	<5,7	-	5,1	-	5,5	4,7	-
Triglycerides (mg/dL)	<100	275	120	86	105	110	101
Low-Density Lipoprotein (mg/dL)	<130	126	-	90,4	107	-	150,3
High-Density Lipoprotein (mg/dL)	>40	43	45,6	36,4	39,7	-	40,1
Total cholesterol (mg/dL)	<200	224	187	144	168	147	211
Fasting blood glucose (mg/dL)	70–100	96	70	88	72	81	83
Insulin (uU/mL)	2–20	54,6	-	-	-	13,18	-
Aspartate aminotransferase (U/L)	15–55	149,46	73	52,9	32,9	30,5	26,4
Alanine aminotransferase (U/L)	5–30	112,11	90	92,1	44,8	33,4	30,1

C.O.I. Disclosure:
A.M. Perez Ramirez: None. **M.L. Porras:** None. **F. Rodriguez:** None. **G.A. Contreras:** None.

EP08.017 Mucopolysaccharidosis IVA: clinical description of a case series in the northeastern region of Colombia

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Background: Mucopolysaccharidosis IVA (OMIM #253000) is an autosomal recessive lysosomal storage disease with an incidence of 1/70,000 to 1/200,000 births. The condition is caused by pathogenic variants in the gene encoding N-acetylgalactosamine-6-sulfatase (GALNS) leading to the accumulation of glycosaminoglycans (GAGs), specifically keratan sulfate and chondroitin

Table 1. Patients clinical features.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Gender	F	F	M	M	F	M	M	F	F
Age	31	29	23	12	19	15	56	51	40
Age of diagnosis	20	19	14	3	7	5	47	41	30
Age of initiation of treatment	21	20	14	3	11	5	47	42	31
Enzymatic activity	0	0	0.07	0,04	0,01	0,21	0,15	0	0,16
Short stature	x	x	x	x	x	x	x	x	x
Increased of anteroposterior thoracic diameter	x	x	x	x	x	x	x	x	x
Scoliosis	x	x	x	x	x	x	x	x	x
Midface Hypoplasia	x	x	x	x	x	x	x	x	x
Short neck	x	x	x	x	x		x	x	x
Genu valgum	x	x		x	x	x	x	x	x
Hypermobility	x	x	x	x	x	x		x	x
Osteopenia		x	x	x	x	x	x	x	x
Corneal Opacity	x	x	x	x	x	x	x		x
Ulnar Deviation	x	x		x	x	x	x		x
Sleep apnea		x	x	x		x	x	x	x
Discopathy		x	x		x	x	x	x	x
Narrowing of intervertebralspaces		x	x	x	x	x		x	x
Kyphosis	x			x	x	x	x		x

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Cardiovascular disease	x			x	x	x	x		x
Hip dysplasia			x	x	x	x		x	x
Restrictive lung disease	x	x	x		x	x		x	
Hearing Loss	x	x	x			x		x	x
Lumbar lordosis	x	x			x	x			x
Macrocephaly	x	x		x		x			x
Depressed nasal bridge	x			x	x				x
Odontoid hypoplasia		x	x		x			x	
Spinal cord compression	x	x	x		x				
Hernia		x				x			

sulfate. This study aims to present a series of Mucopolysaccharidosis IVA cases while highlighting the importance of early diagnosis.

Material and methods: This study includes 9 patients aged 12 to 56 years from the northeastern region of Colombia. Their characteristics fall within the phenotypic range described in the literature (table 1). All cases underwent GALNS enzymatic activity determination in leukocytes.

Results: The enzymatic activity range was 0–0.21 nmol/mg/prot/h (reference value: 2–35.9 nmol/mg/prot/h), leading to diagnosis confirmation between ages 3 and 47. Five adults received late diagnoses despite symptom onset in childhood or adolescence. All patients received a multidisciplinary approach and enzyme replacement therapy with Elosulfase Alfa. However, therapeutic outcomes varied among individuals due to differences in treatment initiation and interruptions.

Conclusion: Medical training must be enhanced to address delayed diagnosis of Mucopolysaccharidosis IVA, allowing timely identification of characteristic findings and appropriate diagnostic testing to prevent disease progression and improve patient outcomes.

C.O.I. Disclosure:

M.L. Porras Piza: None. **A.M. Perez Ramirez:** None. **F. Rodriguez:** None. **G. Contreras:** None.

EP08.018 Isovaleric acidemia due to paternal uniparental isodisomy of chromosome 15

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Background: Isovaleric academia (IVA) is an autosomal recessive disorder, organic aciduria, due to mutations in *IVD* gene encoding the enzyme isovaleryl-CoA dehydrogenase (IVD). IVD dysfunctions lead to accumulation of isovaleryl-CoA and other metabolites, which are toxic to the central nervous system (CNS). The clinical spectrum of IVA varies from asymptomatic form to acute neonatal form. The IVD gene is located on chromosome 15q14-15. Co-occurrence of IVA and other chromosome 15 disorders, such as Angelman syndrome (AS), involving uniparental disomy (UPD) can be seen in rare cases.

Material and methods: We present a 5-year-old girl from second multifetal pregnancy of non-consanguineous parents, delivered by cesarean section at 35 wk of gestation as first twin. The following findings are found in the patient: dysmorphic features (brachycephaly, high forehead, hypertelorism, slightly smoothed supraorbital arches, small and low-set ears, pes equinovarus bilateralis), global developmental delay, normal physical development. The following genetic tests were performed: karyotype - 46,XX; MLPA for microdeletions, subtelomeric deletions and duplications - normal; whole exome sequencing (WES).

Results: WES identified homozygous pathogenic variant c.941C>T (p.Ala314Val) in *IVD* gene, which is genetic finding of IVA, later confirmed by a moderately increased C5 concentration. Homozygosity of c.941C>T in the *IVD* gene predicts mild IVA, which does not explain the clinical phenotype of the patient. The next investigation was UPD analysis, which showed paternal uniparental isodisomy of chromosome 15, associated with Angelman syndrome.

Conclusion: Paternal UPD 15 can rarely lead to co-occurrence of Angelman syndrome and potentially treatable inborn errors of metabolism as IVA.

C.O.I. Disclosure:

T.I. Veleva: None. **D. Avdjieva-Tzavella:** None. **T.N. Delchev:** None.

EP08.019 Mitochondrial genome analysis in patients with suspected monogenic diabetes: expanding the diagnostic yield

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Background: Monogenic diabetes is a heterogeneous group of rare genetic disorders that involves mitochondrial diabetes associated with pathogenic mitochondrial DNA (mtDNA) variants. The diagnosis of mitochondrial diabetes can be particularly difficult due to heteroplasmy and its age-related decline in the blood. The present study aimed to identify pathogenic mtDNA variants in 112 patients referred with suspected monogenic diabetes based on positive family history and clinical presentation and with negative whole exome sequencing (WES) results for nuclear DNA.

Material and methods: DNA was isolated from the blood of patients in the study group using the Maxwell automated system (Promega). Sequencing was performed by applying Illumina DNA Prep to the long-range PCR product of whole mtDNA on the Miseq System (2 × 250bp, Illumina). mtDNA-Server was used for mtDNA analysis and our custom pipeline for variant prioritization. NC_012920.1 was used as the reference sequence.

Results: Causative variants in mtDNA were found in 5/112 (4.46%) patients in the study group. The pathogenic m.3243A > G variant was identified in three patients, while each of the m.6745_15219del and m.12264C > T variants was detected in one patient. Notably, the m.12264C > T variant was previously submitted in MITOMAP with a status of *Reported*. The variant m.6745_15219del represents a large deletion detected in the blood.

Conclusion: The success rate of 4.45% highlights the importance of combining WES with mtDNA analysis in patients referred with suspected monogenic diabetes. However, given the

limitations of blood heteroplasmy, analysis of alternative biological materials such as urine or muscle biopsy could increase diagnostic success rates for mitochondrial diabetes.

C.O.I. Disclosure:

S. Skoczylas: None. **T. Płoszaj:** None. **M. Traczyk-Borszyńska:** None. **K. Gadzalska:** None. **P. Jakiel:** None. **E. Juścińska:** None. **M. Gorządek:** None. **A. Zmyslowska:** None.

EP08.020 Neonatal-OnsetMultiple-acyl-CoA Dehydrogenase Deficiency (MADD):An Unanticipated Early Onset Inborn Errors of Metabolism

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Background: Multiple-acyl-CoA dehydrogenase deficiency (MADD) is a rare autosomal recessive disorder involving compromised fatty acid oxidation and metabolism of amino acids. Clinically, this will translate into acute encephalopathy with hypoketotic hypoglycemia, hepatomegaly, hypotonia, metabolic acidosis and elevated creatine kinase. We report a neonate presenting with sepsis-like illness within 24 hours of life to highlight the importance of early screening for inborn errors of metabolism (IEM), done in tandem with investigations for sepsis.

Material and methods: A late preterm baby girl presented with lethargy and hypoglycemia (2.4mmol/L) at 18 hours of life. She deteriorated fast requiring ventilatory support within a few hours. Clinical examination showed hypotonia without obvious dysmorphism. There was persistent metabolic acidosis, urine organic acid and acylcarnitine profile suggesting MADD. She was commenced on high dose riboflavin, coenzyme Q10, carnitine and a diet restricted in fat and protein, and rich in carbohydrate. Surveillance for other organs involvement revealed hypertrophic cardiomyopathy, lissencephaly and normal kidneys. She had a long stay in NICU due to feeding intolerance, thrombocytopenia, hyperCK-emia and treatment for clinical sepsis without positive cultures. She was subsequently discharged on day 50 of life with perfusor feeding.

Conclusion: Individuals with type I and II MADD typically become symptomatic in the first 24 hours of life. Diagnosis is often delayed because of non-specific clinical presentation that can overlap with many common neonatal conditions. We advocate preforming IEM screening together with septic work-up in neonates without risk of sepsis. Knowing the diagnosis allows early institution of treatment which alters outcome.

C.O.I. Disclosure:

J. Lee: None. **H. Chew:** None. **H. Leong:** None. **S. Chai:** None.

EP08.021 The phenotypic spectrum of Dihydrolipoamide dehydrogenase deficiency in Tunisia

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Dihydrolipoamide dehydrogenase (DLD) deficiency is an ultra-rare autosomal recessive inborn error of metabolism, affecting no less than five mitochondrial multienzyme complexes. It was associated with three major clinical presentations: an early-onset encephalopathic phenotype with metabolic acidosis, a predominantly hepatic presentation with liver failure, and a rare myopathic phenotype. We retrospectively reviewed the clinical and molecular diagnosis of five Tunisian patients with DLD deficiency, from two families originally from the center and south of Tunisia. The age at presentation ranged from 9 months to 15 years. Significant phenotypic variability was observed both between and within families, ranging from a myopathic phenotype in a 15-year-old girl to severe early-onset disease accompanied by neurological sequelae. Notably, hepatic involvement was documented in four patients, presenting as a spectrum from acute hepatic failure to chronic hepatitis. Recurrent episodes of hypoglycemia were reported in one patient. In all cases, a homozygous variant, DLD:c.685G>T (p.G229C), was found. This variant is especially prevalent in the Ashkenazi Jewish population. A study of a possible founder effect of this variant is underway. DLD deficiency is a disorder, with significant phenotypic variability, and comprises a continuum, rather than three distinct clinical presentations.

C.O.I. Disclosure:

M. Guirat: None. **A. Ben Chehida:** None. **S. Guidara:** None. **F. Majdoub:** None. **Y. Lajmi:** None. **H. Kamoun:** None. **I. Boujelbene:** None. **I. Ben Ayed:** None.

EP08.023 A Case of Combined Oxidative Phosphorylation Deficiency 37 associated with a novel homozygous variant in the *MICOS13* gene

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Background: Combined oxidative phosphorylation deficiency type 37 is an extremely rare multisystem disorder caused by biallelic variants in the *MICOS13* gene. It is characterized by hypotonia, failure to thrive, neurologic deterioration and liver dysfunction. Death usually occurs in the first weeks or years of life.

Material and methods: Whole exome sequencing has been performed in a patient with suspected mitochondrial disease. Sanger sequencing was used for segregation analysis.

Results: We report an 11-month-old female patient, born to non-consanguineous parents with no remarkable family history, who presented with generalized hypotonia, hypoglycemia, severe metabolic acidosis, renal failure and hepatocellular dysfunction. The patient had progressive neurologic deterioration and deceased of respiratory failure. Her 5-day-old younger sister was also referred to our department for isolated neonatal hypotonia. Post-mortem whole-exome sequencing revealed a novel homozygous frameshift variant of the *MICOS13* gene (NM_205767.3):c.143dup (p.Ala51Argfs*32), classified as likely pathogenic according to the American College of Medical Genetics and Genomics. Segregation analysis showed the proband's parents and younger sister to be heterozygous carriers. Thus, an appropriate genetic counseling was provided and prenatal diagnosis was proposed for future pregnancies.

Conclusion: Our study expands the mutation spectrum of *MICOS13* and highlights the importance of whole-exome sequencing in the diagnosis of mitochondrial disorders.

C.O.I. Disclosure:

W. Tergui: None. **A. Achour:** None. **M. Zribi:** None. **A. Zanati:** None. **M. Ksentini:** None. **R. Mrad:** None. **M. Trabelsi:** None.

EP08.024 Differentially expressed lncRNAs and microRNAs in visceral fat tissue of obese and non-obese individuals

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Background: Long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) regulate key metabolic processes, including adipogenesis, lipid metabolism, and insulin response [2,3]. However, their roles in obesity remain unclear, with conflicting findings in previous studies [4]. This study examines the expression of selected lncRNAs (ASMER1, SNHG9, P5549, P19461, GAS5) and miRNAs (MIR26A1, MIR222, MIR221, MIR155) in the visceral adipose tissue of obese and non-obese individuals.

Material and methods: Visceral adipose tissue samples were collected from 70 obese and 31 non-obese individuals undergoing elective surgery. Abdominal obesity was defined as BMI ≥ 25.0 kg/m^2 and/or WC ≥ 80 cm (women) or WC ≥ 94 cm (men) [5]. RNA expression was quantified via qPCR, and correlations with clinical markers were assessed using the Mann-Whitney U and Spearman's correlation tests.

Results: expression of ASMER1, miR-222, and miR-221 were undetectable by the used method. P5549 was expressed in a single isoform, while P19461 appeared in two different isoforms. SNHG9, GAS5, and miR-155 showed no significant differences between groups, whereas miR-26A1 was significantly lower (1.5-fold, $P < 0.01$) in obese individuals. GAS5 expression was significantly higher (3-fold, $P < 0.05$) in obese participants with T2D (type 2 diabetes) compared to obese individuals not diagnosed with T2D.

Conclusion: Our findings aligned with previous studies describing miR-26A1 to be downregulated in the visceral fat of obese individuals, and supporting its role in adipogenesis [6,7]. The increase in GAS5 levels in T2D patients contrasts with previous blood-based studies [8,9,10], suggesting a tissue-specific regulatory mechanism that requires further investigation.

C.O.I. Disclosure:

A. Bairqdar: None. **D. Ivanoshchuk:** None. **O. Tuzovskaia:** None. **N. Shirokova:** None. **E. Kashtanova:** None. **Y. Polonskaya:** None. **Y. Ragino:** None. **E. Shakhtshneider:** None.

EP08.025 Biallelic homozygous variant in *NDUFA10* associated with nonsyndromic optic atrophy

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Background: Disturbed mitochondrial function and a predominantly complex I respiratory chain defect is the most frequent cause of optic atrophy. The protein encoded by the NDUFA10 gene is a component of 42 kDa complex I (CI), the first enzyme complex in the electron transport chain of mitochondria. This protein has NADH dehydrogenase and oxidoreductase activity. Biallelic mutations in NDUFA10 have been identified in three cases with Leigh syndrome. Herein we report two siblings with isolated optic atrophy, homozygous missense variant in NDUFA10 and CI + III deficiency.

Material and methods: Exome sequencing was performed using SureSelect Human All Exon V6 Kit. Mitochondrial DNA was screened using Roche Mitochondrial Genome Design enrichment kit. Detected variants were confirmed by Sanger sequencing. Activities of respiratory chain complexes and citrate synthase were analysed spectrophotometrically in lymphocytes.

Results: Two brothers, aged 16 and 18 years were referred with optic atrophy. Ocular examination documented in both siblings bilaterally decreased visual acuity and contrast sensitivity, disturbed colour vision and abnormal retinal nerve fibre layer thickness. No other pathology was found. Both brothers were homozygous carriers of c.1009C>T p.(Arg337Cys) in *NDUFA10* (NM_004544.4), while their parents were heterozygous for this change. No other rare variants in genes associated with optic neuropathy (Panelapp Version 4.0) evaluated as possibly pathogenic were identified. Both affected brothers had markedly decreased activity of complex I + III (30% and 49% compared to controls and parents), suggesting disturbance of complex I function.

Conclusion: The role of *NDUFA10* as a cause of isolated optic atrophy needs to be further investigated.

C.O.I. Disclosure:

P. Liskova: F. Consultant/Advisory Board; Modest; Chiesi. **M. Tesarova:** None. **L. Dudakova:** None. **H. Stufkova:** None. **H. Ptackova:** None. **S. Kelifova:** None. **M. Meliska:** None. **M. Vajter:** None. **H. Hansikova:** None.

EP08.026 A rare variant *MT-ATP6*(NC_012920.1):m.8851T > C (p.Trp109Arg) impairs stability of F_1F_O-ATP synthase: laboratory and clinical findings

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Background: The mitochondrial *MT-ATP6* gene encodes subunit *a* of the F_1F_O-ATP synthase (complex V), a key enzyme of mitochondrial energy metabolism, and extensive phenotypic heterogeneity exists among its pathogenic variants. We studied the impact of a rare *MT-ATP6*(NC_012920.1):m.8851T > C (p.Trp109Arg) pathogenic variant on complex V. The variant was identified in two unrelated families with different clinical phenotypes. Progressive ataxia, spasticity, hearing impairment, and dysarthria were present in three-generation family 1, where the variant was homoplasmic in all symptomatic members. Variable heteroplasmy levels (68 – 100%) and phenotypes, including sensory-motor polyneuropathy, psychomotor retardation, hearing impairment, developmental delay, and Leigh syndrome, were observed in members of a three-generation family 2 (F2).

Material and methods: The variant was detected by mitochondrial genome sequencing in various tissues (blood, urine, buccal cells, hair, skin, muscle) from members of both families. Exome sequencing excluded other pathogenic variants. Functional and electrophoretic analyses were performed on muscle and fibroblast mitochondria obtained from the F2 patient to evaluate the variant's impact on complex V.

Results: In muscle mitochondria, OXPHOS energy-generating capacity indicated diminished OXPHOS activity due to a disturbed complex V. Native electrophoresis revealed a reduced amount of complex V holoenzyme in the muscle and fibroblast mitochondria. Steady-state levels of complex V subunits were unchanged in fibroblasts. 2D-electrophoresis (BN/SDS-PAGE) revealed the presence of accumulated complex V sub-complexes (V*-F1-part with c8-ring and free F1-part) in patient mitochondria.

Conclusion: The *MT-ATP6*(NC_012920.1):m.8851T > C (p.Trp109Arg) variant disrupts mitochondrial complex V function and stability, as demonstrated in both muscle and fibroblast mitochondria.

C.O.I. Disclosure:

K. Lokvencová: None. **H. Štufková:** None. **S. Kohoutová:** None. **E. Vyhnálková:** None. **M. Vyhnálek:** None. **H. Ptáčková:** None. **H. Hansíková:** None. **T. Honzík:** None. **J. Zeman:** None. **M. Tesařová:** None.

EP08.027 Mitochondrial diseases at the Institute for Maternal and Child Health IRCCS 'Burlo Garofolo' of Trieste

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Background: Mitochondrial diseases (MDs) are a group of neuromuscular-metabolic disorders (estimated prevalence 1:4,300 births). Their diagnosis is mainly challenging due to heterogeneous phenotype and the involvement of both mitochondrial (mtDNA) and nuclear (nDNA) genomes. This study describes pediatric patients with suspected or confirmed MD followed at IRCCS Burlo Garofolo since 2000.

Material and methods: We reviewed the clinical and genetic records of 22 probands and three sibling pairs. DNA variants were analyzed using online databases and relevant literature.

Results: MtDNA variants were identified in 11 of 28 patients, all reported and classified as pathogenic or of uncertain significance. However, clinical presentations did not always overlap previous descriptions. In some cases, these differences could be explained by considering the mtDNA variant load and specific affected tissues. In a patient the pathovariant did not explain the phenotype. In addition, 13 patients out of 28 had pathogenic variants in nDNA, with *ACAD9* and *RRMB2* being the most represented, mutated in 2/13 and 3/13 patients, respectively. Five patients remained still undiagnosed.

Conclusion: In our small series, mtDNA alterations were present in approximately one-third of the patients with suspected MDs, confirming the importance of analyzing the mitochondrial genome. Moreover, further investigation through whole exome/genome analysis would allow the evaluation of the possible interaction between an already described mtDNA variant and variants in nDNA that could modulate its effect on the severity of the phenotype. Furthermore, a subject in our cohort with a pathogenic mtDNA variant exhibited a neurodevelopmental disorder, as already reported in the literature.

C.O.I. Disclosure:

M. Bonati: None. **L. Zupin:** None. **E. Lamantea:** None. **N. Zanetti:** None. **A. Magnolato:** None. **F. Baldo:** None. **E. Orzan:** None. **V. Capaci:** None. **F. Celsi:** None. **A. Skabar:** None. **G. Zappulla:** None. **I. Bruno:** None. **M. Zeviani:** None.

EP08.028 Enhanced Phenylketonuria detection rate in Newborn Screening following implementation of Tandem Mass Spectrometry versus ELISA methodology

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Background: Newborn screening (NBS) for Phenylketonuria (PKU) represents a pioneering system for early diagnosis and treatment of genetic disorders. In October 2022, Ukraine expanded its newborn screening program to encompass 21 rare diseases, transitioning from ELISA-based to tandem mass spectrometry-based PKU screening methodology.

Material and methods: Dried blood spot (DBS) samples from newborns were analyzed using tandem mass spectrometry (LC-MS/MS) with Waters Xevo TQD instrumentation and Chromsystems (Germany) reagents. Positive cases underwent confirmatory molecular diagnostic testing. A retrospective analysis of ten-year PKU-NBS data using ELISA methodology was conducted for comparison.

Results: From October 2022, the Lviv Regional Neonatal Screening Center screened 132,210 newborns using tandem mass spectrometry, identifying 44 suspicious cases, of which 24 were confirmed PKU cases (PPV = 0.55). In comparison, ELISA-based screening from 2013 to September 2022 examined 540,862 newborns, yielding 357 suspicious results and 51 confirmed PKU cases (PPV = 0.14). The PKU detection rate in Western Ukraine improved from 1:10,605 to 1:5,508 newborns, attributable to enhanced methodological specificity and implementation of a centralized follow-up system. Molecular analysis revealed p.Arg408Trp as the predominant PAH variant (41.16%), followed by p.Arg158Gln (16.66%), p.Ala403Val (12.5%), p.Tyr414Cys (8.33%), p.Arg252Trp (8.33%), p.Glu280Lys (8.33%), and p.Pro225Thr (4.16%).

Conclusion: Tandem mass spectrometry demonstrates superior efficiency in PKU newborn screening compared to traditional ELISA methodology, significantly improving detection rates and diagnostic accuracy.

C.O.I. Disclosure:

O. Bahniuk: None. **N. Yusechko:** None. **A. Malahova:** None. **M. Malachynska:** None. **H. Makukh:** None.

EP08.029 Case Report: A 20-Year-Old Female with Digenic Familial Hypercholesterolemia

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Background: Familial hypercholesterolemia (FH) is an autosomal dominant disorder characterized by elevated low-density lipoprotein cholesterol (LDL-C) levels and increased cardiovascular risk. It is primarily associated with pathogenic variants in LDLR, APOB, or PCSK9 genes. This report describes a 20-year-old female diagnosed with FH carrying two mutations.

Material and methods: A 20-year-old female with significantly elevated lipid levels was evaluated. Family history was negative for hypercholesterolemia and premature cardiovascular disease. Next-generation sequencing (NGS) identified two heterozygous variants: NM_000527.5:c.1775G>A in the LDLR gene classified as

pathogenic and NM_000384.3:c.10580G>A in the APOB gene classified as pathogenic/likely pathogenic.

Results: Lipid profile revealed markedly elevated total cholesterol and LDL-C levels. The presence of two pathogenic variants suggests digenic FH, often associated with more severe lipid abnormalities and higher cardiovascular risk. The Dutch Lipid Clinic Network diagnostic criteria for FH scored between 5 and 8 points, indicating a possible or probable diagnosis of FH.

Conclusion: The presence of digenic mutations in FH may contribute to a more severe lipid phenotype and increased cardiovascular risk compared to monogenic cases. This highlights the need for early detection and intensified lipid-lowering therapy. Comprehensive genetic testing plays a crucial role in identifying such cases, allowing for personalized treatment strategies. Early intervention with lifestyle modifications and aggressive pharmacologic therapy, including high-intensity statins, ezetimibe, or PCSK9 inhibitors, is essential to mitigate disease progression and reduce long-term cardiovascular complications.

C.O.I. Disclosure:

K. Socha: None. **A. Gach:** None. **T. Kaluzewski:** None. **K. Matuszczyk:** None.

EP08.030 Dominant effect of ABCG5 variants causing sitosterolemia

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Background: Biallelic pathogenic variants in the gene ABCG5 are known to cause autosomal recessive sitosterolemia. This rare metabolic condition is characterized by unrestricted intestinal absorption of cholesterol and plant-derived cholesterol-like molecules, such as sitosterol. Patients with this disorder have very high levels of plant sterols in their plasma. Since analysis of plant sterols in plasma is not regularly done, most patients are diagnosed with hypercholesterolemia and advised to follow a plant-based diet, which is not recommended for sitosterolemia. Therefore, a correct diagnosis is essential for the treatment plan. In this light, the current discussion regarding a dominant effect of pathogenic ABCG5 variant might lead to significant consequences for each patient.

Material and methods: In-silico panel analysis for hypercholesterolemia based on whole exome sequencing (TWES, Twist Human Core Exome with additional customized probes and NGS with NovaSeq Illumina and deep sequencing of mitochondrial DNA) was performed.

Results: We present here patients suggesting a dominant effect of ABCG5 variants causing sitosterolemia.

Conclusion: The increasing reports of monoallelic ABCG5 variants in patients with sitosterolemia suggests dominant effect of ABCG5 variants.

C.O.I. Disclosure:

S. Geuer: A. Employment (full or part-time); Modest; MVZ genetikum GmbH.

EP08.031 Identification of three deep intronic variants in the *PAH* gene in a large cohort of patients with Hyperphenylalaninemia/Phenylketonuria

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Background: The *PAH* gene encodes the phenylalanine hydroxylase enzyme responsible for the conversion of Phenylalanine to Tyrosine. Hyperphenylalaninemia (HPA)/Phenylketonuria (PKU) is an autosomal recessive congenital metabolic disorder caused by *PAH* variants. About 5% of PKU patients remain undiagnosed after Sanger/NGS sequencing and MLPA amplification. To date, increasing numbers of pathogenic deep intronic variants have been reported in a large number of genes associated with HPA/PKU.

Material and methods: Cohort of 244 HPA/PKU patients, positive at neonatal screening and adults with confirmed clinical and biochemical diagnosis, analyzed by:- targeted-resequencing NGS of *PAH* and of 11 genes related to HPA - MLPA analysis to evaluate potential *PAH* rearrangements - Sanger sequencing of the single gene *DNAJC12* and deep intronic portions in *PAH* in patients with a single pathogenic variant identified.

Results: In our cohort, 95.4% resulted compound heterozygous for two pathogenic variants in *PAH*, 0.85% in *DNAJC12*, and 0.45% in *PTS*.Eight cases were identified as heterozygous for a single causative variant previously linked to the clinical phenotype. Three deep intronic variants, located in intron 6 (c.706+601G > A, c.706+843A > G) and intron 11 (c.1199+745T > A), were identified and in silico predicted a potentially causative role for the aberrant splicing defects.

Conclusion: Genetic-molecular analysis of deep intronic regions in the PAH gene is important for genetically unsolved cases of HPA/PKU. The evaluation of these genomic regions can enhance the molecular characterization of patients with HPA/PKU. It is crucial for establishing genotype-phenotype correlations and guiding diagnostic and therapeutic strategies.

C.O.I. Disclosure:

M. Rinaldi: None. **R. Tonin:** None. **F. Marin:** None. **S. Falliano:** None. **L. Ferri:** None. **C. Cavicchi:** None. **E. Procopio:** None. **G. Scaturro:** None. **M. Daniotti:** None. **F. Pochiero:** None. **M. Urban:** None. **C. Meli:** None. **A. Arena:** None. **M. Messina:** None. **R. Guerrini:** None. **A. Morrone:** None.

EP08.032 High Prevalence of metabolic syndrome in children with Down syndrome: A need for new management guidelines

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Background: Down syndrome (DS) is the most common chromosomal abnormality with intellectual disability. Many metabolic issues begin in childhood and children without DS are reported to have high metabolic syndrome (MS) prevalence according to various studies. However, the understanding of the risk of MS in children with DS remains limited.

Material and methods: A cross-sectional study assessed the prevalence of MS in DS children aged 10–18 during 2022–2024. Demographic details and anthropometric measurements were recorded. A fasting blood sample was taken for blood glucose, insulin, and lipid profile. The modified NCEP ATP III criteria were used for classifying MS. We also evaluated insulin resistance (IR) using HOMA-IR and body adiposity pattern with a DEXA scan in DS children aged 6–18.

Results: Seventy-six children aged 10–18 and 38 aged 6–9 were enrolled. The prevalence of MS was 18% with a female preponderance. Dyslipidemia (high triglycerides and low HDL) was observed in 21% and 15.8% of children aged 6–9 and 10–18, respectively. IR was observed in 27.6% and 7.8% of children aged 10–18 and 6–9, respectively. IR showed a positive correlation with BMI whereas no correlation was noted either with MS or dyslipidemia. Total body fat mass showed a positive correlation with MS.

Conclusion: Our study observed a higher prevalence (18.0%) of MS compared to age-matched general population studies (pooled prevalence - 5.0%). Additionally, we noticed a high prevalence of dyslipidemia and IR from 6 years of age. Results indicate a need to review the management guidelines and consider incorporating metabolic workup.

C.O.I. Disclosure:

S. Sundaravel: None. **N. Gupta:** None. **M. Jana:** None. **L. Ramakrishnan:** None. **R. Sharma:** None. **V. Jain:** None. **M. Kabra:** None.

EP08.033 X-linked OTC deficiency misdiagnosed as familial epilepsy: lessons for multi-gene panel design

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Background: Ornithine transcarbamylase (OTC) deficiency is an X-linked urea cycle disorder that can clinically mimic epilepsy. We describe a case in which OTC deficiency explains the sudden death of a young adult and his brother's apparent seizures, misdiagnosed and wrongly treated as epilepsy.

Methods: Whole Exome Sequencing (WES) with evaluation using HPO terms for epilepsy and metabolic diseases was performed on the index patient clinically diagnosed with therapy-resistant epilepsy by a national epilepsy centre. Segregation analysis was conducted using custom-designed primers in the patient's unaffected mother and dizygous twin brother. Post-mortem analysis of the deceased brother is underway.

Results: The hemizygous, maternally inherited pathogenic mutation c.622G>A,p.(Ala208Thr) in the OTC gene was identified. This genetic diagnosis explained the index patient's condition and his brother's's death, which was attributed to hyperammonemia due to a eventually fatal high-protein diet. Dietary treatment for OTC deficiency in the index patient resulted in significant clinical improvement. The OTC mutation was absent in his healthy twin brother, who was released from neurological surveillance.

Conclusion: This case underscores the vital role of including metabolic disorders into the design of multi-gene panels for familial epilepsy, resp. into the evaluation terms of WES. Identification of the X-linked OTC mutation not only explained the older sibling's death but also enabled successful therapeutic intervention for the surviving sibling. These findings emphasize the importance of genetic analysis in complex clinical cases to improve diagnostic accuracy and patient outcomes, and to scrutinize even seemingly ascertained clinical diagnoses.

C.O.I. Disclosure:

A. Stock: None. **A. Koennel:** None. **D. Forster:** None. **M. Reifferscheid:** None. **W. Schulz-Schaeffer:** None. **A. Kahlert:** None. **W. Henn:** None.

EP08.034 Genetic profile of Wilson's disease in a Greek cohort

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Background: Wilson's disease (WD) is a rare autosomal recessive disorder of copper metabolism caused by mutations in *ATP7B* gene, leading to toxic copper accumulation primarily in the liver and brain. Timely diagnosis and intervention are crucial to avoid severe hepatic and neurological complications. Recent studies suggest that WD prevalence may be higher than previously estimated, emphasizing the need for population-specific analyses to understand the disease's impact and optimize early diagnosis.

Material and methods: A total of 2800 individuals were referred to the Laboratory of Medical Genetics for WD testing, from 1995 until 2024. Phenotypic and clinical characterization was performed during pre-test genetic counseling. Serum ceruloplasmin and copper levers were provided prior to genetic analysis. Sanger sequencing was performed for all *ATP7B* coding regions and intron-exon boundaries, followed by CNV analysis with MLPA. Bioinformatic analysis was performed on VarSome Clinical, Franklin, and WilsonGen platforms, with variant classification following ACMG recommendations and ClinGen criteria.

Results: We identified 515 WD carriers and 310 patients. Mean serum ceruloplasmin levels were 11.3 mg/dL in patients and 27.2 mg/dL in carriers, with 17 years as the mean age of diagnosis. The seven most frequent pathogenic variants were: p.His1069Gln (49.4%), p.Arg969Gln (12.2%), p.Leu936X (10%), p.Val845SerfsX28 (7.1%), p.Gln289X (6%), p.X1466Arg (4%) and p.Leu282ProfsX2 (3.1%).

Conclusion: Our findings reveal a diverse mutational spectrum of WD in Greece, with a high prevalence of pathogenic variants. This underscores the importance of population-specific genetic testing in improving early diagnostic efforts and patient outcomes.

Conflict of Interest: None declared.

C.O.I. Disclosure:

T. Brantzos: None. **A. Mitrakos:** None. **M. Tzetis:** None. **K. Kosma:** None. **N. Marinakis:** None. **F. Tilemis:** None. **C. Sofocleous:** None. **P. Makrythanasis:** None.

EP08.035 Comprehensive Screening of 450 Genes in 1152 Patients: Insights into Genetic Variability

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Background: With the advantage of high-throughput sequencing technologies, large-scale genetic screenings have become increasingly feasible and critical for understanding disease etiology. Inborn errors of metabolism(IEM) are a group of genetic disorders caused by defects in enzymes or cofactors.These defects can lead to the buildup of toxic substances or a deficiency of essential

compounds, often affecting multiple organ systems.This study aimed to identify known and novel variants in IEM.

Material and methods: We screened 1152 patients who came to our clinic between 2015 and 2024 with suspicion of various IEM via a targeted next-generation sequencing(NGS) panel covering 450 genes of interest.Bioinformatic analyses included variant calling, annotation, and filtering based on allele frequency and predicted functional impact according to ACMG guidelines.

Results: Of the 450 genes screened, 575 variants were identified in 211 genes, including single-nucleotide variants(SNVs), small insertions and deletions(indels), and potential splice site mutations. We found 396 homozygous and 95 compound heterozygous variants for autosomal recessive diseases, 14 heterozygous variants for autosomal dominant diseases, and 23 hemizygous variants for X-linked diseases.We found 380 known variants and 195 novel variants. Of the 575 variants, 242 were pathogenic, 156 were likely pathogenic, and 177 were variants of unknown significance.

Conclusion: These findings highlight the utility of large-scale genetic screening in uncovering both known and novel variants.The results underscore the importance of comprehensive genomic profiling for improving our understanding of IEM. Further investigation of the newly discovered variants will be essential to elucidate their roles in disease and to translate these insights into clinical practice.

C.O.I. Disclosure:

F. Ergin: None. **B. Çiftçi:** None. **A. Yüce:** None. **R. Öktem:** None. **A. İnci:** None. **&. Okur:** None. **G. Biberoğlu:** None. **L. Tümer:** None. **F. Ezgü:** None.

EP08.036 Challenging diagnosis of a new patient with COX4I1 deficiency: a case involving a de novo variant and a deep intronic mutation

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Background: Genetic alterations in complex IV of the oxidative phosphorylation (OXPHOS) system are among the causes of mitochondrial diseases. Pathogenic variants in *COX4I1* gene, encoding cytochrome c oxidase subunit 4 isoform 1, have been described only in two families with clinical disparity.

Material and methods: The patient was a 10-year-old Spanish boy presenting with progressive motor disorder, normal cognitive skills and Leigh syndrome. Mitochondrial respiratory chain, WES analysis, targeted long read sequencing (LRS) using nanopore technology, cDNA analysis,protein expression by western blot, OXPHOS function (blue native and enzyme activities) and proteomics were also performed. Minigene construction and cloning of COX4I1 transcripts was also done.

Results: We identified two novel *COX4I1* variants, a de novo nonsense substitution (c.64C>T, p.Arg22Ter) and a deep intronic variant (c.73+1511A > G) that led to an 80 bp intronic retention predicted to generate a premature termination codon (p.Glu25-ValfsTer9). Targeted-LRS demonstrated that the variants were in trans. Transient expression of the c.73+1511A > G substitution in HAP1 cells revealed its pathogenic effect as it generates a splicing alteration consistent with the observed intronic retention. Functional studies showed a marked reduction of COX4I1 and other CIV subunits together with an isolated CIV assembly defect. Proteomics corroborated a marked signature consistent with CIV deficiency.

Conclusion: Here we report the third description of COX4I1 deficiency associated with human disease. We identified the first de novo and deep intronic variants in COX4I1 and provided functional data demonstrating that they affected COX4I1 protein levels and subsequently the activity of mitochondrial respiratory chain complex IV.

C.O.I. Disclosure:

L. Gort: None. **O. Ugarteburu:** None. **G. Muñoz-Pujol:** None. **M. Unceta:** None. **J. De Las Heras:** None. **A. Garcia-Ribes:** None. **J. Corral:** None. **A. Arza-Ruesga:** None. **B. de la Morena Barrio:** None. **L. Farré-Tarrats:** None. **A. Ribes:** None. **J. Garcia-Villoria:** None. **F. Tort:** None.

EP08.037 Novel compound heterozygous *PHGDH* variants in siblings with neurodevelopmental disorder, microcephaly, and bilateral cataracts: implications for serine metabolism dysfunction

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Background: Serine metabolism disorders are rare, autosomal recessive conditions linked to *PHGDH* variants, causing phosphoglycerate dehydrogenase deficiency (PHGDHD). This disorder manifests as neurodevelopmental delay, microcephaly, intellectual disability, epilepsy, and bilateral cataracts. We report novel compound heterozygous *PHGDH* variants in two siblings, broadening the genotypic and phenotypic spectrum.

Methods: Whole-exome sequencing (WES) was conducted on an 8-year-old proband and her 15-month-old brother using a trio-based approach. Sanger sequencing confirmed segregation in family members. Clinical evaluation, neuroimaging, metabolic profiling, and lumbar puncture for cerebrospinal fluid (CSF) analysis were performed. Plasma samples were collected for amino acid profiling.

Results: WES identified two novel compound heterozygous *PHGDH* variants (c.562G>T, c.176T>G) in trans in both siblings. Segregation confirmed parental heterozygous carrier status, consistent with autosomal recessive inheritance. According to ACMG guidelines, c.562G>T was a variant of uncertain significance (VUS) (4 points), while c.176T>G was likely pathogenic (6 points). ClinVar (05-Jul-2024) previously classified c.176T>G as VUS. Both siblings exhibited severe developmental delay, microcephaly, dysmorphic features, epilepsy (proband), and bilateral congenital cataracts. Brain MRI showed corpus callosum hypoplasia and structural abnormalities consistent with PHGDHD. CSF and plasma amino acid analysis revealed decreased serine levels in both patients. Serine supplementation therapy is planned.

Conclusion: These novel *PHGDH* variants contribute to serine metabolism-related neurodevelopmental disorders, expanding

the genetic and clinical spectrum of PHGDHD. Early genetic diagnosis is crucial for multidisciplinary management, including serine supplementation. Further studies are needed to assess variant pathogenicity and metabolic intervention responses.

C.O.I. Disclosure:

M. Biela: None. **A. Bodetko:** None. **M. Makowiecka-Wołek:** None. **N. Śmigiel:** None. **A. Gawrońska:** None. **R. Płoski:** None. **M. Rydzanicz:** None. **K. Rutkowska:** None. **R. Śmigiel:** None.

EP08.038 Expanding the clinical spectrum of *POLRMT*-related disorder: A case report with complex movement disorder and two novel variants

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Background: Mutations in the *POLRMT* gene, encoding the mitochondrial RNA polymerase, have been recently associated with a mitochondrial disease characterized by neurological and developmental phenotypes, including progressive optic atrophy, intellectual disability, developmental delay, hypotonia, short stature, and multiple OXPHOS defects, with autosomal recessive or dominant inheritance. To date, only one study has reported on *POLRMT*-related disorder, leaving significant gaps in our understanding of the genetic mechanisms and clinical spectrum of this condition. Here, we report a patient with biallelic variants in trans in *POLRMT*, contributing to the characterization of this rare disorder.

Methods: Trio whole genome sequencing (WGS) was performed to identify potential genetic variants associated with mitochondrial dysfunction. To evaluate the functional impact of identified variants, transcriptomic analysis was performed using RNA sequencing (RNA-seq) on fibroblast samples.

Results: The patient is a 10-year-old female presenting with progressive optic atrophy, complex movement disorder, encephalopathy, and motor and social disabilities. Physical examination reveals generalized choreodystonia, dystonic tremor, bradykinesia, ptosis, and impaired gait. Trio-WGS identified two pathogenic biallelic variants in *POLRMT* (NM_005035.4):c.1291-24A > G and c.2259_2262dup, p.(Lys755Profs*8). RNA-seq confirmed a significant reduction in gene expression, indicating that the POLRMT variants disrupt transcriptional activity.

Conclusion: This report expands the POLRMT-related disease spectrum by identifying complex movement disorder as a novel feature and describing two previously undescribed variants. Our findings highlight the need for additional cases to delineate phenotypic variability and reinforce the diagnostic value of multi-omics approaches in rare mitochondrial disorders.

C.O.I. Disclosure:

V. Martos: None. **M. Alvarez-Barona:** None. **J. Amigo:** None. **R. Rodriguez-Alvarez:** None. **E. Goiricelaya:** None. **G. Bullich:** None. **A. Esteve-Codina:** None. **J. Eiris:** None. **E. Monteagudo-Vilavedra:** None. **J. Ortigoza-Escobar:** None. **B. Sobrino:** None. **F. Barros:** None. **Á. Carracedo:** None.

EP08.039 *PIGG* variants cause a neurodevelopmental disorder with secondary cerebral folate deficiency

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Background: Glycosylphosphatidylinositol (GPI) deficiencies result from pathogenic variants in any of the 22 genes involved in GPI anchor biosynthesis pathway, including *PIGG*. Bi-allelic *PIGG* variants cause neurodevelopmental disorders characterised by seizures, developmental delay and cerebral atrophy. Reduced cell surface expression of GPI-anchored proteins in patient fibroblasts provides functional evidence supportive of pathogenicity. We describe a female patient who presented aged 12 months with seizures, developmental delay, myopathy and cerebral folate deficiency (CFD). Now age 15 years, her symptoms have improved following therapeutic folinic acid, though she remained genetically undiagnosed. Our research aims to establish the genetic basis of her presentation and assess response to folinic acid.

Material and methods: Trio whole exome sequencing (WES) of blood DNA was undertaken. Confocal microscopy investigated the expression of GPI-anchored proteins.

Results: Compound heterozygous loss-of-function *PIGG* (NM_001127178.3) variants were identified, including a novel c.1332+1G > T splicing variant. A homozygous *FOLR2*:(NM_000803.5) c.434C>T, p.(Thr145Met) variant of uncertain significance was also identified. Folate receptors (FOLR1 and FOLR2) are GPI-anchored and whilst no reliable antibody was available to assess FOLR2 expression, immunofluorescent staining of patient fibroblasts confirmed decreased expression of the predominant 5-methyltetrahydrofolate receptor, FOLR1, thereby confirming a defect in GPI-anchor formation which would be consistent with her CFD. Folinic acid can freely diffuse across the choroid plexus without folate receptor-mediated transport, circumventing the effects of the PIGG pathology.

Conclusion: To our knowledge, this is the first report of *PIGG* dysfunction causing reduced FOLR1 expression and CFD, supporting therapeutic folinic acid for this growing group of disorders.

C.O.I. Disclosure:

D.B. Hammadi: None. **C. Wildig:** None. **A. Childs:** None. **K. Pysden:** None. **R.W. Taylor:** None. **R. McFarland:** None. **C.L. Alston:** None.

EP08.040 Rare bi-allelic *COXFA4* variants cause cytochrome *c* oxidase deficiency and paediatric mitochondrial disease

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Background: Cytochrome c oxidase (COX; complex IV (CIV)) comprises of 14 protein subunits originating from both, the nuclear and mitochondrial genome. COX assembly is centred around three conserved catalytic core subunits, followed by sequential assembly of subunits requiring multiple assembly factors. Originally assigned as part of complex I, the protein has been reassigned as a subunit of CIV, and renamed COXFA4. Two unrelated families with bi-allelic *COXFA4* variants have been described in the literature (**PMID: 23746447, 38674434**). Here we present four unrelated families with affected probands demonstrating CIV deficiency. Two probands carry homozygous *COXFA4* splice variants, whilst two others have homozygous deletions encompassing the *COXFA4* gene (NM_002489.4).

Material and methods: Whole cell protein lysates, obtained from patient and control skin and muscle biopsies, were subjected to SDS-PAGE immunoblotting to assess steady-state levels of mitochondrial oxidative phosphorylation (OXPHOS) proteins and COXFA4. RNA-sequencing (RNA-seq), Blue-Native (BN)-PAGE and high-resolution MS-based complexome profiling of patient cells were used to investigate the functional consequences of *COXFA4* variants in patient cells.

Results: RNA-seq confirmed both splice variants caused skipping of exon 2, while SDS-PAGE immunoblotting and complexome profiling demonstrated undetectable COXFA4 steady-state levels. Preliminary BN-PAGE investigations suggest a decrease in CIV holoenzyme assembly, with proteomic analysis highlighting COXFA4 to be a highly dynamic CIV subunit.

Conclusion: The precise molecular consequences of rare *COXFA4* variants remains under investigation, with proteomic studies aimed at unravelling the function of COXFA4 within mammalian cells and providing diagnostic clarity for these families.

C.O.I. Disclosure:

J.K. Leighton: None. **L.S. Taylor:** None. **A. Cabrera-Orefice:** None. **W.L. Macken:** None. **R. Kabiljo:** None. **J. Caberg:** None. **D. Misceo:** None. **E. Frengen:** None. **R.D. Pitceathly:** None. **M. Oláhová:** None. **C.L. Alston:** None. **I. Wittig:** None. **R.W. Taylor:** None.

EP09 Immunology and Hematopoietic System

EP09.001 Interleukin 6 polymorphism rs1800795 in Bulgarian patients with systemic lupus erythematosus

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Background: Systemic lupus erythematosus (SLE) is a multifactorial autoimmune disorder. The disease arises from a complex interplay of genetic predispositions, immunological dysregulation, endocrine influences, and environmental triggers, leading to a breakdown of immunological tolerance to self-antigens. Interleukin-6 (IL-6) plays a crucial role in systemic autoimmunity and pathologic inflammation. The objective of our study was to evaluate the impact of the IL6: c.54-321G > C polymorphism on Systemic lupus erythematosus risk.

Material and methods: Systemic lupus erythematosus (SLE) is a multifactorial autoimmune disorder. The disease arises from a complex interplay of genetic predispositions, immunological dysregulation, endocrine influences, and environmental triggers, leading to a breakdown of immunological tolerance to self-antigens. Interleukin-6 (IL-6) plays a crucial role in systemic autoimmunity and pathologic inflammation. The objective of our study was to evaluate the impact of the IL6: c.54-321G > C polymorphism on Systemic lupus erythematosus risk.

Results: Regarding the frequency of the C-Allele in patients versus the control group no statistical significance was established (33% vs. 34%, p-value = 0,94). Close frequencies in patients compared to controls for the homozygous C/C genotype were observed (7% vs. 11%), not reaching statistically significant difference (p-value = 0,6).

Conclusion: We did not find statistically significant impact of the IL6: c.54-321G > C polymorphism regarding Bulgarian SLE patients. Further studies, including participants of same age range, gender and ethnicity, with larger sample sizes are needed to validate our findings.

C.O.I. Disclosure:

A.M. Syarov: None. **D. Tanev:** None. **I. Dimova:** None. **E.D. Dimova:** None. **R. Robeva:** None. **R. Vazharova:** None.

EP09.002 The Polymorphism IFITM3, rs12252, and COVID-19 severity

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Background: One factor influencing the severity of COVID-19 is the polymorphism of the Interferon-Induced Transmembrane Protein 3 (IFITM3). This study examines whether the rs12252 polymorphism of IFITM3 is associated with severe COVID-19 in our population.

Material and methods: We conducted an observational study on 51 participants with mild and severe cases of COVID-19, examining the correlation between the IFITM3 rs12252 polymorphism, disease severity, and inflammatory marker levels.

Results: Among patients with severe symptoms, 16.1% were found to have the AG genotype. Additionally, those with severe illness exhibited significantly higher levels of ESR, CRP, and fibrinogen compared to those with mild symptoms.

Conclusion: We identified a potential correlation between COVID-19 severity, the AG genotype of IFITM3 rs12252, and clinical parameters. However, large-scale studies are needed to confirm our findings.

C.O.I. Disclosure:

A. Mitroi: None. **A. Mitrea:** None. **C. Brinzan:** None. **G. Cozaru:** None. **E. Dantes:** None.

EP09.003 A Dual Phenotype of SHORT Syndrome and APDS2 Associated with a *PIK3R1* Variant and Concurrent Mosaic Tetraploidy

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Background: SHORT syndrome is characterized by short stature (S), joint hyperextensibility and/or inguinal hernia (H), ocular depression (O), Rieger anomaly (R), and delayed dentition (T) associated with loss-of-function variants in the *PIK3R1* gene. This gene is also linked to an immunodeficiency syndrome known as Activated Phosphoinositide 3-Kinase Delta Syndrome 2 (APDS2).

Material and methods: A 9-year-old girl presented with short stature and growth retardation. Due to concurrent anemia, she was monitored with a preliminary diagnosis of Fanconi anemia. At the age of 19, she was referred to the immunology clinic with recurrent bronchitis (2–3 times per year), persistent wheezing cough, and sputum production. Further investigations revealed hypogammaglobulinemia, leading to a diagnosis of primary immunodeficiency.

Results: The diepoxybutane test, performed to confirm Fanconi anemia, was negative, but 60% mosaic tetraploidy was detected. Whole-exome sequencing identified a heterozygous *PIK3R1* variant, c.1993G>A (G665S) (NM_181523). Segregation analysis in the family showed the same variant in the patient's mother, who was found to have IgA deficiency upon immunological evaluation. In the literature, this variant has been described in a father-son duo, where the child exhibited a phenotype consistent with SHORT syndrome, while the father had a milder presentation with no reported immunological profile.

Conclusion: Previously, the *PIK3R1* c.1425+1G > T variant was reported in three patients with both SHORT syndrome and APDS2 phenotypes. It has been suggested that missense variants in the last exon of this gene may not present with the APDS2 phenotype. This case represents the first reported *PIK3R1* missense variant associated with both phenotypes.

C.O.I. Disclosure:

B. Ak: None. **Ö. Güngör:** None. **A. Aykut:** None. **A. Durmaz:** None. **E. Aslan:** None. **C. Tunakan Dalgic:** None. **R. Gumusburun:** None. **Ö. Ardeniz:** None. **H. Akin:** None.

EP09.004 Robust whole-exome sequencing contribution to the presymptomatic diagnosis of the myh9 mutation, which appeared in a 3-year-old girl

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Background: MYH9-related disease (MYH9-RD) is an autosomal dominant disorder, primarily caused by de novo pathogenic variants. It is characterized by hematologic features from birth, including platelet macrocytosis, thrombocytopenia, and the presence of MYH9 protein aggregates in neutrophil granulocytes. Over time, most affected individuals develop additional extra-hematologic manifestations such as sensorineural hearing loss, renal disease, presenile cataracts, and/or elevated liver enzymes.

Material and methods: We report the case of a 3-year-old Moroccan infant from a non-consanguineous family who presented with isolated thrombocytopenia. A thorough clinical examination and detailed family history review raised suspicion of a rare genetic disorder. Especially the father of the proband has passed away due to similar clinical symptoms in addition to end-stage kidney disease and multiple hematom

Results: Exome sequencing revealed a previously unreported variant in the MYH9 gene, providing a genetic basis for the patient's thrombocytopenia and contributing to the growing body of knowledge about MYH9-related disease.

Conclusion: This case emphasizes the importance of a comprehensive clinical evaluation and family history review of MYH9-related disease.Exome sequencing proved to be an invaluable tool in identifying a novel MYH9 variant, advancing our understanding of the disease and offering potential insights, solving the father's enigma, and providing an early diagnosis and management for the young child.

C.O.I. Disclosure:

S. Bouressas: None. **H. Jelti:** None. **F. Hacht:** None. **A. Lamzouri:** None.

EP09.005 Expression of biomarkers connected with immunological senescence in Bulgarian individuals of active age

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Background: Aging is the major risk factor for common diseases as cardiovascular, metabolic or neurodegenerative. Chronic systemic inflammation is involved in etiology and progression of these conditions. Premature aging has been reported even in young adults and is mainly referred to as "aging of the immune system" (immunosenescence). Immunosenescent individuals are more prone to infectious diseases and even cancer. In the long run, they will have a shorter lifespan and increased mortality rate.

Material and methods: Fifty-five healthy individuals of active age were included in this study. Two pooled samples of RNA isolated from PBMC were created - "control" group (39 persons) and "test" group (16 persons), the latter showing signs of premature aging. RT^2 Profiler PCR Array (Qiagen, CA) was used to analyze the expression of 84 genes of innate and adaptive immunity.

Results: The majority of genes in the "test" group showed increased expression (45 genes). Of them, twenty-three were substantially up-regulated (fold change more than 5) and three (*BCL6*, *IL1beta* and *CCL4*) showed a fold change more than 10. Only three genes showed a decreased expression - *IL23R* (−3.10), *IL5* (−2.66) and *COX-2* (−2.15).

Conclusion: Immunosenescent individuals exhibit expression pattern of immune overactivation as the majority of the analyzed genes were up-regulated. The major role in this activation play chemokines, BCL6, interleukines and toll-like receptors (TLRs). Highly elevated expression of CRP confirms the interrelation between immunological senescence and chronic systemic inflammation. Negatively expressed genes could be interpreted as protective against oxidative stress, tumor formation and environmental antigens.

C.O.I. Disclosure:

D. Nikolova: None. **Y. Todorova:** None. **Z. Hammoudeh:** None. **B. Rukova:** None. **R. Emilova:** None. **M. Aleksova:** None. **V. Koleva:** None. **M. Nikolova:** None.

EP09.006 *DEFA4* as a novel candidate gene in primary immunodeficiency detected in a patient with protein null mutation

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Background: The innate immune response is the first mechanism to protect the host from invading pathogens. Primary immunodeficiency (PI) is a group of disorders with genetic defects in the immune system, and it is estimated that nearly one-third of PI patients remain genetically undiagnosed. Here we present an undiagnosed PI patient harbouring a suspicious *DEFA4* gene deletion.

Material and methods: Trio WES was performed on the affected individual and his Iranian consanguine parents. Sequence data was analysed using GATK and hg38 reference genome, then short variants were filtered based on the ACMG guideline. We also called copy number variations using ExDepth, our proprietary algorithm.

Results: The male proband had been infected with cytomegalovirus transmitted from mother during her pregnancy. After receiving the 6-month vaccine he manifested developmental delay, seizure, and recurrent infections. Finally, he died of severe sepsis at the age of 4.5 years. No significant short variant was detected by exome analysis. However, we found a homozygous deletion of the entire *DEFA4* gene (defensin alpha 4) in the proband, while parents are heterozygous.

Conclusion: *DEFA4* encodes a member of the defensin family of antimicrobial and cytotoxic peptides, hence we propose that the DEFA4 loss of function mutation could be causative. Vaccines may have adverse effects on some PI patients, suggesting that the infant vaccination has led to manifestation of neurological symptoms due to the previous cytomegalovirus infection, and sepsis. Indeed, further immunogenetics studies are needed to validate DEFA4 role in PI disorders.

C.O.I. Disclosure:

Z. Firoozfar: None. **P. Moshtaghian:** None. **M. Montazer Zohour:** None. **M. Reisi:** None. **S. Alavi:** None.

EP09.007 CCL4 as a biomarker of immunosenescence

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Background: Immunosenescence in a dynamic process of acquiring changes in the immune system connected with dysregulated innate and adaptive response. Those changes usually increase with advancing age. However, some young individuals show signs of preterm aging which could be due to specificities in their genetic profile.

Material and methods: Two groups of individuals were analyzed and compare in terms of gene expression of 84 genes associated with innate and adaptive immune response using RT^2 Profiler kit and subsequent software data analysis (Qiagen, CA).

Results: We report altogether 45 of increased expression in the group of people with preterm immunosenescence. Among them, *CCL4* showed the highest up-regulation with a fold change of 15.91.

Conclusion: So far, CCL4 was known as a marker of tumor progression, aggressiveness and metastasis whose production is triggered by pro-inflammatory cytokines. Experiments with mice show that *CCL4* knockout generates anti-aging effects. We report that CCL4 could be considered as a biomarker of immunosenescence in individuals of active age.

C.O.I. Disclosure:

D. Nikolova: None. **Y. Todorova:** None. **Z. Hammoudeh:** None. **B. Rukova:** None. **R. Emilova:** None. **M. Aleksova:** None. **V. Koleva:** None. **M. Nikolova:** None.

EP09.008 Autosomal dominant macrothrombocytopenia: report of new variant in the ACTN1 gen

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Background: Autosomal dominant macrothrombocytopenia or Bleeding Disorder Platelet-Type-15 (BDPLT15) (OMIM #615193) is caused by pathogenic variants in the ACTN1 gene (chr.14q24), which encodes alpha-actinin-1, a structural protein of the cytoskeleton. The condition presents with a clinical spectrum ranging from asymptomatic to severe hemorrhages, with a prevalence of <1/1,000,000. We aim to report a new pathogenic variant in the ACTN1 gene, representing the first case in Latin America.

Material and methods: The patient is a 14-year-old male, born from a third uncomplicated pregnancy, who presents with platelet counts between 100,000–140,000/mm^3, without bleeding episodes. Despite hematology follow-up since age 6, no etiology was established. Given the maternal family history of thrombocytopenia, the patient was referred to genetics. An NGS panel for thrombocytopenia identified a likely pathogenic variant c.2291G>A (p.Gly764Asp) in the ACTN1 gene. Family segregation studies confirmed the variant in the mother, who shows similar symptoms. Complete blood count and peripheral blood smear revealed thrombocytopenia, anisocytosis, and macroplatelets.

Results: ACTN1 alterations affect platelet formation by disrupting megakaryocyte proplatelets due to cytoskeletal dysfunction. Among 148 reported cases worldwide, none presents this specific variant. Review of ACMG criteria showed fulfillment of PP1, PM1, PM2, PP3, and PP4, initially classifying it as likely pathogenic. However, considering the patient's symptoms, family segregation study, and bioinformatics analysis, the variant's pathogenicity was confirmed.

Conclusion: Recognition of BDPLT15 as a potential etiology of chronic thrombocytopenia is crucial, with peripheral blood smear examination serving as a cost-effective diagnostic tool.

C.O.I. Disclosure:

D. Contreras Duque: None. **L.V. Silva Cáceres:** None. **G. Contreras:** None.

EP09.009 Case report of rare autosomal dominant agammaglobulinemia-10

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Background: Agammaglobulinemia is a primary immunodeficiency characterized by profoundly low or absent serum antibodies and circulating B cells as well as early-childhood onset of recurrent infections. Most cases result from X-linked *BTK* deficiency, autosomal forms are less common. We report a novel variant in the *SPI1* gene, which we propose as the cause of autosomal dominant agammaglobulinemia-10 (AGM10) in our proband.

Material and methods: The proband is a 14-year-old male, born full-term to non-consanguineous parents. From the age of 1, he presented with persistent nasal secretion and frequent viral infections. Immunological assessment revealed significantly reduced B lymphocytes and hypogammaglobulinemia. At the age of 3, the patient was diagnosed with primary humoral immunodeficiency, subcutaneous immunoglobulin therapy (SCIG) has been administered. Family history was uninformative.

Results: Whole exome sequencing (WES) revealed a heterozygous frameshift variant in the *SPI1* (NM_003120.3) gene, c.45del (p.Pro16Hisfs*170), classified as VUS based on ACGS 2024/ClinGen criteria PVS1 (strong), PM2 (supporting). Upon further investigation, the variant was confirmed de novo by Sanger sequencing, consistent with previously reported AGM10 cases. Altered protein structure was noteworthy: predicted to escape nonsense-mediated decay, while showing substantial changes to the DNA-binding ETS domain as suggested by bioinformatic tools. This is the first reported case of a c.45del variant in AGM10.

Conclusion: Through the initial analysis, the heterozygous c.45del variant was classified as a VUS. However, additional evidence suggest it to be causative for the patient's symptoms. Further in vitro and in silico analysis are required to fully assess the potential impact of the variant.

C.O.I. Disclosure:

I. Žukauskaite: None. **R. Traberg:** None. **J. Kudzytė:** None. **R. Ugenskienė:** None.

EP09.010 A case report of newborn patient with a novel *NFKB2* mutation inherited from mosaic mother

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Background: Mutations in the nuclear factor-κB2 gene (*NFKB2*) impaire NF-κB pathway are associated with Deficient anterior pituitary with common variable immune deficiency syndrome (DAVID) and CVID. Here, we report a new case with CVID due to variant in the *NFKB2* gene.

Material and methods: DNA was extracted from blood using the salting-out method. Panel sequencing targeting inborn errors of immunity was performed at Invitae Laboratory. TREC/KREC level was determined by q-PCR in laboratory "LeoGENE".

Results: A full-term newborn boy from west of Ukraine was diagnosed with CVID. Patient with severe family history: two previous sibs (a boy and a girl) died shortly after birth from SCID. There is one healthy child from the new partner. All children born before the start of extended neonatal screening in Ukraine. Therefore, an NGS study was conducted. A variant associated with SCID and detected likely pathogenic heterozygous variant c.2595_2596del (p.Asp865Glufs*20) in *NFKB2* gene. TREC/KREC were undetectable (0), indicating impaired T-/B-cell development. The mother is suspected to be mosaic for the same variant, while patient's father and sibling had no this mutation.

Conclusion: To date, the c.2595_2596del (p.Asp865Glufs*20) variant in individuals with NFKB2-related diseases has not yet been reported in the literature. This case suggests that this mutation is pathogenic according to the ACMG criteria (PVS1, PM2, PM4) and causes SCID. Because the mother is mosaic, we hypothesize that this mutation was inherited by the two children from the first marriage of the mosaic mother but is absent in the healthy father and sibling.

C.O.I. Disclosure:

V.S. Kravets: None. **I. Shymanska:** None. **N. Matiytsiv:** None. **H. Makukh:** None.

EP09.011 Single cell transcriptomics and immunomics reveals T cell dysfunction in long-COVID children

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Background: A significant proportion of children being infected with SARS-CoV-2 virus have developed long-COVID syndrome. To date the mechanisms of long-COVID syndrome development are still poorly understood. T cells especially central memory T cells (T_{cm}) play a pivotal role in defense against viral infections.Aim: was to evaluate the transcriptomics and map the T cell receptors of circulating T cells at the single cell level in long-COVID children.

Material and methods: 58 children diagnosed with long-COVID syndrome and 16 aged-matched controls at Semmelweis University's Pediatrics Center's Tűzoltó street Unit between 2021.08.01.- 2023.06.30 were enrolled in deep flow cytometry T-cell immunophenotyping and 8 samples were processed with 10x-based single transcriptomics and immunomics.

Results: The ratio of naive T cells was reduced (13.57% vs 20.32%), meanwhile ratio of Tcm was elevated (29.06% vs. 20.58%) in vaccine naive children with long-COVID syndrome compared to controls. The Tcm concentration in vaccine naive long-COVID patients was 40.05 cells/μL, while the concentration in vaccinated patients was 108.54 cells/μL. TCR epitope mapping revealed, the NSP3 specific memory T cells ratio was decreased (8.69% vs 10.93%) meanwhile the S specific memory T cells ratio was increased (26.81% vs 17.69%) in long-COVID children.

Conclusion: We have identified a reduced naive T cells and increased Tcm ratios compared to healthy controls. The SARS-CoV-2 specific memory T cell epitope pattern was different to matched controls. Based on our results it is likely that the T-cell mediated immune dysfunction is a major component in long-COVID syndrome.

C.O.I. Disclosure:

J.K. Erhardt: None. **É. Pállinger:** None. **Á. Németh:** None. **N. Fekete:** None. **M. Ranyák:** None. **T. Abonyi:** None. **S. Kovács:** None. **L.K. Li:** None. **L. Hegyi:** None. **H. Vágó:** None. **E. Búzás:** None. **C. Bödör:** None. **Á.F. Kovács:** None. **G. Kovács:** None.

EP09.012 Co-occurrence of primary immunodeficiency with a second genetic condition: three independent cases of double diagnosis

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Primary immunodeficiencies (PIDs) are genetically and phenotypically heterogeneous disorders characterized by an inborn increased susceptibility to infections. The clinical variable phenotype making diagnosing of PIDs complex, which is important for the clinical management of the disease. The co-occurrence of PID with other genetic disorders has only been occasionally reported. Here, we describe three independent cases of PIDs, co-segregated with three different genetic conditions, consisting of a patient with Griscelli syndrome and *TMLHE*-related neurodevelopmental presentations such as attention deficit, seizure, abnormal movement and fixation of eyes. The causative homozygous variant which explained the immunologic manifestations was novel. The next double diagnosis had never been reported before: through exome sequencing, an 8-month-girl with recurrent bacterial and viral infections, food allergies, increased serum IgE, encephalopathy, hyperammonemia and severe psychomotor delay, was found to carry two novel truncating variants in the *DOCK8* and *ACADVL* genes. Finally, our investigation identified another two novel truncating variants in *TYR* and *CEBPE* genes in a 15-year-old-male with severe skin abscess, otitis media, albinism, thrombocytopenia and neutropenia.

C.O.I. Disclosure:

A. Karamzade: None. **M. Teimouri:** None. **M. Keramatipour:** None. **S. Talebi:** None.

EP09.013 Clinical and molecular features of Ras-Associated Autoimmune Leukoproliferative Disorder (RALD) in a 2-year-old boy

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Background: Ras-associated autoimmune leukoproliferative disorder (RALD) is a nonmalignant clinical syndrome characterized by splenomegaly, lymphadenopathy and autoimmunity, with features overlapping Juvenile Myelomonocytic Leukemia (JMML). RALD is an extremely rare intrinsic lymphocyte apoptosis defect driven by somatic gain of function variants in the genes of the RAS pathway, particularly KRAS or NRAS, which occur in hematopoietic progenitors or stem cells and are subsequently found in lymphocytes and monocytes.

Material and methods: Here, we describe the case of a 2-year-old male patient. He was referred with hepatosplenomegaly, pancytopenia and acute fever. His general condition was monitored closely and further investigations were undertaken to rule out other possible underlying causes. Genomic DNA was extracted from peripheral blood samples of the affected subject. Whole Exome Sequencing (WES) was performed to investigate germline and somatic variants in JMML/RALD genes.

Results: DNA sequencing identified a novel somatic variant c.194_214dup (p.Ser65_Tyr71dup) in *KRAS* gene. The KRAS somatic variant was found in peripheral blood mononuclear cells in two independent blood samples collected at different times, evoking a diagnosis of RALD, based also on clinical and laboratory results.

Conclusion: We report a new case of *KRAS* mutated RALD. Differentiating RALD from JMML is crucial for determining the appropriate therapeutic approach and prognosis.

C.O.I. Disclosure:

M. Matraxia: None. **M. Di Mauro:** None. **G. Palumbo:** None. **R. De Vito:** None. **R. Alaggio:** None. **A. Pietrobattista:** None. **A. Mosca:** None. **C. Dionisi Vici:** None. **B. Siri:** None. **M. Spada:** None. **G. Spagnoletti:** None. **L. Abballe:** None. **A. Sparaci:** None. **A. Tomaiuolo:** None. **A. Novelli:** None. **E. Agolini:** None.

EP09.014 Atypical initial presentation of hereditary thrombotic thrombocytopenic purpura during a second pregnancy

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Background: Hereditary thrombotic thrombocytopenic purpura (hTTP) typically presents in neonates or in females during their first pregnancy. A prior uncomplicated pregnancy is often considered evidence against hTTP. We report a case in which the first presentation occurred during a second pregnancy, following the termination of the first pregnancy before 8 weeks.

Material and methods: A 31-year-old woman at 26 weeks of second pregnancy presented with fatigue, headache, episodic hypoesthesia, thrombocytopenia, and non-immune hemolytic anemia. Laboratory results showed severely reduced ADAMTS13 activity, low ADAMTS13 antigen levels, and no anti-ADAMTS13 antibodies. She responded well to fresh frozen plasma alone, ruling out immune TTP and suggesting hTTP. Whole-exome sequencing (WES) using next-generation sequencing and *ADAMTS13* gene analysis were performed.

Results: WES identified a compound heterozygous genotype: NM_139027.6(*ADAMTS13*):c.3975dup;1510G > T, comprising a pathogenic frameshift variant c.3975dup, p.Glu1326Argfs*6 (PM3_verystrong, PS3_supporting, PM2_supporting, PVS1_moderate, PP4) and a missense variant c.1510G>T, p.Asp504Tyr, initially classified as a variant of uncertain significance (PM2_supporting, PP3, PP4, PP2, PM3). Sanger sequencing confirmed c.1510G>T as maternally inherited, supporting trans phasing. This led to variant reclassification as likely pathogenic, confirming the hTTP diagnosis.

Conclusion: This case highlights the importance of genetic testing in atypical hTTP presentations, as our patient's first exacerbation occurred only during her second pregnancy. We postulate that her first pregnancy was not advanced enough to trigger disease onset, delaying manifestation. Early genetic testing enables timely diagnosis and management. Prophylactic therapy will be discussed using a shared decision-making approach, considering clinical status and potential risks.

C.O.I. Disclosure:

R. Ramonas: None. **L. Kievišiene:** None. **K. Aleknavičienė:** None. **E. Machtejevienė:** None. **L. Neverbickienė:** None. **S. Žukaitienė:** None. **I. Pocevičienė:** None. **R. Ugenskienė:** None.

EP09.015 Atypical phenotypic pattern of PTPRC (CD45) reveals abnormal maturation of T cells associated with the C77G polymorphism

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Background: The leucocyte common antigen, protein-tyrosine-phosphatase-receptor type-C (PTPRC/CD45), has several isoforms that distinguish naïve (CD45RA) from memory (CD45RO) T-cells.C77G and A138G are two CD45-polymorphisms affecting *PTPRC* gene in human, resulting in CD45RO antigen deficiency (C77G) or in a clear prevalence of T-lymphocytes $CD45RO^+$ subtype (A238G). Assessment of naïve and memory-T-cell is an important marker for scouting various immune disorders. Here we report six cases of children with atypical immuno-phenotype pattern checked for the CD45 genotype.

Material and methods: Lymphocytes were analyzed by multiparameter-flow-cytometry assessing CD45RO/CD45RA antigens on $CD4^+$ and $CD8^+$ T-cells. Genetics analysis was performed using a customized "Clinical Exome" panel on the Illumina NovaSeq6000® platform for the presence of the polymorphisms of the *PTPRC* gene.

Results: Six children showed an atypical phenotypic pattern represented by $CD45RO^+$expression deficiency occurred without the gradual loss of $CD45RA^+$expression. The retained of $CD45RA^+$ expression hesitated in a "double-positive" population in both $CD4^+$ and $CD8^+$ lymphocytes (median $CD45RA^+/CD45RO^+$ percentage was 23.7% and 10.3% of $CD4^+$ and $CD8^+$, respectively). Sequencing analysis identified the presence of C77G-polymorphism in all patients.

Conclusion: In these series cases, we observed a link between the presence of a high percentage of double-positive $CD45RA^+$/$CD45RO^+$ T-lymphocytes and C77G-polymorphism, suggesting a possible role of the genetic background for memory T-cell deficiency and a lack of transitional maturation from naïve-to-memory process. We will continue to enroll evidences in order to translate immunophenotyping assay as a marker for in-depth diagnostic assessment to rule out T-cell-immunity defects or genetic polymorphisms in clinical practice.

C.O.I. Disclosure:

C. Perrone: None. **N. Orlando:** None. **C. Russo:** None. **C. Passarelli:** None. **R. Ruta:** None. **M. Mattiuzzo:** None. **C. Caponi:** None. **S. Piras:** None. **A. Novelli:** None. **C. Perno:** None.

EP09.016 KREC levels in NBS : A Valuable Marker for Early Detection of AR Immunodeficiencies

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Background: Extended neonatal screening enables the detection of inborn errors of immunity at a pre-symptomatic stage. The variability in the number of TREC and KREC molecules is sufficient to detect not only SCID but also other forms of immunodeficiency, including those resulting from impairments in either the T-cell or B-cell compartment.

Material and Method: Genomic DNA was extracted from whole blood using the salting-out method. Exome sequencing was performed at Hannover Medical School. TREC and KREC levels were determined during newborn screening from a dried blood spot (DBS) sample.

Results: Routine neonatal screening revealed that the patient had normal levels of TREC molecules but an absence of KREC molecules in a DBS sample. Repeated tests confirmed these findings. Subsequently, exome sequencing was performed on the patient, revealing a pathogenic variant, c.258del, and a variant of uncertain significance, c.425C>T, in the *IGLL1* gene, which is associated with autosomal recessive agammaglobulinemia. According to ACMG criteria (PM2, PM3, PP3, PP4), variant c.425C>T has 2 moderate and 2 supporting criterions and we can classify this variant as likely pathogenic. Agammaglobulinemia leads to serious infectious complications, early diagnosis allows timely initiation of treatment.

Conclusions: The importance of expanded neonatal screening is confirmed by the fact that it allows detection of not only severe combined immunodeficiency (SCID), but also other forms of immunodeficiency. Determining the levels of KREC molecules is an effective method for detecting disorders B-cell links in the immune system.

C.O.I. Disclosure:

I. Shymanska: None. **O. Arkhipova:** None. **A. Yurchenko:** None. **H. Makukh:** None.

EP09.017 Unveiling the genetic landscape of primary immunodeficiency: Array-CGH and WES discoveries in a cohort of 169 patients in Russian Federation

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Background: To determine the genetic cause in a heterogeneous group of patients with primary immunodeficiency.

Material and methods: Analysis of clinical data and results of WES (157) and Array-CGH (39) of 169 patients with primary immunodeficiency. The first-line DNA test was selected based on the molecular nature of the suspected disease.

Results: The general group consisted of patients with non-specific immunological phenotype or patients with specific manifestations of a certain nosology, but with negative results of targeted DNA diagnostics: 88.5% children, 15.8% adults, mean age 11.2 ± 12.7 $(0.1 \div 62.0)$, sex ratio M(1.0) : W(1.1). The genetic cause of the disease was not detected in 34.4% cases and was identified in 37.7% cases, of which: minority - CNV and aneuploidy (16.4%), and most of it - SNV (41.8% - variants with a dominant type of inheritance; 32.8% - recessive, 9.0% - X-linked). In 27.9% of cases - VUS. The genetic landscape of the affected genes is extremely diverse, with pathogenic variants most frequently clustered in *ATM*, *GATA2*, *NKFB1*, *BTK* and *ELANE* genes. For some patients the immunological phenotype was due to hereditary metabolic diseases (*PUS1, MMACHC, MMUT, CLPB)*. Most of the genetic findings were observed in the group of patients with combined immune deficiencies with associated or syndromic features. For 10 patients pathogenic variants were identified in genes that were not associated with immunodeficiency, but were important for further medical supervision (*GALC, TTN, DLR, LDLR, SDHC, CHEK2, FBN1, RUNX2, RYR2*).

Conclusion: Combining DNA testing methods has shown high efficiency in diagnosing rare diseases.

C.O.I. Disclosure:

O. Novoselova: None. **I. Kazachkova:** None. **N. Isaeva:** None. **R. Bikanov:** None.

EP09.018 Genetic Variants in Hereditary Spherocytosis

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Background: Hereditary spherocytosis is the most common cause of haemolytic anaemia, caused by an abnormal red blood cell membrane that leads to decreased stability and deformability of the RBC membrane, along with a gradual loss of the lipid bilayer in the microvasculature. The classic triad of symptoms associated with hereditary spherocytosis includes jaundice, anaemia, and splenomegaly, although the clinical presentation can differ based on the severity of the condition.

Material and methods: We examined a cohort of approximately 80 patients referred to us with a suspected diagnosis of hereditary spherocytosis. The analysis primarily utilized EDTA blood samples, from which DNA was extracted and sequenced using next-generation sequencing techniques. The resulting sequencing data was processed and analysed according to standard protocols.

Results: In approximately 85% of the patients analysed, we identified a variant that could be classified at least as variant of uncertain significance. Among these, 86% had a variant in one of the well-known genes associated with hereditary spherocytosis: *ANK1* (~15%), *EBP42* (~2.5%), *SLC4A1* (~16%), *SPTA1* (~20%), and *SPTB* (32.5%).

Conclusion: By analysing a cohort of approximately 80 patients we identified genetic variants in a substantial majority of cases. Notably, we observed that a large proportion of these variants were located in genes previously associated with hereditary spherocytosis. This highlights the genetic heterogeneity of the condition and underscores the importance of genetic testing in the diagnosis and management of hereditary spherocytosis.

C.O.I. Disclosure:

R. Lehner: None. **C. Sillaber:** None. **A. Denner:** None. **M. Rybiczka-Tesulov:** None. **M. Gerykova Bujalkova:** None. **M. Pfeifer:** None. **Z. Delissen:** None. **F. Wierzbicki:** None.

EP09.019 Pharmacogenetic study of the *IL-17RA* gene in psoriatic patients

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Background: Psoriasis is a chronic, inflammatory, autoimmune skin disease with systemic effects. The disease affects 2–3% of the global population. Various pharmacogenetic studies have been carried out in order to investigate the different response to the applied treatments. The aim of the present pharmacogenetic study was to identify the single nucleotide polymorphisms (SNPs) of the interleukin *(IL)-17A* subunit gene, *IL-17RA*, which are associated with the response of patients treated with secukinumab. Additional goal was to identify the SNPs that affect the expression of *IL-17RA* gene.

Material and methods: 40 patients received secukinumab for at least 6 months. Genomic DNA was isolated from whole peripheral blood samples. Genotyping of the *IL-17RA* gene followed. Then, the SNPs were checked in order to identify those associated with treatment response. Genotypic analysis, allelic analysis and analysis based on the dominant and recessive allele model were done. An eQTL (expression Quantitative Trait Loci) analysis followed to examine if any of the SNPs affect the expression of the *IL-17RA* gene. The location of SNPs appeared to Ensembl database.

Results: 10 SNPs gave statistically significant results in allelic analysis only. These SNPs are referred as intergenic variants. Of these 10 SNPs only 4 (rs5993915 T, rs78597019 G, rs201584534 G, rs5748974 T) are statistically significant eQTLs for the *IL-17RA* gene.

Conclusion: 4 SNPs can be prognostic markers for secukinumab treatment. As these polymorphisms may related with regulatory elements, further research must be done. Genome-wide eQTL analysis with bigger number of samples will give more credible results.

C.O.I. Disclosure:

K. Mourtou: None. **K. Liadaki:** None.

EP09.020 Investigating genome wide regulators of *CD38* gene, a potential target for Multiple Myeloma immunotherapy

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Background: Multiple myeloma (MM) is the second most aggressive hematopoietic blood cell disorder characterized by accumulation of malignant plasma cells with an average median survival of 3 to 7 years. Immunotherapy with Daratumumab in combination with standard drug treatment has shown promising results but has yet not achieved progression free survival especially for refractory cases associated with low *CD38* gene expression. Therefore, we aim to identify regulators of CD38 surface markers at genome-wide scale.

Material and methods: The genome-wide human CRISPR knock-out library has been used for knocking down genes using electroporation system in high expressing CD38 marker cell line OPM2. The cells were stained with Anti-CD38 to sort affected cell types using flow cytometry. DNA was extracted from sorted cells and single guide ribonucleic acid (sgRNA) sequence representation was determined by Next generation sequencing (NGS).

Results: NGS results show differentially expressed genes regulating CD38 gene expression. Among this gene list a few regulators were known and some are novel. Statistically, significant regulators are being validated in individual experimental set-up for their role in CD38 gene regulation.

Conclusion: Human Genome-wide knock-out screening on high expressing CD38 MM cell identifies known and novel genes regulating CD38 expression. It can be useful in reshaping MM immunotherapy based on *CD38* gene expression levels.

C.O.I. Disclosure:

R. Ajore: None. **B. Nilsson:** None.

EP09.021 Use of polygenic risk scores to assess the contribution of common genetic variation in primary immunodeficiency disorders

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Background: Primary immunodeficiency (PID) refers to a heterogeneous group of congenital immune system disorders with highly variable presentations. Although several monogenic causes

have been identified, they can present with a broad spectrum of symptoms and not all patients carry the variants. Evidence suggests these gaps in understanding of PID aetiology might be explained by a cumulative effect of common genetic variants. This study explores the role of common variants in antibody deficiency-associated PID (AD-PID) through the use of polygenic risk scores (PRS).

Methods: Genotypes of 626 AD-PID patients and 7,672 controls of European ancestry were analyzed, with training-to-testing ratios 80:20 and 60:40. GWAS detecting SNPs associated with AD-PID was performed, followed by clumping and thresholding. PRS model was created (PLINK 1.9, PRSice v2.3.5) and its ability to differentiate cases from controls as well as patients with different genotypes and immunophenotypes was assessed.

Results: The PRS model failed to distinguish AD-PID cases from controls (median AUC: 0.519 for 80:20, 0.516 for 60:40). No significant PRS differences were found between individuals with distinct PID genotypes (monogenic vs. unknown cause: $P = 0.601$; monogenic vs. TACI: $P = 0.223$). The PRS showed no significant correlation with immunophenotypes (Pearson r: −0.11–0.25).

Conclusion: The identified variants do not explain the differences between AD-PID cases and controls, or patient subgroups, likely due to the small effect sizes of common variants and environmental factors influencing the aetiology. Larger studies with greater statistical power are needed to clarify the role of common genetic variants in AD-PID.

C.O.I. Disclosure:

K. Demkova: None. **E.K. Thomas:** None. **P.A. Lyons:** None.

EP09.022 Prevalence and co-occurrence of Southeast Asian Ovalocytosis (SAO) and Thalassemia in Malaysian adolescents: Findings from the national thalassemia screening initiative

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Background: Southeast Asian Ovalocytosis (SAO) and thalassemia are prevalent genetic disorders in Malaysia, posing significant public health challenges. Both conditions have been studied independently, their coexistence and interactions remain underexplored. This study aims to investigate the prevalence and coexistence of SAO and thalassemia among teenagers participating in the National Thalassemia Screening Program in Malaysia.

Material and methods: A cross-sectional study was conducted involving 2204 adolescents aged 16, who were part of the national thalassemia screening initiative. Blood samples were collected and analysed for hematologic parameters, SAO, and thalassemia markers using standard laboratory techniques, including PCR and DNA sequencing.

Results: The prevalence of SAO among the participants was found to be 3.18%, while thalassemia was identified in 16.5% of the cohort. Notably, 0.6% of the adolescents exhibited both SAO and thalassemia. The coexistence of these conditions did not significantly alter the clinical presentation of thalassemia but was associated with distinct hematologic profiles, including increased red cell rigidity and mild haemolytic anaemia.

Conclusion: Although the co-inheritance of SAO does not significantly alter the red blood cell indices, particularly the mean corpuscular haemoglobin (MCH) value, which is a key indicator used in national thalassemia screening, it remains essential to consider this genetic interaction in the broader context of thalassemia management. This study provides new insights into the prevalence and co-occurrence of SAO and thalassemia in Malaysian adolescents. The findings highlight the importance of integrated screening programs and underscore the need for further research to understand the clinical implications of these overlapping genetic disorders.

C.O.I. Disclosure:

N.M. Yasin: None.

EP09.023 Spectrum of primary immunodeficiency disorders in Pakistan - Experience from a single tertiary health care centre

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Background: Primary immunodeficiency disorders (PIDs) represent a group of diseases, affecting the immune system, which predisposes patients to recurrent, chronic, treatment-resistant infections and autoimmunity. High risk of consanguineous leads to high incidence of autosomal recessive (AR) PIDs, however, molecular diagnosis remains largely elusive.

Material and methods: This is a retrospective chart review of patients from Aga Khan University Hospital, Karachi, Pakistan who underwent NGS based panel testing ($n = 74$) or Whole Exome Sequencing ($n = 5$) due to high clinical suspicion of PIDs between 2019 and 2024.

Results: Our study recruited 79 patients (31 female), 49% were born to consanguineous parents. The results on the diagnostic report showed: 34 (43%) P/LP (pathogenic/likely pathogenic) 40 (51%) VUS (variants of uncertain significance) and 5 (6%) negative result. Out of those with P/LP results, 11(32%) patients were only found to be carriers for AR PID. After reverse phenotyping, the result distribution was: 44% P/LP, VUS 20% and 35% negative. The VUS rates had significantly reduced from 51% to 20%, while 30% VUSs were deemed positive. The spectrum of genes was spread across 27 genes and the most common were *DOCK8, RAG1, DLRE1C, ADA, ATM, CYBB* and *IL2RG*.

Conclusion: This study reports a diagnostic yield of 44% which is comparable (but on the higher end) to yield observed in other populations, ranging from 15–46%. As we understand the genetic architecture of our population better, more VUSs will also get resolved as also highlighted by our work.

C.O.I. Disclosure:

H. Asjad: None. **F. Akbar:** None. **S. Kirmani:** None. **F. Qamar:** None.

EP09.024 Clinical case of differential diagnostic between different types of inborn errors of immunity: mosaic variant in STAT5B

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Background: According to classification of inborn errors of immunity (IEI) gain of function somatic variants in *STAT5B* refer to phenocopies of IEI (hypereosinophilic syndrome). Also, they can cause myeloid or lymphoid leukemia (OMIM#102578) what makes it difficult to differentiate.

Material and methods: A male patient born at full term was hospitalized in a severe condition on the 13 days of life with clinical picture of infectious toxicosis. In the hospital, conservative and surgical treatment of abscess of the groin area was performed, during the examination was found a leukocytosis and clinical picture of allergic reaction: eosinophilia, papular rash,

hemocolitis.During antibiotics therapy leukocytosis and eosinophilia are maintained despite the absence of infection site. Bone marrow biopsy did not reveal hemoblastosis. However, the blood tests showed an increase in leukocyte counts and differential diagnosis of hypereosinophilic syndrome and acute myeloid leukemia was regarded.Trio whole genome sequencing (WGS) and targeted high-throughput sequencing (HTS) were performed.

Results: WGS revealed known pathogenic variant NM_012448.4(*STAT5B*):c.1924A>C presumably in heterozygous state which is absent in parents. To determine the status of the variant, various tissue samples were analyzed using HTS. The results of the study confirmed somatic mosaicism with varying alternative allele ratio in different tissues. Taking into account obtained results hypereosinophilic syndrome is the most plausible diagnosis.

Conclusion: This clinical case demonstrates complexity of IEI phenotypes, significance of determination variant's state (somatic or germline) to establish an accurate diagnosis and choose the right treatment tactics and patient's follow-up.

C.O.I. Disclosure:

A. Bobreshova: None. **P. Sparber:** None. **A. Burya:** None. **G. Bronin:** None. **R. Zinchenko:** None. **A. Marakhonov:** None.

EP09.025 A possible novel gene accounting for familial hemophagocytic lymphohistiocytosis in a patient with Gaucher disease:*STXBP3*

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Background: We present a 7-year-old girl with Gaucher disease Type 3 (*GBA*, having biallelic pathogenic variants,p. L483P) born to consanguineous parents, who didn't respond to enzyme and substrate inhibition therapy despite the lack of anti-drug antibodies against enzyme therapy. As bone marrow smear which was done to rule out a coextintent disorder showed hemophagocytic lymphohistiocytosis (HLH) in addition to clinical signs and symptoms such as fever, splenomegaly, bicytopenia, hypertriglyceridemia, hypofibrinogenemia, hyperferritinemia, and hemophagocytosis in the bone marrow and whole exome sequence (WES) analysis was tested.

Material and methods: Next Generation DNA sequencing using the Illumina NovaSeq platform with the QIAseq Human Exome Kit was conducted. Variant classification was evaluated according to the ACMG 2015 guideline.

Results: Biallelic missense variant was detected in *STXBP3* (syntaxin binding protein 3) gene in WES. The patient's parents were carriers. There were no homozygous healthy controls for this variant in population databases and it has been reported as pathogenic in silico analysis databases. The patient died two months after diagnosis due to multiorgan failure.

Conclusion: Although *PRF1, UNC13D, STX11* and *STXBP2* were known to cause familial HLH before this is the first report describing *STXBP3* as a possible additional gene to cause the disease. STX-4 (syntaxin 4) is a SNARE protein that functions together with STXBP3 in the fusion of cytotoxic granules to the membrane in the cytolytic pathway. Our hypothesis suggests that *STXBP3* mutations may impair STX4-mediated fusion, contributing to HLH development. However further functional studies are needed to show the pathophysiological mechanisms.

C.O.I. Disclosure:

M.M. Sari: None. **Z. Kaya:** None. **N. Kan Menku:** None. **A. Yuce:** None. **U. Kocak:** None. **L. Tumer:** None. **F. Ezgu:** None.

EP10 Intellectual Disability

EP10.002 SMARCA5 neurodevelopmental disorder: case report of new variant

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Background: Genes encoding chromatin remodelers and their subunits are associated with several neurodevelopmental disorders. In order to expand knowledge about these conditions, a new variant on the SMARCA5 gene is described.

Material and methods: Case report of a patient with neurodevelopmental disorder, failure to thrive and microcephaly.

Results: Patient, female, 1y, was referred for investigation due to microcephaly and low weight gain. First child of a young, healthy and non-consanguineous couple, with an uneventful pregnancy, she was born by cesarean section due to oligohydramnios at 37 weeks, with Apgar 5/8, 2130g, 45cm and head circumference of 32cm. The neurodevelopmental was slightly delay: she walked with 1 year 3 months and speech was poor, furthermore evolved esophagitis, behavioral sleep disorder, motor coordination and sensory disorder. At physical examination, she presents microcephaly, height and weight below the 3rd percentile, bilateral epicanthus, prominent nose, thin lips, camptodactyly and clinodactyly of the 5th finger, overlapping of the 2nd over the 3rd toe. Echocardiogram, skull radiography, electroencephalogram, transfontanellar ultrasound and SNP-array were normal. Karyotype was 46,XX,inv(3)(P13P25). Whole exome sequencing identified a new SMARCA5 variant GACTGGATTCAAT > G (or c.1879_1890delACTGGATTCAAT). The p.Leu627_Ile630del variant was confirmed by Sanger sequencing in the patient and is not present in her parents.

Conclusion: The neurodevelopmental syndrome associated with the SMARCA5 gene overlaps with conditions caused by variants in different subunits of chromatin remodeling complexes. This case contributes to a better clinical delineation of this ultracare syndrome, with emphasis on low weight and height gain.

C.O.I. Disclosure:

R. Maia: None.

EP10.003 A novel pathogenic variant in *WAC* Gene causes DeSanto-Shinawi syndrome

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Background: Desanto-Shinawi syndrome is an autosomal dominant neurodevelopmental disorder characterized by global developmental delay apparent in infancy or early childhood and associated with characteristic dysmorphic facial features. Pathogenic sequence and copy number variants in the *WAC* gene cause

this syndrome. Here, we present a patient with clinical manifestations of DeSanto-Shinawi syndrome. We aimed to confirm the patient's diagnosis by next generation sequencing analysis.

Material and methods: Genomic DNA was extracted from the patient's peripheral blood sample. Exons and exon-intron transition regions of the candidate genes were investigated by next generation sequencing using Sophia DDM program. Variant information servers dbSNP, ClinVar, Ensembl and ACMG 2015 criteria were used for evaluations.

Results: In the fifth exon of *WAC* gene, c.414_415insTA p.(Ile139*) nonsense variant was detected in heterozygous state. This variant was expected to cause functional effects due to premature ending of the protein translation. It was not reported in large population studies or disease-specific databases.

Conclusion: The detected novel nonsense variant in our study was evaluated as in relation with DeSanto-Shinawi syndrome because of its predicted deleterious effects. To better elucidate a possible association between this syndrome, both variant segregation status in the patient's family and larger population data will be examined and functional analysis of *WAC* gene.

C.O.I. Disclosure:

G. Öner: None. **G. Nacak:** None. **B. Nur:** None. **A. Manguoğlu:** None. **E. Mıhçı:** None.

EP10.004 Helsmoortel-Van der Aa syndrome: Reporting a new pathogenic variant in the ADNP gen

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Background: Helsmoortel-Van der Aa Syndrome (HVDAS) (OMIM#615873) is a rare disease with an estimated prevalence of 1–9 in 100,000 newborns. It is characterized by intellectual disability, autism spectrum disorder (ASD), facial dysmorphism, hypotonia, and congenital cardiac and urinary-tract malformations. It is caused by a pathogenic heterozygous variant or epigenetic signature in the *ADNP* gene, a transcription factor encoding an homeobox domain, which is key to neuronal differentiation (1–3). Hereby, we report a new variant causing the HVDAS.

Material and methods: A 13-year-old female attends genetic consultation for intellectual disability, language and behavioral disorders. She has a family history of two maternal cousins with autism. Physical examination revealed esotropia, minor facial abnormalities such as a prominent forehead, broad nasal bridge with an upturned tip, hypotonia, small hands and feet. Extension studies, karyotype, Prader-Willi/Angelman DNA methylation panel, Microarray-based Comparative Genomic Hybridization (aCGH) and trio-based exome were performed.

Results: Extension studies revealed hypothyroidism and urinary-tract abnormalities, along with a diagnosis of atypical ASD. Karyotype showed no numeric or structural abnormalities, methylation panel was normal, and aCGH ruled out microdeletions and duplications. Trio-based exome identified a de novo heterozygous variant c.896_897insTA (p.Ser300Asnfs*6) in the *ADNP* gene. This variant has not been reported in the literature, however it meets the American College of Medical Genetics criteria to be classified as pathogenic.

Conclusion: A new variant causing this disease was reported. When dealing with a patient with cognitive disability or ASD, it is important to include this syndrome as a diagnostic possibility, given its close association.

C.O.I. Disclosure:

P.A. Espíndola: None. **M. Machado:** None. **A. Castillo:** None. **G.A. Contreras:** None.

EP10.005 Derivation of human embryonic stem cell line from a patient with *CAPRIN1* haploinsufficiency

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IRCCS Giannina Gaslini, GENOVA, Italy.

Background: *CAPRIN1* gene encodes a RNA-binding protein, abundant in the brain where it plays a crucial role, regulating the transport and translation of mRNAs of synaptic proteins. *CAPRIN1* haploinsufficiency causes a neurodevelopmental disorder characterized by language impairment/speech delay, intellectual disability, attention deficit, hyperactivity disorder, and autism spectrum disorder.

Material and methods: To understand the pathogenesis of this disorder and in view of future treatments, we generated human induced pluripotent stem cells (iPSCs) from a patient carrying the c.1744C>T *CAPRIN1* variant.

Results: The line show marker expression for the pluripotency and the capacity to differentiate into the three germ layers.

Conclusion: This cell line will serve as a valuable resource for both basic and translational studies in *CAPRIN1* haploinsufficiency.

C.O.I. Disclosure:

D. Coviello: None. **G. Conteduca:** None. **C. Baldo:** None. **A. Arado:** None. **J. da Silva:** None. **R. Bocciardi:** None. **B. Testa:** None. **S. Baldassari:** None. **M. Mancardi:** None. **L. Timotei:** None. **F. Zara:** None. **M. Malacarne:** None.

EP10.006 A case report of Potocki-Lupski syndrome in an Armenian child

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Background: The patient is from the 1st pregnancy, was born in the 39th week of gestation, with normal biomechanism, the weight was 3450 g and the height was 51 cm. At the age of 3 months, developmental delay was noted[1]. He started to walk at the age of 19 months after the rehabilitative therapy. The patient began to speak at the age of 2,5-3 years. At the age of 2 Fragile X syndrome was suspected, however, the genetic testing results were negative for the disease. The visual impairment was detected at the age of 6,5 years old. At the time of the latest consultation: 7 years old, weight 26 kg (75%), height 122 cm (50%), head circumference 51,5 cm (25%). Physical features include dolichocephaly, an elongated face, small "cup-shaped" ears, and an underdeveloped chin. The patient has mild intellectual, motor developmental delay and behavioral disturbance[2].

Methods: Molecular karyotyping (array CGH)[3] has been performed for the patient and his parents.

Results: The examination has revealed duplication of 17p12p11.2 with 4,8Mb in the region size which includes 52 genes. aCHG testing of patient's parents did not reveal any abnormalities.

Conclusion: In the literature 17p11.2 duplication is described in Potocki-Lupski syndrome, which is a rare chromosomal anomaly, occurring in approximately 1 in 25.000 individuals worldwide. However, only around 50 cases have been described in the medical literature.

C.O.I. Disclosure:

A. Yervandyan: None. **T. Sarkisian:** None. **N. Kostandyan:** None. **A. Gasparyan:** None. **S. Midyan:** None.

EP10.008 Mosaicism for a pathogenic *CHD7* Variant- A case report of a child with with a phenotype unsuggestive of CHARGE syndrome

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Background:CHARGE syndrome is characterized by a pattern of congenital anomalies: coloboma, heart defects, atresia choanae, retardation of growth and/or development, genital hypoplasia, ear anomalies. Heterozygous pathogenic variants in the *CHD7* gene result in a phenotypic spectrum ranging from classic CHARGE syndrome to individuals exhibiting only a subset of CHARGE features.

Additional *CHD7* related features include cranial nerve and vestibular abnormalities, cleft palate, hypothyroidism, tracheoesophageal and brain anomalies, seizures, and renal anomalies. This case report describes a child with a phenotype unsuggestive of CHARGE syndrome with a mosaic *CHD7* variant

Material and methods: A 4-year-old boy was referred to a genetics clinic for developmental delay (developmental quotient 54) and cleft lip and palate. Physical examination revealed normal growth parameters but mild dysmorphic features including male-pattern baldness and facial asymmetry (a consequence of cleft lip and palate repair). External ear morphology was normal.

Results: Genetic testing was performed. Chromosomal microarray analysis was normal, and maternal FMR1 premutation testing was negative. Trio exome sequencing of peripheral blood revealed a heterozygous pathogenic variant in CHD7 (NM_017780.4): c.1552C>T, p.Gln518*, chr8-61655543 C > T (GRCh37), present in 24% of reads (indicating approximately 48% mosaicism). This variant was classified as pathogenic based on PVS1 (very strong), PM2 (supporting) and PS2 (strong- based on a report in ClinVar).

Conclusion: CHARGE syndrome may not be suspected clinically in individuals with mosaic *CHD7* variants.

C.O.I. Disclosure:

R. Sukenik Halevy: None. **R. Goldfarb Yaacobi:** None. **T. Tenne:** None. **D. Lavi Ben Atav:** None. **N. Mevorach:** None. **N. Mevorach:** None.

EP10.009 Broadening the *PHIP*-Associated Neurodevelopmental Phenotype

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Background: Monoallelic damaging variants in *PHIP* (MIM*612870), encoding the Pleckstrin Homology Domain Interacting Protein, have been associated with a novel neurodevelopmental disorder, also termed Chung-Jansen syndrome (CHUJANS, MIM#617991). Most of the described individuals show developmental delay (DD)/intellectual disability (ID), obesity/overweight, and variable congenital anomalies, so the condition can be considered as an ID-overweight syndrome.

Material and methods: We evaluated a child presenting with DD/ID and a craniofacial phenotype reminiscent of a Pitt-Hopkins syndrome (PTHS)-like condition. We performed a clinical exome analysis on his biological sample, as well as an in silico prediction of the obtained data. At the same time, we interrogated the DeepGestalt technology powered by Face2Gene (F2G), using a frontal image of the proband, and clinically reviewed the earlier CHUJANS patients.

Results: In this child, we found a novel *PHIP* pathogenetic variant, which we corroborated through a protein modeling approach. The F2G platform supported the initial clinical hypothesis of a PTHS-like condition, while the clinical review highlighted the lack of the main frequent CHUJANS clinical features in this child.

Conclusion: The unusual clinical presentation of this novel patient resembles a PTHS-like condition. However, a novel variant in *PHIP* has been unexpectedly detected, expanding the phenotypic spectrum of CHUJANS. Notably, PTHS (MIM#610954), which is a different ID syndrome caused by heterozygous variants in *TCF4* (MIM*610954), is not classically considered in the differential diagnosis of CHUJANS nor has been cited in the previous studies. This could support other complex diagnoses and invite further patients' descriptions.

C.O.I. Disclosure:

G. Pascolini: None. **G. Scaglione:** None. **B. Chandramouli:** None. **D. Castiglia:** None. **G. Di Zenzo:** None. **B. Didona:** None.

EP10.010 Rare RORA gene variants and phenotypic variability: Clinical variability in cases with frameshift variant in the RORA gene from the same family

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Background: The RAR-Related Orphan Receptors (RORA, RORB, and RORC) are members of the nuclear receptor gene superfamily. A recessive mutation model in RORA has been linked to a severe cerebellar degenerative model in animal studies, with heterozygote mutations leading to accelerated dendritic atrophy and cell loss. Autosomal variants in the RORA gene have been associated with Intellectual Developmental Disorder with or without epilepsy or cerebellar ataxia. Nevertheless, the clinical understanding of the impact of RORA gene variants in patients is currently limited. Published rare reports connect RORA gene variants with a diverse range of phenotypes, such as Intellectual Disability (ID), epileptic seizures, Autism Spectrum Disorders (ASD), or cerebellar ataxia. These findings reveal substantial variability among reported cases, making the phenotypic impact of RORA gene variants complex.

Methods and Results: This study aims to present genetic and clinical findings from cases carrying RORA variants. Whole exom sequencing and arrayCGH analyses revealed a 15q22 deletion encompassing RORA, as well as nonsense and frameshift variants in the RORA gene.

Conclusion: We have discussed genetic and clinical findings of the cases carrying different type of RORA gene variants. Interestingly, a heterozygous c.683_686del (p.Glu228Valfs*78) variant was detected in four members of the same family caused clinical variability from mild intellectual disability to severe neurodevelopmental delay. The reasons behind the substantial differences in symptoms among these cases remain unclear. This suggests variable expressivity of the mutation, marking the first report of such variability in RORA-related disorders.

C.O.I. Disclosure:

I. Baris: None. **G. Unsel Bolat:** None. **H. Bolat:** None. **S. Citli:** None.

EP10.011 Malan Overgrowth Syndrome: First case report in Colombia

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Background: Malan Syndrome (OMIM #614753) is a rare disease, with a prevalence <1 in 1,000,000 individuals. Its key features are: postnatal overgrowth with advanced bone age, marfanoid habitus, macrocephaly, and facial dysmorphism accompanied by neurodevelopmental delay and intellectual disability. Caused by variants in the *NFIX* gene, generally de novo, this gene is located in chromosome 19p13.13, which contains 11 exons and encodes a nuclear transcription factor (1–2). Hereby, we report the first case of Malan Syndrome in Colombia.

Material and methods: The patient is a young male referred from neuropediatrics due to malformations and moderate intellectual disability at 13 years-old. No abnormalities were identified in the prenatal period. He was initially studied due to macrocephaly and hypotonia. Later, neurodevelopmental delay and spastic diplegia were diagnosed. Physical examination showed marfanoid habitus and facial dysmorphism with long and thin face, synophrys, down-slanted palpebral fissures, prominent forehead and chin. Therefore, Karyotype, Microarray-based Comparative Genomic Hybridization (aCGH) and trio-based exome were performed.

Results: Imaging studies showed an Arnold Chiari type I malformation. Karyotype excluded numeric or structural abnormalities; aCGH ruled out microdeletions and duplications. Finally, at 18 years old a trio-based exome identified a pathogenic heterozygous variant c.515_518del (p.Ile172Lysfs*38) located in exon 2 of the *NFIX* gene, marking it as one of the 80 reported cases worldwide (1–3).

Conclusion: Due to the similarity between several overgrowth syndromes, the study of the *NFIX* gene should be one of the first diagnostic options for patients with an intermediate phenotype between Sotos and Marfan syndrome.

C.O.I. Disclosure:

M. Machado: None. **P. Espindola:** None. **G. Contreras:** None. **A. Castillo:** None.

EP10.012 Novel germline mutation in BCORL1 gene identified in a family with three Shukla-Vernon syndrome patients

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Background: Germline variants in *BCORL1* have been proposed to underlie an X-linked disorder of intellectual disability, dysmorphic features, and behavioral abnormalities, annotated in OMIM as Shukla-Vernon syndrome (OMIM #301029). All reported variants are inherited from mothers who were either unaffected or had mild signs, such as learning disability.

Material and methods: Here, we report on a family with three sons having seizures, intellectual disability, learning difficulties, ataxia, abnormal vision, and abnormal gait. We carried out whole exome sequence analysis of all protein coding genes in the genome for the proband and affected/unaffected family members, coupled with whole exome deletion/duplication (CNV) Analysis.

Results: All patients were found to be hemizygous for BCORL1 c.3366C>T (NM_021946.4), p.(Gly1122=), which is a variant of uncertain significance (VUS). The variant is inherited from the patient's mother, who is heterozygous for the variant. Sanger sequencing confirmed the variant. No other candidate variants were found. This variant is absent in gnomAD, a large reference population database (n > 120,000 exomes and >15,000 genomes). *BCORL1* c.3366C>T, p.(Gly1122=) is a synonymous variant that does not alter the protein sequence, however, in silico splice prediction tools (SSF, MaxEntScan, NNSPLICE) predict this substitution will create a new cryptic donor splice site. The variant may therefore lead to abnormal splicing. Transcriptional analysis is underway to determine this variant's effect on splicing.

Conclusion: We are reporting a novel mutation in *BCORL1* gene. To the best of our knowledge, this variant has not been described in the medical literature or reported in disease-related variation databases.

C.O.I. Disclosure:

A. Alkhateeb: None.

EP10.013 Increased recurrence risk in Phelan-McDermid syndrome: two siblings in an albanian family

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Background: Phelan-McDermid syndrome (PMS) is a rare neurodevelopmental disorder characterized by developmental delay, mild dysmorphisms, autism spectrum disorder and intellectual disability. Most cases are caused by the deletion of the 22q13 genomic region, including the deletion of SHANK3. PMS-*SHANK3* related is an autosomal dominant disorder most often caused by a de novo genetic alteration.

Material and methods: We report two Albanian male siblings, born of nonconsanguineous parents. The 6-year old brother presented intellectual disability, severe speech delay, autistic behaviour and abnormal electroencephalogram discharge. Whole exome sequencing (WES) was performed on genomic DNA obtained from peripheral blood. The 3-year old brother presented language motion development delay, autistic-like behaviour and hyperkinesia. Multiplex ligation dependent probe amplification (MLPA) was performed.

Results: WES identified a 2538 Kb-large loss within chromosomal region 22q13.32q13.33, including the SHANK3 gene.The result was also confirmed by Chromosomal Microarray (CMA) analysis. The obtained result was consistent with a genetic diagnosis 22q13.3 deletion syndrome (Phelan-Mcdermid syndrome). MLPA identified heterozygous deletion at 22q13.33, including the deletion of SHANK3. WES analysis performed in patient's parents did not reveal the deletion. Also cytogenetic analysis in both of them ruled out the carrier status for a possible chromosomal rearrangement. Parental germline mosaicism may be a significant mechanism for the generation of de novo pathogenic 22q13.3 deletion.

Conclusion: An increased recurrence risk in PMS may be more common than currently understood, emphasizing the possibility of parental germinal mosaicism. Prenatal testing and preimplantation genetic testing should always be offered after PMS diagnosis in a proband.

C.O.I. Disclosure:

A. Babameto-Laku: None. **A. Bushati:** None. **B. Basholli:** None. **D. Roko:** None. **A. Gjikopulli:** None. **L. Kollcaku:** None.

EP10.014 The outbreak of ReNU Syndrome: expanding the clinical spectrum

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Background: *RNU4-2*, a component of the major spliceosome, is extremely important for the maturation of messenger RNA, therefore, it appears to be highly expressed during the cerebral development, herein playing a fundamental role. De novo heterozygous variants in *RNU4-2* were recently found to cause a novel neurodevelopmental disorder, known as ReNU Syndrome, mainly characterized by hypotonia and moderate to severe intellectual disability, mostly undermining the language aspect. Moreover, seizures and autism spectrum disorders can be expressed. From a weight-growth point of view, these patients present microcephaly and short stature.

Case Report: The already known de novo variant c.64_65insT in *RNU4-2* was found in two children out of our cohort of patients after a diagnostic odyssey. They both present intellectual disability and share multisystemic clinical findings, consistent with the diagnosis of ReNU syndrome. Both children display peculiar features of the face: strabismus, epicanthus, broad nasal bridge, large and anteverted ears. Bicuspid aortic valve condition and mild aortic stenosis were observed in one patient; meanwhile, sub-nephrotic proteinuria and microhaematuria were found in the other one.

Conclusions: This evidence, concerning the cardiovascular and renal system, represent a new data that enriches the literature; these latest described clinical features might be considered as part of the clinical phenotype of ReNU Sydrome, paving the way for a more defined therapeutic program that fits with the needs of these patients.

C.O.I. Disclosure:

A. De Franco: None. **T. Palazzotti:** None. **G. Marchetti:** None. **M. Mura:** None. **P. Ajmone:** None. **G. Armaroli:** None. **W. Morello:** None. **G. Montini:** None. **C. Agostoni:** None. **P. Di Letto:** None. **A. Torella:** None. **L. Pezzoli:** None. **M. Iascone:** None. **D. Milani:** None.

EP10.015 Fragile X syndrome: A case of premutation mosaicism in a Bulgarian patient

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Background: Fragile X syndrome (FXS) is the most common inherited cause of intellectual disability (ID) and autism spectrum disorder (ASD). This X-linked disorder is associated with an expansion of a cytosine-guanine-guanine (CGG) repeat in the FMR1 (fragile X mental retardation 1) gene. Individuals with larger expansions may have a premutation (55–200 CGG repeats) or a full mutation (more than 200 CGG repeats). Mosaicism in FXS indicates the presence of multiple cell lines with different CGG repeat numbers.

Material and methods: We present a case of a 16-year-old male with features compatible with, but not specific to, FXS. The patient's maternal first cousin has mild intellectual disability. Molecular analysis was performed using triple-primed FMR1 PCR (TP-PCR). The PCR fragments were analysed on an ABI 3130 genetic analyser.

Results: The obtained results by TP-PCR showed a normal allele with 31 ± 1 CGG repeats and a premutation with 85 ± 1 repeats in the mother. The patient exhibited a mosaic pattern with three allele groups: one normal (26 ± 1 CGG repeats) and two premutation alleles with 90 ± 1 and 124 ± 1 CGG repeats. These results are compatible with a Fragile X mosaic case of premutation-associated conditions.

Conclusion: This report emphasizes the importance of DNA molecular testing by TP-PCR for diagnosing FXS in patients with developmental delay and ASD. The variable phenotype in individuals with FMR1 mosaicisms underscores the need for early diagnosis and prompt intervention to improve the quality of life for children with behavioral disorders.

C.O.I. Disclosure:

I. Dimitrova: None. **S. Bichev:** None. **K. Kercheva:** None. **I. Bradinova:** None. **A. Savov:** None.

EP10.016 A novel variant in the *NAPB* as a cause of epileptic encephalopathy and intelectual disability in a 38 year old woman

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Introduction Developmental and epileptic encephalopathy type 107 is a rare syndrome characterized by early-onset epilepsy, intellectual disability, severe psychomotor delay, hypotonia, spasticity, microcephaly, and dysmorphic facial features. Brain abnormalities may include atrophy and white matter alterations.

Case Report We present the case of a 38-year-old woman previously with clinical diagnoses of Rett syndrome because of refractory epilepsy since the neonatal period, experiencing daily seizures. A trio exome study identified the c.713C>G; p.(Ser238)*) variant in homozygosity in the *NAPB* gene, associated with developmental and epileptic encephalopathy type 107, a severe autosomal recessive disorder characterized by early-onset seizures and profound intellectual disability (OMIM#620033). This *nonsense* variant results in a truncated protein. According to ACMG criteria, it is classified as likely pathogenic (PVS1, PP3, PM2).

Discussion This variant has not been previously reported in the literature or population databases. The patient's parents come from a small town in Teruel, which may explain the shared variant. Both presen this variant in heterozygous. They were informed that the initial Rett syndrome clinical diagnosis was due to the lack of genetic testing tools 35 years ago, however, the patient should be considered affected by developmental and epileptic encephalopathy type 107. This results help to genetic and reproductive counselling in her brother.

C.O.I. Disclosure:

N. Padilla: None. **E. Gutierrez:** None. **N. Romero:** None. **J. Sanchez:** None. **S. Izquierdo:** None.

EP10.017 Discovery of a novel variant in the *FBXW7* gene in a patient with global developmental delay

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Introduction: Advances in exome sequencing have improved the identification of genetic variants in patients with intellectual disability and psychomotor delay, facilitating early-age support. Among the 2800+ genes implicated in these disorders, the *FBXW7* gene encodes a protein involved in ubiquitination and degradation of key proteins. Heterozygous pathogenic variants in this gene are associated with developmental delay, hypotonia, and language disorders, with an autosomal dominant inheritance pattern (OMIM#620012).

Case Report: We present the case of a 22-month-old patient under evaluation for global developmental delay. Pregnancy and neonatal screening were normal, but signs of concern appeared at 12 months, including lack of response to their name, absence of language, and motor difficulties. The patient exhibits a distinct phenotype, including thick eyebrows, synophrys, and a broad nose, along with elevated dermatan sulfate excretion in urine. Genetic sequencing identified the c.519_520dup p.(His174Profs66) * variant in exon 3 of *FBXW7* gene, causing a *frameshift* that results in a truncated protein. According to ACMG criteria, this variant is classified as likely pathogenic (PVS1 y PM2).

Discussion: This variant has not been previously reported in the literature or population databases. However, nearby variants classified as pathogenic (P) or likely pathogenic (LP) have been described in ClinVar. This finding explains the patient's clinical presentation. Parental testing is recommended to determine the variant's origin and confirm its diagnostic significance.

C.O.I. Disclosure:

C. Garcia: None. **N. Padilla:** None. **C. Puerto:** None. **R. Gonzalez:** None. **S. Izquierdo:** None.

EP10.018 Chromosomal microarray analysis in undiagnosed Thai children with global developmental delay or intellectual disabilities

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Background: Global developmental delay (GDD) and intellectual disabilities (ID) are significant pediatric neurodevelopmental disorders. Conventional karyotyping has a diagnostic rate of 3%-5.4% and often fails to detect submicroscopic deletions or duplications. Chromosomal microarray analysis (CMA) provides improved resolution, offering a higher diagnostic yield. This study aimed to evaluate the diagnostic yield of CMA in Thai children with GDD/ID.

Material and methods: Forty-eight children with GDD/ID were enrolled in this study. CMA was performed using the CytoScan 750K microarray platform (Applied Biosystems, USA). Copy number variants (CNVs) were classified according to the ACMG/ClinGen 2019 guidelines.

Results: Among the 48 participants, 32 (66.7%) presented with at least one additional congenital anomaly, involving 1–8 organ systems. The most affected systems were neurological (56.3%), growth (50%), craniofacial (47.9%), and musculoskeletal (29.2%). CMA identified abnormal findings in 23 patients (47.9%), including pathogenic or likely pathogenic CNVs in 14 patients (29.1%). Mosaicism was detected in one patient. No significant correlation was found between the number of organ systems involved and abnormal CMA findings. Of five patients with abnormal karyotypes, one had a balanced translocation, but CMA identified a copy number loss in this case.

Conclusion: CMA demonstrated a diagnostic yield of 29.2% in Thai children with GDD/ID, significantly outperforming conventional karyotyping with a 5–10 times higher detection rate. This study highlights the potential of CMA as a superior diagnostic tool and supports its inclusion in the benefits package proposed to the National Health Security Office (NHSO) of Thailand for children with GDD/ID.

C.O.I. Disclosure:

T. Dechpumee: None. **K. Wichajarn:** None. **Y. Chamgramol:** None. **R. Deenonpoe:** None. **K. Intuyod:** None. **M. Thanee:** None. **P. Wichian:** None. **J. Kampan:** None. **A. Rattanathongkom:** None. **K. Kanda Sornkayasit:** None.

EP10.019 A case of autosomal dominant intellectual development disorder type 30 caused by a pathogenic variant in *ZMYND11* gene

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Background: Pathogenic variants in *ZMYND11*, which acts as a transcriptional repressor, have been associated with intellectual disability, behavioral abnormalities, and seizures. Only 27 affected individuals have been reported to date. The associated phenotype (which is not yet fully defined) includes developmental delay, particularly affecting speech, mild-moderate intellectual disability, significant behavioral abnormalities, seizures, and hypotonia. There are subtle shared dysmorphic features, including prominent eyelashes and eyebrows, a depressed nasal bridge with bulbous nasal tip, anteverted nares, thin vermilion of the upper lip, and macrostomia.*ZMYND11* (Zinc Finger MYND-Type Containing 11) is a protein coding gene, located on chromosome 10 (10p15.3). Diseases associated with *ZMYND11* include Intellectual Developmental Disorder, Autosomal Dominant 30, With Speech Delay And Behavioral Abnormalities and Autosomal Dominant Non-Syndromic Intellectual Disability. We present a 3-year-old male patient with facial dysmorphism, psychomotor delay, muscle hypotonia, failure to thrive, cryptorchidism, congenital heart defect and delayed speech development (IQ of 42).

Material and methods: Conventional cytogenetic assay, MLPA for microdeletions, subtelomeric deletions and duplications, as well as a metabolic screening showed normal results. Subsequently we employed Whole Exome Sequencing.

Results: WES established a heterozygous, pathogenic (missense) variant c.1798C>T (p.Arg600Trp) in *ZMYND11*. This variant has been previously reported as disease causing.

Conclusion: A rare case of autosomal dominant intellectual development disorder type 30 caused by a pathogenic variant in *ZMYND11* was confirmed utilizing WES. This finding examplifies the genetic and phenotypic variability of *ZMYND11*-related disorders while expanding our knowledge of these unfamiliar, but noteworthy conditions.

C.O.I. Disclosure:

T.N. Delchev: None. **D.M. Avdjieva-Tzavella:** None. **T. Veleva:** None. **D. Atova:** None. **M. Sredkova:** None.

EP10.020 A novel AHDC1 variant in Xia-Gibbs Syndrome: A rare case report

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Background: Xia-Gibbs Syndrome is a rare neurodevelopmental disorder, with an estimated prevalence of <1/1,000,000, caused by monoallelic de novo pathogenic variants in the AHDC1 gene and characterized by developmental delay, hypotonia, cognitive impairment, epilepsy, respiratory findings, and nonspecific dysmorphic features (MIM:615829; ORPHA:412069). Here, we report a case of Xia-Gibbs Syndrome associated with a novel AHDC1 variant.

Material and methods: Clinical exome sequencing (CES) analysis was carried out on a peripheral blood sample from the proband.

Results: A 5-month-old male patient with recurrent pneumonia and bronchiolitis was referred for suspected primary ciliary dyskinesia and cystic fibrosis. His medical history revealed motor delay, hypotonia, and torticollis. Dysmorphic features included round face, short neck, upslanting palpebral fissures, hypertelorism, prominent-broad forehead, depressed nasal bridge, anteverted nares, attached-upturned ear lobes, and thin vermilion of the lips. Growth parameters were normal. The parents were nonconsanguineous, with no family history of similar symptoms. CES revealed a heterozygous frameshift variant in the AHDC1 gene, namely ENST00000673934.1:c.3611del (p.Leu1204Argfs*2). Parental segregation analysis confirmed that the variant was de novo. This variant, which has not been previously reported in the literature, was classified as "*Pathogenic*" according to the criteria established by the American College of Medical Genetics and Genomics (ACMG) (PVS1, PM2, PM6).

Conclusion: This case report highlights the significance of performing comprehensive genetic testing when unexpected clinical findings are identified during clinical evaluation. Moreover, the identification of a novel AHDC1 variant aims to enhance our understanding of the genotype-phenotype correlation in Xia-Gibbs Syndrome.

C.O.I. Disclosure:

M.S. Yildirim: None. **H. Tarim:** None.

EP10.021 A posible new case of Snijders Blok-Fisher Syndrome

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Background: Snijders Blok-Fisher Syndrome is a rare dominant inherited disorder with a limited number of reported cases in the literature. The implicated gene is *POU3F3*, and most described variants follow a de novo mechanism. Clinical manifestations include mild to severe intellectual disability, developmental delay primarily affecting speech and, to a lesser extent, motor skills, congenital hypotonia, autistic features, and nonspecific facial dysmorphisms. Epilepsy has also been reported in some cases.

Material and methods: We present the case of a seven-year-old girl referred for genetic counseling due to intellectual disability and attention-deficit/hyperactivity disorder (ADHD). The patient also exhibits hearing loss, severe sleep behavior disturbance, and mild facial dysmorphisms, with no family history of similar conditions (parents and sibling are healthy).

Results: Molecular analysis revealed a heterozygous variant in the *POU3F3* gene, (NM_006236.3), c.670C>T; p.(Gln224*), classified as likely pathogenic according to ACMG criteria and following a de novo mechanism. This variant has not been previously reported in the literature.

Conclusions: This case is noteworthy due to the limited number of reported patients, contributing to the phenotypic characterization of Snijders Blok-Fisher Syndrome. The identified variant is novel and located near another variant characterized as pathogenic in 2019 (NM_006236.2:c.668C>A; p.Ser223*).

C.O.I. Disclosure:

S. García-Linares: None. **L. Papay-Ramírez:** None. **F. Castellano-Miñán:** None. **M. Del Aguila-García:** None. **J. Morales-Camino:** None. **M. Martínez-Atienza:** None. **S. Pedrinaci-Rodríguez:** None. **A. Martínez-Cañamero:** None. **G. Alvarez-Corral:** None. **T. De Haro-Muñoz:** None. **A. Poyatos-Andújar:** None.

EP10.022 Exploring USP7 Variants: A Case Study of Intellectual Disability in a 10-Year-Old Girl

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Background: Hao-Fountain syndrome is a neurodevelopmental disorder caused by pathogenic variants in the USP7 gene encoding USP7. In addition to the well-known neurodevelopmental disorder, recent phenotypic findings have been associated, including hyperphagia, increased body weight, higher rates of birth complications, congenital anomalies, and abnormal pain thresholds.

Material and methods: We present the case of a 10-year-old female patient for whom prior molecular studies were negative. A targeted exome analysis was requested based on her phenotypic features (H.P.O.: Premature birth HP:0001622, Hyperactivity HP:0000752, Delayed speech and language development HP:0000750, EEG abnormality HP:0002353, Increased body weight HP:0004324).

Results: Molecular analysis revealed a heterozygous variant in the USP7 gene, NM_001286457.1:c.985_989del, p.E329Lfs*3, classified as likely pathogenic according to ACMG criteria, following a de novo mechanism. This variant has not been previously reported in the literature.

Conclusions: This case may contribute to refining the phenotypic spectrum of patients with Hao-Fountain syndrome. Although the reported variant has not been described previously, it is located within the molecular context of other variants described in the literature as pathogenic and could explain the phenotype presented by the patient.

C.O.I. Disclosure:

L. Papay-Ramírez: None. **S. García-Linares:** None. **M. Del Aguila-García:** None. **F. Castellano-Miñán:** None. **A. Poyatos-Andújar:** None. **J. Morales-Camino:** None. **A. Martínez-Cañamero:** None. **M. Martínez-Atienza:** None. **S. Pedrinaci-Rodríguez:** None. **G. Alvarez-Corral:** None. **T. De Haro-Muñoz:** None.

EP10.023 A rare cause of intellectual disability: Novel mutation of *ALKBH8* gene in two patients with intellectual

developmental disorder, autosomal recessive type 71

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Background: The *ALKBH8* gene, a member of the AlkB family of dioxygenases, encodes the ABH8 protein, which is responsible for modifying tRNAs by methylating the anticodon wobble uridine residues. Variations in the ***ALKBH8*** gene are associated with the phenotype 'intellectual developmental disorder, autosomal recessive type 71' (MIM: 618504) in the OMIM database, characterized by intellectual disability, facial dysmorphic features, and epilepsy. To date, 14 patients from five differnet families carrying variants in the ***ALKBH8*** gene have been reported in the literature. In the present study, we report a Turkish family with two cousins exhibiting clinical features of intellectual disability, macrocephaly, epilepsy, and dysmorphic facial features, who harbor a novel homozygous frameshift variant in the *ALKBH8* gene.

Material and methods: Two affected cousins in this family showed signs of global developmental delay, intellectual disability, Exome sequencing (ES) analysis was performed on the index case (patient 1), who first presented to our clinic. His cousin (patient 2), who exhibited similar phenotypes, and their parents were subsequently evaluated using targeted Sanger sequencing to investigate the variation detected in the index case.

Results: ES analysis revealed a novel homozygous frameshift NM_138775.3: c.1333_1334del (p.Arg445Alafs*8) variant in the *ALKBH8* gene classified as likely pathogenic according to ACMG criteria.

Conclusion: In this case study, we aimed to report a Turkish family with an ultrarare disease associated with mutations in the ALKBH8 gene and to determine the novel variant, and findings inthis family that would ideally contribute to the phenotypic and geno-typic spectrum of this syndrome.

C.O.I. Disclosure:

C. Yilmaz Uzman: None. **S. Gürsoy:** None. **D. Ayyildiz Emecen:** None. **F. Hazan:** None.

EP10.024 Xq22.1 deletion: a new case of syndrome associated with developmental delay and epilepsy in a female

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Background: Deletions in the Xq22.1 region have been linked to intellectual disability, epilepsy and various developmental defects in heterozygous females, while they can be potentially fatal in males.

Material and methods: A CGH-SNP array analysis was performed. A bioinformatics approach was applied for breakpoint determination followed by quantitative real-time PCR assays.

Results: In this report we describe a new female patient presenting with significant developmental delay, intellectual disability, behavioural abnormalities and epilepsy. The patient is the second child of healthy, non-consanguineous parents with an unremarkable family history. The patient showed significant developmental difficulties, including absent speech, limited motor skills and behavioural problems such as poor eye contact and sleep disturbances. CGH-SNP array analysis revealed a de novo deletion of approximately 525Kb in the Xq22.1 arr[hg19] region (101596710_102121846), which overlaps with previously reported deletions and includes critical genes including TMSB15A, NFX4, ARMCX5, GPRASP1, GPRASP2 and BHLHB9. Real-time PCR analysis allowed us to define a 597Kb deletion and characterised the breakpoints.

Conclusion: Our findings reinforce the concept of a novel Xq22.1 microdeletion syndrome, highlighting the critical importance of genetic analysis to understand the mechanisms underlying these complex disorders. This case contributes to the growing body of evidence regarding the clinical implications of Xq22.1 deletion and provides new insights into the pathogenesis of associated neurological and developmental conditions.

C.O.I. Disclosure:

C. Cattani: None. **A. Tursi:** None. **S. Caraffi:** None. **S. Vandelli:** None. **A. Peruzzi:** None. **M. Marinelli:** None. **A. Vezzani:** None. **R. Zuntini:** None. **L. Garavelli:** None.

EP10.025 DIPK2B (DIA1R) a new gene associated to X-linked Intellectual developmental disorder?

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X-linked Intellectual developmental disorder (XLIDD) are associated with X-linked recessive or dominant inheritance mainly resulting in intellectual disability and developmental delay. Males are more severely affected than females who have only one affected gene and tend to show milder symptoms, the exception being skewed X-inactivation. XLIDD accounts for about the 16% of all cases of intellectual disability in males. About 200 genes have been involved in XLIDD of which about 100 have been identified. We report on two brothers with intellectual disability and severe language delay: S. 17 years old and D. 11 years old. Genetic tests performed in S. were FMR1, RASopathies and CGH-array detecting the PAR1 region microduplication (Xp22.33 or Yp11.32), not containing disease causing OMIM genes and a microduplication in the 12p13.33 region, containing part of the gene CACNA1C showing paternal segregation, which was considered of uncertain significance. We finally performed the exome sequencing which identified in both brothers the hemizygous splicing variant c.499-1G > A in the gene DIPK2B (NM_176819.4) showing maternal segregation. DIPK2B (DIA1R) is not a OMIM Disease Causing gene yet, but point mutations described in DIA1R have been associated with X-linked intellectual disability (XLID) and DIA1R deletions have been associated with syndromes with autism spectrum disorder-like traits and/or XLID (PMID: 21264219). Pathogenicity prediction software classified the variant as likely pathogenic since it is located in canonical splice site and the variant is private. We propose the DIA1R gene as a novel gene associated with XLIDD.

C.O.I. Disclosure:

E. Pisaneschi: None. **L. Sinibaldi:** None. **C. Cesario:** None. **D. Verrigni:** None. **S. Petrocchi:** None. **A. Novelli:** None.

EP10.026 MED13L related disorder: a novel de novo missense variant in pediatric case with mild phenotype

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Background: MED13L-related disorder is a condition mainly characterized by intellectual disability, motor developmental delay, speech impairment, hypotonia and facial dysmorphism with or without cardiac defects (MRFACD, OMIM #616789). For most of the cases described, a pathogenic mechanism linked to haploinsufficiency has been hypothesized, although recent studies have reported cases of missense variants for which a pathogenic mechanism of dominant negative type has been hypothesized. Missense mutations have been associated with a higher incidence of epilepsy and a more severe clinical phenotype.

Material and methods: A 13-year-old boy was referred to genetic counseling because of learning disorder associated with emotional disorder, mild cognitive delay (total IQ 65) and mild facial dysmorphisms (hypotelorism, low set ears, bulbous nasal tip). EEG and echocardiogram were negative.Genetic tests were performed on blood sample.

Results: Karyotype (46,XY) and chromosomal microarray analysis were normal. Next-generation sequencing (NGS)-based trio testing identified a novel de novo heterozygous missense variant, c.1989C>G (p.Asn663Lys), in the *MED13L* gene, classified as likely pathogenic according to the American College of Medical Genetics and Genomics classification criteria.

Conclusion: To date, there are still few cases reported in literature of patients with MED13L-related conditions. Case reports are important to better define the *MED13L*-related phenotypic spectrum, to understand genotype-phenotype correlations and the role of missense mutations. Molecular analysis plays an important role in the diagnosis of cases with mild and nonspecific phenotypes.

C.O.I. Disclosure:

G. Mastrogiorgio: None. **D. Verrigni:** None. **M.G. Di Gregorio:** None. **D. Ferretti:** None. **D. Darelli:** None. **M. Canestrelli:** None. **F. Di Lorenzo:** None. **C. Cassadonte:** None. **A. Novelli:** None. **A. Micalizzi:** None.

EP10.027 Molecular insights into Borjeson-Forssman-Lehmann syndrome: A case of a heterozygous female with a novel *PHF6* variant

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Background: Borjeson-Forssman-Lehmann syndrome (BFLS) is a rare X-linked intellectual disability disorder caused by pathogenic variants in the *PHF6* gene. The clinical spectrum includes intellectual disability/developmental delay (ID/DD), truncal obesity, hypogonadism, distinctive facial dysmorphism and features resembling Coffin-Siris syndrome. Although BFLS predominantly affects males, heterozygous females may also display clinical manifestations due to skewed X-chromosome inactivation. Here, we present a heterozygous female patient diagnosed with BFLS, illustrating the clinical and molecular findings associated with this rare disorder.

Material and methods: Whole-exome sequencing (WES) was initially performed, followed by Sanger sequencing for family segregation analysis. Chromosome analysis was performed to evaluate chromosomal abnormalities.

Results: In a 6.5-year-old female patient, clinical findings included ID/DD, horseshoe kidney, and features resembling Coffin-Siris syndrome, including hypertrichosis, bilateral hypoplastic fifth fingers, and prominent sandal gap. Chromosome analysis showed a normal 46,XX karyotype. WES analysis revealed a novel de-novo heterozygous variant, c.740C>G,p.(Ser247Cys), in exon 8 of the *PHF6* gene (NM_001015877.2) classified as a variant of uncertain significance(PM2,PM6,PP3).

Conclusion: In this case, we present a female patient with a heterozygous variant in an X-linked gene, highlighting the complexity of diagnosing X-linked disorders. While X-linked recessive diseases typically affect males, females can also be impacted due to mechanisms such as skewed X-chromosome inactivation. This case emphasizes the importance of considering these mechanisms in variant analysis, particularly in female patients who may exhibit atypical phenotypes. Further studies are needed to clarify the pathogenicity of this variant and its contribution to the patient's phenotype, thereby advancing our understanding of the genetic and clinical spectrum of BFLS.

C.O.I. Disclosure:

M.A. Yücesoy: None. **V. Karaman:** None. **A.D. Aslanger:** None. **Z.O. Uyguner:** None.

EP10.028 An adult case with *GNAO1* complete deletion

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Background: GNAO1 related disorder (GNAO1-RD) has been classified into two categories: 'Developmental and Epileptic Encephalopathy 17' (with variable cognitive impairment, epilepsy) and 'Neurodevelopmental Disorder with Involuntary Movements' (most commonly dystonia). In recent articles, GNAO1 haploinsufficiency presents a milder phenotype compared with missense variants and fewer than five cases with GNAO1 deletion have been described.

Material: We describe a 29-year old boy with normal motor development, language delay and moderate intellectual disability (IQ 50), short stature, arched eyebrows, downward-slanting palpebral fissures, long columella and keloids. We performed the following sequential genetic study based on the clinical suspicion of Rubinstein-Taybi syndrome: normal sequencing of EP300 and CREBBP genes in peripheral blood, urine and saliva normal whole-exome sequencing and negative episignature study. The CGH-array study revealed a pathogenic 2.487Mb deletion in 16q12.2 region, encompassing IRX5, GNAO1 and NUDT21 genes.

Conclusion: The majority of GNAO1-RD have missense variants, although recently complete gene deletions have been described supporting haploinsufficiency mechanism. GNAO1-

haploinsufficiency is associated with a milder phenotype compared to missense variants. Patients with late-onset dystonia and mild intellectual impairment have been reported. The phenotype of our patient is consistent with that of other individuals reported with complete deletions of GNAO1. However, at 29-years old, he has not yet developed movement disorder, except minimal resting hands tremor. Given that fewer than five cases of deletion cases have been described and our patient has not yet presented with dystonia, we recommend close monitoring of our patient, as there have been reports of progressive dystonia developing in adulthood.

C.O.I. Disclosure:

A. Cueto-González: None. **A. Lasa-Aranzasti:** None. **M. Viñas Jornet:** None. **M. Valenzuela-Palafoll:** None. **B. Masotto:** None. **L. Trujillano Lidon:** None. **P. Jolin García:** None. **N. Castells Sarret:** None. **B. Perez Dueñas:** None. **E. García-Arumí:** None. **E. Tizzano:** None.

EP10.029 Potential splicing-altering synonymous variants in the STAG2 gene: A case study of two female patients

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Background: The human STAG2 protein is a component of the evolutionarily highly conserved cohesin complex, involved in various cellular processes like gene expression, DNA replication, heterochromatin formation, sister chromatid cohesion, and DNA repair. Although the role of STAG2 as a DNA-binding subunit is well established, its specific function is still unknown. Pathogenic variants in STAG2 have been described as causes of X-linked developmental disorders. To date, 26 pathogenic variants of STAG2 have been reported. Notably, truncating variants have been reported only in females patients while missense variants have been reported in males. Here, we describe for the first time two female patients with de novo synonymous STAG2 variants localized near canonical splice sites.

Methods: Clinical exome sequencing of the proband and his parents was performed on genomic DNA using the NovaSeq6000 platform (Illumina).

Results: Case 1 carries c.2358G>A; p.Gln786 = variant, which was found in one case in gnomAD 4.1 (AF = 8.815e-7); while case 2 carries the c.2673G>A; p.Lys891 = variant, which is novel. The general pathogenic effect of both variants was assessed using six synonymous-enabled in-silico predictors. Large agreement among tools confirms the loss of both the acceptor (−92 bp) and donor sites of the exon near the c.2358G>A variant, and the loss of the nearby donor site and gain of a new donor site (−68 bp) for c.2673G>A.

Conclusions: These two cases demonstrate how investigating the impact of synonymous variants in genetic diseases could be useful to clarify how these variants affect gene expression and therefore improve the diagnostic rate.

C.O.I. Disclosure:

M. Mucciolo: None. **R. Ruta:** None. **F. Mercadante:** None. **S. Calì:** None. **M. Gnoli:** None. **T. Mazza:** None. **N. Liorni:** None. **L. Sangiorgi:** None. **M. Piccione:** None. **A. Novelli:** None.

EP10.030 Novel variant in *MSL3* leading to the first report of Basilicata-Akhtar syndrome in Bulgaria

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Background: Basilicata-Akhtar syndrome (MRXSBA, OMIM # 301032) is an extremely rare X-linked dominant disorder characterized by global developmental delay, neurocognitive deficits, and systemic complications, with fewer than 50 cases reported worldwide.

Methods: We present a 3-year-old female with intellectual disability and developmental delay. The patient exhibited delayed ambulation (beyond 2 years), absent verbal communication, subtle facial dysmorphisms, increased lower-extremity tone, and bilateral Babinski sign. EEG revealed normal background activity with intermittent high-voltage generalized sharp waves, while MRI demonstrated ventriculomegaly. Genetic testing via conventional karyotyping, *FMR1* repeat expansion analysis and multiplex ligation-dependent probe amplification (MLPA) for subtelomeric chromosomal rearrangements returned negative results.

Results: Whole-exome sequencing (WES) revealed a novel heterozygous missense variant in the *MSL3* gene (c.1440A>T, p.Leu480Phe), with segregation analysis via Sanger sequencing confirming that the variant occurred de novo. The nucleotide change affects a conserved leucine within the highly conserved MRG domain of MSL3, a region essential for the stability and function of the male-specific lethal (MSL) complex.

Conclusion: This case confirms the genetic etiology of Basilicata-Akhtar syndrome in a Bulgarian patient, expanding the disorder's phenotypic and geographic spectrum. The patient's family was subsequently referred to the MSL3 Syndrome Foundation (https://msl3.org). Due to the lack of targeted therapies, the patient was subsequently referred for consistent neurodevelopmental and neurological evaluations.

C.O.I. Disclosure:

I. Tourtourikov: None. **I. Bradinova:** None. **S. Ciurinskiene:** None. **T. Kadiyska:** None.

EP10.031 Expanding the phenotype of a rare 1q21.3 deletion: *UBAP2L* haploinsufficiency and neurodevelopmental disorder

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Background: Neurodevelopmental disorders (NDD) are early-onset conditions causing impairments in functioning due to developmental deficits in cognition, behavior, or motor skills, with a heterogenous etiology. Recently, the haploinsufficiency of *UBAP2L* is described as a cause of a rare neurodevelopmental disorder (UBAP2L-NDD), clinically characterized by cognitive and

behavioral issues, with unspecific dysmorphic features. We present a child with a novel deletion involving the *UBAP2L* gene.

Material and methods: Genetic studies included TP-PCR FMR1 and 180K aCGH (OGT).

Results: A 5-year-old female evaluated in the Genetic Clinic due to Global developmental delay and behavioral issues. She is the first child of a non-consanguineous healthy couple, with no family history relevance. Unremarkable pregnancy and delivery history. At age 2, parents noticed speech delay and unspecific behavior issues; motor-wise with exercise intolerance, fatigue, and with difficulties climbing stairs and running. Physical examination with height and weight within the 50th percentile with mild dysmorphic features. Genetic results showed a deletion: arr[hg19] 1q21.3(154199862-154358076)x1. Parents array was normal.

Conclusion: This is the first case of *UBAP2L*-NDD due to a novel de novo deletion in 1q21.3. There are only 14 patients reported in the literature (Jia et al., 2022; Yang et al., 2024) characterized by ID that ranges from mild to severe, behavior abnormalities, and speech developmental delay. Motor delay and seizures, and mild dysmorphic features are frequently described. Jia et al., (2022) developed a murine model showing that the Ubap2l deficiency leads to social and cognitive impairments and hypothetized that there is a disruption of neurogenesis supporting this association

C.O.I. Disclosure:

N. Baena Díez: None. **E. Zapata-Aldana:** None. **M. Garcia-Puig:** None. **A. Brunet-Vega:** None. **L. Capel-Tardà:** None. **V. Martinez-Glez:** None.

EP10.032 Genomic Variant Analysis by Array-CGH in Patients with Autism Spectrum Disorders and Neurodevelopmental Disorders: a single center experience

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Background: Autism spectrum disorders (ASD) are heterogeneous neurodevelopmental conditions with a strong genetic component. Array-CGH (aCGH) is a key technique for detecting copy number variations (CNVs), which may contribute to the pathogenesis of ASD and related disorders.

Material and methods: We retrospectively describe the array-CGH findings in a cohort of patients with a diagnosis of ASD or related conditions. We analyzed a total of 242 samples addressed from a single institution to our laboratory from March to December 2024. All samples were analyzed by the same platform (GenetiSure Cyto 8 × 60K CGH (Agilent)).

Results: The mean age of the patients was 5 years (range: 1–16 years). Thirty-six patients (14.2%) exhibited at least one CNV, with a higher prevalence in males (66.7%) compared to females (33.3%). 87.9% have been previously identified in association with neurodevelopmental disorders, including ASD and related phenotypes. The remaining 12.1% have not been described before (deletion 16q23.1, duplication 7p15.2, duplication 11q24.3q25, duplication 20q13.33).

Conclusion: Our study confirms the relevance of CNVs in ASD, reinforcing the diagnostic value of aCGH. The male predominance supports the idea of sex-related genetic susceptibility. Identification of known pathogenic CNVs in regions like 16p11.2, 15q11.2-q13.3, and 22q11.21 strenghten their role in neurodevelopmental dysfunction. However, the identification of new CNVs further enhances the spectrum of rearrangements associated with ASD. Identification of CNVs with uncertain clinical significance (VUS), makes it necessary to continue with further studies to understand their actual pathogenetic role and establish clearer genotype-phenotype correlations.

C.O.I. Disclosure:

A. Pranteda: None. **R. Leone:** None. **S. Napoletano:** None. **M. Sessa:** None. **E. Cotroneo:** None. **L. Gigante:** None.

EP10.033 Novel genetic syndromes in a Malaysian cohort of children with intellectual disabilities and global developmental delay: A case series

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Background: Dysmorphism with neurodevelopment delay are common referrals for geneticists. With the advent of diagnostic technologies, more rare diseases are being diagnosed. We aimed to illustrate two patients with rare genetic syndromes diagnosed by whole exome sequencing (WES).

Material and methods: Patient A was first referred at 8 years old for dysmorphism, learning disability and seizures. She is the product of non-consanguineous marriage with uncomplicated antenatal, perinatal and postnatal period. Epilepsy with abnormal EEG was diagnosed at 7 years old. Clinical examination is significant for: blue sclera, downslanting palpebral fissures, mild ptosis, broad bulbous nose, thick lips, long slender fingers and toes with pes planus.

Patient B is a 4 years old girl with dysmorphism and global developmental delay. She was born premature at 33 weeks and diagnosed with atrial septal defect with failure to thrive. Speech domain is markedly delayed. Clinical features are significant for: brownish pigmented hair, hypertelorism, downslanting palpebral fissures, flat and broad nasal bridge, everted upper lip, hyperpigmented patch along Blaschko lines at bilateral lower limbs and long and slender fingers with clinodactyly.

Results: WES of patient A was reported as heterozygous pathogenic variant of *MSL2* NM_018133.4: c796_797del (p.Leu266-ValfsTer5) synonymous with the recently published Karayol-Borrato-Haghshenas syndrome, a rare neurodevelopment disorder characterized by intellectual disabilities and neuropsychiatric conditions. WES for patient B reported a heterozygous pathogenic 19.9Mb duplication identified at Xp22.33p22.12. No similar duplication has been reported before.

Conclusion: WES is highly recommended as a first-tier investigation in children with non-specific dysmorphic features associated with neurodevelopment delay.

C.O.I. Disclosure:

H. Hashim: None. **M. Haniffa:** None. **L. Ngu:** None.

EP10.034 Marfan syndrome associated with intellectual disability and behavioral anomalies: further evidence for the effect of biallelic variants in *FBN1* on phenotypic severity

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Background: Marfan syndrome (MFS) is a rare connective tissue disorder, characterized by the involvement of the cardiovascular, ocular and musculoskeletal systems. Most cases of MFS are caused by mutations in the FBN1 gene, however intellectual disability (ID) is rarely involved.

Material and methods: A non-consanguineous Pakistani family with four affected individuals was recruited. Physical examinations, echocardiograms, and Doppler ultrasounds were performed as part of the clinical assessments. The whole exome sequencing was performed for the index case and Sanger sequencing was carried out in the whole family.

Results: The primary presentation in the affected individuals was ID. Detailed examination revealed that all the affected individuals and their affected mother were tall, had long limbs, craniofacial abnormalities, and displayed low IQ, aggressive and hyperactive behavior. In one affected individual and her affected mother, heart defects, including atrial septal defects and pulmonary hypertension were observed. Genetic analysis revealed two ultra rare missense variants in FBN1 gene (c.1552G>A, p.(Gly518Arg), and c.3046A>G, p.(Thr1016Ala), both of which were predicted to be deleterious. The affected children inherited both variants in a compound heterozygous, one variant from each parent.

Conclusion: This study found rare compound heterozygous missense variants in the FBN1 gene, signifying a possible link between neurodevelopmental outcomes and these mutations in MFS. The role of FBN1 in ID is needed to clarify through further research

C.O.I. Disclosure:

A.U. khan: None. **N.K. Kakar:** None. **S. Malik:** None.

EP10.035 A novel missense *STX1B* variant in a patient with GEFS+ and autism spectrum disorder: expanding a complex neurological spectrum

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Background: Pathogenic variants in syntaxin-1B encoding gene *STX1B* have been linked to a spectrum of epileptic and neurodevelopmental disorders, including Generalized Epilepsy with Febrile Seizures Plus (GEFS+), type 9. Reported cases of individuals presenting with epilepsy, Asperger syndrome, and developmental epileptic encephalopathies highlight the extreme phenotypic variability associated with *STX1B* variants.

Material and methods: We report a female patient presenting with early-onset febrile seizures, hypotonia, developmental delay, and motor dyspraxia, with no familial consanguinity or similar phenotypes reported. Neurodevelopmental evaluation confirmed mild intellectual disability (ID) and autism spectrum disorder (ASD). Previous cytogenetic and microarray analyses excluded chromosomal abnormalities.

Results: A novel de novo heterozygous missense variant, c.623T>A, p.(Ile208Asn) in *STX1B* was identified via Next-Generation Sequencing (NGS) in the patient. The novel variant's de novo origin and multiple lines of computational evidence highlight its potential pathogenicity, warranting further functional studies to elucidate molecular mechanisms.

Conclusion: The molecular finding was compatible with GEFS+ features, including simple febrile seizures, ID, and comorbid ASD. This is the first case to associate *STX1B* with this disorder. This case study underscores the importance of comprehensive genetic evaluation in patients with overlapping neuronal hyperexcitability and neurodevelopmental disorders and advocates for *STX1B* inclusion in diagnostic panels.

C.O.I. Disclosure:

L. Graziani: None. **M.L. Carriero:** None. **C. Minotti:** None. **F. Stregapede:** None. **M. Bengala:** None. **A. Terracciano:** None. **A. Novelli:** None. **G. Novelli:** None.

EP10.036 Investigation of genetic etiology of intellectual disability in 51 Brazilian individuals with suspicion of recessive disorders

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Background: Intellectual disability (ID) and neurodevelopmental delay (NDD) are often associated with rare disorders, with higher prevalence in endogamic populations. Exome sequencing (ES) has proven effective in identifying novel disease genes in these populations. This study aims to uncover the genetic causes of ID with suspicion of a recessive disorder in Brazilian families.

Material and methods: Samples were collected from 51 Brazilian individuals with NDD/ID born from consanguineous couples and/or presenting recurrence of the phenotype in the siblings, and/or presenting multiple regions of homozygosity previously detected in the autosomal genome. ES was performed using a customized capturing kit and the NovaSeq™ 6000 Sequencing System (Illumina©). Variants were classified using Franklin (Genoox©), according to the American College of Medical Genetics recommendations.

Results: We found 13 pathogenic or probably pathogenic variants (eight homozygous and five heterozygous) considered causative of the phenotype in 12 individuals, with a diagnostic yield of 23.5%. In three individuals, the variants explained only part of the phenotype. Novel or ultrarare homozygous variants of uncertain significance (VUS), associated with very rare phenotypes, were identified in other 11 individuals, including the genes *DCHS1*, *TASP1, ZNF407, ANK3, DEAF1, ALG6, TIAM1, AASS, BCAS3*, and *POLR3A*. Novel and homozygous variants were found in two candidate genes: *SNX8* and *CMBL*.

Conclusion: This study contributes to the understanding of genetic mechanisms underlying extremely rare disorders associated with NDD/ID, and to the identification of new genes associated with ID.

C.O.I. Disclosure:

G.R. Correia-Costa: None. **C. Steiner:** None. **T. Félix:** None. **E.M. Ribeiro:** None. **E.L. Mendes:** None. **V. Gil-da-Silva-Lopes:** None. **T.P. Vieira:** None.

EP10.038 Could PCDHGA10 be a potential candidate gene for intellectual disability? An additional case report

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Background: Intellectual disability, neurodevelopmental delay, epilepsy, and dysmorphism are a group of a clinical findings with a wide etiologic spectrum. While many related genes are known, ongoing advances in technology continue to reveal new candidate genes.

Material and methods: A 9-year-old male patient was eveluated for neurodevelopmental delay, intellectual disability, epilepsy, and obesity. He was born of consanguineous parents. His family history was unremarkable. On physical examination, he had symptoms of dysmorphism: midface hypoplasia, long philtrum, small mouth and nose, thin lips, short neck, inverted nipple, fusiform fingers, clinodactyly, and obesity.

Results: Genetic studies including chromosomal analysis, microarray analysis, epilepsy panel, and fragile-X testing revealed normal. Subsequently, clinical exome sequencing (CES)(QIAseq-Actionable-Exome-Kit®) revealed a homozygous missense variant c.820G>A p.D274N in the PCDHGA10 (NM_018913.3) gene.

Conclusion: The PCDHGA10 gene is a member of the protocadherin gamma gene cluster which includes 22 genes divided into 3 subfamilies. These neural cadherin-like cell adhesion proteins most likely play a critical role in the establishment and function of specific cell-cell connections in the brain. Only the PCDHGC4 gene from this gene cluster had been associated with a clinical phenotype[1]. The PCDHGA10 gene has been reported as a candidate gene for intellectual disability based on the detection of homozygous variants in two patients, one of whom reported a missense variant (p.(E275K)) in the same region as our patient and had similar clinical findings[2]. In conclusion, we hypothesise that the patient in this report will help to define the phenotype of the candidate gene.

C.O.I. Disclosure:

M. Zorluer: None. **B. Cavdarli:** None.

EP10.039 Unraveling the impact of *ANKRD11* exonic CNVs in KBG syndrome: functional and epigenetic insights

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Background: KBG syndrome is caused by pathogenic variants in the *ANKRD11* gene and is characterized by dysmorphic features, macrodontia, developmental delay/intellectual disability (ID), skeletal anomalies, short stature, and behavioural disorders. Most of the pathogenic variants causing KBG syndrome are frameshift and nonsense leading to haploinsuffciency. However, a small proportion of missense variants as well as small exonic deletions or duplications have also been reported. For these variants, the characterisation of the functional impact on ANKRD11 function is necessary to determine their clinical significance. We report two patients with clinical features compatible with KBG syndrome carriers of an in frame duplication spanning from exon 3 to exon 4 and of a deletion of exon 2 of the ANKRD11 gene.

Material and methods: MLPA was performed to determine the inheritance pattern. To assess the functional impact of both CNVs, Episign DNA methylation analysis and quantitative real time PCR to measure *ANKRD11* transcript levels were performed. In addition, long PCR was used to characterise the genomic breakpoints.

Results/Conclusions: Exon 2 deletion was maternally inherited (clinical assessment under way). Methylation profiles for both patients were clustering with KBG cases, even though the observed low MVP scores suggest that both variants may have an hypomorphic effect. Quantitative analysis and allele specific expression of the *ANKRD11* gene are being performed to assess whether *ANKRD11* reduced expression levels could lead to partial haploinsufficiency. For the maternally inherited exon 2 deletion comparative analysis of methylation and expression data may clarify the clinical findings.

C.O.I. Disclosure:

N. Spataro: None. **J. Trujillo-Quintero:** None. **C. Manso-Bazus:** None. **A. Brunet-Vega:** None. **N. Baena:** None. **A. Ruiz:** None.

EP10.040 Neurodevelopmental manifestation in SCA19/22: beyond ataxia, enlightening the dark side of the moon

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Background: *KCND3* gene encodes for a potassium channel (Kv4.3) responsible for action potential in neurons and heart conduction tissue. Variants in *KCND3* gene can cause both neurological and cardiac diseases, depending on their effect. Missense loss-of-function variants in *KCND3* gene are associated with SCA19/22. Spino-Cerebellar Ataxias (SCAs) are a group of genetically heterogeneous autosomal dominant diseases whose, intuitively, clinical hallmarks include ataxia, together with other peculiar neurological manifestations. Generally early onset forms of SCA19/22 begin with cerebellar ataxia and oculomotor disorders, possibly later associated with variable movement disorders, neurodevelopmental disorders and seizures.

Case report: Herein we report the case of a 19 years old boy who presented global development delay in childhood and nowadays severe intellectual disability, hyperactivity and behavior anomalies. After a long genetic diagnostic iter, an exome sequencing in trio (WES trio) was performed and it identified the new missense variant c.1094T>A (M365K) in the mutational hotspot of *KCND3* gene. The variant has been classified as likely pathogenic, this allowed the diagnosis of SCA19/22 in our proband, even if he never manifested ataxia in his whole lifetime.

Conclusion: This novel observation goes beyond the literature evidence, describing a case of SCA19/22 that manifests itself even just with neurodevelopmental disorders, without necessarily cerebellar symptoms. Spreading the knowledge about SCA19/22 as a possible cause of NDD in young patients will enable to gain a better differential diagnosis in clinical setting.

C.O.I. Disclosure:

T. Palazzotti: None. **A. De Franco:** None. **G. Marchetti:** None. **A. Di Fonzo:** None. **L. Pezzoli:** None. **M. Iascone:** None. **C. Agostoni:** None. **D. Milani:** None.

EP10.041 Intragenic DLG2 (11q14.1) microdeletion involving the HPin8 alternative promoter in a patient with epilepsy and global developmental delay

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Background: The patient has global developmental delay since birth. The first episode of epileptic seizures was registered at the age of 8. An X-ray revealed spina bifida at the L5 level. MRI

showed signs of hippocampal malrotation and thickening of the parenchymal cystic mucosa of the sphenoid cavity. EEG revealed isolated and clustered peak-wave complexes and sharp waves from the left frontotemporal and frontocentral projection, as well as independent sharp waves from the right projection. Clinical features include retrognathia, mild facial and lumbar hirsutism, speech difficulties, global neurodevelopmental delay, inability to stand on one leg properly.

Material and methods: An aCGH using the SurePrint G3 Human CGH Microarray 8×60K (Agilent Technologies, USA) was performed on the patient and quantitative real-time PCR on both the patient and parents.

Results: The genetic testing revealed a likely pathogenic 87.7 kb partial monosomy of the 11q14.1 of paternal origin. This region contains the DLG2 gene coding a membrane-associated guanylate kinase.

Conclusion: The DLG2 is defined as a large gene, extending over 2.172 Mb. Its numerous isoforms differentially expressed in the brain, especially in the cortex, cerebellum, hippocampus, and striatum. Less than 50 DLG2 deletions have been reported worldwide, most of which are smaller than 100 kb. For the first time DLG2 intragenic microdeletion involving the HPin8 promoter in the intron 8 was identified in our patient. HPin8 is categorized as active promoter in brain tissues and promote a new bona fide DLG2 isoform.

C.O.I. Disclosure:

A. Gasparyan: None. **T.F. Sarkisian:** None. **N. Kostandyan:** None. **A. Yervandyan:** None. **L. Barseghyan:** None. **A.A. Kashevarova:** None. **D.A. Fedotov:** None. **I.N. Lebedev:** None.

EP10.042 A unique *ZNF148* in frame deletion and *MED13L* splice site variant cooccurrence in a child with autism spectrum disorder and intellectual disability

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ZNF148-related syndrome (OMIM #617260) is a recently described neurodevelopmental syndrome, with variable congenital malformations. *MED13L*-related syndrome (OMIM #616789) is characterized by ID, impaired language development, dysmorphic features and variable association of other anomalies. We report on a 9-year-old boy with ASD, mild ID and severe speech impairment. He is the second child of healthy parents with unremarkable family history. He walked without support at 16 months and spoke his first words at 24 months. EEG, brain MRI, ECG, echocardiogram, audiometric and ophthalmologic evaluation were normal. On clinical evaluation, a peculiar facial appearance was noted (hypertelorism, downslanted palpebral fissures, wide mouth with downturned corners and open mouth appearance, widely spaced large teeth with enamel alteration, retrognathia), height, weight and OFC were in the normal range. G-banded karyotype, fragile-X testing, and CMA were negative. Trio-based CES detected an heterozygous c.1420_1422del, p.(Lys474del) de novo variant in *ZNF148* and an heterozygous c.2997-1G > A,p.(?) maternal variant in *MED13L*. Both variants have been currently classified as likely pathogenic (ACMG guidelines). Only 27 patients with *ZNF148*-related syndrome were reported thus far, all displaying nonsense/frameshift variants, mostly located in the last exon, none of them presented an in frame deletion. *MED13L*-related syndrome was reported in more than 100 patients to date, with complete penetrance and variable expressivity. Splice site variants are a known cause of *MED13L*-related syndrome, however, the mother of our proband is healthy and never displayed even slight signs of this syndrome. Functional studies are ongoing and will help to better clarify the role of these variants.

C.O.I. Disclosure:

C. Minotti: None. **L. Graziani:** None. **V. Ferradini:** None. **N. Piumelli:** None. **F. Sangiuolo:** None. **M. Bengala:** None. **G. Novelli:** None.

EP10.043 A Novel UBE2A Splice Variant in a Rare Case of Syndromic Intellectual Disability

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Background: The Nascimento-type X-linked syndromic intellectual developmental disorder (MRXSN) is a rare neurodevelopmental disorder caused by UBE2A mutations or Xq24 deletions. UBE2A encodes an E2 ubiquitin-conjugating enzyme essential for protein degradation and cellular homeostasis. MRXSN is characterized by intellectual disability, seizures, language impairment, dysmorphic features, dermatological findings, and urogenital anomalies.

Case Presentation: A 6-year-old male with epilepsy and global developmental delay was referred for genetic evaluation. His seizures, marked by staring episodes and atonia, began at 11 months and occurred annually. Born to healthy, unrelated parents after an uneventful pregnancy, he exhibited milestone delays, sitting at 18 months and walking with support at 4 years. He never developed functional speech but follows simple commands.Examination revealed obesity, dysmorphic facial features (synophrys, deep-set eyes, wide mouth, short neck, broad nasal bridge), widely spaced nipples, micropenis, toenail onychodystrophy, and hirsutism. EEG showed bilateral frontal paroxysmal discharges.Chromosomal microarray was normal, but whole-exome sequencing identified a hemizygous UBE2A c.241+5G > A variant, classified as likely pathogenic. Segregation analysis confirmed maternal inheritance. RNA isolation and cDNA sequencing from the patient's sample demonstrated that the variant disrupts splicing.

Conclusion: MRXSN, first described in 2006, has been linked to various UBE2A mutations, including splice-site variants. Previously reported splicing variants showed either mild cases without dysmorphic features or severe forms with behavioral problems. Unlike these, our patient exhibits dysmorphic features but no behavioral issues, expanding the known phenotypic spectrum. This case underscores the importance of considering UBE2A-related MRXSN in males with unexplained intellectual disability and highlights the impact of this novel variant.

C.O.I. Disclosure:

E. Kayilioglu: None. **F. Demir:** None. **A. Ceylan:** None. **G. Gulec Ceylan:** None.

EP10.044 Novel *SAMHD1* pathogenic deletion variant causing Aicardi-Goutières syndrome

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Background: Aicardi-Goutières syndrome (AGS) is a rare mostly autosomal recessive disorder that primarily affects the brain, skin, and immune system. AGS is typically caused by mutations in

genes involved in immune response and DNA repair, including TREX1, RNASEH2, SAMHD1. AGS is usually diagnosed in infancy or early childhood with significant developmental delay, seizures, and movement disorders. We set out to identify the molecular basis of AGS in consanguineous Bedouin kindred.

Material and methods: Affected individuals underwent clinical assessment and genotyping, including SNP arrays and linkage analysis using Homozygosity Mapper, followed by whole-exome sequencing (WES) and whole-genome sequencing (WGS) which confirmed segregation.

Results: Through combined homozygosity mapping using SNP arrays and WES, followed by WGS and segregation analysis within the affected kindred, we demonstrate that fully-penetrant AGS can be caused by homozygosity of a 2 KB deletion in SAMHD1(20:36917407-20:36919395; hg38) encompassing exon 7 (of 16 exons) and an adjacent intron.

Conclusion: We show that AGS can be caused by a homozygous deletion within SAMHD1, affecting exon 7 and its adjacent intron, demonstrating the efficacy of WGS in delineating disease-causing deletions and expanding the genotypic spectrum of AGS-causing mutations.

C.O.I. Disclosure:

E. Brav: None. **A. Safran:** None. **T. Poleg:** None. **O. Birk:** None.

EP10.045 Diagnostic challenges of developmental and epileptic encephalopathy originating from the phenotypic spectrum of patients

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Background: Developmental and epileptic encephalopathies (DEE) are a heterogeneous group of devastating neurodevelopmental disorders diagnosed in early childhood and associated with epileptic seizures, severe delay or regression of psychomotor development, and cognitive and behavioral deficits. Genetic factors have a crucial role in the pathogenesis of DEE. Channelopathies make up a significant proportion of DEE cases and pathogenic variants are found in 30–50% of patients with DEE.

Material and methods: Here we present two cases belonging to channelopathies. A 4-year-old male patient with axial hypotonia and severe intellectual disability, and an 11-year-old female patient with mild intellectual disability, autism spectrum disorder and ADHD. Whole-exome sequencing (WES) was performed using Illumina DNA Prep With Enrichment Kit for library preparation and sequenced on NovaSeq 6000 platform (Illumina) with 100bp paired-end chemistry.

Results: WES revealed known disease-causing variants in heterozygous form in the *CACNA1A* gene [c.4291C>T (p.Arg1431*)] in the male patient and in the *KCNB1* gene [c.934C>T (p.Arg312Cys)] in the female patient. Although both genes are responsible for the development of DEE, epileptic encephalopathy and seizures were missing from the clinical picture of both our cases.

Conclusion: Thanks to NGS technology, the number of causative genetic variants identified in rare diseases, including rare epilepsy, is steadily increasing. Since DEE is characterized by a broad clinical spectrum, it makes the diagnosis of DEE challenging. Large-scale phenotype-genotype studies and functional studies help to expand our knowledge in the field of DEE and support the early and quick clinical and molecular diagnosis of patients with DEE.

C.O.I. Disclosure:

J. Bene: None. **A. Till:** None. **B. Patócs:** None. **A. Zsigmond:** None. **K. Hadzsiev:** None.

EP10.046 Heterogeneous Presentation of KBG Syndrome: *ANKRD11* Case Series

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Background: KBG syndrome is a highly variable neurodevelopmental disorder caused by both loss-of-function nucleotide variants and large deletions affecting *ANKRD11*. Due to high clinical heterogeneity, variable expressivity and incomplete penetrance, KBG syndrome may be considered underdiagnosed.

Material and methods: A case series comprising seven patients (3 males and 4 females), aged 9 months to 32 years, is presented focusing on diverse clinical presentations and variable molecular characteristics detected after Whole Exome Sequencing (WES) analysis (Human Exome core 2.0 kit on Illumina NovaSeq 6000 system).

Results: All patients exhibited neurodevelopmental disorders of varying severity, including learning difficulties, autism spectrum disorders, psychomotor delay, and intellectual developmental disorder. Epilepsy was reported in five patients. Each patient showed at least one craniofacial characteristic of the syndrome while other common features, such as short stature and hearing loss, were observed alongside cataracts and white hair tufts that may eventually become characteristic for KBG. Five pathogenic and two likely pathogenic variants were detected in *ANKRD1*. Of those three were de novo and one was inherited from an affected mother. For the remaining three, segregation analysis was not applicable. In a 9-year-old female a chr22:21,066,774-21,570,956 deletion was also detected de novo leading to Noonan syndrome co-morbidity.

Conclusion: Inter and intra-familial clinical heterogeneity, limited reports on older patients as well as the presence of high functioning patients hinder evaluation of KBG prevalence, and/or long-term prognosis. Wider adoption of genetic tests is anticipated to characterize KBG clinical spectrum, improve diagnosis, and pave the way for potential genetic therapies.

C.O.I. Disclosure:

A. Kampouraki: None. **K. Kosma:** None. **D. Veltra:** None. **N. Marinakis:** None. **F. Tilemis:** None. **M. Svingou:** None. **E. Tsentemidou:** None. **V. Christodoulaki:** None. **N. Selenti:** None. **F. Fostira:** None. **G. Niotakis:** None. **I. Nakou:** None. **K. Kekou:** None. **H. Frysira:** None. **S. Kitsiou-Tzeli:** None. **P. Makrythanasis:** None. **C. Sofocleous:** None.

EP10.047 Unveiling Au-Kline syndrome: multidisciplinary insights into a novel HNRNPK variant misdiagnosed as Kabuki syndrome

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Background: *HNRNPK* pathogenic variants cause Au-Kline syndrome (AKS), a neurodevelopmental disorder associated with distinct facial features, intellectual disability, developmental delay, and various systemic abnormalities. Recent advances in DNA methylation and transcriptome profiling provided insights into the phenotypic variability and pathogenic mechanisms of AKS. We validated a novel de novo *HNRNPK* intronic variant identified in a previously misdiagnosed Kabuki syndrome patient.

Material and methods: Variant pathogenicity was ascertained by variant modelling, transcriptomics, DNA methylation, in vitro splicing studies and luciferase assays.

Results: We report the clinical characterization of a Sardinian patient with a AKS mild form due to a novel de novo intronic variant of *HNRNPK* (c.1192-3 C > A). The variant creates an aberrant splice acceptor site, resulting in a frameshift (p.Leu398ValfsTer21) that truncated the KH3 domain of HNRNPK. Minigene studies demonstrated the production of a mutant transcript. Structural modelling revealed disruption of key alpha-helices and beta-sheets within the KH3 domain. Functional assays indicated a partial loss of HNRNPK activity, consistent with the patient's intermediate DNA methylation profile and mild clinical presentation.

Conclusion: We highlight the importance of integrated genomic, epigenomic, and functional approaches in the diagnosis of atypical AKS cases. We emphasize the critical role of HNRNPK pseudogenes in masking transcriptomic analyses validating the pathogenicity of novel variants. More comprehensive structural and functional effects of *HNRNPK* variants will be explored to improve diagnostic strategies for mild AKS phenotypes.

C.O.I. Disclosure:

M. Mingoia: None. **A. Meloni:** None. **S. Sedda:** None. **S. Choufani:** None. **I. Asunis:** None. **G. Gemma:** None. **A. Ammendola:** None. **A. Torabi-Marashi:** None. **E. Di Venere:** None. **G.M. Squeo:** None. **V. Rallo:** None. **M.G. Marini:** None. **P. Moi:** None. **S. Savasta:** None. **R. Weksberg:** None. **G. Merla:** None. **A. Angius:** None.

EP10.048 *FGD1* whole-gene duplication in a child with neurodevelopmental delay and Aarskog-Scott syndrome-like features: a possible clue to triplosensitivity?

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Background: We report on a 5-year-old boy diagnosed with autism spectrum disorder. His physical examination revealed minimal facial dysmorphisms and a shawl scrotum.

Material and methods: Genetic analysis of *FMR1* returned normal results. However, SNP-array identified two maternally inherited hemizygous microduplications on chromosome X: one at Xp11.22 (GRCh37: 50,778,345-50,899,947) and another spanning Xp11.22p11.21 (54,405,542-54,996,659).

Results: The first microduplication did not encompass OMIM genes and was classified as a variant of uncertain significance. The second, at Xp11.22p11.21, included *MAGED2* (MIM*300470) and *FGD1* (MIM*300546).Hemizygous *MAGED2* loss-of-function variants are linked to antenatal transient Bartter syndrome type 5 (MIM#300971), a renal tubular disorder seemingly unrelated to the proband's phenotype. In contrast, *FGD1* variants, particularly loss-of-function ones, are associated with Aarskog-Scott syndrome (MIM#305400), presenting with short stature, hypertelorism, shawl scrotum, and variable features. *FGD1* variants have also been linked to non-syndromic intellectual disability, highlighting the condition's phenotypic variability.To assess the CNV's significance, segregation analysis was conducted on all healthy first-degree relatives of the proband's mother (mother, father, two brothers). None carried the microduplication, confirming its de novo origin.Several genes are dosage-sensitive. *FGD1* haploinsufficiency is well-documented (ClinGen Haploinsufficiency Score = 3), but evidence for triplosensitivity remains scarce, with only a few reports of *FGD1* duplications associated with intellectual disability.

Conclusion: We describe the smallest reported duplication encompassing the entire *FGD1* gene, according to DECIPHER and literature, in association with non-syndromic intellectual disability. This finding supports a possible role for *FGD1* duplication in neurodevelopmental disorders.

C.O.I. Disclosure:

N. Khaleghi Hashemian: None. **D. Gudagnolo:** None. **G. Mastromoro:** None. **B. Torres:** None. **M. Goldoni:** None. **L. Bernardini:** None. **C. Sogos:** None. **A. Pizzuti:** None.

EP10.049 Two *TKT* novel variants are associated with the phenotype of transketolase deficiency

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Background: So far, there is no clear information on the phenotype of transketolase deficiency and the pathogenic variants of *TKT*.

Material and methods:WES was used for searching causative variants, and targeted sequencing were used to determine carry

status. The ELISA technology was used to measure the transketolase in plasma.

Results:A 3-year-old girl got attention due to unexplained delays in intellectual, growth, and language development. Examination revealed mild facial deformities, short stature, stereotyped behavior and poor muscle strength in both lower limbs.As part of etiology study on rare diseases, we performed a WES on patient and found two missense variants in *TKT*, c.1288G>T (p. A430L) and c.575C>T (p. P192L). Database searches revealed that the P192L variant was previously indexed as unknown significance, while the A430L variant remained novel. Using targeted sequencing, we confirmed the carrier status of A430L from father and grandfather and P192L from mother. Thus, the trans status of the patient's two *TKT* variants (compound heterozygotes) was confirmed, and the causative variant of the autosomal recessive disease was also proposed. To investigate the effect of the two variants on *TKT* expression, we measured plasma transketolase concentrations in patients, parents, and grandfathers using ELISA technology. The results showed that the patient's transketolase (2.2 ng/ml) was significantly lower than that of the phenotypically normal parents and Mother(about 3.07 ng/ml) ($P < 0.05$).

Conclusion: This study reports the phenotype of transketolase deficiency and confirms that the A430L and P192L variants of the *TKT* gene are causative factors for loss-of-function effects.

C.O.I. Disclosure:

J. Gao: None. **J. Han:** None. **L. Ma:** None. **X. Yang:** None. **Y. Feng:** None. **Y. Wang:** None. **Y. Kuang:** None. **B. Li:** None. **H. Luo:** None. **Y. Xie:** None.

EP10.050 A case of de novo partial trisomy 13q: clinical, cytogenetic, and molecular characterization of a complex multisystemic phenotype

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Background: Partial trisomies of chromosome 13 long arm (13q) are rare chromosomal abnormalities leading to complex and variable phenotypes. They frequently arise from balanced parental translocations or inversions, while de novo duplications are not common. A literature review suggested that phenotype differs between proximal and distal 13q partial trisomies. Proximal 13q trisomy is typically associated with strabismus, facial dysmorphisms, persistence of fetal hemoglobin, and congenital malformations. Distal 13q trisomy is characterized by polydactyly, hemangioma, facial dysmorphisms, and ocular abnormalities.

We report on the case of a 2-year child with a de novo partial 13q trisomy, associated with psychomotor developmental delay, somatic features, and severe respiratory disorders.

Material and methods: The diagnostic workup included blood cells karyotype analysis, Fluorescence In situ Hybridization (FISH) analysis, SNP-array analysis and FMR1 molecular analysis.

Results: FMR1 analysis resulted normal while the cytogenetic techniques revealed de novo duplications in four different 13q regions (13q11q12.3, 13q14.2q31.1, 13q31.1q31.2, 13q34), comprehensively spanning 50 Mb. These alterations defined a partial 13q trisomy, causative of the clinical phenotype. The patient exhibited psychomotor delay, generalized hypotonia, absence of language, somatic features, astigmatism, and respiratory crises with cyanosis.

Conclusion: The precise cytogenetic and molecular characterization confirmed the de novo origin of the rearrangement, excluding an elevated increased reproductive risk. Even though this case highlights the role of de novo 13q duplications in the pathogenesis of complex, multisystemic phenotypes, further studies are needed to improve the genotype-phenotype correlations and to explore the molecular mechanisms underlying the abnormalities associated with partial 13q long arm trisomies.

C.O.I. Disclosure:

L. Sinibaldi: None. **F. Lo Bascio:** None. **A. Lombardo:** None. **G. D'Elia:** None. **E. Sallicandro:** None. **A. Del Popolo:** None. **G. Viscogliosi:** None. **M. Dentici:** None. **R. Capolino:** None. **A. Novelli:** None. **M. Digilio:** None.

EP10.051 A Novel Homozygous *LINGO-1* Variant in a Consanguineous Bangladeshi Family: Expanding the Neurodevelopmental Phenotype

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Background: LINGO-1 is a key regulator of oligodendrocyte differentiation and survival, playing a role in several central nervous system and psychiatric disorders. We describe a novel homozygous missense variant in the *LINGO-1* gene in a consanguineous Bangladeshi family with three affected children, presenting with a neurodevelopmental phenotype.

Materials and methods: Genetic testing was performed on Family F3424, comprising three affected siblings and two unaffected first-cousin parents. Whole-exome sequencing identified a homozygous *LINGO-1* variant (NM_032808.6:c.1157G>A; p.Arg386Gln). Bioinformatics analyses, including conservation scores, gnomAD constraint metrics ($z = 4.00$), and in silico prediction tools, were used to assess pathogenicity. Clinical features were reviewed to evaluate genotype-phenotype correlations.

Results: The affected siblings exhibited a spectrum of neurodevelopmental disorders, including microcephaly, global developmental delay, intellectual disability, epilepsy, feeding difficulties, bladder and bowel incontinence, mobility impairments, and behavioural issues. The *LINGO-1* variant affects a highly conserved residue, with computational models predicting a deleterious impact on protein function. Despite classification as a variant of uncertain significance, segregation analysis supports disease association.

Conclusion: This case series highlights a potential role for *LINGO-1* in human neurodevelopmental disorders. The identified variant segregates with disease in an autosomal recessive manner and shares characteristics with previously reported *LINGO-1* alterations, suggesting a common pathogenic mechanism.

C.O.I. Disclosure:

D. Barnes: None. **N. Lakhani:** None.

EP10.052 Assessing the genetic yield of isolated autism spectrum disorder referrals via microarray

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Background: Autism is a specific neurodevelopmental disorder with a strong genetic component and known phenotypic features, presenting on a spectrum of severity. No specific mutation or genetic imbalance has been identified as causative, although various recurrent copy number variants (CNVs) are thought to contribute, often with variable penetrance and expressivity. In the past, NHS diagnostic testing has been carried out using microarray platforms; however, due to lack of specific genetic findings, NHS England (NHSE) will no longer fund microarray testing for patients with isolated autism. Therefore, we aim to establish the impact of these changes, through the assessment of in-house microarray data from patients referred for isolated autism.

Material and methods: In total, 758 referrals were identified from microarray data generated between 2008–2024, with isolated autism. CNVs were re-assessed using the current ACGS Best Practice Guidelines for Variant Classification.

Results: CNVs were detected in 40 patients from 758 cases (5.3%), including recurrent susceptibility regions with variable penetrance (2.4%), sex chromosome imbalances (1.2%), CNVs in *NRXN1* (0.5%) and incidental findings (0.4%). Six referrals (0.8%) reported two CNVs. None of these CNVs is considered likely to be of clinical diagnostic utility, due to incomplete penetrance or a lack of confirmed clinical association with autism.

Conclusion: Our data shows that a fully penetrant genetic association for autism remains uncertain, bringing in to question the clinical value of testing patients with isolated autism via microarray, and providing support to the decision of NHSE to remove isolated autism referrals from the National Genomics Test Directory.

C.O.I. Disclosure:

O. Addis Jones: None. **S. Bint:** None. **A. Pendlebury-Watt:** None. **C. Ogilvie:** None.

EP10.053 The role of *YY1* mutations in X-chromosome inactivation: insights from three new cases of Gabriele-de Vries syndrome

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Background: Gabriele-de Vries syndrome (GDVS) is a syndromic intellectual disability disorder caused by de novo pathogenic *YY1* variants. *YY1*, a ubiquitously expressed transcription factor, plays a pivotal role in the X-chromosome inactivation (XCI) process. Recent studies from our group have linked de novo *YY1* variants to skewed XCI in females with GDVS. In this study, we describe the clinical and molecular characteristics of three newly identified female patients with GDVS.

Material and methods: Segregation analysis was performed by Sanger sequencing. The XCI patterns of the patients were evaluated by assessing differential methylation at two X-chromosome *loci*: *AR* and *RP2*.

Results: While the patients exhibited several clinical features consistent with those previously reported for this syndrome, they also demonstrated significant clinical heterogeneity, including additional signs, namely hypertrichosis and atopic dermatitis, and uncommon signs, such as dysarthria. Two novel *YY1* mutations were identified. Analysis of blood samples revealed mild to moderate XCI skewing in all three patients.

Conclusion: These findings further support a role for *YY1* mutations in influencing XCI patterns. This study broadens the clinical and molecular spectrum of GDVS and provides new insights into the involvement of mutated *YY1* in the XCI process.

C.O.I. Disclosure:

B.M. Kurashima: None. **L.M. Carvalho:** None. **S.S. Costa:** None. **M. Castro:** None. **F. Cammarata-Scalisi:** None. **M.C. Moreira:** None. **C.B. Santos-Rebouças:** None. **A.C. Krepischi:** None.

EP10.054 Weiss-Kruska syndrome: clinical and molecular description of five new unrelated cases

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Background: Weiss-Kruska syndrome (WKS) is a rare genetic disorder characterized by developmental delay (DD), intellectual disability (ID), metopic ridging, and distinctive facial features (ptosis, downslanted palpebral fissures, arched eyebrows, exaggerated cupid´s bow). Additional findings include cardiac, auditory, gastrointestinal, and limb anomalies. It is caused by heterozygous pathogenic variants in *ZNF462*, deletions of 9p31.2 involving *ZNF462*, or chromosomal rearrangements disrupting *ZNF462*. This study aims to present a comprehensive analysis of five unrelated patients diagnosed with WKS through retrospective evaluation.

Material and methods: We describe 5 unrelated patients with WKS, all carrying different genetic alterations involving *ZNF462* gene. Patient 1: SNP-array CytoScan 750K chip (ThermoFisher Scientific) (GRCh37/hg19). Patient 2 and parents: Agilent G4827A (array CGH ISCA v2, 8 × 60K) (GRCh37/hg19). Patient 3, 4, 5: whole-exome sequencing. Parents: Sanger sequencing.

Results:

Patient	Age	Sex	Phenotype	Genetic Findings	Inheritance
1	49	F	Severe ID, epilepsy, cataracts, facial dysmorphism	7.9 Mb 9q31.1q31.3 deletion	Parental studies pending
2	9	M	Mild DD, autism spectrum disorder (ASD), facial dysmorphism	1.6 Mb 9q31.2 deletion	De novo pathogenic
3	19	F	Mild ID, facial dysmorphism	ZNF462: NM_021224.5:c.6163C>T; p.R2055*	De novo pathogenic
4	15	M	Moderate ID, ASD, facial dysmorphism, umbilical hernia	ZNF462: NM_021224.5:c.628del; p.V210Yfs*20	De novo pathogenic
5	22	F	Mild ID, facial dysmorphism, congenital heart defect	ZNF462: NM_021224:c.3715C>T; p.Arg1239Ter	De novo pathogenic

Conclusion: WKS exhibits phenotypic variability, in particular among patients with different genetic alterations. While 9q31 deletions share overlapping clinical features with single nucleotide variants in *ZNF462*, they may be associated with a more severe phenotype due to the loss of multiple genes that potentially interact with *ZNF462*, influencing phenotypic expression. These findings provide evidence regarding molecular mechanisms underlying WKS, offering a basis for future studies aimed at exploring genotypic differences and their clinical correlations in greater depth.

C.O.I. Disclosure:

B. Masotto: None. **A. Cueto-González:** None. **M. Valenzuela-Palafoll:** None. **A. Cisneros-Sala:** None. **A. Lasa-Aranzasti:** None. **C. Trujillano-Lidon:** None. **P. Jolín-García:** None. **P. Muñoz-Cabello:** None. **A. Ros-Peña:** None. **M. Pauta-Puig:** None. **B. García-Jimenez:** None. **P. Fernandez Alvarez:** None. **A. Herencia-Ropero:** None. **M. Codina-Solà:** None. **M. Torres-López:** None. **N. Castells-Sarret:** None. **M. Viñas-Jornet:** None. **E. García-Arumi:** None. **I. Blanco:** None. **E. Tizzano:** None.

EP10.055 Whole Exome Sequencing in 135 Patients with Intellectual Disability and Autistic Spectrum - Experience of SYNLAB Genética Médica Porto

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Background: Intellectual disability (ID) and autism spectrum disorder (ASD) belong to neurodevelopmental disorders and occur in ~1% of the general population. ID affects the individual, its family, and the community, therefore being an important socio-economic problem in healthcare. Approximately 30–50% of ID cases are due to a genetic cause, and 25–30% of these are due to genetic variants in single genes.Autism spectrum disorder (ASD) generally becomes apparent after the first year of life and it has been reported in an increasing number (2.2–2.7%) of children. Whole Exome Sequencing (WES) has increasingly become used to rapidly identify variants associated with these two entities that often co-exist. In this study, we retrospectively studied 135 patients referred to our center.

Material and methods: WES analysis was performed in all cases. Bioinformatic analysis was focused on our panel for ID and ASD and mainly on the targeted phenotype. In all cases the bioinformatic analysis included copy number variants (CNVs) analysis.

Results: We identified 40 pathogenic/ likely pathogenic variants with a detection rate of 29,4% (41/136). In 69 cases (50%) variants of uncertain clinical significance (VUS) were also detected.

Conclusion: Due to disease heterogeneity, identifying the aetiology of ID and ASD remains challenging. Our diagnosis rate is high and many of these patients were undiagnosed for a long time. This study highlighted the importance of WES in the diagnosis of ID/ASD. Our study strongly supports the value of this technology, especially WES, in solo or trios, as an effective first-choice diagnostic tool.

C.O.I. Disclosure:

N.B. Salgueiro: None. **L. Castro:** None. **A. Conceicao:** None. **M. Cardoso:** None. **J. Goncalves:** None. **E. Garcia:** None. **S. Barros:** None. **M. Freitas:** None. **J. Mata:** None. **A. Gavina:** None. **M. Reis-Lima:** None.

EP10.056 Dual genetic mutations in Verheij and Coffin-Siris syndromes: A case report of a complex diagnosis

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Background: Thanks to advancements in next-generation sequencing technologies, the coexistence of multiple genetic disorders in affected individuals has been found to be much more frequent than previously thought, making definitive diagnosis increasingly challenging. In this study, we present a patient diagnosed with both Verheij syndrome and Coffin-Siris syndrome. The clinical findings of the patient carrying de novo variants in *PUF60* and *ARID1B* genes simultaneously will be evaluated.

Material and methods: Whole-exome sequencing (WES) was performed to identify the potential genetic cause of the index patient's clinical findings. The identified variants were confirmed by next-generation sequencing (NGS), and their pathogenicity was assessed according to the ACMG criteria. Targeted variant analysis in family members was also performed using NGS.

Results: The 2-year-old female patient presented with decreased body weight (−3.18 SD), short stature (−4.07 SD) variable dysmorphic features, coloboma, hypotonia, motor developmental delay, idiopathic intracranial hypertension, and cardiovascular anomalies. Her physicians initially suspected mucopolysaccharidoses but WES revealed two loss-of-function variants: NM_078480.3: c.51del (p.Ser18ProfsTer42) variant in the *PUF60* gene and a NM_001374828.1: c.1198G>T (p.Gly400Ter) variant in the *ARID1B* gene. Parental analyses revealed that both variants have occurred de novo.

Conclusion: This case contributes to expanding the clinical spectrum of Verheij syndrome and Coffin-Siris syndrome while highlighting the co-occurrence of rare genetic disorders in shaping clinical findings. It underscores the importance of considering multiple genetic conditions when a single variant cannot fully explain a patient's phenotype. Additionally, WES emerges as a critical tool for diagnosing such complex disorders and guiding the management of similar cases.

C.O.I. Disclosure:

B. Caliskan: None. **O. Unal uzun:** None. **H. Bas:** None.

EP10.057 Methylation mosaicismin a male with FMR1 full mutation and mild Fragile X Syndrome

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Background: Fragile X syndrome (FXS) is the leading inherited cause of intellectual disability (ID) and single-gene cause of autism. While most FXS patients have complete methylation of the FMR1 gene, some have methylation mosaicism (a proportion of methylated cells and others unmethylated) or size mosaicism (some cells with premutation and others with premutation). Male individuals carrying partially methylated full mutated alleles tend to have milder learning disabilities and behavioral problems than those with a methylated full mutation.

Material and methods: Here we describe a 25-year-old boy, followed at Bambino Gesù Children's Hospital, initially for attention problems in school and mild neurocognitive delay, who underwent X-Fragile molecular analysis. We performed amplification of the CGG

repeat sequences of the FMR1 gene promoter by RP-PCR (Repeat Primed PCR) with AmplideX FMR1 PCR Kit, Asuragen and fragment study by Applied Biosystems 3500xl capillary sequencer. MS-MLPA (Methylation-specific - Multiplex ligation-dependent probe amplification) assay was performed to detect the methylation status of the promoter of the FMR1 gene.

Results: The analysis by RP-PCR revealed the presence of a full mutation allele of >200 CGG repeats; by the MS-MLPA assay, partial methylation of the FMR1 gene promoter was observed.

Conclusion: These results, consistent with the clinical indication for investigation, suggest that partial methylation may be associated with less severe manifestations of FXS.

C.O.I. Disclosure:

P. Bontempo: None. **G. D'Elia:** None. **E. Agolini:** None. **C. Alicata:** None. **D. Vecchio:** None. **S. Cetola:** None. **K. Bejo:** None. **A. Novelli:** None.

EP10.058 Glucose stimulation of Ftsj1 deficient hepatocytes induces expression of proteins involved in protein folding in the endoplasmic reticulum

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Background: Familial forms of intellectual disability (ID) affect around 1% of the population and up to 50% of the cases are due to genetic defects. Over 100 ID causing genes have been found on the X chromosome alone and one of these genes is FTSJ1, a ubiquitously expressed and evolutionarily conserved tRNA 2′-O-methyltransferase. The related pathomechanism is still unclear, but Ftsj1-deficient mice show altered behavior and reduced hippocampal long-term potentiation in combination with reduced body mass, fat and lean mass and increased glucose tolerance, pointing towards an involvement of glucose metabolism.

Material and methods: As the liver is the major glucose metabolizing organ, we isolated and cultivated primary hepatocytes from Ftsj1-deficient and WT mice and created RNA and protein abundance profiles from untreated, glucose/pyruvate starved, and glucose stimulated cells using RNA-Seq and mass spectrometry, respectively.

Results: There was no significant difference between Ftsj1-deficient and WT hepatocytes at the transcriptional level. However, glucose stimulation induced expression (adjusted $p < 0.01$) of several proteins involved in protein folding in the endoplasmic reticulum (ER) of Ftsj1-deficient hepatocytes.

Conclusion: As protein folding in the ER requires glycosylation of the newly synthesized polypeptides, it is likely that in Ftsj1-deficient hepatocytes the glucose used for stimulating the cells is also used to counter problems with protein folding. As the brain has the largest repertoire of expressed proteins, it is reasonable to assume that correct folding of certain proteins may be partially impaired there as well, which could be related to the observed ID phenotype in FTSJ1 deficient patients.

C.O.I. Disclosure:

L.R. Jensen: None. **D. Thalheim:** None. **V. Rönnpagel:** None. **A. Tzvetkova:** None. **L. Hagenau:** None. **S. Michalik:** None. **E. Hammer:** None. **C. Wolke:** None. **U. Lendeckel:** None. **O. von Bohlen und Halbach:** None. **M. Grube:** None. **A. Kuß:** None.

EP10.059 New cases of recently identified genetic syndromes with intellectual disability

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Background: Syndromes with intellectual disabilities (SID) encompass a variety of rare genetic disorders characterized by significant clinical and genetic diversity, overlapping manifestations, and variable clinical presentation. Integrating next-generation sequencing into clinical practice allows for rapid and effective genetic diagnosis of rare monogenic forms and identification of novel genes for SID. However, limited clinical data hinders their classification as clinical and genetic entities. We present genetically verified clinical cases of recently reported genetic forms of SID.

Materials and methods: We performed whole exome sequencing of patients with SID and analyzed the data using a comprehensive gene panel for developmental disorders. Sanger sequencing was used to validate the identified variants and assess their segregation within families.

Results: We identified potentially-pathogenic variants in *SEMA3E, NLGN2, IQSEQ3* and *CLTCL1* genes without disease associations in the OMIM database, and single literature reports linking them to SID. Distinct genetic variants were found in the *SEMA3E* gene in two unrelated patients, each presenting different clinical features. So far, clinical phenotypes linked to *SEMA3E* include CHARGE syndrome and severe intellectual disability with regression. One of our patients showed characteristics of CHARGE syndrome, while another had severe intellectual disability with regression and seizures. To our knowledge, epileptic seizures have only been described in mouse models experimentally, and not as part of the clinical spectrum of *SEMA3E*.

Conclusion: Our data expand the clinical and genetic spectrum of newly identified SID, helping to delineate their clinical phenotype, which is crucial for the correct genetic diagnosis, prognosis, and genetic counseling in families.

C.O.I. Disclosure:

N. Ivanova: None. **K. Mihova:** None. **T.N. Delchev:** None. **M. Sredkova:** None. **V. Bojinova:** None. **D. Avdjieva-Tzavella:** None. **A. Jordanova:** None. **I. Dimova:** None. **R. Kaneva:** None.

EP10.060 Fifty Years of Clinical Experience with Prader-Willi Syndrome: A Single-Center Study

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Background: Previous studies demonstrated that early diagnosis

delayed the onset of obesity in patients with Prader-Willi syndrome (PWS). However, the age of diagnosis did not affect the age when patients developed an appetite and activity of seeking food. Besides hyperphagia, behavioral problems are common in PWS. We need to consider problems that cannot be solved by early diagnosis and GH therapy. To consider this issue, we decided to look back on 50 years of PWS practice.

Material and methods: We performed a retrospective and literature review evaluating data from 88 patients aged 0–57 years with PWS who visited the Aichi Developmental Disability Center Central Hospital from 1971 to 2024.

Results: Two were born in the 1960s, nine in the 1970s, 17 in the 1980s, eight in the 1990s, 20 in the 2000s, 25 in the 2010s, and seven in the 2020s. Although the age of diagnosis has been accelerated by the dissemination of genetic testing, age of first visit to our department tends to be delayed. GH therapy was introduced in more than half of the cases born after the 1990s, and the starting age tended to be earlier. BMI in early childhood tended to be lower in recently born cases, while BMI after puberty did not correlate. There were cases at all ages that were difficult to manage in terms of severe obesity and behavioral problems.

Conclusion: There may be problems that cannot be solved by early diagnosis and GH therapy alone, and further study is needed.

C.O.I. Disclosure:

S. Otsuji: None. **H. Ueda:** None. **N. Nakamura:** None. **T. Uehara:** None. **S. Mizuno:** None. **S. Hayashi:** None. **M. Inaba:** None.

EP10.061 Novel genetic insights into *MED13L* syndrome: a four case series

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Background: *MED13L* encodes a protein that is one of the subunits of the mediator complex regulating transcriptional activity of genes and plays an important role in brain and heart development. Deletions and loss of function variants in *MED13L* gene are associated with autosomal dominant impaired intellectual development and distinctive facial features with or without cardiac defects. Here we present four patients with *MED13L* haploinsufficiency causing this disorder.

Material and methods: Whole exome sequencing (WES) was performed for a boy with psychomotor delay, hearing impairment, dysmorphic features as well as for a girl with Pierre Robin sequence, mild dysmorphic features and speech delay. In two boys with intellectual disability and additional features array CGH was conducted.

Results: A heterozygous, likely pathogenic variant NM_015335.4:c.4213del in *MED13L* was identified in a 3-year old boy. In a second patient- a 2-year old girl, WES revealed likely pathogenic variant NM_015335.5:c.4136C>T in *MED13L*. A pathogenic deletion involving exons 5–14 of the *MED13L* gene was reported in a 2-year old patient using array CGH. The fourth case was a 13-year old boy with ID, speech delay and heart defect in whom array CGH revealed a deletion encompassing 5–7 exons of *MED13L*.

Conclusion: Loss of function variants and deletions involving *MED13L* has been described as causative for intellectual disability and another various complications. All four patients exhibited clinical features of MED13L syndrome. These molecular findings expand our understanding of disease variability and show that both WES and aCGH are effective tools in diagnosing this syndrome.

C.O.I. Disclosure:

A. Kucinska: None. **U. Wysocka:** None. **L. Kepczynski:** None. **M. Piotrowicz:** None. **K. Polatynska:** None. **A. Gach:** None.

EP10.062 Trio vs. singleton exome sequencing in NDD diagnosis: insights from diagnostics in Latvia

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Background: Neurodevelopmental disorders (NDDs) affect approximately 2–4% of the general population. Exome sequencing (ES) is the first-line genetic testing method for diagnosing NDDs. Although ES has a diagnostic yield of 30–50%, many patients remain without a clear diagnosis. Trio analysis can be used to establish a more definitive diagnosis and reduce analysis time.

Material and methods: We performed germline exome sequencing for 344 NDD cases, either singleton (238) or trio (106), with analysis of NDD panels (N = 2,418 genes) with SNVs and CNVs, typically followed by open ES for trio, and compared diagnostic outcomes between both approaches.

Results: Singleton exome analysis established a definitive molecular diagnosis in 39% (92/238) of cases and in 26% (63/238) diagnosis remained uncertain due to VUS. Trio exome analysis established a definitive molecular diagnosis in 41% (43/106) of cases and in 11% (12/106) diagnosis remained uncertain due to VUS. Among resolved trio cases, 70% (30/43) of pathogenic variants were de novo and 30% (13/43) were inherited: autosomal dominant variants were inherited in 16% of cases - 12% (5/43) from a heterozygous parent and 4% (2/43) from a low-level mosaic parent. Additionally, 12% (5/43) were X-linked and 2% (1/43) were autosomal recessive homozygote.

Conclusion: Trio ES did not significantly improve the diagnostic yield for NDDs over singleton ES, but significantly reduced the proportion of uncertain diagnoses due to VUS. Additionally, our results from trio ES highlight the importance of analysing inherited autosomal dominant variants, with 16% of pathogenic variants inherited from heterozygous or mosaic parent.

C.O.I. Disclosure:

K. Bukele: None. **L. Bārdiņa:** None. **L. Voložonoka:** None. **A. Zariņa:** None. **L. Freimane:** None. **A. Eihmane:** None. **D. Kidere:** None. **U. Čonka-Priedeslaipa:** None. **I. Grīnfelde:** None. **I. Mičule:** None. **M. Mašinska:** None. **D. Ločmele:** None. **L. Skrabule:** None. **M. Jurčenko:** None. **I. Piruška:** None. **I. Nartiša:** None. **D. Rots:** None.

EP10.063 *GTF2I* heterozygous variants at the Williams-Beuren syndrome's locus cause a neurodevelopmental disorder

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Background: Williams-Beuren syndrome (WBS) is a well-known neurodevelopmental disorder caused by a copy-number loss at the 7q11.23 locus. Although the 1.5–1.8 Mb recurrent deletion carries several genes of interest, no single gene has been identified in which pathogenic variants cause a neurodevelopmental phenotype. At this locus, *GTF2I*, encoding the general transcription factor II-I, has been considered as the main candidate gene for the cognitive and behavioral phenotype of WBS, based on clinical observations of cases with atypical 7q.11.23 deletions and on functional studies in mice and humans.

Material and methods: Individuals with a neurodevelopmental disorder were identified through a multi-center collaboration using GeneMatcher and the ERN-ITHACA network. These patients remained undiagnosed following genome or exome sequencing. Clinical evaluations were performed in each participating center.

Results: We identified six unrelated individuals with de novo variants in *GTF2I* (1 nonsense, 2 splicing, 1 missense, 1 indel, 1 intragenic deletion). They all presented with global developmental delay and facial dysmorphic features, with speech delay and/or autistic features in four cases. The effect of the two splicing variants was confirmed by RNA-sequencing. In another cohort of undiagnosed patients, we identified another adult patient with the main neurodevelopmental features of WBS and a loss of *GTF2I* expression identified by RNA-sequencing.

Conclusion: Pathogenic heterozygous *GTF2I* variants cause a neurodevelopmental disorder characterized by global developmental delay with facial dysmorphic features, resembling the phenotype observed in individuals affected with WBS.

C.O.I. Disclosure:

J. Jury: None. **T. Besnard:** None. **W. Deb:** None. **A. Toutain:** None. **P. Gueguen:** None. **A. Bruel:** None. **A. Bouman:** None. **D. Veenma:** None. **T.S. Barakat:** None. **L. Do Souto Ferreira:** None. **P.J. Zwijnenburg:** None. **S. Schuhmann:** None. **G. Vasileiou:** None. **P. Blanc:** None. **S. Bézieau:** None. **B. Cogné:** None. **B. Isidor:** None.

EP10.064 Novel Bi-Allelic *LGI3* Variants and Intellectual Developmental Disorder: Clinical and Genetic Insights from Four New Families

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Background/Objectives: Bi-allelic loss-of-function variants in *LGI3* (MIM: #608302) have been implicated in intellectual developmental disorder with muscle tone abnormalities and distal skeletal defects (IDDMDS). To date, only 16 patients from eight unrelated families have been reported. In this study, we expand the genetic and phenotypic spectrum of IDDMDS by identifying four novel homozygous *LGI3* variants from four unrelated consanguineous families, comprising seven clinically affected patients.

Methods: Using whole exome and genome sequencing, we identified four clinically relevant variants in the *LGI3* gene. To confirm segregation, family members were screened using Sanger sequencing.

Results: Clinical features varied widely, but the common symptoms include global developmental delays (7/7 patients), microcephaly (6/7 patients), distinctive skeletal deformities (6/7 patients), failure to thrive (6/7 patients), intellectual disabilities of varying severities (5/7 patients), and facial dysmorphism (5/7 patients). Interestingly, we observed microcephaly, short stature, and failure to thrive in 6 patients, while it was observed in only 1 out of 16 patients in the literature. Four novel biallelic *LGI3* variants were identified in our study, including a stop-gain variant (c.397C>T, p.Arg133*), two frameshift variants (c.841_850delGTGCACTGCA, p.Val281Serfs49, and c.1358del, p.Gly453Valfs171), and one missense variant (c.415A>C, p.Thr139Pro).

Conclusion: This study expands the mutational and phenotypic spectrum of *LGI3*-related disorders, providing valuable insights into the underlying mechanisms of IDDMDS and contributing to a deeper understanding of this rare condition.

C.O.I. Disclosure:

M. Ali: A. Employment (full or part-time); Modest; Arcensus GmbH. **M. Mustafa:** None. **E. Jarvis:** A. Employment (full or part-time); Modest; Arcensus GmbH. **N. Rana:** None. **I. Iqbal:** None. **M.B. Toosi:** None. **P. Sarraf:** None. **S. Akhondian:** None. **E. Ghayoor Karimiani:** None. **A. Rad:** A. Employment (full or part-time); Modest; Arcensus GmbH. **G. Oprea:** A. Employment (full or part-time); Modest; Arcensus GmbH.

EP10.065 Identification of a missense variant in *RAB11FIP1 g*ene in a family with intellectual disability

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Background: Intellectual disability (ID) affects 1–3% of the population globally. Prior studies have estimated the involvement of more than 1500 genes, but despite the application of next-generation sequencing many cases/families are still unresolved. This study aimed to identify the genetic cause in a Pakistani family with three individuals suffering from severe ID presented with developmental delay, facial dysmorphism and psychological impairment.

Material and methods: We performed homozygosity mapping and exome sequencing to identify disease-causing variants followed by a knockdown of the gene. In the embryonic mouse brain (at E14.5) *Rab11fip1* was knocked down with shRNA using in-utero electroporation followed by the assessment of neuronal migration pattern.

Results: Exome sequencing of one affected individual was performed and data analysis identified a potential missense variant (c.451A>C, p.Asn151His) in exon 2 of *RAB11FIP1*. The gene is present within the HBD region mapped on chromosome 8. The variant co-segregated with the ID phenotype and is predicted to be rare and pathogenic. The role of *RAB11FIP1* in normal neurodevelopmental processes was further explored by knockdown of its homologue in the developing mice brain. RAB11FIP1 is involved in the trafficking of beta-1 integrin at the periphery of growth cones, and manipulation of this protein affects integrin level and axon growth. In this study, the knockdown of *Rab11fip1* caused a significant decrease in neuronal migration strongly suggesting that reduced expression of *Rab11fip1* affects neuronal function in mice.

Conclusion: It can be concluded that loss of *Rab11fip1* affects neuronal functioning and could be a potential cause of ID in this family.

C.O.I. Disclosure:

M.A. Malik: None. **V. Khan:** None. **E. Mientjes:** None. **A. Acharya:** None. **Y. Elgersma:** None. **S.M. leal:** None. **M. Ansar:** None.

EP10.066 Identification and functional characterization of a novel *PAK3* missense mutation associated with intellectual disability

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Background: The Rho GTPases effector PAK3 is a serine-threonine kinase regulating multiple signaling pathways, including the MAPK cascade. PAK3 is predominantly expressed in the brain, contributing to neurogenesis [1,2]. Kinase activity-independent PAK3 function has been reported [3,4].In families with non-syndromic X-linked intellectual disability (NS-XLID), pathogenic *PAK3* mutations have been shown to impair catalytic activity of the kinase [5]. We previously functionally characterized the PAK3^{K389N} mutant implicated in NS-XLID [6]. Here, we biochemically profile a novel variant (p.Y439C) identified in a child affected with severe developmental delay, dystonic movements, severe feeding difficulties and seizures.

Material and methods: The novel missense *PAK3* mutation was identified by exome sequencing. Protein levels, activation, and PAK3 downstream signaling events (i.e. MAPK activation) were analyzed upon the stimulation of the Rho GTPase CDC42 in cells overexpressing PAK3 *wild type* (WT), and the p.K389N and p.Y439C mutants.

Results: Biochemical studies show that p.Y439C decreases the full activity of the kinase, concomitantly reducing the negative feedback-induced degradation of CDC42 and PAK3 itself. Similarly to the p.K389N variant, p.Y439C enhances the downstream MAPK signaling pathway with respect to the WT protein.

Conclusion: Our study expands the molecular and clinical spectrum of *PAK3* pathogenic variants and suggests that, beside the loss of function behavior, dysregulation of multiple signaling pathways contributes to the pathogenic mechanism.

C.O.I. Disclosure:

E. Zara: None. **M. Barone:** None. **M. Tondo:** None. **A. Gardham:** None. **M. Tartaglia:** None. **S. Coppola:** None.

EP10.067 Evaluation of MLPA in cases with intellectual disability, developmental delay with or without congenital anomalies: Pilot study of 332 North Indian cases

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Background: Chromosomal abnormalities are a major cause of intellectual disability (ID),developmental delay (DD), and congenital anomalies. While karyotyping is cost-effective and accessible, it cannot detect copy number variations smaller than 5 Mb. Chromosomal microarray (CMA) overcomes this limitation but remains costly in resource-constrained settings.

Methods: This study evaluated the utility of multiplex ligation-dependent probe amplification (MLPA) as an intermediate diagnostic approach. An optimal de Vries score cutoff was also determined to guide test selection based on clinical presentation. A total of 332 children with DD/ID, with or without facial dysmorphism and congenital anomalies, were analyzed using MLPA probe set P245. Clinical data, including birth history, facial dysmorphism, malformations, and family history, were recorded, and the de Vries score was calculated for all participants.

Results: The overall MLPA detection rate was 13.5% (45/332 cases). DiGeorge syndrome (22q11.21 deletion) was the most common finding (3.3%, 11 cases). Other abnormalities included 15q11.2 deletions (Angelman/Prader-Willi syndromes, 3.6%, 12 cases), Williams-Beuren syndrome (3.0%, 10 cases), Wolf-Hirschhorn syndrome (1.8%, 6 cases), and 1p36 deletion syndrome (1.2%, 4 cases). The optimal de Vries score cutoff for recommending MLPA was 3, with a sensitivity of 82.2% and specificity of 66.7%.

Conclusion: MLPA is an affordable and effective alternative to CMA for diagnosing children with DD/ID, especially in resource-limited settings. This study represents the largest Indian cohort evaluating MLPA for detecting chromosomal abnormalities.

C.O.I. Disclosure:

D. Kaur: None. **D. Srivastava:** None. **P. Panigrahi:** None.

EP11 Neurogenetic and Psychiatric Disorders

EP11.001 Dravet syndrome: an atypical case revealed by whole-exome sequencing

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Background: Dravet syndrome is a rare epileptic encephalopathy that begins early in life and is resistant to treatment. It manifests within the first months of life with convulsive seizures, often triggered by fever, and progresses to a polymorphic epilepsy associated with developmental delay, language impairment, motor incoordination, and behavioral disorders, ultimately leading to intellectual disability. Pathogenic variants in the *SCN1A* gene are identified in more than 85% of cases, causing dysfunction of the *NaV1.1* sodium channels and impaired GABAergic signaling.

Materials and methods: We report the case of a 3-year-and-5-month-old girl referred to our genetics department by her neurologist for epileptic seizures suggesting a genetic origin. Clinical and electroencephalographic evaluations were performed, followed by whole-exome sequencing to establish an accurate diagnosis.

Results: Molecular analysis revealed a heterozygous missense mutation in the *SCN1A* gene (c.2839G>A; p.Val947Met), confirming

the diagnosis of Dravet syndrome. However, the uniqueness of this case lies in its atypical clinical presentation, characterized by milder severity, a relatively late onset of symptoms, and a favorable response to treatment, with an absence of seizures.

Conclusion: This case highlights the phenotypic variability of Dravet syndrome and underscores the importance of genetic diagnosis, even in the presence of an atypical clinical presentation. Identifying a pathogenic *SCN1A* variant enables tailored patient management and genetic counseling for families.

C.O.I. Disclosure:

K. Dafir: None. **O. Cherkaoui Rhazouani:** None.

EP11.002 From Pre to Post : The diagnostic importance of following the patient from the womb to the postnatal period

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We present a case of a 4-year-old girl presented with tonic-clonic seizures and a prenatalhistory of ventriculomegaly, which later resolved; She has global developmental delay andneuroimaging showed hypoplasia of the corpus callosum; malrotation of the cerebellum;reduced white matter representation; and an enlarged supratentorial ventricular system.The proband was tested with clinical exome using next-generation sequencing on aNextSeq 550dx with DNA Prep for Enrichment and TruSight One 2.0 Expanded byIllumina. Bioinformatic analysis and variant filtering were performed using DRAGENEnrichment 4.2.4 and Geneyx analysis software. Different variants were selected since thepresented phenotype was common to several Neurodevelopmental Disorders (NDD).However, one particular finding gave us the final clue, leading to the identification of thede-novo heterozygous variant PPP2R1A: c.656C>T (PS4 moderate, PP3 supporting, PM2supporting, PM6 moderate, PP2 supporting) classified as Likely Pathogenic for Houge-Janssens syndrome. This gene encodes the regulatory subunits of protein phosphatase2A, an enzyme that is present in the heterotrimeric form, that plays a fundamental role inthe development and functionality of the brain. The phenotype associated with thissyndrome includes global developmental delay, epilepsy, ADHD, and anomalies identifiedin neuroimaging, such as hypoplasia or aplasia of the corpus callosum, hypoplasia of thecerebellum, and ventriculomegaly; phenotypes that perfectly overlap with those presentedin our case. This case report underlines the importance of identifying and reportingphenotypes during in-utero development. In this case, the indication of ventriculomegalyduring pregnancy provided the crucial clue that led to the diagnosis.

C.O.I. Disclosure:

A. Cianflone: None. **N. Callegari:** None. **G. Porta:** None. **F. Orsini:** None. **A. Filannino:** None. **E. Meroni:** None. **A. Bassani:** None. **A. Tassan:** None. **V. Ferri:** None. **R. Valli:** None. **R. Righi:** None.

EP11.003 A novel homozygous *LAMA1* variant in a patient with cerebellar hypoplasia and ocular findings

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Background: *LAMA1*-related disorders are rare autosomal recessive conditions characterized by cerebellar malformations and ocular abnormalities. We present a 6-year-old female patient born to consanguineous parents with cerebellar and ophthalmologic findings associated with a homozygous nonsense mutation in *LAMA1*.

Material and methods: The patient underwent detailed clinical, neurological, and ophthalmological evaluations. Serial brain MRI scans were performed, and clinical exome sequencing (CES) targeting 5500 genes was conducted using the Illumina NextSeq platform. The identified variant was classified according to ACMG guidelines.

Results: The patient presented with esotropia, myopia, amblyopia, and restricted lateral gaze movement. Neurological examination revealed normal motor and cognitive development. Brain MRI demonstrated cerebellar vermis hypoplasia (more pronounced in the inferior portion), cerebellar hemisphere hypoplasia, cerebellar cortical dysplasia, and multiple subcortical cerebellar cysts. Additional findings included a dilated fourth ventricle, mega cisterna magna, periventricular leukomalacia, lateral ventricular dilatation, and a thin corpus callosum. CES analysis identified a novel homozygous nonsense mutation in *LAMA1*:c.317G>A (p.Trp106*), classified as likely pathogenic based on ACMG criteria. The variant is consistent with the observed phenotype, including cerebellar and ocular manifestations.

Conclusion: Our findings support the association of *LAMA1* mutations with cerebellar and ocular developmental abnormalities and expand the phenotypic spectrum of *LAMA1*-related disorders. Early genetic diagnosis is essential for proper clinical management and genetic counseling.

C.O.I. Disclosure:

S. Öztürk: None. **O. Dikbas:** None. **A. Tosun:** None. **Z. Manav Yigit:** None. **G. Bozkurt:** None.

EP11.004 Detection a novel mutation in CSNK1E gene by next-generation sequencing

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Background: Advances in the genetics of neurological diseases have provided new insights into their pathogenesis. This study reports a novel finding in a gene not previously annotated in major databases, potentially linked to neurological disorders, highlighting opportunities for further research and the development of diagnostic strategies.

Material and methods: A 2-year-old patient exhibited severe acute encephalomyelitis, flaccid tetraparesis with significant motor deficits, pseudobulbar palsy, and multiple organ failure. To explore genetic causes, whole genome sequencing of a blood sample was conducted using next-generation sequencing (NGS) on the DNBSEQ-G400 platform, achieving a mean coverage depth of 44x. Data were analyzed using an in-house bioinformatics pipeline.

Results: A previously unreported splice site variant (c.1078+2T > C) in the CSNK1E gene was identified. Loss-of-function variants in CSNK1E, particularly at splice sites, are associated with epileptic encephalopathies, West syndrome, and autism spectrum disorders. The potential impact of CSNK1E gene variants on other neuropsychiatric conditions warrants further investigation.

Conclusion: These findings suggest that *CSNK1E* mutations may contribute to neurological and psychiatric disorders. The study's significance lies in the scarcity of clinical data on this gene in existing databases, marking a critical step toward understanding its role in nervous system pathologies.

C.O.I. Disclosure:

V. Udalova: None. **I. Kanivets:** None. **E. Zinina:** None.

EP11.005 Pathogenic KCNH2 Variant in Monozygotic Twins with autism spectrum disorders (ASD) and long QT syndrome (LQTS)

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Background: Pathogenic variants in the *KCNH2* gene, which encodes the Kv11.1 potassium channel, are classically associated with Long QT Syndrome Type 2 (LQT2). Emerging evidence also suggests possible links between *KCNH2* dysfunction and neurodevelopmental disorders.

Methods: Whole-exome sequencing (WES) was performed on the monozygotic twin sisters to identify potential pathogenic variants associated with neurodevelopmental or cardiac disorders. Chromosomal microarray analysis (array-CGH) was conducted to exclude pathogenic copy number variations (CNVs). Cardiac evaluation included a standard 12-lead ECG and 24-hour Holter monitoring to detect QT interval prolongation and subclinical arrhythmic events. Neurodevelopmental assessments were performed using standardized autism spectrum disorder (ASD) screening tools and speech development evaluations.

Results: We describe monozygotic twin sisters carrying the pathogenic frameshift variant c.2959_2960delCT (p. Leu987fs*) in *KCNH2* gene, inherited from their asymptomatic father. Both twins presented with autism spectrum disorder (ASD) traits and speech delay. Cardiac evaluation through 24-hour Holter monitoring revealed intermittent QT prolongation and non-sustained ventricular ectopy, although neither twin exhibited overt arrhythmic symptoms. In contrast, the father remained asymptomatic despite carrying the same variant, suggesting incomplete penetrance or the influence of additional genetic or environmental modifiers.

Conclusion: These findings emphasize the need for comprehensive familial screening and multidisciplinary evaluation of KCNH2 variant carriers. Even in the absence of apparent cardiac symptoms, carriers may experience neurodevelopmental disturbances and subclinical arrhythmias. Larger studies are warranted to elucidate the pathophysiological mechanisms linking KCNH2 dysfunction to neurodevelopmental phenotypes, especially in light of this new genotype-phenotype association.

C.O.I. Disclosure:

K. Margiotti: None. **M. Fabiani:** None. **C. Zangheri:** None. **A. Cima:** None. **F. Monaco:** None. **C. Alì:** None. **M. Barone:** None. **A. Viola:** None. **A. Mesoraca:** None. **C. Giorlandino:** None.

EP11.006 Double trouble: the value of sibling clinical examination in neurodevelopmental disorders

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Background: Neurodevelopmental disorders (NDDs) are a group of diverse conditions affecting brain growth and function. Autism spectrum disorder (ASD) and attention-deficit/hyperactivity disorder (ADHD) are the most common NDDs, with different symptoms and severity, isolated or syndromic forms. The parental understanding of NDDs can be difficult. Among relatives of affected individuals with NDDs their phenotype is not recognized. Clinical history and findings remain of utmost importance when formulating the diagnostic impression of a neurodevelopmental disorder by the specialist.

Material and methods: In this work we present 16 quartet families (families with 2 or even 3 affected siblings) with clinical findings from group of neurodevelopmental disorder diagnosed in a period of 6 months in our Genetics Department. Initially the families had appointments for apparently a single affected offspring, but the family history and clinical examination of other members of the family identified the second/third affected child. The most common reasons for referral to Genetics were: delayed or impaired speech/language development, behavioral problems (aggression, hyperactivity), attention deficit, sleep disturbance, social skills deficits, additional anomalies.

Results: A rigorous characterization of the clinical phenotype of the siblings combined with selection of different genetic laboratory algorithms were crucial to orientate all referred patients and new suspected siblings with for a precise genetic diagnosis and for understanding the molecular mechanisms to improving treatments.

Conclusion: The geneticist recommends combining sibling pairs appointments mainly when there is a clinical suspicion of ASD or other neurodevelopmental disorders. The identification of the genetic etiology of the disease has important ramifications for genetic counseling and patient management.

C.O.I. Disclosure:

V. Plaiasu: None. **D. Ozunu:** None. **G. Motei:** None. **I. Apostol:** None. **L. Ghita:** None. **C. Trutescu:** None.

EP11.007 SHANK3gene variants in autism, novel mutations and common clinical features

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Background: Neurexins and neuroligins are cell surface proteins critical for the proper formation and functioning of synaptic connections. Presynaptic signals by neurexins increase the signal transduction capacity of neuroligins at the postsynaptic membrane. Shank proteins are large, multifunctional proteins that couple these signals and regulate synaptic organization. The SHANK3 gene is associated with autism spectrum disorder (ASD), a neurodevelopmental disorder characterized by difficulties in social interaction, communication problems, and limited interests. This study aims to examine two ASD patients carrying novel variants in SHANK3.

Material and methods: In this study, we evaluated two ASD patients who underwent Clinical Exome Sequencing (CES) on the Illumina NextSeq platform. Their genetic data were analyzed using Sophia Genetics DDM V4, and they were found to carry a disease-related variant in SHANK3 according to the ACMG classification.

Results: Two novel variants c.5126C>G p.(Ser1709Cys) and c.1234_1249delinsTCC (p.Thr412Serfs*39) were identified in SHANK3. In both cases, common findings such as language developmental delay, stereotyped movements, learning disability, limited interests, social communication disorder, and lack of eye contact were noted. One patient exhibited drug-resistant seizures, while the other showed developmental regression as distinguishing features.

Conclusion: Our study highlights the role of SHANK3 variants in the pathogenesis of ASD and underscores the contribution of genetic testing to clinical diagnosis. Novel variants in SHANK3 may help us better understand the genetic basis of ASD. Larger-scale future studies will provide deeper insights into the genetic diversity of ASD and its molecular mechanisms.

C.O.I. Disclosure:
O. Dikbas: None. **B. Sapmaz:** None. **M. Ayanoglu:** None. **Z. Manav Yigit:** None.

EP11.008 Expanding the phenotypic spectrum of *KAT6B*-related disorders: A case report of a novel variant and brain glioma

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Background: The *KAT6B* gene encodes a histone acetyltransferase involved in chromatin remodelling and gene expression regulation. Heterozygous pathogenic variants in *KAT6B* are associated with overlapping disorders: Say-Barber-Biesecker-Young-Simpson syndrome (MIM #603736) and Genitopatellar syndrome (MIM #606170). Both are characterized by varying degrees of global developmental delay (DD)/ intellectual disability (ID), hypotonia, feeding difficulties, and genital and skeletal anomalies. Additional features may include congenital heart defects, gastrointestinal and renal anomalies, hearing loss, thyroid dysfunction, and dental anomalies.

Subject and methods: Our patient is a 7-year-old boy presenting with DD, moderate ID, facial dysmorphic features, strabismus, hypotonia, feeding difficulties, cryptorchidism, a hypoplastic scrotum, and skeletal anomalies. Clinical exome sequencing was performed for the patient and his healthy parents using the TruSight One Sequencing Panel and NextSeq550 sequencing system (Illumina Inc., USA).

Results: A novel de novo likely pathogenic variant was identified in the proband in *KAT6B* (NM_012330.3: c.4443_4452del, p.Thr1482ValfsTer64). Clinical re-evaluation following the genetic diagnosis confirmed the presence of typical *KAT6B*-related features. However, in addition to these expected features, the patient was also diagnosed with a brain glioma, a finding that has not been previously reported in individuals with *KAT6B* neurodevelopmental disorders.

Conclusion: *KAT6B* is involved in chromatin remodelling, and mutations in chromatin-related genes have been linked to tumorigenesis. Some histone acetylation genes, including *KAT6B*, play a role in tumor suppression. A loss-of-function variant in *KAT6B* might predispose the patient to glioma development, but further studies are needed to establish a direct link between *KAT6B* mutations and glioma formation.

C.O.I. Disclosure:
K. Vulin: None. **A. Measic:** None. **M. Miklos:** None. **A. Bobinec:** None. **I. Sansovic:** None. **L. Morozin Pohovski:** None. **L. Odak:** None.

EP11.009 Presence of a novel likey pathogenic variant in *KAT5* gene and association with neurodevelopmental disorder

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Introduction Variants in the *KAT5* gene are associated with an autosomal dominant inheritance pattern and the development of a neurodevelopmental disorder characterized by a broad spectrum of cognitive dysfunctions, including: Severe global developmental delay, severe intellectual disability, developmental regression, absent or impaired speech, seizures, hypotonia, sleep disturbances (OMIM#619103). The gene encodes the histone acetyltransferase KAT5 (HTATIP) enzyme, which is involved in chromatin remodeling, transcription, DNA repair, and apoptosis, playing a key role in signal transduction. Variants in *KAT5* gene are linked to a neurodevelopmental disorder characterized by global delay, severe intellectual disability, and behavioral abnormalities.

Case Report A 3-year-old boy has been followed by neurology since September 2023 due to a neurodevelopmental disorder with autism and communication difficulties. Born in Spain to non-consanguineous Nicaraguan parents, he exhibited developmental delay from his first year of life. M-CHAT screening (score: 13, high risk) indicated a global maturational level of 8 months. Clinical features include absent propositional language, lack of response to his name, repetitive behaviors, intense tantrums, hyperactivity, and feeding difficulties. He currently attends a special education classroom. After an unremarkable aCGH, trio exome sequencing identified a likely pathogenic de novo heterozygous variant, c.1115A>G, p.(Tyr372Cys), in exon 9 of *KAT5* gene.

Discussion This variant has not been previously reported in the literature or population databases. However, nearby variants classified as pathogenic (P) or likely pathogenic (LP) have been described in ClinVar. Based on ACMG criteria (PM2, PP2, PP3, PS2), this variant is classified as likely pathogenic, supporting the patient's clinical presentation.

C.O.I. Disclosure:
T. Benitez: None. **N. Padilla:** None. **C. Marti:** None. **M. Miramar:** None. **S. Izquierdo:** None.

EP11.010 A novel variant of NOTCH4 gene as a genetic susceptibility to schizophrenia?

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Background: NOTCH4 has been linked to neuronal development, and the NOTCH4 signaling pathway is recognized as being pivotal in cell fate determination. While an association between the NOTCH gene and schizophrenia has been reported, the results have been inconsistent.The gene that codes NOTCH4 is located in the major histocompatibility complex (MHC) region of 6p21.3 in humans. The MHC region has been hypothesized to play an important role in the aetiology of schizophrenia, in addition to its critical function in regulating the immune system.The present study aims to identify genetic variants associated with schizophrenia and their association with immunity in a non-consanguineous African family.

Material and methods: The present study analysed three subjects, including two siblings diagnosed with schizophrenia and one subject with an immune disease, in addition to an unaffected

mother.Next generation sequencing of whole genome was performed in trio.

Results: The results obtained are as follows: The female subject, aged 36, exhibits acute schizophrenia and immune thrombocytopenia. The brother, aged 32, has been diagnosed with chronic, resistant schizophrenia and hypertrophic cardiomyopathy.

Two variants of unknown significance (VUS) have been identified in the heterozygous state in the NOTCH4 gene: c.395G>A (p.(Arg132His)) in both children with schizophrenia, and c.1459G>A (p.(Gly487Arg)) in both children as well as in the asymptomatic mother. The latter has been classified as pathogenic by all bioinformatic softwares.

Conclusion: The two children are therefore NOTCH4 composite heterozygotes.To date, this is the first report of potential pathogenic variants of the NOTCH4 gene even though classified VUS3, in a familial form of schizophrenia.

C.O.I. Disclosure:

I. Nkam: None. **O. Dufrenne:** None. **C. Verebi:** None. **O. Belfquih:** None. **A. Macone:** None. **A. Pelissolo:** None. **R. Tamouza:** None. **T. Bienvenu:** None. **M. Leboyer:** None.

EP11.011 First molecular diagnosis of Familial Adult Myoclonus Epilepsy type 3 in an Italian family: genetic detection of TTTCA-repeat expansion in *MARCHF6*

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Background: Familial Adult Myoclonus Epilepsy (FAME) is a genetically heterogeneous syndrome linked to TTTCA-repeat expansions in different genes, varying by geographic region. FAME3, caused by an expansion in *MARCHF6* intron one, has been reported in European families but not in Italy. This study presents the first molecular diagnosis of FAME3 in an Italian family

Material and methods: A 44-year-old woman (proband) and her 75-year-old mother underwent clinical and genetic evaluation. Repeat-primed PCR (RP-PCR) was performed to detect expansions in *SAMD12*, *STARD7*, and *MARCHF6* genes.

Results: Neurological evaluation confirmed a clinical diagnosis of FAME in both individuals. The proband experienced upper limb jerks at 40, followed by worsening tremors, dizziness, and seizures over four years. Her mother developed hand tremors at 35, with seizures occurring 15 years later and symptoms worsening over 35 years. RP-PCR revealed *MARCHF6* intronic TTTCA-expansion in both, confirming FAME type 3

Conclusion: This study identifies the first Italian case of FAME3 with a confirmed molecular diagnosis. While FAME2 has been reported in Italy, this case highlights the importance of TTTCA-motif detection for accurate diagnosis and provides further evidence of genetic heterogeneity in FAME

C.O.I. Disclosure:

M. Talarico: None. **I. Sammarra:** None. **A. Giugno:** None. **M. Trimboli:** None. **F. Fortunato:** None. **A. Gambardella:** None.

EP11.012 First two Saudi Children Presented with childhood Onset of MYORG Gene Primary Familial Brain Calcification

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Background: Primary familial brain calcification (PFBC), is one of the rare causes of bilateral basal ganglia calcification.It caused by the mutation of many genes with either autosomal dominant or autosomal recessive mode of inheritance.The MYORG (myogenesis-regulating glycosidase) gene mutation is one of the causes of PFBC, in an autosomal recessive mode. The usual and typically onset of PFBC symptoms is ranges between 35–50 years old including (verbal impairment, movements disorder and basal ganglia calcification).

Material and methods: Retrospectively we did a whole exome sequence in the patient of 12 years of age, presented with typical symptom and signs of PFBC.We also reviewed all reported cases of PFBC syndromes.

Results: In our 12 years old child, who are showing clinical and radiological feature of PFCB, We did the whole exome sequence analysis. The result revealed a homozygous likely pathogenic variant mutation (c.1060_1062del;p.Asp354del) in exon2 of MYORG gene.Subsequently we did segregation in the family. The result revealed that her asymptomatic younger sister (9 years old) has the same homozygous mutation in MYORG gene.We did Brain CT for her and showed symmetrical calcification involving the deep nuclei, bilateral frontal and cerebellar grey-white matter junction.

Conclusion: This is the first two Saudi patient presented with childhood onset of symptomatic PFBC with MYORG mutation.To our knowledge and reported cases of PFBC, only four cases of PFBC presented in childhood.Our recommendation is to consider segregation testing for the children of family with a MYORG gene mutation patients.

C.O.I. Disclosure:

M.A. Bawazir: None. **Y.H. Housawi:** None. **R. Alhazmi:** None. **A.M. Al Hashem:** None.

EP11.013 Blood-based microRNAs as the potential biomarkers for Alzheimer's disease: evidence from a systematic review

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Background: Alzheimer's disease (AD) is a neurodegenerative disorder that progresses over time and is identified by the development of neurofibrillary tangles and amyloid deposits in the brain. Mounting evidence has revealed that microRNAs (miRNAs) are significantly involved in AD progression, and may be used as promising biomarkers for diagnosis and prognosis.

Material and methods: A comprehensive search was performed across various databases (PubMed, EMBASE, Web of Science, Scopus, Google Scholar, Cochrane, and ProQuest), starting from its inception and ending in January 2023. The criteria for inclusion consisted of original research studies written in English, which utilized Real-Time PCR to analyze miRNA expression in the blood, serum, or plasma of AD patients and healthy controls. The extracted data included the miRNA(s) investigated, dysregulation status, study type, human sample(s), and major findings. The search produced 608 records, which after careful examination, resulted in 48 suitable articles for data extraction. The research revealed a wide range of sample types used, with whole blood (39.59%) and serum (27.09%, including serum-exosome at 4.17%) emerging as the most prominent. The compiled dataset featured 4001 AD patients and 3886 healthy controls, revealing intricate regulatory patterns among 83 up-regulated (35.78%), 66 down-regulated (28.44%), and 83 not significantly altered (35.78%) miRNAs.

Results: Our results demonstrated that specific circulating miRNAs are consistently dysregulated in AD and could serve as non-intrusive biomarkers for the identification, prognosis, and prediction of cognitive decline.

Conclusion: miRNAs could serve as non-intrusive biomarkers for the identification, prognosis of AD.

C.O.I. Disclosure:

M.S. khaniani: None. **F. fattahi:** None. **S. Mansoori Derakhshan:** None.

EP11.014 A case of hypomyelinating leukodystrophy type 7 caused by a compound heterozygous mutation in the POLR3A gene in a Yakut family

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North-Eastern Federal University named after M.K. Ammosov, Yakutsk, Russian Federation.

Background: Hypomyelinating leukodystrophy type 7 (OMIM #607694) is an autosomal recessive neurodegenerative disorder characterized by childhood onset of progressive motor decline manifest as spasticity, ataxia, tremor, and cerebellar signs, as well as mild cognitive regression. It is caused by mutations in the *POLR3A* gene, localized on chromosome 10q22.3. Other features may include hypodontia or oligodontia and hypogonadotropic hypogonadism.

Material and methods: A 12-year-old girl, Yakut ethnicity, the only child in the family, is under observation at the Medical and Genetic Center in Yakutsk (Russia). Sequencing of the clinical exome was performed on an Illumina NextSeq-500 sequencer with subsequent validation of the results by Sanger sequencing on an ABI Prism 3500 DNA analyzer (Applied Biosystems).

Results: The child was observed by a neurologist since the age of 2 with complaints of periodic tremors of the body and hands, clumsiness in movements, weakness in the hands and general weakness. From the age of 11, deterioration of vision, memory loss, stumbles when walking, and fine motor skills impairment were noted. The examination revealed cerebellar syndrome, dysarthria, tremors of the head and trunk, short stature, underweight, oligodontia, delayed puberty. According to MRI of the brain, signs of leukodystrophy were revealed. The presence of a compound mutation in the POLR3A gene was revealed: c.3014G>A (p.Gln1190Ter) in exon 27, c.3568C>T in exon 23 (p.Arg1005His).

Conclusion: Despite the rarity of the disease, geneticists and neurologists need to be alert for this pathology. The use of NGS made it possible to establish the exact cause of the disease.

C.O.I. Disclosure:

P.I. Golikova: None. **G.D. Moskvitin:** None. **A.L. Sukhomyasova:** None. **I.A. Nikolaeva:** None. **E.E. Gurinova:** None. **A.E. Yakovleva:** None. **N.R. Maksimova:** None.

EP11.015 Advances and perspectives in epilepsies of genetic origin, case report

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Background: The PCDH19 gene was initially identified in 2008 as responsible for epilepsy and mental retardation limited to females. In 2009, the first mosaic deletion of the gene was described in a male patient, revealing a unique X-linked inheritance pattern. Both heterozygous females and males with mosaicism are affected.- Dravet syndrome is a rare and severe childhood epilepsy, some variants of the PCDH19 gene, encoding protocadherin 19, are associated with a Dravet syndrome-like phenotype, known as DS-like

Material and methods: This case describes a 15-year-old male patient with a clinical picture since the age of 10 months consisting of generalized tonic-clonic seizures, accompanied by moderate intellectual disability with compromised behavioral and adaptive skills, the product of non-consanguineous parents

Results: Given the age of presentation, characteristics, progression and the need for multiple anticonvulsants, de novo epilepsy of genetic origin was suspected, so whole exome sequencing and copy number variant (CNV) analysis of 110 genes associated with refractory epilepsy was performed, the results showed a pathogenic hemizygous variant in the PCDH19 gene

Conclusion: Genetic factors are the major contributors to the cause of epilepsy in up to 80% of epileptics. Research to elucidate the genetic landscape of epileptic and developmental encephalopathies contributes to the elucidation of molecular pathogenesis and the development of personalized targeted therapies in these disorders. Since epilepsy and cognitive disorders have advanced in the knowledge of their genetic cause, it is important a specific diagnosis, in order to establish targeted and personalized treatments

C.O.I. Disclosure:

C.L. Delgado Salcedo: None. **L.J. Moreno Giraldo:** None.

EP11.016 Unraveling the genetic complexity of neurodevelopmental disorders: insights from autosomal recessive and de novo variants in a consanguineous family

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Background: Neurodevelopmental disorders (NDDs) are genetically and clinically heterogeneous conditions caused by disruptions in brain development. Utilizing multiple analytical pipelines and platforms enhances variant detection and improves diagnostic accuracy. Here, we aimed to investigate the genetic basis of an undiagnosed NDD case by applying diverse approaches.

Material and methods: A consanguineous family, including first-cousin parents, an affected male proband, and unaffected siblings was recruited. The family history included a sibling and cousins, all with neurodevelopmental symptoms who died in early childhood. Trio whole-exome sequencing (WES) was performed for the proband and parents via Oxford Gene Technology, supplemented by whole-genome SNP genotyping-based linkage and CNV analyses. WES data were analyzed using an in-house pipeline and the Genomize-SEQ platform.

Results: Given the family history and parental consanguinity, the initial analysis prioritized autosomal recessive inheritance, identifying homozygous missense variants in *DOCK8* and *LAMC3*. To account for de novo variants, we extended the analysis, identifying additional candidates in *ASH1L*, *ATAD3A*, and *COL12A1*, all linked to NDDs. *ATAD3A* and *COL12A1* were excluded due to maternal inheritance. The *ASH1L* variant appeared de novo via IGV visualization. Validation and segregation with Sanger sequencing are ongoing.

Conclusion: Although the family history suggested an autosomal recessive pattern, we identified a likely pathogenic de novo *ASH1L* variant. This may explain the proband's phenotype, while other affected relatives could have distinct homozygous variants. The *ASH1L* variant may contribute to a complex phenotype resulting from multiple genetic factors.

C.O.I. Disclosure:

S. Susgun: None. **Y. Kesim:** None. **B. Salman:** None. **E. Yucesan:** None. **F. Tuncer:** None. **M. Calik:** None. **G. Altiokka Uzun:** None. **U. Ozbek:** None. **S. Ugur Iseri:** None.

EP11.017 Digging with two ANDS: exploring phenotypical variability in ANKS1B microdeletions

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Background: Neurodevelopment Disorders (NDD) are caused by Single Nucleotide Variants (SNV) as well as Copy Number Variations (CNV). Heterozygous microdeletions encompassing the entire ANKS1B gene (OMIM: 607815), or partial deletions affecting specific exons, have been associated with ANKS1B Neurodevelopmental Syndrome (ANDS). Gene haploinsufficiency leads to a reduction in AIDA-1 (APP Intracellular Domain Associated-1) expression at neural synapses, clinically manifesting as autism spectrum disorder (ASD,) verbal apraxia, and motor coordination difficulties. Here, we present two patients exhibiting phenotypic heterogeneity associated with this NDD condition, thus sharing a peculiar telecant-like eyelids conformation. Additionally, one of the children exhibited language delay in her clinical history.

Material and methods: Following pre-test genetic counselling, peripheral blood samples were collected, and SNP-array (SNPa) analysis was performed.

Results: SNPa analysis revealed ANKS1B microdeletions in both children: -the first patient carried a ~ 200 Kb microdeletion spanning exons 5–9 (arr[GRCh37]12q23.1(99994118_100184812) x1), -the second patient carried a similar CNV, ~ 118 Kb, including exons 5–8. Parental segregation analysis was conducted revealing a de novo CNV in one case, while in the other, the CNV was inherited from the father.

Conclusion: The genomic analysis confirmed ANKS1B microdeletions in both patients, each presenting with a variable clinical phenotype. Given the limited number of reported cases with 12q23.1 microdeletions involving ANKS1B, and phenotypic heterogeneity observed in affected individuals, we aim to discuss these two newly identified cases to enhance our understanding of ANDS. Our goal is to further characterize the condition and identify common clinical features to facilitate improved diagnosis and management.

C.O.I. Disclosure:

S. Lattarulo: None. **M. Piglionica:** None. **M. Iacoviello:** None. **Y. Krylovska:** None. **S. Martino:** None. **C. Simone:** None. **E. Meneleo:** None. **M. Stasi:** None. **M. Chetta:** None. **N. Resta:** None. **N. Bukvic:** None.

EP11.018 Late-onset autosomal recessive spastic ataxia caused by a homozygous p.V723M pathogenic variant in the AFG3L2 gene

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Background: Heterozygous mutations in AFG3L2, a mitochondrial ATP-dependent metalloprotease, are well known to cause autosomal-dominant spinocerebellar ataxia type 28 (SCA28), which is characterized by very slowly progressive gait and limb ataxia. In contrast, homozygous AFG3L2 missense mutations typically cause early-onset spastic ataxia type 5 (SPAX5), which includes severe progressive myoclonus and epilepsy. Recently, patients harboring the c.2167G>A (p.V723M, NM_006796.3) mutation together with either a truncating or another established SPAX5 mutation in trans have been reported in adult-onset slowly progressive ataxia, thereby extending the phenotypic spectrum of biallelic AFG3L2 mutations.

Material and methods: Exome sequencing (Twist Comprehensive Exome + Mitochondrial Panel) and analysis of multigene panel for ataxia.

Results: Here we report a 55-year-old Austrian woman with unsteadiness from age 50, who presented with gait ataxia, upper and lower limb dysmethria, dysarthria as well as mild lower extremity spasticity and dystonic posturing of the left leg during walking. Neuropsychological evaluation showed deficits in information processing, concentration and working memory. MRI gave evidence of pronounced cerebellar atrophy, DAT-imaging did now show dopaminergic basal ganglia deficits. Genetic testing revealed a homozygous p.V723M mutation in the AFG3L2 gene. Family history was negative, the patient's parents were not known to be related, suffered from Parkinson's disease and dementia and died at age 82 and 90.

Conclusion: A homozygous p.V723M AFG3L2 mutation has not been previously reported. The clinical picture of our patient, affected by mild, late-onset spastic ataxia rather corresponds to SCA28 than to SPAX5 and confirms that p.V723M acts as a hypomorphic variant.

C.O.I. Disclosure:

R. Praschberger: None. **C. Überbacher:** None. **S. Burkhard:** None. **B. Mühlegger:** None. **M. Witsch-Baumgartner:** None. **S. Boesch:** None. **J. Zschocke:** None. **S. Rudnik-Schöneborn:** None.

EP11.019 Brain small vessel disease 1 type in Ukrainian child with multiple anomalies, global developmental delay and structural epilepsy

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Background: Brain small vessel disease with or without ocular anomalies (OMIM 175780) is congenital collagenopathy, associated with COL4A1 gene, autosomal-dominant (AD) inheritance, clinically has porencephaly, seizures, intellectual disability, spasticity and ocular anomalies.

Material and methods: Genetic counselling of 2 years old boy with walking delay, seizures, visual impairment, developmental

and speech delay has been conducted. Child was born from second pregnancy, complicated by viral infection, second delivery, complicated by arterial hypertension. Prenatally on the 18–19th week - polyhydramnios. Ophthalmological diagnosis - congenital anomalies of the eye (partial atrophy of optic nerves discs, capsular cataract). Neurological findings: brain congenital anomaly (porencephaly), hypoxic encephalopathy, spastic tetraparesis, structural epilepsy, developmental delay. Instrumental methods: electroencephalogram - epileptiform interictal activity, multi-regional complexes in the right fronto-central and parietal-temporal loabs; brain MRI - porencephaly, atrophic-gliotic changes of the right frontal lobe. Phenotype: microcephaly, minimum threshold of stigmatization. Hereditary history is not burdened. NGS-based molecular testing and standard chromosome analysis have been used for differential diagnostics.

Results: Standard karyotyping result - without pathology. Using NGS one possibly pathogenic heterozygous variant c.1793_1795del (p.Gly598del) of the gene COL4A1 has been detected, genetic diagnosis of congenital brain small vessel disease, AD, de novo variant has been confirmed.

Conclusion: Presence of multiple anomalies, developmental, speech and motor delay, neurological symptoms require genetic counseling with molecular testing to clarify the genetic diagnosis, to predict the inheritance of the disease, to figure out the treatment and monitoring plan of the patient.

C.O.I. Disclosure:

N.A. Pozhar: None. **T. Orlova:** None. **V. Galagan:** None. **V. Serafymovych:** None. **H. Fedushka:** None. **M. Filozop:** None. **O. Skrypnichenko:** None.

EP11.020 Lamb-Shaffer Syndrome: first case reported with the c.567delA (p.Thr191Leufs*5) variant in the SOX5 Gene

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Background: Lamb-Shaffer Syndrome (LSS) (OMIM #616803) is a genetic neurodevelopmental disorder caused by haploinsufficiency of the *SOX5* gene, located on chromosome 12p12.1. LSS is characterized by global developmental delay, behavioral alterations (including autism and hyperactivity), and dysmorphic facial features. This syndrome has a prevalence of <1/1,000,000.

Material and methods: A 13-year-old patient, with no parental consanguinity, had an uncle with epilepsy, an aunt with cognitive disorder, and a cousin with autism. The patient exhibited developmental delay and, at 18 months of age, experienced an episode characterized by perioral cyanosis and hypotonia. She was evaluated by a neurologist, who performed an electroencephalogram that revealed right parietal focal epilepsy. At age 7, she was diagnosed with a cognitive disorder. Upon referral to a genetics specialist, minor facial anomalies were identified, but no specific syndrome was diagnosed. A trio exome sequencing study was ordered.

Results: The heterozygous de novo variant c.567delA (p.Thr191Leufs*5) in the SOX5 gene was identified. Variants located within the HMG domain are classified as pathogenic because this domain constitutes the central functional region of the transcription factor. Although the variant identified in this patient has not been previously reported in the literature, it is classified as pathogenic according to ACMG criteria. In a cohort of previously described patients, a nonsense variant located near the one reported here was associated with similar phenotypic features.

Conclusion: A novel variant in the SOX5 gene causing LSS is reported. Understanding this condition is essential to establish a prognosis, facilitate interdisciplinary management, and provide genetic counseling.

C.O.I. Disclosure:

L.V. Silva: None. **D. Contreras:** None. **G. Contreras:** None.

EP11.021 Analysis of *APOE* ε2, ε3 and ε4 haplotypes in healthy controls, patients with Alzheimer's disease, frontotemporal, and unspecified dementia from Bulgaria

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Background: The E2, E3 and E4 alleles of apolipoprotein E (*APOE*) are respectively considered as protective, neutral and risk genetic factors for the development of the most common form of dementia-Alzheimer's disease (AD). In the present study we have compared, for the first time, the occurrence of *APOE* haplotypes in individual DNA samples of patients with different forms of dementia, healthy controls and cognitively healthy centenarians from Bulgaria.

Material and methods: We have compiled data for 36 dementia patients: 10 with AD, 9 with early-onset AD (EOAD), 6 with frontotemporal dementia (FTD) and 11 with non-AD, non-FTD i.e. unspecified dementia (UD). Eighteen of the patients were analyzed by next generation sequencing after long-range PCR of *APOE*. The remaining 18 patients and the 67 controls (average age 39,9 years) were tested by real-time PCR genotyping, whereas centenarians were whole genome sequenced. Obtained genotype counts were compared pairwise by Fisher's test.

Results: The E2 allele has occasional presence among dementia patients and controls (5–10%), E3 is the only allele found in centenarians and E4 prevails among AD and EOAD patients (>40%). The pairwise genotype comparisons (Table) were significant only between healthy controls and AD and EOAD patients (p-value 0.0377 and 0.0109, respectively).

Conclusion: The E4 allele plays role in the etiology of AD, which is not the case with UD and FTD; whereas healthy mental aging is not associated with the E2 allele. This findings should be further evaluated in larger samples.

APOE genotypes in the analyzed samples

	AD	EOAD	FTD	UD	Controls	Centenarians
E2/E3	0	1	1	1	7	0
E2/E4	1	0	0	1	0	0
E3/E3	3	1	5	5	52	16
E3/E4	5	6	0	3	8	0
E4/E4	1	1	0	1	0	0

C.O.I. Disclosure:

S. Karachanak-Yankova: None. **L. Balabanski:** None. **R. Vazharova:** None. **M. Atanasoska:** None. **S. Yaneva:** None. **O. Antonova:** None. **V. Spasova:** None. **S. Peykov:** None. **M. Stancheva:** None. **A. Ahmed:** None. **D. Serbezov:** None. **S. Mehrabian:** None. **D. Belejanska:** None. **M. Petrova:** None. **L. Traykov:** None. **S. Hadjidekova:** None. **D. Tocncheva:** None.

EP11.022 Case report: A truncating *HNRNPH2* variant in a female patient with cognitive regression

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Background: The *HNRNPH2* gene encodes a protein crucial for RNA processing and transport. X-linked *HNRNPH2* pathogenic variants cause a Rett-like neurodevelopmental disorder (NDD) characterized by developmental delay, intellectual disability, growth delay, and epilepsy. Additional features include developmental regression, strabismus, acquired microcephaly, stereotypic movements, and psychiatric conditions such as anxiety and autism. Most patients harbor de novo missense variants in females or males. These gain-of-function variants often affect regions near or within the nuclear localization signal (NLS), leading to protein mislocalization and impaired RNA metabolism. Here we report a female with a truncating *HNRNPH2* variant and an atypical clinical presentation.

Material and methods: A 16-year-old female presented with childhood-onset cognitive decline, autism spectrum disorder, poorly controlled epilepsy, macrocephaly, relatively short stature, and no dysmorphic features. Chromosomal microarray (CMA) and Fragile X testing were normal, prompting trio exome sequencing.

Results: A de novo *HNRNPH2* variant, c.969del; p.(Asp324Metfs*38) (NM_019597.5), was identified. This frameshift in exon 2 within the RNA recognition motif (RRM) domain truncates ~20% of the protein, likely impairing function. This and other loss-of-function (LOF) variants are absent from population databases (e.g., gnomAD). Previously, only missense variants were associated with disease in females, while recently, truncating variants in males were linked to milder NDD and growth retardation were reported.

Conclusion: This case expands the spectrum of *HNRNPH2*-related disorders, highlighting the first reported truncating variant in a female. Further studies are essential to elucidate the pathogenicity of truncating variants and their phenotypic impact.

C.O.I. Disclosure:

G. Shalev-Malul: None. **S. Bratman-Morag:** None. **T. Abdalla Moady:** None. **T. Paperna:** None. **K. Weiss:** None.

EP11.023 Pathogenic variant in *OGT* resulting in marked reduction of enzyme activity in a child with severe developmental delay and hepatoblastoma

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Background: O-GlcNAc transferase (OGT) and its antagonist O-GlcNAcase (OGA) regulate a highly conserved protein post-translational modification involved in metabolic sensing. Pathogenic variants in the encoding gene OGT cause an X-linked disorder presenting developmental delay, hypotonia, intellectual disability and dysmorphic features. Here, we report a child with a previously unreported *OGT* pathogenic variant who developed hepatoblastoma.

Material and methods: *OGT* variant was detected by exome sequencing. Global O-GlcNAcylation and enzyme expression were evaluated on cells derived from the proband or in cells expressing wild-type (WT) or mutant (Pro942Leu) OGT. Enzyme activity was investigated in vitro using different substrate glycosylation assays.

Results: The proband was found to have cardiomyopathy, microcephaly, and severe developmental delay. At the age of 15 months he was diagnosed with hepatoblastoma that caused death at 3 years. A de novo variant p.(Pro942Leu) in *OGT* was found by exome sequencing and classified as likely pathogenic by ACMG criteria. Western blot on patient-derived fibroblasts showed slightly reduced O-GlcNAcylation and reduced OGA expression. Murine embryonic stem cells expressing the mutant OGT showed compensatory reduction of OGA likely resulting in unchanged global O-GlcNAcylation. Purified P942L OGT showed significantly reduced activity in vitro.

Conclusion: Pathogenic variants in *OGT* are responsible for an intellectual disability syndrome reported in less than 20 cases. To date, no patients have been reported with hepatoblastoma or other malignancies. Although the occurrence of hepatoblastoma in the proband might be coincidental, the role of O-GlcNAcylation in cancer suggests that marked reduction in OGT actvity might increase susceptibility to cancer development.

C.O.I. Disclosure:

A. D Alessio: None. **H. Yuan:** None. **I. Boffa:** None. **L. Soria:** None. **B. Attianese:** None. **N. Cullinan:** None. **S. Lynch:** None. **D. van Aalten:** None. **M. O'Sullivan:** None. **S. Basse Hansen:** None. **N. Brunetti-Pierri:** None.

EP11.024 Expanding the allelic and clinical heterogeneity of movement disorders linked to defects of mitochondrial ATP synthase

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Background: Mitochondrial ATP synthase (ATPase) defects are an emerging yet not fully understood cause of neurodevelopmental disorders characterized by abnormal movements. This study aims to expand the understanding of the phenotypic and mutational spectrum of movement disorders associated with ATPase subunit-encoding genes *ATP5F1A* and *ATP5F1B*.

Materials and methods: We recruited regionally distant patients who had undergone genome or exome sequencing. Fibroblast cultures from two patients were established to perform RNA sequencing, immunoblotting, and ATPase activity assays.

Results: We identified a patient with developmental delay, myoclonic dystonia, and spasticity carrying a heterozygous frameshift variant c.1404del (p.Glu469Serfs*3) in *ATP5F1A*. This patient's cells showed significant reductions in *ATP5F1A* mRNA, ATPase α-subunit expression, and ATPase activity. Additionally, we discovered a novel deleterious missense variant c.1252G>A (p.Gly418Arg) in *ATP5F1A*, present in three patients from two families with hereditary spastic paraplegia (HSP). This variant affects a critical intersubunit communication site. A third variant, c.1074+1G > T, occurred in the donor splice site of *ATP5F1B*, resulting in exon skipping and reduced *ATP5F1B* mRNA levels, as well as impaired ATPase activity. The associated phenotype included cerebral palsy (CP) with prominent generalized dystonia.

Conclusion: Our findings confirm and broaden the role of dominant *ATP5F1A* and *ATP5F1B* variants in neurodevelopmental movement disorders. *ATP5F1A*- and *ATP5F1B*-related ATPase diseases should be considered a potential cause of dystonia, HSP, and CP.

C.O.I. Disclosure:

P. Harrer: None. **M. Krygier:** None. **M. Krenn:** None. **V. Kittke:** None. **A. Saparov:** None. **V. Pichon:** None. **M. Malbos:** None. **C. Scherer:** None. **I. Dzinovic:** None. **M. Skorvanek:** None. **R. Kopajtich:** None. **H. Prokisch:** None. **S. Silvaieh:** None. **A. Grisold:** None. **M. Mazurkiewicz-Bełdzińska:** None. **J. de Sainte Agathe:** None. **J. Winkelmann:** None. **J. Necpal:** None. **R. Jech:** None. **M. Zech:** None.

EP11.025 First report of a familiar *MYCBP2* pathogenic variant: expanding the knowledge of neurodevelopmental disorders

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Background: Over the past five years, the *MYCBP2* has emerged as a potential candidate gene associated with neurodevelopmental disorders (NDDs). In 2023, a newly characterized condition, termed *MYCBP2*-related developmental delay with corpus callosum defects (MDCD), has been reported in eight individuals presenting corpus callosum abnormalities, developmental delay, intellectual disability, epilepsy and autistic features. To date, all reported cases have been associated with distinct de novo variants. Here, we describe the first family with three affected members carrying a novel pathogenic *MYCBP2* variant.

Material and methods: A mother and her two sons were referred to our laboratory for genetic testing. The phenotypic characteristics of our three patients included intellectual disability, speech impairment, facial dysmorphism, microcephaly and seizures (presented only in the mother). We performed WES on the three affected family members using Twist Human Core +RefSeq+Mitochondrial Panel on Illumina Nova Seq 6000.

Results: We have identified a novel loss of function variant in *MYCBP2* [NM_015057.5 c.7311del, p.(Leu2438Trpfs*3)] in heterozygous state in the mother and her two sons. The variant causes a frameshift, leading to NMD and C-terminal truncation, eliminating key domains (RING and TC) essential for ubiquitin ligase activity and interactions with other cellular components, particularly in processes related to synaptic regulation.

Conclusion: Here, we present the clinical manifestation of *MYCBP2*-related developmental delay in three patients of a same family. Our study further supports the critical role of *MYCBP2* in the pathogenesis of NDDs and contributes to the genotype/phenotype correlation of a recently recognized MDCD syndrome.

C.O.I. Disclosure:

I. Maleva Kostovska: None. **P. Noveski:** None. **E. Sukarova-Angelovska:** None. **D. Plaseska-Karanfilska:** None.

EP11.026 A second case study: neurodevelopmental disorder linked to rare INTS8 gene mutation

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Introduction: In 2017, a family was identified with a rare neurodevelopmental disorder characterized by spasticity and cerebellar hypoplasia, attributed to a compound heterozygous mutation in the *INTS8* gene. This report presents the second documented case of this unique condition. **Patient summary** A 6-year-old girl, third child in her family, referred for diagnostic clarification due to significant developmental delays. The patient was born prematurely at 34 weeks via emergency cesarean section and required mechanical ventilation during the first day of life, followed by care in the neonatal pathology unit with a prior diagnosis of perinatal CNS injury. Her developmental milestones were delayed: she began rolling over at 6 months, sitting at 1 year, and walking at 2.5 years. At age 3, she was diagnosed with atonic-astatic cerebral palsy, delayed speech/language development and stereotypical behaviors. At age 4, she started experiencing "freezing" episodes, and an EEG revealed diffuse epileptiform activity. On physical examination, her height was 109 cm, weight 14.9 kg, and head circumference 49 cm. Notable dysmorphic features included a low hairline, hypertelorism, epicanthic folds, a broad nasal bridge, and hirsutism on the back and arms. Episodes of tachypnea with apnea were also observed. **Genetic investigation** Genetic studies revealed a karyotype of 46,XX. Whole-exome sequencing identified a previously unreported pathogenic variant in the splice acceptor site of intron 1 of the *INTS8* gene, detected in a homozygous state, resulting in a nucleotide deletion (NM_017864.4: c.131-7_131-2del).

Conclusion: This case highlights the importance of genetic analysis in understanding rare neurodevelopmental disorders.

C.O.I. Disclosure:

V.V. Zabnenkova: None. **M.S. Petukhova:** None. **O.P. Ryzhkova:** None.

EP11.027 A Novel TUBA1A Variant Associated with Lissencephaly in a Pediatric Patient: A Case Study

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Background: A 5-year-old boy presents with complex congenital brain malformation characterized by lissencephaly, hypoplasia of the temporal lobes and cerebellum, Dandy-Walker variant, and ventricular enlargement. He has severe combined intellectual and motor disabilities, symptomatic epilepsy with neonatal seizures (well-controlled on valproate), and Hirschsprung's disease, along with significant failure to thrive and multiple nutritional deficiencies. Chromosomal analysis at the karyotype level and the Array CGH profile were normal. Massively parallel sequencing of a custom panel revealed no pathogenic variants on *RET* gene.

Material and methods: Whole-exome sequencing (WES) was conducted using the SSELXT HS Human All Exon V8 + NCV (Agilent) on the NextSeq 500/550 DX (Illumina), with bioinformatic analysis performed by GENOVESA (Bioxys) using a virtual panel focusing on lissencephaly (HPO: 0001339).

Results: WES identified a heterozygous "de novo" variant in the *TUBA1A* gene HGVS/HGVp:TUBA1A(NM_006009.4): c.520G>A p.Ala174Thr; dbSNP:- classified as class 4 (deep reads 37), and negative for mutations in other lissencephaly-associated genes, confirmed by Sanger sequencing.

Conclusion: Pathogenic variants in the *TUBA1A* gene are linked to Lissencephaly 3 (OMIM:611603) with autosomal dominant inheritance. Clinical features include microcephaly, profound intellectual disability, spastic tetraplegia, and uncontrolled tonic-clonic seizures. These findings help explain some of the phenotypic features observed in this patient.

C.O.I. Disclosure:

M. Langova: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Supported by Ministry of Health, Czech Republic – conceptual development of research organization (Thomayer University Hospital - TUH, 00064190). **K. Brozova:** None. **N. Grosup Friedova:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Supported by Ministry of Health, Czech Republic – conceptual development of research organization (Thomayer University Hospital - TUH, 00064190). **R. Kejkulova:** None. **J. Tajtlova:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Supported by Ministry of Health, Czech Republic – conceptual development of research organization (Thomayer University Hospital - TUH, 00064190).

EP11.028 The case of microduplication Xp11.23p11.22 in a child with frequent tonic-clonic seizures

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Background: The dupXp11.23p.11.22 syndrome was first described in 2009. Clinically, it can manifest as epilepsy, clonic-tonic seizures, speech development disorders, autism spectrum disorders, mental retardation.

Material and methods: clinical case, clinical-genealogical, molecular and cytogenetic research methods.

Results: The girl, one year old with frequent tonic-clonic seizures (up to 9 seizures per day), delayed physical and psychomotor development was referred to a geneticist to exclude hereditary disorders. From the first months of life, a delay in stato-kinetic development was observed. During the examination, the child is communicative, smiles, confidently holds the body in a sitting position, does not stand independently, does not walk, does not show motor initiative, pronounces individual sounds, syllables, but does not speak words. MRI of the brain: stable macrocrania that does not develop, with moderate paracerebral effusion of a benign type. Phenotype: macrocephaly, high convex forehead, overhanging upper eyelid, asymmetrical almond-shaped palpebral fissures, epicanthal fold, narrow upper lip, sharp chin, oval face, low-set dysmorphic auricles, short neck, hypertelorism of the nipples, flat feet, clinodactyly of the 5th toe. According to the results of genetic studies, a microduplication of the short arm of the X chromosome was detected: karyotype 46,X,dup(X)(p11.23-p11.22); Array-CGH arr[grch37] Xp11.23-Xp11.22 (44215686_50657312)x3, duplication size: 6.4 Mb, in which 28 pathological genes are involved. The child was diagnosed with: Chromosomal aberration. Microduplication syndrome Xp11.23p11.22.

Conclusion: The child was recommended to be observed by a pediatric neurologist and receive rehabilitation therapy. Array-CGH was recommended to the closest relatives to exclude mosaic carriership or carriership of the duplication in an inactivated state.

C.O.I. Disclosure:

V. Antsupova: None. **I. Lastivka:** None. **O. Polodiyenko:** None. **L. Sheiko:** None. **L. Brisevac:** None. **V. Ostapchuk:** None. **I. Ushko:** None.

EP11.029 Familial nonprogressive microcephaly caused by a novel splice variant in the*VRK1 gene*

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Introduction: The *VRK1 gene* encodes a member of the vaccine-related kinase family of serine/threonine protein kinases and is widely expressed in human tissues, especially in actively dividing cells. Several phenotypes, including pontocerebellar hypoplasia, spinal muscular atrophy and hereditary motor and sensory neuropathies, have been associated with mutations in the *VRK1 gene*. The full spectrum of the phenotypes and their associations with various mutations affecting the function of *VRK1* is unclear.

Cases: We present two brothers, now five and 23 months old, with isolated microcephaly born after full-term pregnancy. The parents are young and healthy. Brain MRI showed a small neurocranium with inferior vermian hypoplasia in both brothers. The older brother had a hypoplastic corpus callosum, and agenesis of the corpus callosum was observed in the younger brother. Brothers have delayed motor development with normal cognition and severe nonprogressive microcephaly. The older brother sat at 20 months and now he is walking with support. Clinical exome sequencing was performed and a novel

homozygous pathogenic null variant in the *VRK1 gene c.1160-2A > C* was detected. Familial segregation analysis showed that the parents are carriers, and confirmed that the younger brother is homozygous.

Conclusion: It is important to consider mutations in the *VRK1 gene* during the evaluation of patients with severe nonprogressive microcephaly and delayed motor development. The homozygous or compound heterozygous mutations in the *VRK1 gene* may lead to a spectrum of clinical phenotypes ranging from mild to severe. Our finding extends different mutations of *VRK1 gene* with clinical phenotype associated with microcephaly.

C.O.I. Disclosure:

M. Vidakovic: None. **K. Gotovac Jercic:** None. **M. Oroz:** None. **F. Stipoljev:** None. **S. Huljev Frkovic:** None.

EP11.030 The Effect of Amyloid Beta 1-42 and 1-40 on the Expression of Mitochondrial DNA-Encoded Genes

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Background: Amyloid beta 1-42(Aβ1-42) and 1-40(Aβ1-40) are derived from APP via β- and γ-secretase cleavage. Aβ1-42,being more hydrophobic and fibrillogenic, drives amyloid plaque formation in Alzheimer's disease (AD).Aβ1-40 is the most abundant Aβ peptide, followed by Aβ1-42. Aβ1-42 localizes to mitochondria,though its role is unclear.Mitochondrial gene expression changes in AD suggest mtDNA involvement.This study investigates the dose-dependent effects of Aβ1-42 and Aβ1-40 on mtDNA-encoded gene expression in HEK cells.

Material and methods: HEK 293T cells were treated with 0.1μM and 1μM Aβ1-42 as well as Aβ1-40.RNA isolation was performed at 24 and 48 hours.qRT-PCR analyzed 13 respiratory complex genes,2 mitochondrial rRNA,and 3 mitochondrial tRNA genes.Statistical analysis was conducted using one-way ANOVA in GraphPad Prism 8.

Results: At 24 hours after Aβ1-42 treatment,MTND3, MTND4,MTND4L,MTND5,and MTATP6 expression levels differed significantly among groups,with a decrease in the 0.1μM-treated group.At 48 hours, MTND4L,MTCO2, and MTATP8 were significantly altered among the three groups,where MTCO2 expression decreased in the 0.1μM group, while MTND4L and MTATP8 expression levels decreased in the 1μM group compared to controls.In contrast,Aβ1-40 treatment caused no significant changes at 24 hours.However,at 48 hours, MTCO2 expression increased in the 0.1μM-treated group,while MTND3,MTND4, MTCO2,MTCO3, MTATP6,MTATP8,and MTtRNA2 expression levels increased in the 1μM group.However, a statistically significant decrease in MTND4L expression was observed in the 1μM Aβ1-40-treated group compared to the control group.

Conclusion: The observed changes in mitochondrial gene expression following Aβ1-42 and Aβ1-40 treatment suggest their role in regulating mtDNA-encoded genes.This study lays the foundation for understanding Aβ mediated changes in mtDNA gene expression and its implications in AD pathology.

C.O.I. Disclosure:

B.N. Bilge: None. **Z. Yurttaş:** None. **T. Çamoğlu:** None. **B. Şengül Yediel:** None. **S. Ildiz:** None. **N. İlhan:** None. **E. Dursun:** None. **D. Gezen Ak:** None.

EP11.031 Unraveling amyloid peptide effects on cellular transcriptome, an RNA-Seq approach

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Background: Amyloid precursor protein cleavage by beta-secretase (BACE) and gamma-secretase generates peptides of 37–43 amino acids, including amyloid beta 1–42 (Aβ1-42) and Aβ1-40. Aβ1-42 is a key pathological component, and its physiological roles have gained attention. This study explores the effects of amyloid peptides on cellular gene expression using RNA sequencing and functional enrichment analysis.

Material and methods: HEK cells were treated with varying doses of a BACE1 inhibitor, with 200 nM for 24 hours identified as the most effective concentration via western blot. Total RNA was isolated from treated and DMSO controls cells, followed by sequencing on the Illumina NovaSeq 6000 platform. RNA-Seq data were analyzed using nf-core/RNAseq v3.17.0 within the NEXT-FLOW v24.10.2 framework. Differentially expressed genes (DEGs) were identified using DEseq2 v1.4.2 in RStudio, and functional enrichment analysis was performed with the DAVID tool. Protein-protein interaction networks were constructed using stringApp v2.2.0 and cytoHubba v0.1 in Cytoscape v3.10.13.

Results: A total of 62 DEGs were identified between BACE1 inhibitor-treated and DMSO-treated cells. These DEGs were primarily associated with 17 clusters, mainly related to cell adhesion, signaling, and endoplasmic reticulum function.

Conclusion: Our findings suggest that amyloid peptides influence multiple cellular functions. The involvement of DEGs in cell signaling and adhesion pathways may provide insights into early neurodegenerative processes. These results contribute to a better understanding of the molecular mechanisms underlying amyloid-related pathologies.

This study was supported by Istanbul University-Cerrahpaşa BAP (ID:37406) and TÜBİTAK (ID: 219Z179).

C.O.I. Disclosure:

T. Çamoğlu: None. **Z. Yurttaş:** None. **O. Duzenli:** None. **E. Yucesan:** None. **E. Dursun:** None. **D. Gezen-Ak:** None.

EP11.032 Investigation of the interaction of Aβ1-40 with mitochondrial DNA under physiological conditions

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Background: Amyloid beta 1-40(Aβ1-40) is the most abundantly produced amyloid peptide, generated through the proteolytic cleavage of the APP protein.Although Aβ1-40 is less associated with Alzheimer's Disease (AD), it is present in small amounts within Aβ1-42 plaques. Mitochondrial dysfunction is a key cellular process in AD, with mitochondrial gene expression changes playing a crucial role. Additionally, Aβ aggregation has been observed in mitochondria under pathological conditions.While Aβ is known to interact with the promoter regions of certain target genes,the ability of Aβ1-40 to interact with DNA—an amyloidogenic protein characteristic—remains poorly understood. Given that Aβ1-40 is abundantly produced in cells and its mitochondrial functions are not well characterized,it may influence mitochondrial gene expression by interacting with mitochondrial

DNA(mtDNA).The aim of our study is to investigate the interaction between Aβ1-40 and mtDNA under physiological conditions.

Material and methods: In our study, the physical interaction between Aβ1-40 and mtDNA was investigated in SH-SY5Y cells using the chromatin immunoprecipitation (ChIP) method.Following the ChIP assay, DNA fragments were analyzed by qPCR using specific primer pairs covering the mitochondrial genome.

Results: Our findings indicate that, under physiological conditions,the Aβ1-40 peptide interacts with a region within the mtDNA-D-Loop, albeit with a very low signal(fold enrichment).Additionally, Aβ1-40 was found to interact with approximately 16 specific regions outside the D-loop within the mitochondrial genome

Conclusion: Our study demonstrates that Aβ1-40, can associate with certain regions of mtDNA.Although the effect of this interaction on mitochondrial gene expression remains unknown, this finding reveals a novel characteristic of Aβ1-40.This study was supported by TÜBİTAK(ProjectID: 219Z179).

C.O.I. Disclosure:

Z. Yurttaş: None. **T. Çamoğlu:** None. **E. Dursun:** None. **D. Gezen Ak:** None.

EP11.033 Genetic influences on separation-individuation: Examining dopaminergic, serotonergic, and oxytocinergic pathways in emerging adults

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Background: Separation-individuation is a fundamental process in developmental psychology and is linked to emotional, psychological and psychosocial functioning. While the outcome of the process is considered primarily dependent on the nature of the child-parent relationship, genetic factors might also play a role. The present study examined several polymorphisms, within genes in relevant neuromodulatory pathways, in relation to aspects of separation-individuation in emerging adulthood.

Material and methods: We included polymorphisms in the dopamine pathway *COMT* (rs4680, rs165815), serotonin pathway *TPH2* (rs1843809, rs7305115, rs4570625, rs4290270) and oxytocin pathway *OXTR* (rs53576, rs968389, rs2268498). The sample comprised 332 emerging adults ($M_{age} = 21.97$ years, $SD = 2.48$, 56.9% female), who provided buccal swabs and completed a measure of separation-individuation in emerging adulthood in relation to mothers and fathers respectively.

Results: Our main findings include SNP-SNP interaction effects between several polymorphisms. These include: *COMT* rs4680 x rs165815 on self-reliance in mothers ($F = 5.375$; $p = .021$; $\eta p^2 = .017$) and fathers ($F = 4.339$; $p = .038$; $\eta p^2 = .014$), *TPH2* rs4290270 x rs4570625 on levels of perceived intrusiveness in relation to mothers ($F = 11.173$, $p = .001$, $\eta p^2 = .020$) and fathers (($F = 6.400$, $p = .012$, $\eta p^2 = .035$) as well as *OXTR* rs53576 x rs2268498 on perceived intrusiveness ($F = 3.863$, $p = .050$, $\eta p^2 = .012$) in mothers and *TPH2* rs4290270 x rs7305115 in fathers ($F = 9.341$, $p = .002$, $\eta p^2 = .029$).

Conclusion: The study was the first to identify potential molecular genetic effects in separation-individuation.

C.O.I. Disclosure:

T. Glavac: None. **M. Barbo:** None. **M. Ravnik Glavac:** None. **M. Zupančič:** None. **V. Dolžan:** None.

EP11.034 De novo, previously unreported splice donor region variant beyond +1, +2 in EHMT1 associated with Kleefstra syndrome type 1: A case report

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Background: Kleefstra syndrome type 1 (KS1) is a neurodevelopmental disorder caused by heterozygous pathogenic variants in EHMT1. The syndrome is characterized by hypotonia, developmental delay, and distinctive facial features. Here, we report a case of KS1 confirmed by the identification of a de novo splice-altering variant in EHMT1, out-side the +1, +2 donor site, expanding the spectrum of genetic findings associated with this disorder.

Material and methods: Whole exome sequencing was performed on a 12-month-old female patient referred for genetic evaluation due to neonatal hypotonia and facial dysmorphism. Neonatal period was complicated by the intraventricular and cerebellar hemorrhage. Family history was complicated by the intellectual disability and epilepsy in older sister.

Results: A rare heterozygous de novo splice-altering variant, EHMT1(NM_024757.5):c.2018+3A > G, was identified. Prediction tools (SpliceAI score 0.68) suggest a loss of donor splice site function, potentially leading to exon 12 skipping and frameshift. The variant was absent from population databases (gnomAD v.4.1.0) and unreported in ClinVar. A previously classified pathogenic variant (c.2018+1G > A) affecting the same splice site supports the pathogenicity of our finding. Parental testing confirmed its de novo origin, reclassifying it as likely pathogenic. Whole exome testing in the older sister was normal.

Conclusion: This case further supports the role of EHMT1 splice-altering variants in KS1 and highlights the diagnostic utility of WES in genetically unexplained hypotonia. Our findings contribute to the growing knowledge of EHMT1 genotype-phenotype correlations.

C.O.I. Disclosure:

L.P. Przesor: None. **L. Kepczynski:** None. **K. Polatynska:** None. **Z. Krepsztul-Baczewska:** None. **U. Wysocka:** None. **T. Kaluzewski:** None. **A. Kucinska:** None. **M. Piotrowicz:** None. **A. Gach:** None.

EP11.035 *KLHL20* and neurodevelopmental disorders: Further evidence supports the phenotypic association of a recurrent variant

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Background: The *KLHL20* gene encodes an E3 ubiquitin ligase adaptor involved in neurodevelopmental processes. Despite its role in synaptic function, its association with human neurodevelopmental disorders remains largely uncharacterized. Here, we report a heterozygous de novo variant, identified in this gene in two unrelated patients presenting distinct yet overlapping clinical manifestations. This study aims to highlight the potential pathogenicity of this variant and strengthen its association with human disorders.

Material and methods: Two unrelated pediatric patients underwent whole-exome sequencing following presentations of neurodevelopmental abnormalities. Clinical data, including neurological and developmental phenotypes, were analyzed to establish genotype-phenotype correlations. The confirmation of

the candidate variant and familial segregation analyses were assessed using next-generation sequencing. Potential effect of the variant was assessed through ACMG guidelines.

Results: The 8-year-old male patient exhibited epilepsy and psychomotor delay, while the 9-year-old female patient presented with febrile convulsions, arteriovenous malformation, and neurodevelopmental delay. The NM_014458.4: c.1069G>A p.(Gly357Arg) variant was identified as the potential cause of their diseases. The variant is absent from population databases and predicted to be deleterious by the majority of computational prediction tools. This variant has recently been proposed as disease-causing in a number of patients with neurodevelopmental disorders, intellectual disability, epilepsy, and autism spectrum disorder, supporting its pathogenic role.

Conclusion: Our findings provide further evidence of *KLHL20*'s involvement in neurodevelopmental disorders and establish c.1069G>A p.(Gly357Arg) as a recurrent pathogenic variant. These cases emphasize the association of *KLHL20* with epilepsy and developmental delay and contribute to the expanding phenotypic association of this gene.

C.O.I. Disclosure:

T. Ersoy: None. **H. Bas:** None. **F. Alehan:** None.

EP11.036 The role of *SCN1A* and *SCN1B* in Severe Myoclonic Epilepsy in Infancy

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Background: Epilepsies encompass a broad group of disorders with diverse genetic and clinical presentations. Among infant-onset forms, Dravet Syndrome - known as Severe Myoclonic Epilepsy in Infancy (SMEI) - emerges in the first year of life with prolonged focal or generalized clonic seizures and cognitive impairment, often linked to *SCN1A* variants. Within the SMEI spectrum, Severe Myoclonic Epilepsy in Infancy-Borderline (SMEB) shares many clinical features but follows a less typical or milder course, highlighting the variability within this group of epileptic disorders.

Material and methods: AmpliSeq custom panel was designed to target the coding regions of 39 epilepsy-related genes. Library preparation was performed using the Ion AmpliSeq library Kit plus, followed by template preparation with the Ion PGM Template OT2 200 kit and Ion OneTouch-2 Instrument. Sequencing was conducted on the Ion PGM™ system using the Ion PGM sequencing kit (Thermofisher). Filtering tools and in silico analyses were applied to identify potentially damaging variants, subsequently validated via Sanger sequencing.

Results: We identified five heterozygous pathogenic variants (p.E1021X, p.F807L, p.R1636X, p.R1886G, p.T1286I) in *SCN1A* gene in patients with SMEI. Moreover, two distinct variants were observed in patients with SMEB: p.E1502del was found in *SCN1A* gene, and p.P188A was identified in *SCN1B* gene. These variants were predicted to be pathogenic according to all prediction software evaluated (PolyPhen2, PROVEAN, Mutation Taster).

Conclusion: Variants in *SCN1A* and *SCN1B*, encoding sodium channel subunits, reinforce their involvement in SMEI and SMEB. Our findings provide valuable insights to understand the complex molecular mechanism underlying the epilepsy disorder.

C.O.I. Disclosure:

A. Felicetti: None. **M. Gagliardi:** None. **R. Procopio:** None. **F. Fortunato:** None. **I. Sammarra:** None. **G. Annesi:** None. **A. Gambardella:** None.

EP11.037 Further delineation of Nabais Sa-de Vries Syndrome Type 2: a boy with a novel de novo dominant-negative *SPOP* variant

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Background: Heterozygous missense variants in *SPOP*, encoding the speckle-type pox virus and zinc finger protein, have been reported in ten affected individuals with neurodevelopmental disorder (NDD) and hypotonia, the Nabais Sa-de Vries Syndrome (NSDVS). While affected individuals with dominant-negative variants show macrocephaly, sleeping disorders, cardiovascular and endocrinological anomalies and characteristic dysmorphism (NSDVS type 2),three individuals with gain-of-function variants present with microcephaly, hearing impairment and different facial dysmorphism (NSDVS type 1).

Methods: Diagnostic trio-exome sequencing was performed in a boy with NDD and his healthy parents.

Results: A novel de novo heterozygous missense variant with predicted dominant-negative effect (c.413G>A; p.(Arg138His)) was identified at an amino acid residue where a different missense change was reported to have a dominant negative effect. At age 3 years he presented with developmental delay (walking age 2 years), especially language delay (speaks only a few words at age 3 years), central muscular hypotonia, hyperopia, celiac disease, diabetes mellitus type 1, recurrent infections recurrent ear infections, treated with paracentesis), and obstructive sleep apnoe disorder. Facial dysmorphism included a prominent forehead, thin eyebrows, hypertelorism, epicanthic folds, downturned corners of the mouth, slightly deep-set rotated ears, and hypoplastic toenails.

Conclusion: The boy showed overlapping clinical findings with the affected individuals with NSDVS type 2 reported in literature, but also additional, previously unreported findings like Diabetes mellitus type1 and celiac disease. Our findings therefore contribute to a further characterization of the molecular and phenotypic spectrum of *SPOP*-related neurodevelopmental disorders.

C.O.I. Disclosure:

H. Gaspar: None. **V. Rusu:** None. **D. Braun:** None. **C. Zweier:** None.

EP11.038 The prevalence of neurodevelopmental disorders in Smith-Magenis syndrome: a PRISMA compliant systematic review

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Background: Smith Magenis Syndrome is either due to a deletion in 17p11.2 locus or to a pathogenic variant in RAI1 gene and is associated with neurodevelopmental disorders (NDDs). We performed a systematic review assessing prevalences of autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD), intellectual disability disorder (IDD) and specific learning disabilities in Smith Magenis Syndrome. We described methods of assessment and phenotype-genotype correlation.

Material and methods: Main electronic databases were searched on January 31st, 2024. We considered original articles

published in peer-reviewed journal and performed quality assessment.

Results: 1941 articles were identified and 14 were included in this review for a total of 451 patients. 6 studies (n = 220 patients) assessed ASD, with prevalences ranging from 14% to 95%, and pooled prevalences of 43% and 80% for deletion and RAI1 samples respectively. 6 studies assessed ADHD (n = 174 patients). Only one study used standardized clinical criteria, reporting 100% prevalence. Other studies assessed impulsivity, hyperactivity, attention deficit with pooled prevalences of 78%, 76% and 96% respectively. 9 studies reported IDD, ranging from 67% to 100%. For deletion carriers, pooled prevalence was 93%, mostly moderate (43%), while 80% was reported for RA1 carriers, mostly mild (69%). Specific learning disabilities were described for 9% of deletion 17p11.2 patients.

Conclusion: IDD, ASD and ADHD are highly associated with Smith Magenis syndrome. RAI1 carriers are more frequently affected by ASD and ADHD despite a higher cognitive level. This systematic review highlights the need to systematically assess all NDDs in Smith Magenis syndrome.

C.O.I. Disclosure:

P. Boiroux: None. **M. Babinet:** None. **C. Demily:** None.

EP11.039 A *SIK1* and *HDAC8* pathogenic variants carrying young female with a relatively mild phenotype: a case report

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Background: *SIK1* codes for a kinase part of a signal transduction pathway regulating nuclear gene expression and when pathogenically variated causes a form of autosomal dominant severe epileptic encephalopathy (DEE30), while *HDAC8* codes for a histone deacetylase and when pathogenically variated causes an X-Linked dominant form of Cornelia de Lange Syndrome (CDLS5). We report a young female presenting with focal epilepsy, moderate ID, and some dysmorphisms, with negative family history, carrying variants in *SIK1* and *HDAC8*.

Material and methods: NGS was performed, on peripheral blood extracted DNA, using a Twist Custom Panel kit on the NovaSeq6000 platform, analyzing a panel of 30 genes associated with Cohesinopathies/Chromatinopathies.

Results: The panel found de novo heterozygosity for the intronic c.164+5G > A variant in *HDAC8*, and heterozygosity for a variant in a gene outside the panel, the c.1039G>T:p.Glu347Ter nonsense *SIK1* variant, inherited from the proband's father, and later found in her grandfather. The variants were classified as pathogenic (Class 5 ACMG) and are described in the literature: the *HDAC8* variant in a large family segregating with XLD CDLS5; the *SIK1* variant in a 3-month-old deceased of untreatable epilepsy and as object of a functional study.

Conclusion: Our proband is seemingly affected by two rare syndromes: epilepsy not being a feature of *HDAC8*-related CDLS, in our proband it's likely caused by the variant in *SIK1*, while ID and dysmorphisms are typical of CDLS5. Additionally, we found that, differently from previously reported, penetrance in *SIK1*-related DEE30 may be incomplete, as the variant segregates in healthy relatives.

C.O.I. Disclosure:

F. Casciani: None. **M. Gnazzo:** None. **M. Dentici:** None. **F. Lo Bascio:** None. **A. Del Popolo:** None. **M. Carriero:** None. **N. Pietrafusa:** None. **N. Specchio:** None. **G. Novelli:** None. **A. Novelli:** None. **M. Digilio:** None. **L. Sinibaldi:** None.

EP11.040 Genetic analysis of *GBA* gene in a cohort of patients with Parkinson's Disease

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Background: Multiple studies have shown that glucocerebrosidase activity is lower in patients carrying *GBA* mutations and in sporadic Parkinson's Disease (PD) cases, suggesting a broader role for *GBA* in pathogenesis. Here, we reported the presence of mutations in *GBA* gene in a cohort of 171 Italian PD patients.

Material and methods: Next generation sequencing was performed using the Ion PGM-Dx sequencer with an AmpliSeq custom panel, designed to sequence the coding regions of 30 genes, which were previously reported to contain disease-causing mutations in patients with Parkinson or parkinsonism.

Results: A novel missense mutation c.1515G>T (p.K505N) was identified in a proband, a 66 years-old man who developed signs of PD in his right arm at the age of 62. The sequencing analysis confirmed the segregation of the mutation in his sister and his first cousin, both affected by PD. The most common *GBA* mutations, p.L483P, p.N409S, p.E365K, p.H294Q, p.I200N, were observed in nine PD patients.

Conclusion: The p.K505N identified in a PD family has not been previously reported in ClinVar, dbSNP and gnomAD, and according to the classification based on the PM1, PM2, PP1, PP3 ACMG criteria (ACMG, guidelines), it is classified as "likely pathogenic." A synonymous variant has already been described in the same position, rs7673733402. Nine PD patients' carriers of the already described mutations (p.L483P, p.N409S, p.E365K, p.H294Q, p.I200N) have also observed. Therefore, our data can confirm that mutations in this gene are the strongest genetic risk factor for Parkinson's disease.

C.O.I. Disclosure:

M. Gagliardi: None. **R. Procopio:** None. **A. Felicetti:** None. **M. Morelli:** None. **G. Arabia:** None. **A. Quattrone:** None. **A. Gambardella:** None. **A. Quattrone:** None. **G. Annesi:** None.

EP11.041 Expanding the clinical and molecular spectrum associated with *CNTNAP2* biallelic variants

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Background: Biallelic variants leading to *CNTNAP2* deficiency result in a unique neurodevelopmental disorder, characterized by developmental delay, onset of seizures within the first two years followed by developmental regression, moderate to severe intellectual disability and variable manifestations of autism spectrum disorder and behavioural abnormalities.

Material and methods: A thorough longitudinal evaluation of the phenotype has been carried out, allowing for the enhancement of the clinical picture and its evolution in association with the molecular data. Then, trio whole exome sequencing (WES) analysis was performed, followed by a thorough review of the available literature.

Results: WES analysis identified two novel compound heterozygous nonsense variants in *CNTNAP2* in a 13 years old boy. The two variants introduce premature stop codons: the first, inherited from the healthy father, is located at position c.1162G>T, (p,Gly388Ter), while the second one, inherited from the healthy mother, affects the position c.3697dupC (p.Leu1233ProfsTer14). The proband presented a phenotype characterized by behavioural disorder, developmental delay and cognitive impairment, in the milder end of the *CNTNAP2* spectrum compared to most of the cases described. However, in contrast to all previously reported patients, the child developed epilepsy around 4 years of age.

Conclusion: The phenotypic spectrum associated with biallelic variants of *CNTNAP2* is still expanding and a genotype-phenotype correlation is not yet possible because the exact pathological mechanism and thus the origin of the wide phenotypic variability is still being investigated. Therefore, the collection of comprehensive clinical and molecular data from new patients is important to address this issue.

C.O.I. Disclosure:

E. Paccagnella: None. **D. Pasquetti:** None. **S. Zampieri:** None. **G. Girotto:** None. **M. Carrozzi:** None. **L. Musante:** None.

EP11.042 Identification of genetic variants associated with catatonia in psychiatric patients

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Background: Catatonia is an underdiagnosed, severe psychomotor syndrome that is highly comorbid with psychiatric conditions such as schizophrenia and bipolar disorders. While several studies have highlighted the role of genetics in psychiatric diseases, genetic studies specifically focused on catatonia remain extremely limited.

Material and methods: To investigate the genetics of catatonia, Whole Genome Sequencing (WGS) was performed on 11 patients diagnosed with catatonia associated with schizophrenia or bipolar disorder.

Results: Our analysis identified several candidate genetic variants, particularly in genes associated with the GABAergic pathway (GABRA6, CHAT, and CACNA1A), supporting the hypothesis of GABAergic dysfunction in catatonia. Additionally, we found variants in genes involved in developmental processes (RELN, ZFHX3, FZD8, and HOX genes), suggesting the syndrome's complexity and potential neurodevelopmental origins.

Conclusion: This preliminary study provides insights into the genetic underpinnings of catatonia, highlighting potential GABAergic and neurodevelopmental contributions. Further studies are needed to validate these genetic associations and explore their functional consequences.

C.O.I. Disclosure:

W. Ghoul: None. **N. Zhukova:** None. **A. Grujic:** None. **M. Moyal:** None. **Q. He:** None. **M. Krebs:** None. **G. Rouleau:** None. **B. Hennart:** None. **A. Amad:** None. **B. Chaumette:** None.

EP11.044 Genomics in intellectual disability: Identifying molecular etiologies with next-generation sequencing

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Background: Intellectual disability is a neurodevelopmental disorder caused by genetic and non-genetic factors that affects 1–2% of the general population. While non-genetic and environmental factors can also be effective in etiology, genetic causes play a major role, especially in severe intellectual disability. Especially with the technological developments in the last decade, new genetic etiologies have been defined and new perspectives have been brought to the underlying biological mechanisms. With all these developments, a diagnostic algorithm has been created.

Material and methods: We included 15 patients who we evaluated with intellectual disability, whose chromosomal microarray and Fragile X tests were normal and who did not have any structural brain anomalies in our study. DNA was isolated from the patient's sample and enriched from genomic DNA with the Nanodigmbio kit.All variants reported to be disease causing in databases and variants with minor allele frequency (MAF) < 1% according to gnomAD, ExAC, KGP, dbSNP databases were evaluated.

Results: We performed a copy number change-based next-generation sequencing analysis test to determine the underlying molecular etiology. We determined the molecular cause of intellectual disability in 12 of our patients. The genes we identified included SIN3A, ALG1, KAT6A, BRPF1, STIL, BPTF, CCNK, SMAD4, WDR62, and ANKRD11.

Conclusion: In our region, where the frequency of consanguineous marriage is high, single gene defects were detected in 80% of our patients, and extended family segregations were planned. Considering social characteristics, next-generation sequencing can also be preferred as the first step in determining the etiology of intellectual disability.

C.O.I. Disclosure:

F. Maden bedel: None. **N. Bilgec:** None.

EP11.045 Exome analysis on skin biopsy in unsolved cases with neurodevelopmental disorders: molecular confirmation of suspected mosaicism

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Background: Whole exome sequencing (WES) is the first-tier method in the genetic diagnosis of neurodevelopmental disorders (NDDs). However, approximately 50% of the patients remain undiagnosed. Growing evidence indicates mosaicism as a possible explanation for unsolved cases.

Material and methods: WES of skin-derived DNA, longitudinal phenotyping.

Results: We report two cases with NDD and epilepsy and negative WES results of blood DNA. The first patient is a 23-years old female. She presented facial dysmorphisms, hypomelanosis of Ito and postnatal macrocephaly. At 3-years of age, she developed epilepsy. Cortical malformations were documented. She now presents with profound intellectual disability and autistic behavior. The second patient is a 5-years old boy who developed epilepsy at

six-months of age. Developmental milestones were delayed. In the second year of life macrocephaly became evident and a progressive extension of hypopigmented striae was observed, first noted as isolated sign at four-months of age. He now presents with moderate-severe cognitive impairment. WES of skin biopsy derived DNA revealed a pathogenic somatic variant in *mTOR* (NM_004958.4: c.4448G>A, p.Cys1483Tyr) in both patients. Germline and brain somatic *mTOR* variants have been implicated across a continuum of neurodevelopmental disorders ranging from Smith-Kingsmore syndrome to hemimegalencephaly and focal cortical dysplasia.

Conclusion: The present study demonstrates the importance of considering mosaicism in NDD cases and the value of WES analysis of skin-derived DNA for identifying genetic defects. Moreover, the findings contribute to the existing body of knowledge on the clinical manifestation of somatic *mTOR* variants, thereby providing valuable insights for clinical practice.

C.O.I. Disclosure:

L. Musante: None. **E. Paccagnella:** None. **F.M. Murru:** None. **S. Zampieri:** None. **M. Bin:** None. **G.M. Di Marzio:** None. **P. Costa:** None. **M. Carrozzi:** None. **G. Girotto:** None. **C. Zanus:** None.

EP11.046 Novel REEP1 variant expands the spectrum of Hereditary Spastic Paraplegia type 31

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Background: Hereditary spastic paraplegia type 31 (HSP31) (OMIM #610250) is an autosomal dominant neurodegenerative disorder caused by alterations in the REEP1 gene, which encodes a protein essential for the endoplasmic reticulum and mitochondria. Identifying novel variants in REEP1 may contribute to a better understanding of HSP31 pathogenesis and improve diagnostic accuracy.

Material and methods: Whole exome sequencing (WES) was performed on PBMCs from a patient with lower limb spasticity and hyperreflexia. The bioinformatics analysis was conducted in duplicate to ensure accuracy. BWA MEM alignment and GATK variant annotation were performed using the GRCh38 reference genome.

Results: Bioinformatics analysis consistently identified a novel heterozygous frameshift deletion in REEP1 (g.chr2:86282237delA, c.38delT, c.(37–39)atafs, p.(Ile13AsnfsTer15)). This variant induces a frameshift starting at codon 13, resulting in the introduction of a cryptic stop codon 15 amino acids downstream. These alterations lead to significant disruption of the protein's structure and function. The predicted impact of the variant is HIGH (Conservation Scores phyloP100: 7.434), suggesting severe impairment of REEP1. The variant is not listed in population databases, lacks RefSeq and ClinVar IDs, and is absent from NCBI databases, indicating it is previously undescribed. The clinical features of the proband align with HSP31, further supporting the variant's pathogenicity.

Conclusion: This study identifies a novel REEP1 deletion (p.(Ile13AsnfsTer15)) associated with HSP31, expanding the spectrum of disorder-causing variants. Its absence in databases underscores the need for further investigation and submission. WES of additional family members is planned to assess the segregation of the identified variant.

C.O.I. Disclosure:

V. Saroyan: None.

EP11.047 ADCY5-related distonia mimicking cerebral palsy: The importance of whole exome sequensing in atypical motor disorders

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Background: Cerebral palsy (CP) is a prevalent diagnosis for children exhibiting delayed motor development. However, certain genetic mutations, such as those in the ADCY5 gene, can present with clinical features that closely resemble CP, leading to potential misdiagnoses. Accurate identification of these genetic disorders is crucial, as they necessitate distinct therapeutic approaches.

Material and methods: We present a case involving a 4-year-9-month-old male with a history of prematurity and perinatal asphyxia, initially diagnosed with quadriplegic CP due to motor delay, myoclonus and dystonia. Standard physiotherapy was initiated; however, the atypical clinical presentation and lack of expected progress prompted further genetic evaluation. Whole exome sequencing (WES) was performed to investigate potential genetic etiologies.

Results: WES identified a pathogenic mutation in the ADCY5 gene, confirming a diagnosis of ADCY5-related dyskinesia. This condition is characterized by hyperkinetic movement disorders, including chorea, dystonia, and myoclonus, which can be exacerbated by stress and fatigue. Notably, the patient's initial symptoms mimicked those of CP, leading to the initial misdiagnosis.

Conclusion: This case underscores the importance of considering genetic etiologies in patients with atypical motor development, especially when clinical features deviate from typical CP presentations and standard therapies yield limited improvement. Early genetic testing, including WES, should be contemplated to facilitate accurate diagnosis and guide appropriate management strategies. Recognizing ADCY5-related dyskinesia is vital, as it may respond to specific treatments, such as caffeine and other medications, tailored to the individual's symptomatology.

C.O.I. Disclosure:

S.A. Todorova: None. **H. Ivanov:** None. **I. Sotkova-Ivanova:** None.

EP11.048 Essential Tremor: A disorder driven by primary GABA dysfunction?

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Background: Essential tremor (ET) is one of the most common neurological disorders, primarily characterized by a postural or kinetic tremor that starts asymmetrically and progressively involves bilaterally towards contralateral sides. While usually seen as arm tremors, some patients also experience tremors in areas like the head. Genetic and molecular mechanisms in ET are poorly understood due to genetic heterogeneity, variable expression, and overlapping phenotypes, and our goal is to identify the molecular etiology in affected families.

Material and methods: Exome sequencing was conducted for five patients from three families, including three from one family

as a trio. Sanger sequencing is used for confirmation and segregation. Additionally, repeat variations in *NOTCH2NLC* and *GIPC1* were screened by using an *in-house* designed TP-PCR assay.

Results: Heterozygous/novel missense variants in *GABRR2* and *FTL* were found in Family-I with titubation and liver disease, in *GABBR1* in Family-II with titubation and hand tremor, and in *GABRP* in Family-III with bilateral rest and postural tremor. GABA-receptor subunit knockout mice exhibit tremors and motor incoordination resembling human ET.

Conclusion: This study highlights the GABAergic dysfunction in the major motor pathway and potential targets for pharmacotherapy of ET. Identifying molecular mechanisms is key to improving ET management and treatment.

C.O.I. Disclosure:

A. Aghayev: None. **G. Bagirova:** None. **M. Erdogdu:** None. **N. Akcakaya:** None. **Z. Uyguner:** None.

EP11.049 Congenital Ataxia with Progressive Cerebellar Atrophy, Camptodactyly and Hypertrichosis (CAPCACH): a novel recognizable phenotype for NALCN heterozygous variants

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Background: Non-selective sodium leak channel (NALCN) protein encoded by NALCN gene is of key importance for neuronal cell excitability. Previous reports showed that biallelic NALCN pathogenic variants cause infantile hypotonia with psychomotor retardation and characteristic facies 1 (IHPRF1) while monoallelic variants lead to congenital contractures of the limbs and face, hypotonia, and developmental delay (CLIFAHDD). In our work we aimed to expand heterozygous NALCN-related clinical spectrum, presenting two affected individuals and a literature review.

Material and methods: We describe two new unrelated subjects harboring mono-allelic NALCN pathogenic variants identified through clinical exome sequencing and review current literature of other heterozygous NALCN patients.

Results: The c.3542G>A (p.Arg1181Gln) and the novel c.3423C>A (p.Phe1141Leu) heterozygous missense variants were disclosed in two subjects manifesting a similar phenotype characterized by congenital ataxia with progressive cerebellar atrophy, camptodactyly and hypertrichosis of the arms (CAPCACH). Other *NALCN* subjects with overlapping features have already been reported. Combination of these clinical and neuroimaging findings suggests the definition of the new CAPCACH phenotype.

Conclusion: We expand the heterozygous *NALCN*-related clinical spectrum from the more severe CLIFFAHDD to the milder CAPCACH phenotype. These conditions should be considered in differential diagnosis of syndromic congenital ataxias, and presence of camptodactyly and/or hypertrichosis may represent peculiar diagnostic clues.

C.O.I. Disclosure:

G. Piccolo: None. **J. Sartorelli:** None. **G. Garone:** None. **M. Mattiuzzo:** None. **M. Dentici:** None. **L. Sinibaldi:** None. **M. Digilio:** None. **A. Novelli:** None. **F. Nicita:** None. **L. Travaglini:** None.

EP11.051 The correlation between microduplication 17q21.31 and speech delay, learning difficulties and ADHD in children

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Background: The prevalence of speech and language delays in children is in significant increase. Some of these are also diagnosed with attention deficit and hyperactivity disorder - ADHD and/or learning difficulties. Approximately 5–15% of children in the general population may have some form of the above-mentioned conditions that represent a serious obstacle interfering with their social, cognitive, and academic development.

Material and methods: Our study presents a closer understanding of rare microduplication of the 17q21.31 chromosomal region (occurring in 1 in 1,000 individuals) with consistent specific neurodevelopmental disorders. Although the underlying mechanisms of this microduplication remain to be elucidated we compare the known literature findings with our case study of 10 children analysed with array-CGH using the ~45 Kb Agilent Genome CGH Microarray 44B combined with CGH Analytic 3.4.40 software.

Results: The microduplication encompassed the KASNL1 gene and displayed symptom similarities to the classical Koolen-de-Vries syndrome, including lower level of recognition memory, associative learning, memory problems and speech delay. Our study adds additional evidence for this potential association to already existing literature and available data indicates that this chromosomal variation significantly influences the neural underpinnings of these critical cognitive and communication skills.

Conclusion: The implications of this research are significant, as early identification of children with 17th chromosome duplications may allow for targeted interventions and support services to be implemented, potentially mitigating the negative impacts of speech and language delays on a child's overall development and academic success.

C.O.I. Disclosure:

R. Janevski: None. **F. Boceska:** None. **Z. Moneva Ackovska:** None. **N. Arsovski:** None. **M. Kacka:** None. **S. Markovska Simoska:** None.

EP11.052 Genetic Diagnostics and Personalized Treatment in Pyridoxine-Dependent Epilepsy: From Palliative Care to Targeted Intervention

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Background: We report a case involving a 4-month-old boy whose epileptic seizures began eight days post-birth. Initial response was observed only with high doses of phenobarbital (30 mg/kg). As seizures progressed, the patient required continuous sedation with midazolam. Despite comprehensive assessments, including electroencephalography, brain ultrasounds, ophthalmological exams, and cerebrospinal fluid analysis, the cause remained unidentified. The result of array-CGH analysis was normal.

Methods: We performed massively parallel sequencing on a custom epilepsy panel (Custom Tier kit, Sure Agilent ID 3368871, 223 genes). Sequencing was executed with the NextSeq 500/550

DX (Illumina, GeneTiCA), and GENOVESA (Bioxys) software facilitated data analysis.

Results: Massively parallel sequencing identified two pathogenic variants, confirming the patient as a compound heterozygote for the *ALDH7A1* gene variants: c.1489+5G > A (rs368820286) and c.1279G>C (rs121912707). These findings were confirmed through Sanger sequencing. These variants are known to cause early-onset, vitamin B6-dependent epilepsy.

Conclusion: Pyridoxine-dependent epilepsy is a rare neurometabolic disorder characterized by recurrent intractable seizures resistant to anti-epileptic medication. Rapid genetic diagnosis, in collaboration with the Department of Palliative Medicine, was pivotal in treatment success. Pyridoxine supplementation, paired with a low-lysine diet and arginine, immediately improved the patient's condition, halting seizures and fostering favorable psychomotor development. While neurological assessments indicate ongoing hypertonic syndrome with an opisthotonic tendency, these symptoms are diminishing.

C.O.I. Disclosure:

N. Grosup Friedova: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Supported by Ministry of Health, Czech Republic – conceptual development of research organization (Thomayer University Hospital - TUH, 00064190). **J. Tajtlova:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Supported by Ministry of Health, Czech Republic – conceptual development of research organization (Thomayer University Hospital - TUH, 00064190). **R. Kejkulova:** None. **K. Brozova:** None. **M. Langova:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Supported by Ministry of Health, Czech Republic – conceptual development of research organization (Thomayer University Hospital - TUH, 00064190).

EP11.053 Two cases of pantothenate kinase-associated neurodegeneration in Rivne region of Ukraine

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Background: Pantothenate kinase-associated neurodegeneration (PKAN, OMIM #234200) is caused by biallelic pathogenic variants in *PANK2*, leading to progressive extrapyramidal dysfunction due to iron accumulation in the basal ganglia. PKAN has two clinical subtypes: classic, characterized by early onset and rapid progression, and atypical, with later onset and slower progression. A hallmark of PKAN is the "eye-of-the-tiger" sign on T2-weighted brain MRI. We report two unrelated cases of PKAN caused by homozygous variant c.1561G>A (p.Gly521Arg).

Material and methods: Patient 1: Sequence and deletion/duplication analysis of 1,190 genes (Invitae). Patient 2: WES followed by the analysis of sequence and copy number variants of 412 genes (Movement Disorders Gene Panel), courtesy of the Laboratory of Genome Diagnostics, Radboud University Medical Center, NL.

Results: Patient 1: A male proband, first evaluated at age 2 for motor and speech delay, ataxia, and toe-walking. Initial brain MRI showed no pathological findings; by age 5, repeated MRI revealed the "eye-of-the-tiger" sign. His condition deteriorated, with progressive dystonia, rigidity, contractures, dysphagia, and loss of ambulation. Patient 2: A 12-years-old female proband presented with nyctalopia, slowed speech, clumsiness, and mild limb hypertonia. MRI showed the "eye-of-the-tiger" sign. She remains ambulatory with no cognitive impairment.

Conclusion: The c.1561G>A (p.Gly521Arg) variant is prevalent in PKAN patients of European ancestry; homozygosity typically presents with classic PKAN. In this work, what we consider an atypical presentation of PKAN is reported in Patient 2. Further follow-up is needed to clarify Patient 2's clinical trajectory and possibly expand our understanding of genotype-phenotype correlations in PKAN.

C.O.I. Disclosure:

H. Havriushenko: None. **Y. Kulikovskyy:** None. **O. Tsizh:** None. **L. Yevtushok:** None.

EP11.054 Genome diagnosis of spinocerebellar ataxia type 4: a case report from childhood to adulthood

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Background: Spinocerebellar ataxia type 4, linked to the *VPS13D* gene, is an autosomal recessive neurological disorder with variable age of onset. Symptoms typically include ataxic gait, spasticity, oculomotor abnormalities, abnormal movements, and cerebellar atrophy on brain imaging. Global developmental delay and mild intellectual disability have also been reported. The diagnostic odyssey delays appropriate genetic counseling for affected individuals and their families.

Methods: A 4-year-old female was initially evaluated in genetic consultation. After several years of multidisciplinary medical follow-up and genetic testing, including *OPA1* analysis and advances in genetic knowledge, the diagnosis was confirmed at age 19 through genome sequencing (SeqOIA-FMG2025, France).

Results: The patient walked independently at 21 months, with initial loss of stability. Spastic paraplegia was diagnosed at age 5. At age 6, bilateral optic atrophy was discovered, with visual acuity reduced to 3/10. Initial brain MRI at age 4 was normal, but follow-up at age 12 showed moderate global cerebellar hypoplasia and white matter abnormalities. By age 15, progressive loss of walking ability led to daily wheelchair use. Her intellectual development remained normal. The diagnosis of spinocerebellar ataxia type 4, linked to compound heterozygosity of the two variants c.2650dupA and c.12350C>T in the *VPS13D* gene ended the medical odyssey, and led to a reassuring genetic counseling for the patient and her sister.

Conclusion: This genome diagnosis explains the clinical course of this early-onset case, with the exception of optic atrophy, which is not previously reported and may be a novel symptom of this rare disease.

C.O.I. Disclosure:

A. Cospain: None. **A. Riou:** None. **L. Damaj:** None. **T. Attie-Bitach:** None. **C. Dubourg:** None. **S. Odent:** None.

EP11.055 Identification of a Novel TRIO Gene Variant in a Pediatric Patient with Severe Developmental Delay

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Background: A 4-year-old male patient has been monitored since infancy for severe psychomotor developmental delay and significant central hypotonia. Development has progressed slowly without regression, accompanied by early feeding difficulties, including swallowing and chewing disorders. Clinical examination revealed relative macrocephaly and mild dysmorphic features: high forehead with frontal bossing, a high nasal bridge, and a thin upper lip. MRI findings showed delayed periventricular myelination, hypoplasia of the corpus callosum, and agenesis of the septum pellucidum. Conventional cytogenetic analysis revealed a normal male karyotype, while array CGH was also normal.

Material and methods: Blood samples were collected from the patient and parents. Whole exome sequencing (WES) was performed using the SSELXT HS Human All Exon V8 + NCV (Agilent) on the NextSeq 500/550 DX (Illumina). Bioinformatic analysis utilized a virtual panel targeting postnatal microcephaly (HPO:0005484) conducted by GENOVESA (Bioxys).

Results: Steinert's disease (myotonic dystrophy type 1) was excluded. WES identified a heterozygous "de novo" variant in the *TRIO* gene HGVS/HGVp: TRIO(NM_007118.4):c.3232C>T p.Arg1078Trp; dbSNP:- categorized between class 4 and 5 based on pathogenicity (deep reads 32). This variant was confirmed by Sanger sequencing.

Conclusion: Pathogenic variants in the TRIO gene are linked to Intellectual Developmental Disorder, Autosomal Dominant 44 (OMIM:61706) and 63 (OMIM:618825), characterized by growth disorders, macrocephaly/microcephaly, developmental and speech delays, behavioral abnormalities, and rare seizures. These genetic findings are believed to explain the phenotype observed in this patient, highlighting the importance of WES diagnostics in unsolved cases.

C.O.I. Disclosure:

J. Tajtlová: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Supported by Ministry of Health, Czech Republic – conceptual development of research organization (Thomayer University Hospital - TUH, 00064190). **R. Kejkulova:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Supported by Ministry of Health, Czech Republic – conceptual development of research organization (Thomayer University Hospital - TUH, 00064190). **N. Grosup Friedova:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Supported by Ministry of Health, Czech Republic – conceptual development of research organization (Thomayer University Hospital - TUH, 00064190). **K. Brozova:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Supported by Ministry of Health, Czech Republic – conceptual development of research organization (Thomayer University Hospital - TUH, 00064190). **M. Langova:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Supported by Ministry of Health, Czech Republic – conceptual development of research organization (Thomayer University Hospital - TUH, 00064190).

EP11.056 Monogenic and structural variants in autism spectrum disorder: ınsights into cases with defined underlying causes

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Background: Autism spectrum disorder (ASD) is a neurodevelopmental condition strongly influencedby genetic factors, which contribute to its complex etiology. This study aimed to evaluate epilepsy, micro-/macrocephaly, neuromotor retardation, mild facial dysmorphism, and additional findings in cases of ASD withgenetically clarified etiopathogenesis to enhance understanding of the diagnostic journey.

Material and methods: We reevaluated the clinical and genetic findings of patients with autism, focusing on those with well-defined and genetically determined etiopathogenesis. Seven of the 18 patients were analyzed using array-CGH, FMR1 analysis, or Sanger sequencing for recognizable syndromes, while the remaining 11 cases underwentexome sequencing (ES).

Results: Dysmorphic facial features were observed in 11 individuals, gross motor impairment in nine, epilepsy ineight, microcephaly in four, overgrowth in three, and macrocephaly in two. The cytogenetic anomalies includedduplications at 16p12.2-p11.2 and 15q11.2-q13.1 and a deletion at 15q13.2. In addition, three patients werediagnosed with Fragile X syndrome and one with Sotos syndrome. Monoallelic variants were detected in *MECP2*,*CHD8, PTEN, CUL3, RNF2*, and *SCN2A* genes. Moreover, a mosaic variant of the *KMT2C* gene was identified inone individual.

Conclusion: Findings in this report highlight the presence of dysmorphic features, motor impairments, and cranialanomalies in patients with ASD and a genetically clarified etiopathogenesis, emphasizing the importance ofcomprehensive clinical and genetic evaluations in the diagnostic process. Notably, ASD has a complex geneticarchitecture caused by monogenic and structural variants, which can be observed in both syndromic cases withdistinct phenotypes and in non-syndromic individuals with milder manifestations.

C.O.I. Disclosure:

H. Atraqchi: None. **G. Bulut:** None. **B. Ozsait Selcuk:** None. **M. Yücesoy:** None. **G. Toksoy:** None. **S. Akbas:** None. **D. Durmaz:** None. **Z. Uyguner:** None. **B. Karaman:** None. **A. Aslanger:** None.

EP11.057 Compound Heterozygosity for an *ATM* Splice-Altering Variant and a Large Exonic Deletion in Ataxia-Telangiectasia: The Critical Role of CNV Analysis

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Background: Ataxia-telangiectasia (A-T) is a severe autosomal recessive disorder caused by biallelic pathogenic variants in *ATM*. The disease manifests with progressive cerebellar ataxia, telangiectasias, immunodeficiency, and increased cancer susceptibility. While single-nucleotide variants (SNVs) are well-characterized, intragenic deletions are often underdiagnosed, particularly when they overlap with SNVs, leading to misinterpretation of sequencing data.

Material and methods: Whole-exome sequencing (WES) with CNV analysis was performed in a 13-year-old male presenting with early-onset ataxia, cerebellar atrophy, conjunctival and skin telangiectasias, and immunodeficiency.

Results: Whole Exome Sequencing identified two pathogenic *ATM* variants in trans. c.6095G>A p.(Arg2032Lys), a pathogenic SNV located in the last nucleotide of exon 41, predicted to disrupt

splicing by altering the donor site. Experimental studies (PMID: 10330348) support exon skipping, leading to frameshift and nonsense-mediated decay. CNV analysis revealed a heterozygous deletion encompassing exons 17–47 (c.2467-10_6975+11del), fully overlapping the second allele at the c.6095G>A site, resulting in partial gene loss. The variant appeared homozygous in sequencing reads (100% alternate allele frequency) due to the deletion of the corresponding exon.

Conclusion: This case highlights the critical role of CNV detection in diagnosing A-T. Without comprehensive analysis, large deletions may lead to erroneous zygosity classification, compromising diagnostic accuracy, including further parental testing. Systematic inclusion of CNV analysis in WES testing is essential for precise genetic diagnosis and clinical management of A-T patients.

C.O.I. Disclosure:

K. Polatynska: None. **L. Kepczynski:** None. **T. Kaluzewski:** None. **A. Gach:** None.

EP11.058 Identification of the novel *MAPT* p.(Asp750Val) variant in three brothers presenting with atypical and levodopa-responsive Parkinsonism

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Background: Heterozygous pathogenic variants of *MAPT* gene, coding for microtubule-associated protein, are responsible of a spectrum of autosomal dominant parkinsonisms, so-called tauopathies, characterized by behavioral, cognitive and motor symptoms. Unlike Parkinson's Disease, patients with tauopathies do not show neuronal α-synuclein aggregates and do not respond to dopaminergic treatments.

Material and methods: Index-case was a 60-year-old man, from Sri Lanka, with a 5-year history of parkinsonism, with resting tremor, motor impairment and freezing and a positive family history (one brother - 58 years - and one sister - 55 years - suffering from the same conditions). Though never evaluated, also their mother was reported to have motor dysfunction. The three brothers underwent neurological and neuropsychological tests, imaging, skin biopsy and whole-exome sequencing analysis (WES).

Results: Neurological imaging showed severe nigrostriatal degeneration; neuropsychological tests revealed mild executive function deficits, preserved global cognition and moderate anxiety/depression; skin biopsy analysis revealed the presence of α-synuclein oligomers. WES identified a novel heterozygous variant c.2249A>T, p.(Asp750Val) in *MAPT* gene, classified as likely pathogenic by in silico prediction. Same clinical and genomic findings were shared with the other two affected brothers, supporting a pathogenic role for the nucleotide variant, associated with an atypical presentation of tauopathy. All three brothers benefited from dopaminergic therapy.

Conclusion: This report describes a novel likely pathogenic variant in *MAPT* gene in three members of a family with uncommon dominant levodopa-responsive parkinsonism and highlights the clinical heterogeneity of *MAPT*-related phenotypes. Further experiments are ongoing to clarify the mechanism of pathogenicity.

C.O.I. Disclosure:

M. Ferrara: None. **G. Brescia:** None. **M. Balzo:** None. **L. Magistrelli:** None. **E. Contaldi:** None. **G. Pezzoli:** None. **P. Finelli:** None. **S. Tabano:** None.

EP11.059 From VUS to pathogenic: reclassifying a*KDM6B* variant through clinical and genomic collaboration

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Background: Gene expression is epigenetically regulated through DNA methylation and chromatin modifications. Histone H3K27 demethylation, mediated by specific histone demethylases such as KDM6B, plays a pivotal role in this process. In 2019, pathogenic variants in *KDM6B* (17p13.1), encoding an H3K27 demethylase, were linked to Stolerman Neurodevelopmental Syndrome (NEDSST), characterized by neurodevelopmental delay, coarse facial features, and mild distal skeletal anomalies.

Material and methods: A 20-month-old male was referred for genetic evaluation due to hypotonia, large anterior fontanelle, sunsetting eyes, and mild weakness without tendon reflex loss. Additional features included sleep apnea, feeding difficulties, gastroesophageal reflux, and dysmorphic features. Brain MRI revealed subarachnoid space enlargement. From the family history the 47-year-old mother presented with similar facial characteristics, in addition to type 2 diabetes, learning difficulties in childhood as well as psychiatric issues and a suicide attempt. Whole Exome Sequencing (WES) analysis (Human Exome core 2.0 kit on Illumina NovaSeq 6000 system) was performed.

Results: Two variants were identified and initially classified as variants of uncertain significance (VUS) in *HNRNPH1* (paternal inheritance) and *KDM6B* (maternal inheritance) respectively. KDM6B variant (NM_001080424.2:c.3879+1dup) was characterized as de novo after maternal grandparental testing. Phenotype-matching tools (HPO phenomizer, Face2Gene) failed to recognize a *KDM6B related disease*, which was subsequently identified by PubCaseFinder with a 74.2% probability. Reassessment led to reclassification of the *KDM6B* variant as likely pathogenic.

Conclusion: Identification of a novel likely pathogenic KDM6B variant, illustrates the pleiotropic effects of KDM6B-related disorders and highlights the critical role of multidisciplinary collaborative data sharing in variant interpretation.

C.O.I. Disclosure:

K. Kosma: None. **A. Kampouraki:** None. **T. Liakopoulou-Tsitsipi:** None. **N. Marinakis:** None. **F. Tilemis:** None. **V. Christodoulaki:** None. **E. Tsentemidou:** None. **N. Selenti:** None. **M. Tzetis:** None. **C. Solokleous:** None. **P. Makrythanasis:** None.

EP11.060 Clinical and genetic characterization of three patients with *DCX*-related lissencephaly, including one with a novel pathogenic variant

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Background: Lissencephaly (LIS) is a spectrum of severe brain malformations caused by a neuronal migration defect during fetal development. Children with LIS display developmental delay/intellectual disability (DD/ID) and epilepsy. We present three patients with LIS and pathogenic variants in the *DCX* gene.

Subjects and methods: Patient (P) 1 is a 7-year-old girl with subcortical band heterotopia (SBH), pharmacoresistant epilepsy, developmental delay (DD), moderate intellectual disability (ID), selective mutism, and spastic diplegia. P2 is a 12-year-old girl with SBH, DD, mild ID, and minor neuromotor dysfunction (MND). P3 is a 9-year-old boy with pachygyria-SBH, DD, mild ID and MND.

Clinical exome sequencing was performed in all three patients and available family members using the TruSight One Sequencing Panel and MiniSeq/NextSeq550 sequencing systems (Illumina Inc., USA).

Results: Heterozygous/hemizygous pathogenic variants in *DCX* (NM_000555.3) were identified: P1 has nonsense variant c.508C>T, p.Arg170Ter, P2 has novel de novo frameshift variant c.567_577del, p.Asp189GlufsTer and P3 has missense variant c.775C>T, p.Arg259Cys.

Conclusion: The *DCX* encodes doublecortin, a protein crucial for neuronal migration. Variants leading to loss-of-function/haploinsufficiency of *DCX* are known to cause X-linked dominant LIS. Males typically present with a more severe phenotype, including classic LIS and profound DD/ID, while females exhibit variable presentation, ranging from asymptomatic to milder SBH phenotype. Interestingly, our male patient (P3) has a mild clinical phenotype. This may be related to the type of variant (missense) or its position within the C-terminal region of the protein. A mild phenotype has previously been reported in another male patient with this variant.

C.O.I. Disclosure:

A. Meašić: None. **K. Vulin:** None. **A. Bobinec:** None. **M. Miklos:** None. **A. Tripalo Batoš:** None. **L. Odak:** None.

EP11.061 A rare type of leukodystrophy presenting with multisystemic symptoms

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Background: Some neurological diseases can cause multisystemic symptoms that may arise at different ages, making diagnosis challenging. Perinatal asphyxia can sometimes obscure the symptoms of an underlying neurological condition, complicating the diagnostic process. Leukodystrophies are rare genetic disorders that primarily affect the white matter of the central nervous system. Clinical evaluation, neuroradiology, laboratory tests, and genetic testing play a crucial role in diagnosing these conditions.

Material and methods: We present the case of a young woman with mild intellectual disability who required resuscitation at birth due to severe asphyxia. Her psychomotor development was mildly delayed, and ataxia, high-degree myopia, and dental problems developed later. Brain MRI scans showed white matter abnormalities and cerebellar-dominant atrophy, initially thought to be related to perinatal asphyxia. Later, during an endocrinological examination for primary amenorrhea, hypogonadotropic hypogonadism was diagnosed, and hormone replacement therapy was initiated. At 19, she developed epilepsy. Genetic testing, including karyotyping, array CGH, and tests for various ataxias, showed no abnormalities. As her symptoms progressed, a brain MRI at age 32 suggested the possibility of a rare genetic leukodystrophy.

Results: Multigene NGS Leukodystrophy Panel identified two known pathogenic variants in the *POLR3B* gene, consistent with the patient's phenotype. Heterozygous carrier status was confirmed in her parents, revealing a compound heterozygous state in the patient.

Conclusion: POLR3-related leukodystrophy is a rare, multisystemic, autosomal recessive genetic disorder. Diagnosing it requires a comprehensive review and periodic reevaluation, especially when progression or new symptoms occur. Anamnesis of perinatal asphyxia in adults should be evaluated with caution.

C.O.I. Disclosure:

K. Dorogi: None. **A. Till:** None. **N. Tiszlavicz:** None. **R. Szalai:** None. **K. Hadzsiev:** None.

EP11.062 Further evidence supporting the role of MAPT in monogenic forms of amyotrophic lateral sclerosis

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Background: Pathogenic variants in the *MAPT* gene represent a known cause of a class of AD neurodegenerative disorders, including frontotemporal dementias (FTD) with or without parkinsonism, as well as AD and AR progressive supranuclear palsy-1. While sporadic reports of amyotrophic lateral sclerosis (ALS) in patients with *MAPT* pathogenic variants exist, a definitive association between ALS and the *MAPT* gene has yet to be established.

Material and methods: Following the identification of NM_001377265.1:c.2266C>T (p.Pro756Ser) variant in a pre-symptomatic 50-year-old patient, we performed a retrospective search in our internal sequencing database to identify further cases with ALS and this variant.

Results: We identified three additional patients carrying the identical *MAPT* variant. Two patients (ages 55 and 59) had been referred for WES due to ALS, while one (age 45) presented with spastic tetraparesis. The variant, previously referred to in older literature as c.1090C>T (p.Pro364Ser) is pathogenic. It has been reported in at least five individuals with variable clinical features, including FTD with or

without parkinsonism and/or motor neuron disease; however, none of these individuals were reported to present as ALS.

Conclusion: While the potential association between the c.1090C>T variant and ALS remains to be fully elucidated, two of our four patients with this variant have ALS. Currently, the *MAPT* gene is infrequently considered in patients referred for genetic testing for ALS, and it is not consistently included in ALS gene panels. Further research is needed to assess the possible contribution of *MAPT* variants to ALS and expand the known phenotype spectrum.

C.O.I. Disclosure:

B. Golob: None. **A. Maver:** None. **B. Peterlin:** None.

EP11.063 Case report: A novel missense variant in the *UNC80* gene in trans with a known pathogenic variant in a patient with UNC80 deficiency

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Background: The *UNC80* gene encodes a large UNC80 protein, an important part of the NALCN sodium channel in neuronal cell membranes. Pathogenic variants in *UNC80* disrupt the function and stability of voltage-gated ion channels and lead to nerve conduction impairment. Biallelic mutations in *UNC80* cause a severe autosomal recessive neurodevelopmental disorder known as UNC80 deficiency (IHPRF2; MIM #616801).

Subjects and methods: The 14-month-old boy, born to healthy non-consanguineous parents, was admitted for genetic evaluation due to severe global developmental delay, hypotonia, strabismus, prominent neurocranium and facial dysmorphic features. The patient and his parents underwent the clinical exome sequencing analysis using peripheral blood samples with the TruSight One Sequencing panel on the NextSeq 550 System (Illumina Inc., USA).

Results: Trio analysis revealed compound heterozygous variants in the *UNC80* gene (NM_032504.2): a paternal heterozygous missense variant c.4040A>C, p.Gln1347Pro, classified as a VUS (Variant of Unknown Significance) and a maternal pathogenic heterozygous nonsense variant c.7447C>T, p.Arg2483Ter.

Conclusion: UNC80 is a highly conserved part of the UNC80-UNC79 subcomplex with a unique and intertwined geometry. The c.4040A>C variant substitutes polar glutamine with non-polar proline within a repeating helical motif, which could, according to some in sillico predictors, affect protein folding. Although the variant c.4040A>C is classified as a VUS, the patient's clinical features strongly suggest UNC80 deficiency. Furthermore, its presence in trans with a pathogenic variant suggests that this variant is likely disease-causing. Additional clinical and experimental data may provide further insight, potentially leading to the reclassification of the VUS as likely pathogenic or pathogenic.

C.O.I. Disclosure:

M. Miklos: None. **K. Vulin:** None. **A. Bobinec:** None. **A. Measic:** None. **L. Odak:** None.

EP11.065 Elucidating the genetic etiology of childhood epileptic encephalopathies: a preliminary study

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Background: Epileptogenesis refers to the process in which a normal brain undergoes a series of cellular, molecular, structural, or functional changes over time, transforming into an epileptic brain that is capable of generating spontaneous and persistent seizures. The underlying genetic causes play a significant role in this pathophysiological heterogeneous group. Classical and current genetic techniques are employed to investigate the genetic etiology of epileptic encephalopathy, effectively examining single gene defects and copy number variations. This study explores the genetic factors involved in the etiology of epileptic encephalopathy and serves as a resource for advanced genetic testing.

Material and methods: Between 2012 and 2024, data from 88 patients with epilepsy were selected from a cohort of 1,311 patients in the multiple congenital anomalies and intellectual disability group and evaluated using the array-based comparative genomic hybridization (a-CGH) method.

Results: The analysis revealed anomalies in 14 of the 88 (15.9%) patients. The most frequently detected copy number variation (CNV) was located in the 16p/16q region in five cases, followed by the 1p/1q region in two cases, with changes observed in the 2q, 3p, 6q, 11p, 17p, 22q, and Xp regions in subsequent cases.

Conclusion: The findings obtained through a-CGH highlight potential chromosomal anomalies associated with epileptic encephalopathy. Our advanced whole-exome sequencing (WES) analysis will further investigate the underlying mechanisms of this condition in detail. Genetic variants in epileptic encephalopathies, a severe group of epilepsy disorders, are expected to play a significant role in future disease classification systems.

C.O.I. Disclosure:

G. Bulut: None. **B. Özsait Selçuk:** None. **A. Dilruba Aslanger:** None. **G. Toksoy:** None. **H. Maraş Genç:** None. **E. Pembegül Yıldız:** None. **B. Karaman:** None.

EP11.066 Novel NSD2 variants in Rauch-Steindl Syndrome: Report of two cases expanding the clinical spectrum

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Background: Rauch-Steindl Syndrome is a rare autosomal dominant disorder characterized by growth retardation, dysmorphic facial features, short stature, and varying degrees of motor developmental and speech delay. *NSD2* encodes a transcriptional regulatory protein with histone methyltransferase activity, playing a role in gene expression during embryonic development. Heterozygous pathogenic variants in this gene are responsible for the clinical manifestations of Rauch-Steindl Syndrome.

Material and methods: Genetic analysis was performed using next-generation sequencing, and targeted variants were confirmed by Sanger sequencing.

Results: Case 1, a 9-year-old female, was referred due to motor and speech delay, learning difficulties, gait disturbances, frequent falls, and early menarche. Prenatal history includes intrauterine growth retardation. Physical examination revealed microcephaly, high-arched palate, irregular dentition, and 4th-5th toe clinodactyly of the left foot. Case 2, a 12-month-old male, was referred due to failure to thrive, developmental delay, dysmorphic facial features, and pes equinovarus, who was born from nonconsanguineous parents. His physical examination revealed short stature, microcephaly, dysmorphic facial features, and bilateral 4th-5th toe clinodactyly. Two novel heterozygous variants were identified in *NSD2*: a missense variant, c.3887G>A, p.(Cys1296Tyr), and a frameshift variant, c.1577_1580del, p.(Arg526Thrfs*71). c.3887G>A was confirmed as de novo via Sanger sequencing.

Conclusion: This study expands the genotypic and phenotypic spectrum of Rauch-Steindl Syndrome by identifying two novel *NSD2* variants. The cases highlight the importance of considering this syndrome in individuals with developmental delay, growth retardation, and dysmorphic features. Our findings emphasize role of *NSD2* in disease etiology and the value of next-generation sequencing in rare disorder diagnosis.

C.O.I. Disclosure:

R. Savas: None. **Z. Manav Yigit:** None. **A. Mengübas Erbas:** None. **R. Deveci Sevim:** None. **E. Goksoy:** None. **A. Abaci:** None. **A. Anik:** None.

EP11.067 Doctor

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Background: ALKKUCS, OMIM # 617822, is an autosomal recessive severe neurodevelopmental disorder characterized by arthrogryposis, brain abnormalities associated with cerebral parenchymal underdevelopment, and global developmental delay. Most affected individuals die in utero or soon after birth. Additional abnormalities may include hypotonia, dysmorphic facial features, and involvement of other organ systems, such as cardiac or renal. The few patients who survive have variable intellectual disability and may have seizures. We hereby report a 1 day old female neonate, with bilateral microphthalmia, ankyloblepharon, absent vermis, clenched fest, anterior placement of anus, sacral dimple with hair tuft, ASD II, and talipes equinovarus, her WES has confirmed Alkuraya-Kucinskas Syndrome.

Material and methods: Whole-exome sequencing was utilized for the proband, and variants were filtered, annotated, and classified. Candidate variants were validated by Sanger sequencing of the proband and her family.

The literature was reviewed; the prognosis among different regions and the variant types were analyzed. Phenotypic delineation among different families and variants and studying genotype- phenotype correlation.

Results: We have reviewed the literature and found that it is an extremely rare diagnosis found in 26 total patients world wide up to current date.

Conclusion: In this study we report our young patient with splicing homozygous variant causing Alkuraya-Kucinskas syndrome, we compared our case to all other reported cases, such studies of rare diseases are needed to have a better understanding of gene function, impact of different types of variants with their resultant phenotype.

C.O.I. Disclosure:

H. AlAkeel: None. **F. AlMutairi:** None.

EP11.068 A novel Adult Neurogenetics Clinical Service: A review of the first 3 years

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Background: In 2018, the Ministry of Health, Singapore, announced a moratorium describing the minimal clinical requirements for genetic counselling in patients undergoing diagnostic genetic testing from 2020. In preparation to meet these requirements, we developed an adult clinical genetics service that started in September 2020 and has since expanded. In this study, we describe the attendance, testing take up, and outcomes of clinical genetic testing in an adult neurogenetics clinical service.

Material and methods: A retrospective review of all patients who attended the neurogenetics clinic from September 2020 to September 2023 was performed. Information on demographic factors, phenotype, source of referral, and whether testing was performed, and results of testing were extracted from the medical records.

Results: A total of 454 patients (mean age 54 ± 12, 48% Female) were seen in the first three years of this service (67 in Y1, 156 in Y2, and 231 in Y3). Of these, 267 (58%) proceeded with genetic testing. Cost (64%), and futility (36%) were the main reasons not to proceed with genetic testing. Panel based testing was the most common test (71%), followed by single gene (25%), and then whole exome or genome testing (4%). In the 267 patients who underwent testing, 110 (41%) had a pathogenic/ likely pathogenic finding, 42 (16%) had negative results, and 115 (43%) had variants of unknown significance.

Conclusion: In the first Adult Neurogenetics Clinical Service in Singapore, 58% of patients referred underwent clinical genetic testing. Of these, 41% had a pathogenic or likely pathogenic result.

C.O.I. Disclosure:

K. Narasimhalu: D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Modest; Takeda.

EP11.069 Brain single-cell transcriptomics identifies co-expressed gene networks in epilepsy disorders with *TBC1D24*, vATPase and vSNARE pathogenic variants as targets for precision medicine

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Background: Epileptic disorders affect 70 mio. individuals, with high levels of clinical variability and genetic heterogeneity that challenge diagnosis, treatment and patient management. Despite anti-epileptica (AED) availability, 1/3 of patients is refractory to existent medication. For the development of new, better treatments it is thus crucial to understand individual disease characteristics as well as finding shared pathways as inroads to treatment.

Material and methods: Our overall aim is to gain mechanistic insight into epilepsy syndromes and develop advanced in silico model systems of excitatory and inhibitory neurons for personalized medicine. We will take advantage of scientific advances in single-cell transcriptomics and bioinformatics analysis in order to inform n-of-1 observations to evaluate patient responsiveness to small compound drugs.

Results: We have tested animal models of epileptic disorders using transgenic *Drosophila* disase models reared on drug compounds. Next, we identified co-expression gene networks based on human patient as well as animal brain single-cell transcriptome data with the aim to unravel cell-type specific contributions and to identify novel connections between epilepsy syndromes.

Conclusion: These results will allow us to identify targeted precision medicine strategies for refractory epileptic disorders based on existing and novel AEDs for patients, with a focus on the role of pathogenic changes cause by variants in the gene *TBC1D24* as well as pathogenic variants in vATPase and vSNARE genes.

C.O.I. Disclosure:

K. Lüthy: None. **C. Germeys:** None. **F. Kaiser:** None.

EP11.070 Early onset FXTAS and *CHD8* related IDD in a family with male siblings of different phenotypes

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Background: *FMR1* premutation, and occasionally grey zone (GZ) alleles, are considered linked to fragile X-related disorders (FXRDs) including the late-onset tremor/ataxia syndrome (FXTAS) and Fragile X-associated neuropsychiatric disorders (FXAND). *CHD8* pathogenic or likely pathogenic variants are associated with autosomal dominant intellectual developmental disorder.

Material and methods: Male siblings are herein discussed to highlight genetic and phenotypic heterogeneity in the same family. Patient 1 (P1) is a 26 years old male presenting with encephalopathy and cerebellar ataxia and patient 2 (P2) his 21 years old brother with autism. *FMR1* triplet repeat primed PCR (Adellgene®FragileX) and Whole Exome Sequencing (WES), using Human Exome core 2.0 kit on Illumina NovaSeq 6000 system, were performed. Bioinformatic analysis used Franklin platform and all variants were classified according to ACMG and ClinGen recommendations.

Results: Hemizygosity of an *FMR1* GZ allele (49 pure CGG) was identified and initially deemed as non-causative. Further investigation revealed mosaic presence of alleles up to 120 CGG repeats only in P1 for whom WES failed to detect any additional causative variants, while the known CHD8:c.6730C>T pathogenic variant was identified in heterozygosity in P2.

Conclusion: Patients 1 and 2 share hemizygosity for an *FMR1* grey zone allele. However, mosaic expansions to premutation, consistent with an FXRD, was recorded only in P1 with the cerebellar ataxia. In contrast, although a possible impact of GZ may not be excluded, *CHD8* variant is considered causative of autism in P2. Co-existing molecular alterations in a family may interfere with differential diagnoses and should never be overlooked.

C.O.I. Disclosure:

D. Veltra: None. **K. Kekou:** None. **E. Anagnou:** None. **G. Tsikalakis:** None. **S. Giannopoulos:** None. **G. Tsivgoulis:** None. **J. Traeger-Synodinos:** None. **P. Makrythanasis:** None. **C. Sofocleous:** None.

EP11.071 In silico analysis of *PSEN1* and *PSEN2* missense SNPs in early-onset Alzheimer's disease

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Background: Alzheimer's disease (AD) is characterized by the accumulation of amyloid-β (Aβ) plaques and tau tangles. Presenilin 1 (PSEN1) and Presenilin 2 (PSEN2) are essential components of the gamma-secretase complex, responsible for cleaving amyloid precursor protein (APP) to produce Aβ peptides. Variations in PSEN1 and PSEN2 have been associated with early-onset Alzheimer's disease (EOAD). Mutations in these genes can be loss-of-function (LoF) or gain-of-function (GoF), both showing pathogenicity through different mechanisms. LoF mutations impair gamma-secretase activity, leading to an imbalance in Aβ production. Additionally, LoF mutations may exacerbate tau phosphorylation, accelerating neurodegeneration. Understanding these mutations, structural and functional consequences is essential for unraveling their role in AD pathogenesis.

Material and methods: SNP data and protein sequences for PSEN1 and PSEN2 were retrieved from dbSNP and Ensembl. Protein structures were modeled using AlphaFold and Phyre2. The pathogenicity of SNPs was assessed using SIFT,PolyPhen-2,PredictSNP1/2,MetaSNP,FATHMM,SNPs&GO, and CADD. Structural and stability changes were analyzed with DynaMut2, MutPred, MUpro, mCSM, mCSM-Membrane,INPS-MD,HOPE, and Missense3D. Evolutionary conservation analysis performed with PANTHER and ConSurf.

Results: Our initial findings indicate that rs1203424298 in PSEN1, rs781543554 and rs756899463 in PSEN2 may affect protein stability as well as functionality. Structural modeling and stability predictions further support the potential impact of these variants on the protein conformation.

Conclusion: These alterations could impair gamma-secretase efficiency, potentially contributing to EOAD. This in silico study highlights potentially pathogenic PSEN1 and PSEN2 variants that may have a role in EOAD. Further experimental validation is needed to confirm their impact and possible therapeutic approaches.

C.O.I. Disclosure:

M.F. Açik: None. **M. Alaylioglu:** None.

EP11.072 Germline mosaicism in a rare neurodevelopmental disorder associated with *MAST1* variants: Clinical and genetic insights

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Background: The *MAST1* gene encodes microtubule-associated serine/threonine-protein kinase 1, which is essential for neuronal development and function. Variants in *MAST1* have been linked to rare neurodevelopmental disorders characterized by global developmental delay, intellectual disability, and characteristic brain malformations. Due to the limited number of reported cases, the clinical spectrum and inheritance patterns remain incompletely understood. Here, we identified an in-frame deletion

in three affected siblings, absent in both parents, suggesting germline mosaicism.

Material and methods: Whole-genome sequencing was performed on the proband, followed by targeted next-generation sequencing of family members. In silico analyses and structural modeling assessed the variant's functional impact, and clinical phenotypic characterization was conducted.

Results: The proband exhibited developmental delay, hypotonia, intellectual disability, and mental deterioration. One affected sibling had seizures and intellectual disability, while the other presented a similar phenotype without seizures. The NM_014975.3: c.844_846del; p.Ile282del variant, absent in population databases, affects a highly conserved residue. A PROVEAN score of −12.175 and structural modeling suggest a deleterious effect. Other in-frame deletions in this region have been linked to deleterious effects, and the variant has been identified in another patient. It was confirmed in other affected siblings, while both parents and an unaffected sibling tested negative, suggesting parental germline mosaicism.

Conclusion: These findings highlight the potential for germline mosaicism in families with atypical inheritance patterns and multiple affected children. By providing a detailed clinical and genetic characterization of this family, we contribute to the expanding phenotypic spectrum associated with this rare neurodevelopmental disorder.

C.O.I. Disclosure:

H. Rao: None. **M. Ocal:** None. **H. Bas:** None.

EP11.073 Case Report: A Novel *PPT1*:c.677T>G Variant in a Adult Form of Neuronal Ceroid Lipofuscinosis-1

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Background: Neuronal ceroid lipofuscinoses (NCLs) are a group of inherited, progressive, degenerative disorderscharacterized clinically by cognitive decline, epilepsy, and vision loss due to retinal degeneration. Neuronalceroid lipofuscinosis-1 (CLN1) is an autosomal recessive disease caused by mutations in the *PPT1* gene, whichencodes palmitoyl-protein thioesterase-1.We report a 16-year-old male with a novel *PPT1* c.677T>G variant linked to adult CLN1. He exhibitedprogressive neurological decline, including loss of speech, inability to walk, intellectual disability and psychiatricsymptoms such as mania. Brain MRI revealed cerebral and cerebellar atrophy, corpus callosum thinning, and anarachnoid cyst. His brother showed flapping tremors, myoclonus, and dysarthria, though neither sibling hadepilepsy. The parents are first cousins with no other family history of neurodegenerative disorders.

Material and methods: Genomic DNA was extracted from the patient's peripheral blood, and whole-exome sequencing (MGI T7Twist Human Comprehensive Exome Kit) was performed to identify disease-associated variants. Clinical,neurological, and radiological evaluations were conducted.

Results: Genetic analysis revealed a novel homozygous *PPT1* c.677T>G (NM_000310.4) (p.Val226Gly) variant in theaffected patient. This variant has not been reported in public databases and was predicted to be pathogenicbased on bioinformatic analysis. Neurological examination indicated progressive deterioration, and brain MRIfindings supported diagnosis.

Conclusion: This case expands the spectrum of *PPT1* mutations and highlights an atypical presentation of CLN1,characterized by the absence of epilepsy, and the presence of an arachnoid cyst. These findings underscore thecritical importance of genetic testing in the diagnosis of neurodegenerative disorders, even in the absence ofclassical features.

C.O.I. Disclosure:

P.A. Toker: None. **Z. Anarbaeva:** None. **H. Ercelebi:** None. **Y.K. Terzi:** None. **T. Sezer:** None. **Z. Celik:** None.

EP11.074 KIF1A variants in two patients presenting with NESCAV syndrome

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Background: Heterozygous *KIF1A* variants have been associated with neurodegeneration and spasticity with/without cerebellar atrophy or cortical visual impairment syndrome (NESCAVS). One of the main characteristics among affected individuals are: cognitive impairment, progressive spasticity, profound developmental delay, intellectual disability, speech delay or absence, and behavioral problems.

Material and methods: Clinical exome sequencing (CES) was performed in two probands with clinical presentations of cerebral palsy and additional neurological abnormalities. DNA libraries were prepared using TruSightOne kit and sequenced on MiSeq/NextSeq550 systems. Data analysis was performed with BaseSpace Variant Interpreter and curated online databases.

Results: In both our patients, CES analysis revealed pathogenic heterozygous variants in *KIF1A* gene considered responsible for their clinical presentation. In P1 we detected a missense variant c.356T˃C,p. (Ile119Thr) that could not be found in curated databases. To our knowledge this is the first time this variant has been described in a patient with NESCAV syndrome. In P2 we found a missense variant c.757G>A, p.(Glu253Lys) that has already been described in patients with this disorder.

Conclusion: We believe that detected pathogenic missense variants in *KIF1A* gene (I119T and E253K) are responsible for the clinical presentation of NESCAV syndrome in our patients. Next generation sequencing methods are an important tool in elucidating the genetic causes among children with severe neurological impairments who were previously thought to have cerebral palsy as a result of perinatal brain injury. Establishing the correct diagnosis in these patients is crucial for adequate patient care and proper genetic counseling of the affected families.

C.O.I. Disclosure:

A. Bobinec: None. **K. Vulin:** None. **I. Sansovic:** None. **A. Measic:** None. **L. Morozin-Pohovski:** None. **L. Odak:** None.

EP11.075 Establishment and characterization of induced pluripotent stem cells from patients with 22q11.2 Duplication Syndrome

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Background: 22q11.2 Duplication Syndrome (22q11.2DupS), caused by microduplication of the q11.2 region on chromosome 22, is associated with a high risk of developing neurodevelopmental disorders (NDDs), including autism spectrum disorders, and attention-deficit/hyperactivity disorder. On the other hand, in individuals affected by schizophrenia, 22q11.2DupS occurs less compared to the general population, suggesting that this microduplication could have protective effect against schizophrenia. Thus, 22q11.2DupS represents an adequate model system for studying the molecular mechanisms underlying NDDs.

Material and methods: iPSCs were generated by reprogramming peripheral blood mononuclear cells using CytoTune™-iPS 2.0 Sendai Reprogramming Kit. Established iPSC lines were characterized by examining their morphology, genomic integrity, pluripotency and the ability to differentiate into cells of three germ layers.

Results: iPSCs were generated from three patients with an inherited form of 22q11.2DupS, their mothers that carry the microduplication, and three healthy individuals. Genotyping showed that some of the iPSC lines have additional copy number variations. All generated iPSC lines express pluripotency markers and are capable of differentiating into the cells of three germ layers (ectoderm, mesoderm, and endoderm).

Conclusion: iPSCs were successfully generated from patients with 22q11.2DupS, their carrier mothers, and healthy individuals, providing a valuable tool for gaining insights into the molecular mechanisms underlying NDDs.

C.O.I. Disclosure:

N. Kovacevic Grujicic: None. **J. Kostic:** None. **D. Drakulic:** None. **M. Peric:** None. **I. Simeunovic:** None. **G. Cuturilo:** None. **O. Petter:** None. **A.J. Harwood:** None. **M. Stevanovic:** None.

EP11.076 Comparison of hetero- and homodimeric recombinant protein variants of aromatic amino acid decarboxylase (AADC) - kinetic parameter-based complex effects

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Background: AADC deficiency is a rare neurometabolic disease. AADC is encoded by *DDC* gene and catalyses the decarboxylation of L-3,4-dihydroxyphenylalanine to dopamine and 5-hydroxytryptophan to serotonin. Using whole exome sequencing, we identified 4 likely pathogenic variants in *DDC* gene in patients with AADC deficiency: p.Pro47Ala/p.Arg447Cys and p.Cys261Phe/p.Gly354Ser. The molecular mechanism associated with these variants was not investigated so far.

Material and methods: Recombinant heterodimeric AADC enzymes were overexpressed in *E. coli* BL21 Codon Plus and purified with the use of affinity chromatography and FPLC. Measurements of enzymatic activity were carried out by monitoring the rate of serotonin formation from substrate using Ion Pair Reversed Phase HPLC with photodiode detection.

Results: Both heterodimeric proteins had an impaired enzymatic activity to different degrees. p.Pro47Ala/p.Arg447Cys exhibited only about 10% of WT activity, with K_m increased by 1,6-fold, indicating that its catalytic efficiency was impaired mostly due to catalysis disturbances. Intriguingly, p.Cys261Phe/p.Gly354Ser showed only moderately affected activity, resulting in 42,11% of WT activity, while K_m was decreased by 0,62-fold leading to catalytic efficiency of almost 70% that of the WT. Moreover, in the homozygous system, the activity of p.Cys261Phe was only moderately affected, whereas p.Gly354Ser was almost completely inactive.

Conclusion: The impact of pathogenic variants on the AADC activity is more pronounced in p.Pro47Ala/p.Arg447Cys than in p.Cys261Phe/p.Gly354Ser. The differences in the activity of hetero- and homodimeric proteins result not only from the type of the point mutation but also from the functional properties of the protein complexes and the cooperative effects between different subunits.

C.O.I. Disclosure:

K. Ślusarczyk: None. **K. Kuśmierska:** None. **J. Kamińska:** None. **J. Sykut-Cegielska:** None. **K. Szymańska:** None. **J. Poznański:** None. **J. Drożak:** None. **A.M. Rygiel:** None.

EP11.077 Genome sequencing in patients with developmental and epileptic encephalopathy unsolved by ES

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Introduction: Early onset epilepsies, especially developmental and epileptic encephalopathies (DEE), are severe epilepsies of childhood with early onset of usually drug-resistant seizures and developmental delay or regression. Even if targeted gene panel and/or exome sequencing (ES) became standard examination, many patients remain unsolved.

Patients and methods: We performed genome sequencing (GS) in a cohort of 15 DEE patients unsolved by ES. GS libraries were prepared with IlluminaTruSeq PCR free library kit. GS data were processed following GATK-DRAGEN workflow for calling germline SNVs/Indels and GATK-SV workflow for CNV variants.

Results: GS enabled to finally diagnose one patient. First, a well-known pathogenic missense variant in *ALDH7A1* gene was identified with ES. The second variant was found via CNV analysis of genome data which detected the deletion of exon 17 of *ALDH7A1* gene. Biallelic variants in *ALDH7A1* cause pyridoxine-dependent epilepsy, but patient's phenotype was not typical and the deletion of the whole exon 17 was missed by standard CNV analysis of ES data. After getting genetic diagnosis, high doses of pyridoxine significantly decreased the seizure frequency in the patient.

Conclusion: Although NGS methods represent robust diagnostic tool, the genetic diagnosis is not always easy to find. The NGS diagnostics in the presented patient included targeted gene panel testing, ES and several subsequent ES reanalysis. Even after genome analysis of both SNVs, Indels and CNVs we were able to get genetic diagnosis. GS thus may help to obtain genetic diagnosis in selected patients who remained unsolved after ES.

C.O.I. Disclosure:
L. Sedlackova: None. **A. Musilova:** None. **K. Sterbova:** None. **P. Lassuthova:** None.

EP11.078 Identification of Ser71Arg mutation in *RAB32* gene in familial Parkinson's disease from Southern Italy

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Background: Parkinson's disease (PD) is one of the most common neurodegenerative diseases, characterized by degeneration of dopaminergic neurons in the substantia nigra pars compacta. Recently, p.Ser71Arg variant in RAB32, encoding a small GTPase that interacts with LRRK2, has been described as a high-risk factor for PD.

Material and methods: We sequenced RAB32 in 300 unrelated PD patients from Southern Italy with a positive family history for PD, and in 300 healthy subjects from the same geographic area using an ABI 3500 Genetic Analyzer.

Results: Sanger sequencing analysis identified 7 patients carrying the c.213C>G (p.Ser71Arg) heterozygous rare missense variant in exon 1 of RAB32. Segregation analysis found the same variant in 4 family members without PD diagnosis, but all of them showed some non-motor signs suggestive of prodromal PD.

Conclusion: The p.Ser71Arg variant showed a frequency of 2.33% in our PD cohort, which is significantly higher than the 0.0%-0.7% range commonly reported in other international studies. Our findings align with the higher prevalence (2.3%) observed in a cohort of 130 familial PD cases from Canada and Tunisia. The high prevalence of the p.Ser71Arg variant in our cohort can be attributed to Calabria being a genetic isolate due to its geographical characteristics, which have promoted a high rate of consanguinity and limited immigration over the last three centuries. Moreover, its location in Southern Italy, near Tunisia, suggests that RAB32 is a notable contributor to familial PD in the Mediterranean region, underscoring the importance of RAB32 gene analysis in these patients.

C.O.I. Disclosure:
R. Procopio: None. **M. Gagliardi:** None. **A. Felicetti:** None. **J. Buonocore:** None. **M. Talarico:** None. **A. Quattrone:** None. **A. Quattrone:** None. **G. Annesi:** None.

EP11.079 Establishment of a patient-derived induced pluripotent stem cells with 22q11.2 microdeletion: a model system for studying neurodevelopmental disorders

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Background: Neurodevelopmental disorders (NDDs), including autism spectrum disorders, intellectual disability and schizophrenia, represent a public health challenge in modern societies. However, molecular mechanisms underlying NDDs are still unknown. 22q11.2 Deletion Syndrome (22q11.2DS), caused by microdeletion 22q11.2, is one of the syndromes with a high risk for NDDs; it is one of the strongest known risk factors for development of psychiatric illness and one of the highest known genetic risks for schizophrenia.

Material and methods: Peripheral blood mononuclear cells from patients with 22q11.2DS and control subjects were reprogrammed using CytoTune™-iPS 2.0 Sendai Reprogramming Kit. Characterization of generated iPSC lines were done by analyzing their morphology, genomic integrity, pluripotency and the ability to differentiate into three germ layers.

Results: Peripheral blood mononuclear cells from seven patients with 22q11.2 microdeletion and three healthy controls were reprogrammed. Genotyping revealed that some of the iPSC lines contain additional CNVs. All generated iPS cell lines express markers of pluripotency and have ability to differentiate into three germ layers.

Conclusion: iPSC lines derived from patients with 22q11.2DS and healthy controls were successfully established. They represent a powerful model system for studying molecular mechanisms underlying NDDs.

C.O.I. Disclosure:
D. Drakulic: None. **N. Kovacevic-Grujicic:** None. **M. Peric:** None. **O. Petter:** None. **I. Simeunovic:** None. **J. Kostic:** None. **G. Cuturilo:** None. **A.J. Harwood:** None. **M. Stevanovic:** None.

EP11.080 Experience in the clinical and genetic diagnosis of a series of Algerian patients with hereditary spastic paraplegias

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Background: Hereditary spastic paraplegias (HSPs) are neurodegenerative diseases. Their diagnosis is challenging owing to their exceptional clinical and genetic heterogeneity.

Material and methods: Using whole exome sequencing, we investigated the genetic cause of 29 patients from 16 Algerian families clinically diagnosed with HSP.

Results: We successfully identified pathogenic variants in 11 HSPs-causing genes (*ATL1*, *SPAST*, *SPG11*, *GBA2*, *AP4M1*, *AP4S1*, *DDHD2*, *CYP2U1*, *ATP13A2*, *HPDL*, and *AMFR*) with a diagnostic rate of 81.2%. The diagnosed families (n = 13/16) mainly presented autosomal recessive inheritance due to the high consanguinity rate in Algeria. Five novel causative variants were identified in the homozygous state: the nonsense variant *GBA2*:c.1351C>T/p.Arg451Ter in sisters with the standard SPG46 phenotype; the frameshift variant *SPG11*:c.6530_6534del in siblings with a complicated HSP phenotype without thin corpus callosum; the missense variant *HPDL*:c.862T>C/p.Tyr288His variant in a patient with complicated HSP; the splice site variant *AMFR*:c.707+1G > A in a patient with a complicated HSP form extending the clinical spectrum of SPG89; and the missense variant *SPAST*:c.1076T>C in sisters with a pure HSP overlapping with the classical SPG4

phenotype associated with dominant pathogenic variants in the same gene. In one family, reverse phenotyping revealed a case of Kufor-Rakeb syndrome after *ATP13A2* variants were identified, showing the importance of dialogue between clinicians and geneticists.

Conclusion: This is the first study consisting exclusively of Algerian HSP families. Our findings broaden the clinical and genetic spectrum of HSPs, and confirm high heterogeneity in Algeria. These results also underline the efficiency of WES as a diagnostic tool for HSPs.

C.O.I. Disclosure:

G. Stevanin: None. **R. Boulariah-Hadjou:** None. **M. Islam Kediha:** None. **F. Mochel:** None. **J. De Sainte-Agathe:** None. **L. Ali-Pacha:** None. **W.A. El Khedoud:** None. **M. Messaoud-Khelifi:** None.

EP11.081 Utility of genetic testing for diagnosing hATTR patients, results from a European and Middle East genetic testing program

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Background: Hereditary transthyretin amyloidosis (hATTR), a rare autosomal dominant disorder caused by *TTR* gene mutations, is characterised by non-specific symptoms often leading to delayed diagnosis. This genetic testing program evaluated the diagnostic yield and efficacy of single gene sequencing with patient eligibility criteria for hATTR.

Material and methods: GeneAct, a pharma-sponsored no-charge genetic testing service in Europe and the Middle East, utilised Sanger sequencing of *TTR* gene with a patient eligibility criteria. Briefly, patients had to be above 18 years of age with family history of hATTR or experiencing symptoms suggestive of the disease. Diagnostic yield was defined as one Pathogenic (Path) or Likely Pathogenic (LPath) variant in the *TTR* gene.

Results: A total of 2,713 samples were analysed from Jan 2022 till May 2024, from 22 countries. Genetic diagnosis was established in 93 patients with Path or LPath variants resulting in a 3.4% diagnostic yield. Furthermore, 8 Variants of Unknown Significance (VUS) were detected. Mean age at testing for all patients was 65 and for patients with a variant was 57 years. Twenty five unique Path/LPath variants were found in patients. In patients found to have a variant in the *TTR* gene, cardiac and neuropathy manifestation were amongst the most common reported symptoms.

Conclusion: The genetic testing program achieved a 3.4% diagnostic yield, identifying 25 unique pathogenic variants, with 93 patients reaching a genetic diagnosis. This underscores the importance of genetic testing for early identification of hATTR, especially in patients with cardiac and neuropathy symptoms.

C.O.I. Disclosure:

B. Streubel: None. **A. Singh:** A. Employment (full or part-time); Significant; Alnylam Pharmaceuticals. **M. Theaudin:** None. **D. Kasper:** A. Employment (full or part-time); Significant; ARCHIMEDlife. **K. Frascello:** A. Employment (full or part-time); Significant; Alnylam Pharmaceuticals. **K. Frascello:** A. Employment (full or part-time); Significant; Alnylam Pharmaceuticals. **A. Bondue:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Fonds De La Recherche Scientifique, Fonds pour la Chirurgie Cardiaque, Fonds Erasme pour la Recherche Scientifique. F. Consultant/Advisory Board; Modest; Alnylam Pharmaceuticals, Amicus Therapeutics, Bristol Myers Squibb, Pfizer, Sanofi-Genzyme.

EP11.082 Genetic testing for neurological movement disorders using NGS: insights from Latvia's experience

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Background: movement disorders (MOV), including ataxia, Parkinsonism, tremors, dystonia, and chorea, are characterized by abnormal motor control due to central nervous system dysfunction. Genetic factors are increasingly recognized as significant contributors to the pathophysiology of these disorders. This study aims to evaluate the effectiveness of molecular genetic testing in diagnosing genetic movement disorders and compare its relevance in children versus adults.

Material and methods: germline exome sequencing with SNV/indel, CNV and STR calling was performed on 124 MOV patients, including 113 singleton cases (91.13%) and 11 trio cases (8.87%). A movement disorder panel (N = 784) was used for 55 patients (44.35%), while 69 patients (55.65%) received additional panels based on clinical symptoms.

Results: genetic testing resolved 24 cases (19.35%) through SNVs/indels (17/24), CNVs (3/24), and STRs (4/24), while 100 cases (80.65%) remained unsolved. Among the unsolved cases, 82 (66.13%) had no reported findings, and 18 (14.51%) had variants of uncertain significance or autosomal recessive pathogenic variants in a heterozygous state. The diagnostic yield was similar for children and adults (24.49% (12/49) vs. 16% (12/75), respectively)) ($p = 0.25$).

Conclusion: genetic testing showed no significant difference in diagnostic rate by age. CNV and STR detection from exome increased diagnostic yield for ~30% and highlights how exome data can identify additional diagnoses with integrating additional tools.

C.O.I. Disclosure:

A. Zarina: None. **L. Bardina:** None. **E. Berga-Svitina:** None. **D. Enkure:** None. **I. Nartisa:** None. **K. Bukele:** None. **I. Adamena:** None. **A. Eihmane:** None. **D. Kidere:** None. **L. Volozonoka:** None. **I. Micule:** None. **I. Grinfelde:** None. **G. Taurina:** None. **D. Locmele:** None. **M. Masinska:** None. **R. Insberga:** None. **M. Jurcenko:** None. **A. Kalnina:** None. **I. Piruska:** None. **L. Skrabule:** None. **D. Rots:** None.

EP11.083 Unveiling m^5C effector proteins: Epitranscriptomic drivers in brain disorders

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Background: RNA methylation is a post-transcriptional modification regulating RNA, including ribosomal activity and translation, via RNA-binding proteins known as effector proteins. Among

these, 5-methylcytosine (m^5C) is abundant in brain RNA, including mRNA. Brain diseases affecting cognition, such as Mild Cognitive Impairment (MCI) and Alzheimer's disease (AD), often involve dysregulated RNA processing.

Material and methods: To explore changes in the m^5C RNA methylation system in neurodegenerative disorders, we analysed RNA-seq data from human brain samples in the Aging, Dementia, and Traumatic Brain Injury (TBI) Study. We compared the expression of m^5C effector proteins across AD, TBI, and age-matched controls, and correlated the data with Braak and CERAD stages. Using confocal microscopy, we also examined NSUN5's abundance, a key m^5C rRNA effector protein, in brain tissue from MCI and healthy controls.

Results: AD and TBI samples showed altered expression of m^5C effector proteins. NSUN6 expression was significantly reduced in AD across two brain regions and in TBI cases, while NSUN7 expression increased in the hippocampus, correlating with CERAD stages. Braak scoring revealed additional changes in NSUN6, NSUN7, and ALYREF across two brain regions. NSUN5 protein levels were significantly higher in controls compared to MCI and primarily localized in pyramidal cells.

Conclusion: Our findings highlight the dysregulation of m^5C RNA methylation in neurocognitive disorders, emphasizing its critical role in brain function. Mutations in m^5C-related genes are linked to neurological conditions, suggesting that m^5C effector proteins influence synaptic processes and neuronal health. Targeting m^5C modifications may offer therapeutic and biomarker opportunities in cognitive diseases.

C.O.I. Disclosure:

A. Perez-Grovas Saltijeral: None. **H.M. Knight:** None.

EP11.084 *ATXN1* and *ATXN2* intermediate-length expansions in non-SCA neurodegenerative disorders

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Background: Spinocerebellar ataxia 1 (SCA1) and 2 (SCA2) are a group of rare, neurodegenerative disorders caused by abnormally expanded CAG repeats in *ATXN1* and *ATXN2*, respectively (normal ranges for *ATXN1* and *ATXN2* are 6–32 and 13–31, respectively). CAG expansion of >38 repeats in *ATXN1* cause SCA1. Alleles with >33 CAG repeats in *ATXN2* cause SCA2. Interestingly, intermediate alleles, not associated with SCAs, have recurrently been described as risk factors or modulators for other neurodegenerative diseases, but consensus still awaits.

Material/Methods: Whole genome was sequenced using Illumina Novaseq 6000 from prefrontal cortex of 150 brain donors without previous genetic testing but clinical and anatomopathological diagnoses for various neurodegenerative disorders, none of which was SCA. Incidental findings of *ATXN1* and *ATXN2* expansions were analysed using *Stripy*, which provides a graphical display of motive-repeat alignments by combining both *Expansion Hunter* and *REViewer* tools. Results were validated by PCR and TP-PCR using the Imegen® SCAs Kit.

Results: We identified 16 and 17 patients of various neurodegenerative disorders with intermediate expansions of *ATXN1* and *ATXN2*, respectively. Two of them showed intermediate alleles for both genes. Our data further supports the role of intermediate expansions of *ATXN1* and *ATXN2* as risk factors for various non-SCA, neurodegenerative disorders.

Conclusion: Intermediate expansions of *ATXN1* and/or *ATXN2* may be relevant predisposing factors for several non-SCA neurodegenerative disorders, therefore genetic testing shall be considered for more accurate diagnosis in some cases. It is worth reiterating that this series of patients had anatomopathologically-confirmed diagnoses, which is typically missing in previous studies.

C.O.I. Disclosure:

F. Alvira-Iraizoz: None. **Ó. Teijido Hermida:** None. **M. Miranda Pérez:** None. **D. Cobos Hermosa:** None. **A. Lavin Remón:** None. **F. Capell Pascual:** None. **A. Almanza Goicoechea:** None. **D. Armendia Giron:** None. **Á. Alonso Sánchez:** None. **M. Erro Aguirre:** None. **V. García Solaesa:** None.

EP11.085 Genotype variability in congenital Hereditary Spastic Paraplegia: a single-center study

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Hereditary spastic paraplegia (HSP) are genetically and clinically heterogeneous inherited, slowly progressive neurological disorders characterized by primary involvement of the corticospinal tracts (CST). The early-onset (or congenital) forms of HSP are often defined as "cerebral palsy mimics" since symptoms begin in early childhood and manifest as spastic di- or tetraplegia and neurodevelopmental disorder. The rarity and heterogeneity of these disorders make their diagnosis challenging. In this single-center study, we focus on the outcomes and diagnostic detection rate of congenital HSP patients from a cohort of 102 consecutive HSP cases. The discussion highlights the genetic variability in cases of sporadic congenital HSP that tested positive through a series of molecular analyses, particularly those requiring further investigation via whole exome sequencing (WES). This approach has shed light on the etiopathogenetic role of 17 novel variants across nine different genes (*FOXG1*, *GRIN2B*, *HPDL*, *LRP2*, *RBMX*, *SPART*, *TBCD*, *ZBTB11*, and *ZC4H2*) in 13 patients with congenital complex HSP. Notably, most of these genes are not conventionally classified as HSP-related or included in the SPG classification on the Online Mendelian Inherited in Men catalog. We emphasize the importance of detailed genotype-phenotype correlations and the diagnostic potential of a highly specialized translational approach in clinically selected cases lacking molecular confirmation, thereby expanding our understanding of the genetic variability of congenital forms of HSP.

C.O.I. Disclosure:

A. Piluso: None. **V. Colona:** None. **J. Sartorelli:** None. **A. Sparaci:** None. **G. Vasco:** None. **A. Novelli:** None. **F. Nicita:** None. **L. Travaglini:** None.

EP11.086 Association of miRNA expression in plasma derived extracellular vesicles with Alzheimer's disease biomarkers

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Background: To support established cerebrospinal fluid (CSF) biomarkers used in diagnostics, miRNAs enriched in extracellular vesicles (EVs) are a promising source of non-invasive biomarkers of Alzheimer's disease dementia (AD) and mild cognitive impairment (MCI). Our aim was to assess the association of target miRNA expression in EVs from plasma (plasma-EVs) with biomarker levels (amyloid beta (Aβ) and tau protein from CSF) in AD and MCI patients.

Material and methods: Our study included 39 AD patients, 20 MCI patients with CSF biomarker levels characteristic for AD and 20 MCI patients with normal CSF biomarker levels. Sucrose cushion ultracentrifugation (sUC) was used for enrichment of small EVs and nanoparticle tracking analysis for determination of EV size and concentration. The expression of target miRNAs (hsa-miR-146a-5p, hsa-miR-451a, hsa-miR-375-3p and hsa-miR-107) in plasma-EVs was determined with qPCR using TaqMan™ Advanced miRNA Assays.

Results: Median concentration of plasma-EVs was 4.60×10^{10} (3.48×10^{10}–6.42×10^{10}) particles per ml, with a median mean size of 141.60 (134.50–155.80) nm. There were no significant differences in EV concentration and size between AD and MCI patients. In the entire cohort, higher expression of miR-375-3p in plasma-EVs was associated with higher CSF total tau ($p = 0.027$) and p-tau$_{181}$ ($p = 0.018$). When only AD patients were included in the analysis, higher expression of miR-146-5p was associated with higher CSF total tau ($p = 0.050$), while higher expression of miR-107 was associated with higher p-tau$_{181}$ ($p = 0.040$).

Conclusion: We observed important associations of miR-146a-5p, miR-375-3p and miR-107 from plasma-EVs with AD CSF biomarkers that support their potential as circulating, non-invasive AD biomarkers.

C.O.I. Disclosure:

D. Vogrinc: None. **M. Holcar:** None. **M. Lenassi:** None. **M. Gregorič Kramberger:** None. **A. Emeršič:** None. **S. Čučnik:** None. **V. Dolžan:** None. **K. Goricar:** None.

EP11.087 Effect of LCT-13910 C-T and LCT-22018 G-A polymorphisms associated with adult-type lactose intolerance on multiple sclerosis development

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Background: Multiple sclerosis (MS) is a complex inflammatory neurodegenerative disease influenced by genetic and environmental factors. Observational studies suggest a link between milk intake and neurodegenerative disease. Lactose exhibits immunomodulatory properties due to microbiota alterations that may contribute to brain-gut axis inflammation. Lactase persistence (LP), the ability to digest lactose into adulthood, is determined by genetic variants. Approximately 75% of the global population loses this ability over time, while others retain it due to LCT-13910C > T (rs4988235) and LCT-22018G > A (rs182549) polymorphisms in the MCM6 gene, an enhancer of lactase (LCT) transcription. This study aimed to investigate the influence of LCT-13910C > T and LCT-22018G > A polymorphisms on MS susceptibility and disease progression.

Material and methods: A total of 320 MS patients (82 men and 238 women) and 350 matched healthy donors from Croatia, with no family history of MS, were genotyped using the PCR-RFLP method.

Results: A significantly higher frequency ($P = 0.001$) of the LCT-13910T and LCT-22018A alleles was observed in MS patients (42.2% and 43.6%) compared to controls (33.6% and 35.0%). Homozygosity for LCT-13910TT and LCT-22018AA was associated with an increased MS risk (OR = 2.09, 95% CI = 1.36–3.23; OR = 2.05, 95% CI = 1.35–3.12; $P = 0.001$). Furthermore, MS patients carrying the LCT-22018A polymorphism had a higher disease progression index (0.42 vs. 0.33; $P = 0.046$).

Conclusion: Our findings indicate that lactase persistence genotypes LCT-13910TT and LCT-22018AA are associated with a risk of MS in our patients, while LCT-22018G > A polymorphism may influence clinical expression. Precise mechanisms underlying this association require further investigation.

C.O.I. Disclosure:

I. Stankovic Matic: None. **D. Janko-Labinac:** None. **J. Nekić:** None. **N. Starčević Čizmarević:** None.

EP11.088 MED13L Haploinsufficiency Syndrome: Insights from a Comprehensive Cohort Study

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Background: MED13L haploinsufficiency syndrome (MED13L) is a rare condition characterized by intellectual disability, language impairment and dysmorphisms, and can be associated with cardiac defects. MED13L is a regulator of transcriptional processes. The aim of this study is to clinically and molecularly characterize a cohort of patients followed at a tertiary care hospital and compare the findings with existing literature.

Material and methods: Descriptive, retrospective study conducted through clinical record review and literature analysis.

Results: The cohort included 12 patients with MED13L (aged 4–25 years). Point mutations in the *MED13L* gene were identified in most cases (9/12), followed by intragenic deletions (3/12), all of which were de novo. Facial dysmorphisms were common, with the most frequent features being bulbous nasal tip (6/10) and a prominent/bulging forehead (4/10). Approximately 60% of patients presented hypotonia, with delayed gait acquisition, and on the neuropsychological profile, ADHD (5/12) and autistic spectrum traits (5/12) were predominant. Expressive language delay was present in all patients (3 being non-verbal), with most showing a more preserved comprehension. Brain MRI in 75% of patients revealed non-specific findings (subarachnoid cysts, dilated Virchow-Robin spaces, and ventricular enlargement). Regarding extra-neurological involvement, only one patient had a cardiac condition (pulmonary stenosis). Other findings included orthopedic deformities (7/9), strabismus (5/10) and skin manifestations (5/8).

Conclusion: We observed a broader clinical spectrum with milder manifestations, and lower prevalence of autistic spectrum disorder, epilepsy and cardiac defects compared to what is described in the literature. A thorough characterization enhances clinical understanding and provides valuable information for management and prognosis.

C.O.I. Disclosure:

L. Pias-Peleteiro: None. **A. Gutierrez-Rodriguez:** None. **M. Pereira Soares:** None. **M. Bolasell Girgas:** None. **S. Lotz-**

Esquivel: None. **P. Montserrat:** None. **M. O'Callaghan:** None. **C. Ortez Gonzalez:** None. **L. Martorell Sampol:** None. **D. Yubero:** None. **J. Armstrong:** None. **M. Serrano Gimare:** None. **A. Martinez-Monseny:** None.

EP11.089 Genetic similarity between Autism Spectrum Disorder and comorbid brain disorders

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Background: A range of brain disorders are comorbid with Autism Spectrum Disorder (ASD), such as Epilepsy, Intellectual Disability or Anxiety. Previous evidence suggested a shared genetic influence between ASD and several brain disorders. In this study, we sought to further substantiate the genetic similarity between ASD and a set of comorbid brain disorders.

Material and methods: We constructed a network of 29 brain disorders based on their genetic similarity, estimated from the Jaccard coefficient between disease pairs. The Leiden algorithm was used to identify network disease communities. In 3,881 ASD cases from a whole-genome sequencing dataset, we further searched for de novo loss-of-function (LoF) Single Nucleotide Variants (SNVs) in genes shared by the disease communities.

Results: Through network analysis we identified three disease communities, including a heterogeneous community that is genetically more similar to ASD, and that also includes Epilepsy, Bipolar Disorder, Attention-Deficit/Hyperactivity Disorder combined type, and some disorders in the Schizophrenia Spectrum. Several of the genes shared by diseases communities are strong ASD candidate genes, such as *SHANK3*, *SCN2A, ASH1L* or *CHD2*, and harbour the highest number of rare de novo LoF SNVs in ASD patients.

Conclusion: This study provided further evidence for a shared genetic architecture between ASD and several other brain disorders, including some frequent comorbidities of ASD. It also showed that ASD patients have rare de novo LoF variants in genes associated with frequent comorbid disorders, and identified genes that overlap between brain disease communities.

C.O.I. Disclosure:

J. Vilela: None. **H. Martiniano:** None. **A.R. Marques:** None. **J.X. Santos:** None. **C. Rasga:** None. **G. Oliveira:** None. **A.M. Vicente:** None.

EP11.090 Genetic variant identification in malformations of cortical development: a reanalysis of whole exome sequencing data

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Background: Malformations of cortical development (MCD) represent a rare, diverse, and heterogeneous group of neurogenetic disorders, often presenting with developmental delay, cerebral palsy, or epilepsy. The genetic and clinical heterogeneity, coupled with their rarity, presents significant diagnostic challenges. Here, we aimed to re-evaluate the data of patients diagnosed with MCD who could not be molecularly diagnosed through initial whole exome sequencing (WES) analysis.

Material and methods: A cohort of 20 MCD patients, for whom no molecular diagnosis was established in the initial WES analysis, underwent comprehensive reanalysis. Raw sequencing data were re-aligned to the hg38 reference genome to improve variant calling accuracy and alignment precision. Additionally, an alternative commercial data analysis tool (SEQ) was employed for comparative variant interpretation. Reverse phenotyping was performed based on the identified candidate genes to refine genotype-phenotype correlations.

Results: Reanalysis provided a molecular diagnosis in 60 % (12/20) of cases. Biallelic variants were identified in *SAMHD1, ESAM, TIAM1*, *MAN1B1*, and *NPHP4*, while monoallelic variants were identified in *POGZ*, *IRF2BPL, COL4A1*, *MACF1, CSTF2*, and *PIK3R2*.

Conclusion: Systematic reanalysis of WES data significantly enhances diagnostic yield in MCD by identifying previously overlooked variants, refining genotype-phenotype correlations, and contributing to a better understanding of the underlying molecular mechanisms.

C.O.I. Disclosure:

B. Kasap: None. **C. Yalcinkaya:** None. **N. Gunes:** None. **D. Uludag Alkaya:** None. **S. Saltık:** None. **E. Sengenc:** None. **B. Tuysuz:** None. **M. Sayitoglu:** None.

EP11.091 The two faces of pediatric SCA2

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Background: Spinocerebellar ataxia type 2 (SCA2) is an autosomal dominant neurological disease usually described in adults. Expanded CAG repeats in the *ATXN2* gene can lead to pediatric onset. This study aims to describe the natural history of SCA2 in children.

Material and methods: We analyzed clinical and genetic data from 22 children with SCA2 across 17 institutions and compared them to 20 previously reported cases and to pediatric SCA7.

Results: Unlike pediatric SCA7, which exhibits a continuum within the phenotypic spectrum, the phenotype of pediatric SCA2 can be divided into two distinct groups based on CAG repeat size and age. In the infantile group ($n = 9$), developmental delay and seizures were prominent features, along with cerebellar and cerebral atrophy. In the juvenile group ($n = 13$), the disease was a cerebellar degeneration similar to adults. A threshold of 88 ± 4 CAG repeats distinguished the infantile group from the juvenile group. Pediatric SCA2 type was independent of parental origin, SCA2 was maternally inherited in 22%, including three infantile presentations.

Conclusion: This large cohort of pediatric SCA2 disease provides the first comprehensive description, which characteristics differ from those of SCA7. Pediatric SCA2 is recognizable yet challenging to diagnose with whole-genome sequencing. Genetic counseling for families should consider the significant proportion of maternal transmission.

C.O.I. Disclosure:

N. Rive Le Gouard: None. **M. G. Bah:** None. **G. Coarelli:** None. **A. Heinzmann:** None. **A. Fauret:** None. **J. de Sainte-Agathe:** None. **C. Cazeneuve:** None. **A. Gerasimenko:** None. **D. Gras:** None. **Y. Capri:** None. **M. Renaud:** None. **B. Brais:** None. **C. Grenenko:** None. **A. Masurel:** None. **P. Berquin:** None. **F. Jobic:** None. **J. Metreau:** None. **K. Deiva:** None. **A. Afenjar:** None. **V. Gravrand:** None. **A. Lannuzel:** None. **M. Anheim:** None. **T. Geis:** None. **U. Hehr:** None. **J. Madan Cohen:** None. **B. Desnous:** None. **A. J.a kievit:** None. **N. Bahi-Buisson:** None. **D. Rodriguez:** None. **F. Renaldo:** None. **C. Cances:** None. **D. Devos:** None. **C. Angelini:** None. **C. Goizet:** None. **C. Ewenczyk:** None. **A. Durr:** None. **C. Mignot:** None.

EP11.092 8 years experience in the evaluation of adult epilepsy in a tertiary genetics clinic in Basque country: results and implications for clinical practice

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Background: We explore the characteristics of adult epilepsies and epileptic encephalopathies in a large population of Bizkaia province in Basque Country, through multidisciplinary approach during 8 years in a third-level Clinical Genetics Clinic.

Material and methods: Patients >15 y.o. from 2017 to 2024 were evaluated by clinical geneticists after 3 Adult Epilepsy Units confirmed diagnosis of epilepsy without or with very limited previous genetic testing. Conventional cytogenetic, array-CGH, epigenetic, clinical and whole exome sequencing (singleton or trio) were performed according to pre-test genetic evaluation and counselling, and written informed consent was obtained. Demographic information, clinical features, cytogenetic and molecular findings were analyzed, including change of diagnosis or drug treatment according to genetic test results. Post-test counselling was performed, eventually including incidental findings if patients or tutors agreed to be acknowledged about them.

Results: Global rate of positive (definite or probably pathogenic findings) testing was around 42%, with an additional 5.5% of VOUS variants identified, including a strong candidate in a gene belonging to lysine acetyltransferase (KAT) group. Results vary according to subtype of epilepsy, ranging from no molecular cause found in three subgroups groups to the highest positive rate around 67% seen in syndromic epileptic encephalopathies, especially when exome trio was performed. Neurologists changed therapy according to test findings in 12% of cases. 5% incidental findings were managed by geneticists.

Conclusion: Multidisciplinary approach allowed better detection of genetic bases of adult patients with epilepsy in our population, allowing lower VOUS detection and higher positive rates than those described in literature.

C.O.I. Disclosure:

C. Catalli: None. **M. Escalza Cortina:** None. **P. De Ceballos Cerrajería:** None. **I. Llano Rivas:** None. **A. Marinas Alejo:** None. **I. Garamendi Ruiz:** None. **M. Agundez Sarasola:** None. **B. Gener Querol:** None.

EP11.093 Novel de novo mutations in early onset epilepsy from whole genome sequencing of a Middle Eastern cohort

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Background: Epilepsy is one of the most common pediatric neurological disorders, affecting 1–2% of children worldwide (1). However, its etiology remains unknown in most cases, especially in underrepresented Middle Eastern populations. A study on childhood epilepsy in Qatar found that 55% of children underwent genetic testing, with 11% diagnosed with a genetic cause (2). As genetic testing becomes more accessible, especially for children with early-onset epilepsy, the proportion of genetically diagnosed cases is expected to rise (3).

Material and methods: We recruited 79 families with at least one child affected by epilepsy and performed whole-genome sequencing for all recruited individuals. Genetic analysis was performed for a pilot of 18 families using either Congenica or Exomiser. Rare variants were filtered based on in silico prediction scores, phenotype association, and modes of inheritance, and classified using ACMG guidelines.

Results: Among the 18 families analyzed, 4 children with intractable early-onset epilepsy (<2 years of age) were found to have de novo variants in epilepsy-associated genes (2 pathogenic and 2 likely pathogenic). Both pathogenic variants are novel and actionable as they lie in voltage-gated channels, and the seizures were controlled with the use of sodium channel blockers.

Conclusion: Our study highlights the role of de novo mutations in early-onset epilepsy in Middle Eastern populations. The novel pathogenic variants expand the mutational spectrum and emphasize the need for genetic studies. While epilepsy in Arab populations is often linked to recessive inheritance, our findings stress the importance of considering de novo variants in clinical evaluations.

C.O.I. Disclosure:

Z. Siddig: None. **R. Farhad:** None. **R. Abouelhassan:** None. **M. Tauro:** None. **H. Naeem:** None. **M. Ghorbani:** None. **R. Benini:** None. **Y. Mokrab:** None.

EP11.094 Variants in *SPOUT1* are associated with epileptic encephalopathy, and severe global developmental delay

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Background: SPOUT1, a member of the SPOUT family of methyltransferases, contains a catalytic domain crucial for RNA methylation. This study investigates the role of bi-allelic missense variants in *SPOUT1* and their association with refractory seizures and severe global developmental delay in affected infants.

Material and methods: Whole-genome sequencing (WGS) was conducted on affected individuals, followed by segregation analysis within families to confirm variant inheritance. In silico structural modeling was utilized to predict the impact of identified *SPOUT1* variants on protein stability. Immunoblotting of patient-derived fibroblasts quantified SPOUT1 protein levels, while immunofluorescence assessed subcellular localization and nuclear speckle integrity.

Results: WGS identified three homozygous missense variants in *SPOUT1* in three families: c.292G>A (p.Gly98Ser), c.1058C>T (p.Thr353Met), and c.836T>C (p.Phe279Ser). Structural modeling predicted that these variants destabilize the SPOUT1 protein, potentially leading to its degradation. Immunoblotting revealed significantly reduced SPOUT1 protein levels in fibroblasts carrying the G98S variant compared to controls. Immunofluorescence showed abnormal nuclear speckle organization in patient fibroblasts, likely contributing to the observed neurological symptoms. The F279S variant replaces a bulky, hydrophobic residue with a small polar side chain, creating an unoccupied cavity and further destabilizing the protein, suggesting a shared pathogenic mechanism across variants.

Conclusion: This study provides the first direct evidence linking SPOUT1 deficiency to neurodevelopmental disorders characterized by refractory seizures and severe global developmental delay. The findings highlight SPOUT1's critical role in nuclear speckle organization and RNA processing, offering valuable insights into the molecular mechanisms underlying epilepsy and related disorders, and laying the groundwork for future therapeutic exploration.

C.O.I. Disclosure:

A. Mesika: None. **L. Kalfon:** None. **G. Nadav:** None. **A. Litmanovich:** None. **S. Ben Haroush:** None. **N. Cohen Kfir:** None. **E. Shuster Biton:** None. **H. Ladany:** None. **D. Savitzki:** None. **B. Ben-Zeev:** None. **R. Abou Jamra:** None. **T. Hershkovitz:** None. **M. Dessau:** None. **T.C. Falik-Zaccai:** None.

EP11.095 Characterizing Direct and Indirect Parental Genetic Effects on Child Behavioural Problems

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Background: Childhood mental health problems can be caused both directly via genetic inheritance and indirectly by parent-derived genetic nurture effects[1]. To better understand genetic mechanisms underlying the influences of parental psychiatric disorders and related mental traits on child's behavioural problems, we studied two European cohorts using trio-based genomic and epidemiological data.

Material and methods: Generation R[2,3] (GenR3 and GenR4, N = 1649, Dutch) and Prediction and Prevention of Preeclampsia and Intrauterine Growth Restriction[4] study (PREDO, N = 249, Finnish) were investigated. For each cohort, we calculated polygenic scores (PGSes) for 13 psychiatric disorders and 3 associated traits (educational attainment, alcohol and cigarette consumptions) from child (PGSc), mother (PGSm), and father (PGSf) in each family. We used these PGSes as predictors for child's internalizing, externalizing, and total behavioural problems, measured by the CBCL/6-18 and calculated as summative t-scores. We conducted linear regression in two models leveraging each individual PGS and trio PGSc-PGSm-PGSf, respectively. We also applied logistic regression leveraging the individual PGS and the trio PGSes on dichotomized child's total problem t-scores (cutoff ≥60), respectively. Finally, we used inverse variance-weighted fixed effect method to meta-analyze cohort-level results.

Results: Multiple individual child's and maternal PGSes predicted child's behavioural problems. Interestingly, PGSf-neuroticism negatively predicted child's externalizing problems above PGSc itself, while PGSm-insomnia predicted a higher risk for child's clinically significant behavioural problems.

Conclusion: While most psychiatric traits showed direct genetic transmission, paternal neuroticism and maternal insomnia may convey genetic nurture effects on child behavioural problems. We have been recruiting more cohorts to further expand the current study.

C.O.I. Disclosure:

L. Tian: None. **M. Shahisavandi:** None. **M. Lahti-Pulkkinen:** None. **J. Tuhkanen:** None. **C.A. Cecil:** None. **K. Räikkönen:** None. **A. Neumann:** None. **J. Lahti:** None.

EP11.096 *HTRA1* Gene-Related Cerebral Arteriopathy with Subcortical Infarcts and Leukoencephalopathy: A Single-Center Experience

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Background: Biallelic variants in the *HTRA1* gene are associated with the rare CARASIL disease. Heterozygous *HTRA1* variants cause autosomal dominant cerebral small vessel disease (CADASIL type 2). Although CARASIL shares imaging findings similar to CADASIL, it differs by presenting with significant memory loss and extracerebral manifestations (e.g., alopecia, connective tissue involvement).

Material and methods: The mutation spectrum and clinical manifestations of patients diagnosed with germline *HTRA1* mutations through the clinical exome panel at the Department of Medical Genetics, Ege University were analyzed in a retrospective manner.

Results: Five families with *HTRA1* variants who visited the Ege University Medical Genetics outpatient clinic were evaluated. The mean age at presentation was 43.2 years, and the mean age of symptom onset was 29.4 years. The clinical manifestations included recurrent ischemic strokes, transient ischemic attacks, headaches, cognitive decline, gait and speech disturbances, numbness, incontinence, depressive symptoms, pseudobulbar signs, and seizures. Earlier disease onset (ages 24 and 25) was observed in two families with biallelic *HTRA1* variants. Cranial MRI scans showed white matter hyperintensities. Three heterozygous variants were identified in the *HTRA1* gene: one missense, one nonsense, and one splice-site variant. Additionally, two homozygous missense variants were found in two families with consanguinity. The *HTRA1* variant c.496C>T (p.Arg166Cys) was previously reported, while the c.249C>A (p.Cys83*), c.1235C>T (p.Ala412Val), c.1040T>G (p.Val347Gly), and c.1120+1G > A variants were newly discovered in this study.

Conclusion: By presenting our single-center experience, this study contributes to the existing body of literature, facilitating a more comprehensive understanding of genotype-phenotype correlations and informing future diagnostic and therapeutic approaches.

C.O.I. Disclosure:

F. UĞuzdoĞan: None. **A. Aykut:** None. **D. Evyapan akkuŞ:** None. **Ö. Ekmekçİ:** None. **F. Gökçay:** None. **H. Akin:** None. **A. Durmaz:** None.

EP12 Neuromuscular Disorders

EP12.001 Late-onset Tay-Sachs disease: a rare and atypical presentation resembling motor neuron disorders

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Background: A 31-year-old patient presented with limb weakness, difficulty climbing stairs, gait disturbances, and muscle cramps. Neurological examination revealed moderate proximal lower limb flaccid paraparesis, and electromyography indicated anterior horn cell involvement at the lumbosacral level. Spinal muscular atrophy (SMA) is the most common anterior horn cell disease (AHCD). Approximately 95–98% of SMA cases result from homozygous deletions of exon 7 in *SMN1*, while 2–5% are due to point mutations or small insertions/deletions. Detecting point mutations is challenging due to the presence of its highly homologous pseudogene, *SMN2*. Given this distribution, a stepwise genetic testing approach was recommended.

Material and methods: MLPA analysis of *SMN1* gene revealed two copies of exons 7–8 in *SMN1* (point mutation of *SMN1* has not been performed). Whole-exome sequencing identified the previously reported variant rs121907954 c.805G>A (p.Gly269Ser) in a homozygous or hemizygous state in exon 7 of the *HEXA* gene.

Results: The p.Gly269Ser variant in the *HEXA* gene is associated with late-onset Tay-Sachs disease (LOTS), a rare form of the disease characterized by progressive neurological symptoms. Typically, Tay-Sachs disease manifests in infancy (3–6 months) with hypotonia and developmental regression, followed by seizures, blindness, and spasticity, leading to death by 2–4 years. In contrast, our patient had a slowly progressive limb weakness, gait disturbances, and muscle cramps, mimicking neuromuscular disorders.

Conclusion: This case highlights the importance of DNA-based diagnostics for differentiating LOTS from other neuromuscular disorders. Late-onset Tay-Sachs disease can present atypically, resembling anterior horn cell diseases. LOTS is exceptionally rare, particularly in non-Ashkenazi Jewish populations.

C.O.I. Disclosure:

N. Andreeva: None. **D. Ferdman:** None. **I. Kazachkova:** None. **K. Danko:** None. **N. Isaeva:** None. **R. Bikanov:** None.

EP12.002 From VUS to Pathogenic A Case Report on Nemaline Myopathy

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Background: The NEB gene encodes nebulin, which constitutes 3–4% of total myofibrillar protein. Mutations in this gene are associated with autosomal recessive Nemaline Myopathy 2. Although hundreds of disease-causing variants of the NEB gene have been identified, some mutations remain classified as variants of uncertain significance (VUS).

Material and methods: An 11-year-old boy with weakness in the lower extremities presented for genetic consultation. There was no known family history of genetic conditions. He exhibited abnormal walking patterns, fatigue, pain, a positive Gower's sign, and tibial muscle pseudohypertrophy. Initially, muscular dystrophy was suspected. Whole exome sequencing was performed.

Results: Primary findings revealed that the patient was heterozygous for NEB p.Tyr2024Cys and NEB p.Leu1503Trp. Del/dup (CNV) analysis was negative. The analysis was also negative for secondary findings. Both NEB p.Tyr2024Cys and NEB p.Leu1503Trp variants are classified as VUS (variants of uncertain significance). Considering the patient's clinical manifestations and

the phenotype-genotype correlation, these VUS may potentially be reclassified as clinically significant or likely pathogenic.

Conclusion: In this case, it is essential to consider the genotype-phenotype correlation for timely diagnosis and to guide appropriate treatment and rehabilitation. Additionally, performing specific studies in the parents is important for future reclassification and for better understanding the genetic inheritance pattern. Reclassification should be carried out properly as new information becomes available, which is also critical for family planning decisions in the future.

C.O.I. Disclosure:

N. Vardosanidze: None. **L. Margvelashvili:** None. **O. Kvlividze:** None. **D. Agladze:** None.

EP12.003 A Novel Heterozygous Variant in *DRD2* as a Cause of Movement Disorder

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Background: Dopamine is a key neurotransmitter in the central nervous system. The dopamine D2 receptor (D2R), a G protein-coupled receptor, is predominantly expressed in the basal ganglia, which regulates movement. It is also involved in emotion, reward, and has been implicated in disorders like alcoholism and schizophrenia. Variants within the transmembrane domains (TM5 and TM6) of DRD2 have been linked to an autosomal dominant disorder featuring chorea and dystonia. Heretofore only two pathogenic de novo *DRD2* variants (p.Ile212Phe, p.Met374Arg) have been reported.

Material and methods: Exome sequencing (TRIO-ES) was performed on a 3-year-old patient with walking difficulties (three-point gait, foot rotation) and frequent falls. Neurological examination revealed pyramidal syndrome and a persistent Babinski reflex, with cognitive, social, and emotional development above expectations. Motor functions showed no improvement despite rehabilitation.

Results: A heterozygous missense variant, c.610G>A (p.Val204Ile), was found in the *DRD2* gene. This variant was not present in the parents, suggesting it is de novo. It was absent in gnomAD (v4) and an in-house database of 12,000 Polish exomes. No other likely pathogenic variants were identified. The p.Val204Ile variant is located in the fifth transmembrane domain, a core binding site for D2R agonists.

Conclusion: This study reports a novel heterozygous variant in *DR D2*, which may cause movement disorders. Despite insufficient in silico evidence for pathogenicity, the rarity of the variant and its location in a functional domain suggest its potential role in the observed phenotype. Further functional studies are warranted.

C.O.I. Disclosure:

G. Kostrzewa: None. **K. Rutkowska:** None. **A. Pollak:** None. **M. Rydzanicz:** None. **M. Franaszczyk:** None. **R. Ploski:** None.

EP12.004 Case report of a rare *CAPN3* gene variant affecting RNA splicing and causing calpainopathy

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Background: Calpainopathy is characterised by symmetric and progressive weakness of proximal limb-girdle muscles. It has several phenotypes while pelvifemoral limb girdle dystrophy is the most common and manifest from 12 to 30 years of age. Despite clear phenotype, diagnosis can be difficult, especially in cases when only one *CAPN3* gene pathogenic variant is found.

Methods: We report 51 year old female who was diagnosed with progressive muscular dystrophy since the age of 13, with proximal muscles more affected. From age of 16 patient had difficulty climbing stairs, had an abnormal gait. Symptoms progressed with radiating leg pain and at 46 years patient lost ambulation. Creatine kinase was increased up to 600 UI. Whole exome sequencing (WES) was performed for patient and neuromuscular gene panel was analysed.

Results: WES identified two variants: NM_000070.3 (*CAPN3*):c.550del which is one of the most common pathogenic variants for calpainopathy, and NM_000070.3(*CAPN3*):c.1536+3A > G. Patients' father was unavailable for testing. Patients' mother was carrier of c.550del variant, suggesting variant *trans* phasing. Variant c.1536+3A > G was classified as variant of uncertain significance (VUS), because it was rare and no homozygous occurrences were seen in GnomAD (PM2_supporting), SpliceAI predicted donor loss (PP3), and variant was in *trans* with other pathogenic variant (PM3). Patients' RNA was extracted from lymphocytes and cDNA was analysed revealing an increased amount of abnormally spliced RNA and decreased amount of wild-type product compared to control samples.

Conclusion: According to clinical and molecular data we confirm that *CAPN3* c.1536+3A > G variant affects splicing and causes calpainopathy.

C.O.I. Disclosure:

R. Jonikas: None. **M. Šukys:** None. **R. Ugenskienė:** None.

EP12.005 Enhancing O-Mannosyl glycans on alpha dystroglycan for treating Fukuyama muscular dystrophy

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Fukuyama congenital muscular dystrophy (FCMD) is a severe, intractable genetic disease that affects the skeletal muscle, eyes, and brain and is attributed to a defect in alpha-dystroglycan (αDG) O-mannosyl glycosylation. We previously reported that the basic polycyclic compound Mannan-007 (Mn007) restored αDG glycosylation in brain and muscle models and partially rescued the abnormal radial glial (RG) migration observed in cortical organoids. However, mechanism was unknown. In this report, we investigated the mechanism by which Mn007 enhances glycan production. Using single-cell RNA sequencing analysis and an HPLC assay on HEK cells, we found that Mn007 enhances the enzymatic activity of O-mannosyl glycosyltransferase.

C.O.I. Disclosure:

M. Taniguchi-Ikeda: None. **M. KoyanagiAoi:** None. **S. Mizumoto:** None. **Y. Harada:** None. **T. Maruyama:** None. **T. Aoi:** None.

EP12.006 Molecular genetic markers of neuromuscular disorders in children

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Background: Neuromuscular disorders are a heterogeneous group of diseases, including both hereditary and acquired forms characterized by disorders of the structure and a function of the muscle tissue. Early detection of hereditary forms of neuromuscular disorders is important for the initiation of the timely treatment.

Material and methods: The study included 25 patients aged from 1 to 16 years (an average age - 7.64 ± 4.66 years), of whom 20 were boys and 5 were girls. All patients underwent clinical, biochemical and genetic examinations. Genetic testing was performed using molecular genetic methods (the Lumbar-limb muscular dystrophy panel and the genome-wide sequencing).

Results: The manifestation of the disease was observed at the age of 5 months to 3 years. Molecular genetic analysis revealed pathogenic variants in the following genes: *SPTAN1, RYR1, TRAP, LAMA2, INF2, DMD, KIF5A, TRIM32, ASXL3, ANO5, QRICH1, POLG, KIF1B, PRX, FKBP14, SMN2, NKX2-1, TTN, ACADM*. The most common mutations were in genes associated with the progressive Duchenne/Becker muscular dystrophy (*DMD*) (n = 3) and myopathies associated with anotazine deficiency (*ANO5*) (n = 2). Genetic pathology in a myopathy syndrome was found in 76% (n = 19).

Conclusion: 76% of neuromuscular disorders have a genetic cause. Detection of mutations in genes associated with neuromuscular disorders allows accurate diagnosis and selection of an individual therapeutic strategy.

C.O.I. Disclosure:

O.I. Gumeniuk: None. **M.E. Lobanov:** None. **Y.V. Chernenkov:** None. **O.S. Groznova:** None.

EP12.007 A strange paraparesis: when the obvious sign is missing

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Background: Cerebrotendinous Xanthomatosis (CTX) is a rare autosomal recessive lipid storage disease usually presenting in childhood, caused by biallelic mutations in the CYP27A1 gene, resulting in cholesterol deposition in various tissues. Symptoms include gastrointestinal and heart problems, juvenile cataract, seizures, movement disorders, cognitive decline. Tendon xanthomas are often a clinical clue.

Material and methods: neurological examination, genetic testing.

Results: This female patient started complaining about difficulty in walking at age 44; within 1 year she developed cognitive decline with a slowly progressive course. Early milestones and past medical history were unremarkable; at age 17, she underwent eye surgery for bilateral cataract; she had gluten intolerance. Her sister had adult onset spastic paraparesis and dementia with behavioral problems starting during the 4th decade and celiac disease and died at 54 from pancreatic cancer. Patient's brain MRI showed irregular signal of corpus callosum and of and cervical medulla, with white matter alterations, mild cortical and subcortical atrophy. Blood test revealed mild increase in triglyceride, cholesterol and bilirubin levels with liver steatosis. Neurological examination showed cognitive decline, paraparetic-ataxic gait, difficulty in standing with feet together, no tremor nor dysmetria in the upper limbs, bilateral bradykinesia in lower limbs with right predominance, hyperreflexia with pyramidal signs, spasticity in the lower limbs, bilateral pes cavus, normal cranial nerves, a single xanthelasma around the right eye. Genetic testing revealed a homozygous *CYP27A1* mutation (NM_000784.4:c.825G>A;p.(Trp275*)).

Conclusion: neurological, adult onset CTX is an ultra-rare, potentially treatable disorder that should not be missed, since symptoms can improve with Chenodeoxycholic acid.

C.O.I. Disclosure:

I. Gabbiato: None. **V. Fumini:** None. **G. Bonato:** None. **B. Savini:** None. **L. Bresciani:** None. **P. Santurelli:** None. **L. Salviati:** None. **A. Antonini:** None. **N. Vitturi:** None. **M. Carecchio:** None. **D. Zuccarello:** None.

EP12.008 The genetic neonatal screening for spinal muscular atrophy in Romania - results of a two years pilot project

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Background: Spinal muscular atrophy (SMA), severe neurodegenerative disorder of childhood, benefits from three disease-modifying treatments, all available for Romanian patients. While SMA is considered nowadays a real neurogenetics urgency, children with diagnostic of SMA need to be treated as soon as possible, preferably in presymptomatic state for the best results. This objective can be achieved by newborn screening (NBS), already applied for more than two thirds of newborn population in European Union.

Material and methods: Our pilot project, first NBS genetic screening in our country, covers twenty-five maternities and approximately 12–15% of Romanian newborn population. More than 45 000 samples were analyzed in two year and a half time period, from August 2022, for homozygous deletion of exon 7 in SMN1 gene with a validated Melt Assay method, kit SALSA MC002 SMA Newborn Screen (MRC Holland).

Results: Eight cases were detected positive and all were confirmed by second-tier genetic confirmatory test (MLPA), indicating until now, a high incidence of SMA.

Conclusion: As 2025 is the year targeted to be the time when NBS for SMA will be available for all newborns in UE, we strongly advocate for including the first neuromuscular disease, SMA, in the National Program for Newborn Screening.

C.O.I. Disclosure:

E. Neagu: None. **E. Bercu:** None. **O. Balanescu:** None. **M. Nicut:** None. **D. Vasile:** None. **F. Grigoras:** None. **S. Shelby:** None. **C. Bercu:** None. **M. Leanca:** None. **A. Mirea:** None.

EP12.009 Congenital fiber-type disproportion myopathy in LZTR1-related Noonan Syndrome: expanding the phenotype

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Background: Noonan syndrome (NS) is a heterogeneous genetic disorder characterized by distinct phenotypic features, including congenital heart defects, facial anomalies, short stature, and learning difficulties. It is most caused by mutations in genes involved in the RAS signaling pathway, such as *PTPN11*, *SOS1*, *KRAS*, *NRAS*, and *RAF1*. Recently pathogenic variants in *LZTR1* were linked with clinical features of NS. Myopathy is not a classical hallmark of NS but has been reported for few NS patients with *PTPN11* pathogenic variants.

Methods: We collected clinical data in a patient presenting with congenital hypotonia, cardiomyopathy, mild dysmorphism and intellectual deficiency. Molecular investigations were performed with primary analysis of 1478 genes using Whole Exome Sequencing (WES), followed by Whole Genome Sequencing (WGS).

Results: A 30-year-old man born of consanguinous parents of Libanese origins, presented with congenital muscle hypotonia, typical myopathic elongated facies, double chambered ventricule, cardiomyopathy and slight aortic dilatation. Cardiomyopathy resolved around the age of 4 years while myopathy did not, but remain stable. Additionally, the patient has mild cognitive impairment (TIQ 52). Muscle biopsy at age 2 demonstrated muscle fibers disproportion. Physical examination showed growth delay during childhood with generalised underdeveloped muscle bulk. Some facial features were suggestive of NS. WES failed to identify pathogenic variants. However WGS revealed a homozygous deep intronic variant, c.1943-256C > T, p.(Thr648fs*36) already reported as pathogenic.

Conclusion: This is the first report of a Noonan syndrome patient related to pathogenic variants in *LZTR1* presenting with congenital fibers disproportion myopathy which expands the phenotypic spectrum of *LZTR1* related NS.

C.O.I. Disclosure:

C. Marconi: None. **F. Masclaux:** None. **J. Blouin:** None. **J. Lobrinus:** None. **J. Fluss:** None. **R. van heurck:** None.

EP12.010 A three-year spinal muscular atrophy newborn screening program in a Spanish region

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Background: Spinal muscular atrophy (SMA) is a hereditary condition defined by biallelic pathogenic variants in the survival of motor neuron 1 (*SMN1*) gene, with homozygous deletion of exon 7 being responsible for approximately 95% of cases. The *SMN1* deficiency results in progressive muscle weakness, particularly affecting proximal muscle groups. Early diagnosis through newborn screening (NBS) and the commencement of treatment before symptom onset are vital for optimizing health outcomes in individuals affected by this condition.

Material and methods: We carried out a screening trial in the Valencia community between October 2021 and August 2024. This screening involved the extraction of DNA from dried blood spots (DBS) and was conducted by a multiplex quantitative polymerase chain reaction assay aimed at the c.840C position within exon 7 of the *SMN1* gene to identify homozygous *SMN1* deletions. Genetic confirmation was achieved using multiplex ligation-dependent probe amplification (MLPA) and the AmplideX PCR/CE *SMN1/2* Plus kit.

Results: A three-year prospective study on population-based newborn screening was conducted at La Fe University and Polytechnic Hospital in Valencia. So far, 31,560 DBS samples were examined, resulting in four positive cases, which corresponds to an incidence rate of 1 in 7,890. Genetic methods provided consistent results for the copy number of *SMN1* and the survival motor neuron 2 (*SMN2*) gene in all patients.

Conclusion: In line with other projects, this study demonstrate how newborn screening is necessary for the presymptomatic identification of SMA patients in order to increase their survival and quality of life.

C.O.I. Disclosure:

A. Berzal-Serrano: None. **B. García-Bohórquez:** None. **E. Aller:** None. **T. Jaijo:** None. **I. Pitarch-Castellano:** None. **D. Rausell:** None. **G. García-García:** None. **J. Millán:** None.

EP12.011 Pathogenic variants of the *CAPN3* gene associated with autosomal recessive limb-girdle muscular dystrophy type 2A (LGMD2A)

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Background: LGMD2A also known as calpainopathy is the most common form of limb-girdle dystrophy, that caused by mutations in the gene encoding the protein calpain 3 and is characterized by selective atrophy and weakness of the muscles of the proximal extremities and muscles of the upper and lower girdle. The aim of the study was to analyze the genetic testing of sequences of 153 genes associated with skeletal muscle diseases in patients with suspected limb-girdle dystrophy.

Material and methods: Clinical-genealogical and method of differential diagnosis was performed in 48 patients observed at the Medical Genetics Center with suspected limb-girdle dystrophy. Genetic sequence analysis of *CAPN3* gene performed by "Invitae" (USA).

Results: The sequencing data of the *CAPN3* gene sequence in the study group were analyzed and 6 pathogenic homozygous variants were identified. Among them, a homozygous variant of Exon 4 (c.550del (p.Thr184Argfs*36); rs762960425, ExAC 0.05%) was found in four families. This sequence change creates a premature translational stop signal in *CAPN3* gene and result in an absent or disrupted protein product. We also identified 1 family case of homozygous pathogenic variant of *CAPN3* Exon 8 (c.1043del (p.Gly348Valfs*4); rs781013226, ExAC 0.01%) and 4 isolated heterozygous cases of minor pathogenic variants with autosomal recessive inheritance.

Conclusion: The analysis of the *CAPN3* gene sequence among patients with suspected limb-girdle dystrophy showed a high frequency (12,5%) of pathogenic variants leading autosomal recessive limb-girdle muscular dystrophy type 2A.

C.O.I. Disclosure:

B. Tretiak: None. **K. Bakum:** None. **O. Semeryak:** None. **H. Akopyan:** None. **D. Zastavna:** None.

EP12.012 The early manifestation of hereditary spastic paraparesis SPG4 in a pediatric patient

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Background: Hereditary spastic paraparesis is a group of genetically heterogeneous disorders. The most common type of HSP is SPG 4 with AD inheritance. Onset and severity of disability in SPG4 varies. SPG4 is characterized by slowly progressive muscle weakness and spasticity in the lower half of the body.

Material and methods: Exome sequencing, Sanger sequencing, Western blot

Results: A 2-year-old boy was referred for genetic testing for gait impairment. The neurologist suspected central spastic diparesis. A brain MRI showed no causes for the symptoms. The exome sequencing revealed the variant c.131C>T, p.(Ser44Leu) in the *SPAST* gene (NM_014946.4) in the homozygous state. This variant is currently described as a phenotype modifier. When present in the trans position with another pathogenic variant in the *SPAST* gene, it leads to disease onset at a younger age. A heterozygous deletion of all 17 coding exons of the *SPAST* gene was confirmed by MLPA. The size of the deletion is at least in the range of Chr2(GRCh38):g.32063421-32156397 (c.-411-c.*1901 according to NM_014946.3). MLPA revealed the same heterozygous deletion in the mother. The variant c.131C>T, p.(Ser44Leu) was found in heterozygous state in the father. The diagnosis of SPG4 was confirmed in several other family members. To support the diagnosis in the family, spastin protein levels were determined by Western blot on peripheral blood mononuclear cells.

Conclusion: The finding of SPG4 in a pediatric patient with very early disease onset may be due to the presence of the c.131C>T variant, which has been described as a modifier of the phenotype.

C.O.I. Disclosure:

A. Pecková: None. **J. Laštůvková:** None. **D. Šafka Brožková:** None. **V. Čejnová:** None. **J. Krejčíková:** None. **F. Sardina:** None. **C. Rinaldo:** None. **A. Uhrová Mészárosová:** None.

EP12.013 Clinical and histopathological manifestations of desminopathies: From dominant to recessive inheritance in 10 cases

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Background: Desminopathies are a rare, clinically heterogeneous form of myofibrillar myopathies, presenting with distal, proximal, or scapuloperoneal phenotypes, mandatory cardiac involvement, and eventual respiratory failure. They result from dominant and, rarely, recessive *DES*-gene mutations. Histologically, desminopathies feature excessive desmin-accumulation, Z-disk alterations, and myofibrillar disintegration. Dominant mutations are identifiable via onset in early adulthood distal leg muscle weakness, MRI and histopathological features, whereas recessive mutations remain poorly characterized.

Material and methods: This study included 10 patients. All underwent clinical examination; six had needle-EMG, seven nerve conduction velocity tests, six CK analyses, and eight muscle MRI. Additionally, eight underwent cardiac assessment, and *DES* sequencing via Sanger sequencing was performed in eight. Whole-genome sequencing, FSHD genetic testing, and tibialis anterior biopsy were conducted in two patients.

Results: A classical distal desminopathy phenotype was observed in eight patients (5-males, 3-females) from three unrelated families. Sanger sequencing identified dominant missense mutations in *DES* (one de novo). Two siblings (ages 13 and 20) with shoulder girdle weakness and FSHD-like phenotype tested negative for FSHD but harbored a homozygous recessive *DES* mutation via WGS. MRI showed sartorius and adductor magnus atrophy, sparing the biceps, semitendinosus, and tibialis anterior muscles. Histochemical analysis revealed mild myopathy, up to 59%-centrally nucleated fibers, and near-total desmin absence. Electron microscopy identified rimmed vacuoles and granulofilamentous inclusions.

Conclusion: Age of onset, weakness pattern, MRI, and histopathology differ significantly between dominant and recessive desminopathies. In early-onset recessive/sporadic cases with an FSHD-like phenotype and sartorius/adductor magnus atrophy, initial *DES*-gene screening via Sanger sequencing is recommended.

C.O.I. Disclosure:

K.S. Shirokova: None. **A.V. Budnevskiy:** None. **D.A. Atiakshin:** None. **I.A. Chekmaryova:** None. **T.E. Khalilova:** None. **O.A. Tikhonova:** None. **R.E. Tokmachev:** None. **V.V. Shishkina:** None. **S.A. Kurbatov:** None.

EP12.014 A novel homozygous variant in *ALDH18A1* detected in two unrelated families in Saudi Arabia with adult-onset spastic paraplegia 9B

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Background: The initially reported cases of autosomal recessive spastic paraplegia 9B (MIM#616586) (SPG9B) were characterized by early onset of complex spastic paraplegia, intellectual disability (ID), and dysmorphic features. The first reported adult-onset spastic paraplegia was out of a Japanese cohort in 2018, characterized by a spastic gait and ID. Here, we present the first cases of SPG9B identified in Saudi Arabia.

Methods: Three females from two unrelated families were evaluated for adult-onset progressive lower limb spasticity; all three were initially misdiagnosed with multiple sclerosis. Symptoms began in their early to mid-20s. MRI brain revealed bilateral cerebral subcortical nonspecific T2 hyperintense foci in all three patients. In light of the disease's progression and MRI findings, molecular testing was done for all three patients.

Results: All three were found to be homozygous for a missense variant in the *ALDH18A1* gene, c.1073C>T p.(Pro358Leu); there is a single heterozygous submission in ClinVar, a phenotype not reported and classified as a variant of unknown significance (VOUS). This variant has not been previously reported in the literature and is absent from controls (or at extremely low frequency if recessive) in public and local databases. Computational prediction tools provided conflicting predictions.

Conclusion: We report the first cases of SPG9B in Saudi Arabia with adult-onset spastic paraplegia, normal cognition, and abnormal MRI findings. The homozygous variant identified in all three patients offers supportive evidence of its pathogenicity, given its low allele frequency and phenotypic consistency with SPG9B. Further functional studies are underway.

C.O.I. Disclosure:

N.S. Alsaleh: None. **M. Almijmaj:** None. **A. Abulaban:** None.

EP12.015 Expanding the Molecular Spectrum of Alpha-Sarcoglycanopathy (LGMD R3) in Moroccan Patients: Insights from Next-Generation Sequencing

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Background: Limb-girdle muscular dystrophies (LGMDs) represent a genetically and clinically diverse group of neuromuscular disorders. One such subtype, limb-girdle muscular dystrophy due to alpha-sarcoglycan deficiency (LGMD R3), is caused by mutations in the *SGCA* gene (17q21.33). This autosomal recessive condition typically manifests in childhood with progressive weakness of the pelvic and/or scapular girdle muscles and calf hypertrophy, exhibiting considerable clinical variability both within and between families. This study aims to broaden the molecular spectrum of the *LGMD* genes by identifying pathogenic variants, thereby enhancing patient management and genetic counseling for affected families.

Material and methods: We investigated ten unrelated families presenting with clinical and paraclinical features of LGMD, after excluding dystrophinopathies in boys and LGMD R5 associated with the recurrent mutation *SGCG*:c.525delT in both boys and girls. Molecular analysis was performed using Next-Generation Sequencing (NGS) to identify genetic variants associated with the disease.

Results: Seven distinct variants in the *SGCA* gene were detected across the cohort, including four previously reported variants and three novel variants predicted to be pathogenic based on bioinformatic tools.

Conclusion: Our findings expand the known molecular spectrum of alpha-sarcoglycanopathy in the Moroccan population, specifically the LGMDR3 subtype, which is relatively rare in this demographic. These results provide important insights for genetic counseling and clinical management of affected families.

C.O.I. Disclosure:

Y. Rahmuni: None. **O. Batta:** None. **J. Lyahyai:** None. **I. Cherkaoui Jaouad:** None. **Z. Rchiad:** None. **O. Askander:** None. **A. Sefiani:** None. **I. Ratbi:** None.

EP12.016 Caveolin 3 (CAV3) variant in a complex case of drug-induced neuromuscular disorder

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Background: A 57-year-old male with a history of exercise-induced muscle stiffness, fatigue and myalgia, frequent urinary infections, and stomatitis. Symptoms started during treatment with Telbivudine. Biochemical and immunological analyses were performed, which showed normal CPK levels, but elevated inflammatory markers IL-6, IL-8 and IL-10. The patient was referred for whole exome sequencing (WES) for evaluation of suspected late-onset neuromuscular disorder.

Material and methods: WES was performed using the Celemics Whole Exome Sequencing Panel. Alignment, variant calling and annotation were performed via the Franklin (http://franklin.genoox.com) platform.

Results: Upon analysis of a neuromuscular disorder panel, a heterozygous variant of uncertain significance was identified: *MYH2*: c.5755G>A, p.Ala1919Thr, NM_0175346. The variant was excluded, due to its association with early onset congenital myopathy-6 with ophthalmoplegia (CMYO6). Reanalysis of the data detected a well-known heterozygous missense variant in the *CAV3* gene (c.233C>T, p.Thr78Met, NM_033337.3), associated with limb-gridle muscular dystrophy 1C (LGMD-1C), corresponding to the patient's clinical manifestations.

Conclusion: Genetic testing is an important part of the diagnostic evaluation of neuromuscular disorders. However, data interpretation can be extremely challenging. Variants such as the *CAV3*:c.233C>T can be easily missed due to their high frequency in the population and the conflicting reports in the literature. We hypothesize that Telbivudine has triggered rhabdomyolysis in a subject in which the CAV3 variant may present a risk factor for decreased muscle fiber integrity and signaling. Further research is essential in order to clarify the clinical relevance of such variants and enhance diagnostic decision-making.

C.O.I. Disclosure:

D. Madzharova: None. **I. Tourtourikov:** None. **I. Bogdanova:** None. **D. Vassilev:** None. **T. Kadiyska:** None.

EP12.017 Diagnostic utility of repeat number expansion analysis in ataxia patients

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Background: Ataxia is a clinically and genetically heterogeneous neurodegenerative disorder, with Spinocerebellar Ataxia (SCA) being one of the most common inherited forms. Accurate genetic diagnosis is crucial for early intervention and genetic counseling. While next-generation sequencing-based methods have

revolutionized molecular diagnostics, repeat expansions in ataxia related genes remain undetectable using this method alone.

Material and methods: This study aimed to evaluate the diagnostic utility of repeat expansion analysis by examining the ATXN1, ATXN2, ATXN3, CACNA1A, ATXN7, ATXN8, ATXN10, TBP, PPP2R2B, and FXN genes in patients presenting with ataxia. The repeat numbers detected in the tests of ataxia patients were evaluated based on gene-specific threshold values, and the results were compiled.

Results: The repeat number analysis of 28 patients from different age groups and sexes was examined. While 18 patients had normal repeat numbers and 8 patients tested positive for pathogenic repeat expansions, 2 patients had a heterozygous repeat expansion in the *FXN* gene with an unidentified second variant. Diagnostic increased repeat numbers were identified in *FXN* (4 patients), *ATXN1* (2 patients), *ATXN2* (1 patient), and *TBP* (1 patient) genes.

Conclusion: These findings highlight the complementary role of repeat expansion testing alongside exome sequencing. While exome sequencing is valuable for detecting point mutations, it is insufficient for diagnosing repeat expansion disorders, necessitating specialized genetic screening for SCA.

C.O.I. Disclosure:

H. Seker: None. **H. Bas:** None.

EP12.018 *Understand the association of PGC-1alpha polymorphism aspects and mitochondrial membrane potential in Parkinson's disease (PD) patients*

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Background: Peroxisome proliferator-activated receptor-γ co-activator (PGC)-1alpha is a transcriptional co-activator of antioxidant genes and a master regulator of mitochondrial biogenesis. Parkinson's disease (PD) is associated with oxidative stress and mitochondrial dysfunction and recent work suggests a role for PGC-1alpha. We hypothesized that the rs8192678 PGC-1alpha SNP may influence risk or age of onset of PD. We also analysed the impact of PGC-1alpha polymorphism could impact the levels of ROS among PD patients.

Methods: Using a standardised questionnaire, PD patients and control group of about 64 subjects selected for this study. The subjects include $n = 32$ PD patients and $n = 32$ control subjects who were sex and gender matched. Further the genomic DNA samples from PD patients and age-matched controls were analysed by sanger sequencing, followed by statistical analyses of the association of each SNP, alone or in combination, with risk or age of onset of PD. Further, the ROS levels were analysed using the flowcytometry assay.

Results: We found that the genetic abnormalities in PD patients showed some synonymous type followed by missense mutations in PGC-1alpha (rs8192678) GG variant more significantly. In this study, we also found there is significant levels of oxidative stress due to release of free radicals.

Conclusion: In conclusion, this is the first study to examine the association between PGC-1 alpha SNP and PD patients from Punjab, India. Investigations need to be performed for identifying the individual factors involved in PD progression in various ethnic backgrounds and we suggest that further research is mandatory to detect other possible associations among PD.

C.O.I. Disclosure:

M. Balachandar: None.

EP12.019 Pregnancy outcome in a woman with spinal muscular atrophy (SMA): A case report

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Background: Spinal muscular atrophy (SMA) is a neurodegenerative disease due to homozygous mutation in *SMN1* gene. SMA type 1 is the most fatal and presents early in infancy while type 4 presents in adulthood with mild clinical features. Women with milder version of SMA are known to be able to reproduce however their pregnancy outcome is not widely reported.

Case presentation: Our patient is a 32 year old lady with SMA who was referred for genetic counseling while being pregnant at 21 weeks. She reported progressive weakness since childhood and diagnosis of SMA was only confirmed at 30 years old by MLPA genetic analysis showing homozygous deletion of *SMN* exon 7 and 8. Premorbidly she required wheel chair for most of her activities. This is the second pregnancy of the patient with the first resulted in first trimester miscarriage. Fetal scans were normal. The baby was delivered at 31 weeks of gestation via emergency LSCS for breech in labor with the birth weight of 1000 grams. Baby was intubated transiently for mild respiratory distress syndrome. Meanwhile, our patient was intubated for one day and discharged home after 5 days. Echocardiogram and lung function test were not reported. During follow up, she was found to have reduced ambulatory function, increased weakness and suffered from constipation.

Conclusion: Multidisciplinary management is essential for pregnant women with SMA. Detailed assessment of respiratory function pre and post labor, early physiotherapy and regular rehabilitation services following birth are crucial to reduce complications and improve muscle strength.

C.O.I. Disclosure:

H. Hashim: None. **M. Haniffa:** None.

EP12.020 Epidemiological Analysis of Duchenne and Becker Muscular Dystrophy in the Republic of Bashkortostan (Russian Federation)

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Background: X-linked recessive Duchenne and Becker muscular dystrophies (DMD and BMD) are caused by mutations in the dystrophin gene (DMD; Xp21.2-p21.1). These disorders are characterized by progressive muscle weakness. The aim of this study was to provide an epidemiological analysis of DMD and BMD in the Republic of Bashkortostan, Russia.

Material and methods: A population-based registry was established to collect comprehensive data on patients with DMD and BMD in the Republic of Bashkortostan. Data was collected through patient interviews, family history questionnaires, and clinical examinations by neurologists and neurogeneticists. DNA diagnosis was performed for 110 patients.

Results: The registry currently contains information on 124 patients with DMD/BMD, including 86 children and 38 adults (January 1, 2025). Among these patients, 96 have DMD and 28 have BMD. 37 cases (30%) are familial, 86 cases (70%) are sporadic. The overall prevalence of DMD/BMD in the Republic of Bashkortostan was found to be 12.4 per 100,000 population.

Ethnic differences in prevalence were observed, with Tatars having the highest prevalence (4.3 per 105), followed by Russians (3.4 per 105) and Bashkirs (2.5 per 105). The mutation spectrum in the DMD gene was characterized by a high frequency of large deletions (46%), large duplications (12%), and nonsense mutations (18%).

Conclusion: This study provides valuable insights into the epidemiological characteristics of DMD and BMD in the Republic of Bashkortostan. The established registry serves as a resource for clinical research and can facilitate the development of targeted interventions and improvements in patient care.

C.O.I. Disclosure:

S. Yarushina: None. **E. Gaisina:** None. **R. Magzhanov:** None. **O. Schagina:** None. **E. Saifullina:** None. **E. Saifullina:** None.

EP12.021 Digenic inheritance in the *SRPK3/TTN* genes as a cause of progressive skeletal muscle myopathy and cardiomyopathy in a young boy

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Background: 15-year-old boy was resend to reanalysis data because of worsening his motor skills and proximal and axial muscle weakness. From childhood he suffers from progressive bioptically verified structural myopathy type central core disease and other minor anomalies: chest deformity, right-sided scoliosis, bilateral Achilles tendons shunts and right ventricle cardiomyopathy. Previous SNP microarray and karyotype were normal and although neuromuscular NGS panel found two significant variants in *CHRNB1* gene c.199-2A > C and in *TTN* gene c.46609_46610del, subsequent DNA analysis of his healthy parents and sister showed his father and sister also carry these familial variants and therefore these findings do not explain the underlying cause of patient´s neuromuscular phenotype.

Material and methods: Patient´s DNA was analyzed within a unknown origin disease group of pediatric patients who underwent whole-exome sequencing through the Rare Disease Research Study. Genomic DNA extracted from leukocytes of the patient, his parents (trio) and sister was used for WES on the Novaseq 6000 platform (Illumina).

Results: WES confirmed the previously reported p. pathogenic variant c.46609_46610del p. (Gln15537AspfsTer3) in *TTN* gene and revealed hemizygous deleterious variant in *SRPK3* gene c.257G>T, p.(Gly86Val). Digenic *SRPK3/TTN* inheritance has recently been described and explains fully patient´s phenotype. Changes in *SRPK3* encoding the X-linked serine/arginine protein kinase 3 lead to a progressive early skeletal muscle myopathy onset only when in combination with heterozygous variants in *TTN* gene.

Conclusion: This case report highlights the advantages of WES which can identify the hitherto hidden underlying cause of a serious disease where NGS panel has failed.

C.O.I. Disclosure:

A. Hladikova: None. **D. Grecmalova:** None. **M. Zikanova:** None. **L. Noskova:** None. **S. Kmoch:** None.

EP12.022 Haplotype analysis of the most prevalent *GBA1* pathogenic variants in Italy

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Background: Biallelic mutations on Glucocerebrosidase (GBA1) gene cause Gaucher disease (GD), the most common lysosomal storage disease; heterozygote variants represent the most frequent genetic risk factor for developing Parkinson disease (PD). This study aims to define the haplotype context of the two most prevalent GBA1 pathogenic variants in Italy (N370S/p.Asn409Ser and L444P/p.Leu483Pro), identify potential ancestral founder haplotypes linked to each variant tracing their genetic history within the Italian population.

Material and methods: **T**welve short polymorphic markers (SNPs and microsatellites) spanning 600 kB of the GBA1 locus (1q22) were analyzed in patients' DNA using automated capillary electrophoresis. The study cohort included 100 GBA1 variant carriers from Italy (N370S n = 51, L444P n = 35).

Results: All N370S carriers exhibited a shared haplotype, suggesting a single ancestral origin. In contrast, L444P carriers displayed three distinct haplotypes (designated as 1, 2, and 3). Geographical cluster analysis revealed that haplotype 1 was widespread across Italy, while haplotype 2 was predominantly found in central Italy. In southern Italy, haplotype 1 was present in 75% of cases, while haplotype 3 accounted for 25%.

Conclusion: Our findings suggest a single ancestral founder haplotype for the N370S variant, whereas the L444P variant exhibits greater genetic heterogeneity, being associated with multiple haplotypes. These results provide novel insights into the genetic history of GBA1 variants in the Italian population and may enhance the understanding of genotype-phenotype correlations and mechanisms contributing to PD risk.

C.O.I. Disclosure:

S. Lucchiari: None. **G.P. Comi:** None. **S.P. Corti:** None. **S. Pagliarani:** None. **A. Di Fonzo:** None. **E. Monfrini:** None.

EP12.023 Health-Related Quality of Life in Children with Neuromuscular Diseases in Georgia: A Multi-Domain Analysis Using PROMIS

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Background: Neuromuscular diseases (NMDs) are a broadly defined group of disorders affecting the peripheral nervous system. NMDs are characterized by their chronic nature, often progressive disability, significant comorbidities, and limited treatment options, all of which influence health-related quality of life (HRQL). Our study represents the first assessment of HRQL in children with NMDs in Georgia.

Material and methods: The study involved 51 patients aged 5 to 17 years. Patients and their parents were recruited through physician network and rare disease organizations. HRQL was assessed using the standardized Patient-Reported Outcomes Measurement Information System (PROMIS). Five domains - anxiety, depression, fatigue, pain interference, and physical function - were evaluated using pediatric and parent proxy profile instruments. Intercorrelations were done to examine the relationships between HRQL, demographic factors, and experiences of patients with NMDs.

Results: Mean HRQL scores were significantly poorer across all five PROMIS domains when compared to normative data indicating substantial impairments among pediatric patients with NMDs. The intercorrelation analysis revealed significant associations between HRQL scores and various demographic and disease-

specific factors, underscoring the multifaceted impact of NMDs on children's lives.

Conclusion: Children with NMDs in Georgia experience significantly reduced HRQL. Our study highlights the need for comprehensive care approaches that address the psychological and physical challenges experienced by these patients. Further research should explore targeted interventions to improve HRQL and support systems for affected individuals.

C.O.I. Disclosure:

K. Bregvadze: None. **E. Phagava:** None. **L. Abashishvili:** None. **T. Tkemaladze:** None.

EP12.024 Clinical and genetic characteristics of hereditary motor and sensory neuropathy type VIC in the Republic of Sakha (Yakutia)

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Background: Hereditary motor and sensory neuropathy type VIC (HMSN6C) is an autosomal recessive axonal sensorimotor peripheral neuropathy. HMSN6C is caused by homozygous mutation in the PDXK gene on chromosome 21q22. Previously, we detected a mutation in the PDXK gene by clinical exome sequencing in a Yakut family. In the world have been described 6 patients from 3 unrelated families with hereditary motor and sensory neuropathy type VIC. The purpose of the study is to describe the clinical and genetic characteristics of hereditary motor and sensory neuropathy type VIC in the Republic of Sakha (Yakutia).

Material and methods: All patients were examined by a neurologist, geneticist and ophthalmologist. For molecular genetic diagnostics, we used the PCR-RFLP method.

Results: The study included 65 patients out of 85 in whom we found a pathogenic variant c.659G>A (p. Arg220Gln) in the PDXK gene in a homozygous state. The gender composition was 35 men and 30 women. The mean age at disease debut was 10,3 years. The first symptoms such as frequent stumbles, falls, steppage were observed in all patients. Impaired walking and running was due to peripheral neuropathy. Neurological examination revealed the following clinical signs: distal muscle weakness and atrophy in hands and legs, pes cavus, hammertoes, areflexia. Optic atrophy was found in 45 (69,2%) patients.

Conclusion: A distinctive clinical feature of hereditary motor and sensory neuropathy type VIC is the presence of optic atrophy in most patients.

C.O.I. Disclosure:

A.A. Maksimova: None. **A.L. Sukhomyasova:** None. **N.R. Maksimova:** None.

EP12.025 Novel Bi-allelic *FRA10AC1* Variants in Neurodevelopmental Disorder: Implications for Growth Retardation, Dysmorphic Facies, and Corpus Callosum Abnormalities

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Background: The FRA10AC1 protein, part of the spliceosome, is crucial for recognizing and interacting with splice sites. Recently, *FRA10AC1* bi-allelic variants were associated with neurodevelopmental disorder with growth retardation, dysmorphic facies, and corpus callosum abnormalities (NEDGFC). Here, we report five cases from three unrelated consanguineous families, all exhibiting similar neurodevelopmental symptoms.

Material and methods: Using whole genome (WGS) or whole exome sequencing (WES), we identified clinically relevant variants in *FRA10AC1*. Segregation of *FRA10AC1* variants was assessed using Sanger sequencing.

Results: In family 1, two siblings aged 9 and 15 years exhibited muscle weakness, growth delay, gait imbalance, and language impairment. Family 2 had two affected siblings who presented with development and motor delay, intellectual disability, hypotonia, dysmorphism, rocker bottom feet, and brain abnormalities. The younger siblings were additionally presented with autistic behavior and genital anomalies and passed away at age 1.5. Family 3 included one affected patient aged 13 years, with development and motor delay, intellectual disability, hypotonia, behavioral issues, dysmorphism, skeletal, brain, cardiac and ocular abnormalities.We identified three rare homozygous *FRA10AC1* (NM_145246.5) variants in the affected individuals: a novel frameshift variant (c.464del, p.(Lys155fs*8)) in Family 1, a novel missense variant (c.550G>C, p.(Glu184Gln)) in Family 2, and a previously reported missense variant (c.481 C > T, p.(Arg161*)) in Family 3. Family data showed segregation of these variants with the disease.

Conclusion: This work contributes to a more comprehensive understanding of the role of *FRA10AC1* in neurodevelopmental disorders. Further functional characterization is needed to determine the disease mechanism.

C.O.I. Disclosure:

A. Simões: A. Employment (full or part-time); Modest; Arcensus GmbH. **M. Ali:** A. Employment (full or part-time); Modest; Arcensus GmbH. **S. Rafique:** None. **N. Hadi:** None. **M.S. Zaki:** None. **M. Abdel-Hamid:** None. **J. Gburek-Augustat:** None. **R. Jamra:** None. **G. Oprea:** A. Employment (full or part-time); Modest; Arcensus GmbH.

EP12.026 Evaluation of the AmplideX® SMA Plus Kit for comprehensive *SMN1/SMN2* analysis in Spinal Muscular Atrophy

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Background: Spinal muscular atrophy (SMA) is a hereditary disorder causing progressive motor weakness and atrophy, with copy number variations in *SMN1* and *SMN2* influencing disease onset and severity. Recent studies have suggested the potential impact of specific modifiers: the *SMN2* c.859G>C variant, linked to a milder phenotype, and the *SMN1* variants c.*3 + 80T > G and c.*211_*212del, associated with *SMN1* duplication and silent carrier status. However, there is no general consensus in the

literature on their significance. Accurate and urgent molecular testing is crucial for clinical management of SMA patients, where targeted therapies can significantly improve outcomes, and for preconceptual carrier screening to assess reproductive risk. This study aims to evaluate the performance of the Amplidex®SMA Plus Kit (Asuragen, CE-IVD) for *SMN1/SMN2* analysis.

Material and methods: Nineteen DNA samples from diagnostic testing and carrier screening were analyzed using the AmplideX®SMA Plus Kit, a single-reaction PCR-based assay. Capillary electrophoresis was performed on an ABI3730XL instrument, and results were analyzed using AmplideX®PCR/CE Reporter software. Results were compared with the MRC-Holland SMA-P021 MLPA assay.

Results: The AmplideX®SMA Plus Kit accurately determined *SMN1* and *SMN2* copy numbers (0, 1, 2, 3, and >4 copies). The *SMN2* c.859G>C variant was identified in one sample. No *SMN1* duplication variants (c.*3 + 80T > G or c.*211_*212del) were detected. Validation on additional samples is ongoing.

Conclusion: The AmplideX®SMA Plus Kit demonstrated a streamlined workflow with accurate results for *SMN1/SMN2* analysis. Despite higher consumable costs compared to the MRC-Holland MLPA assay, the short turnaround time (<4 hours) provides significant added value.

C.O.I. Disclosure:

L. Claeys: None. **A. De Jaegher:** None. **J. Anckaert:** None. **X. Leroy:** None. **T. Reyniers:** None. **M. Yöruk:** None. **E. De Baere:** None. **K. Claes:** None.

EP12.027 Variants in the mitochondrial genome as a cause of inherited peripheral neuropathy and hereditary spastic paraplegia

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Background: The mitochondrial genome consists of 37 essential genes. Mutations in several of these mitochondrial genes, including *MT-TK* and *MT-ATP6*, can cause neurodegenerative disorders characterised by psychomotor regression and a wide range of neurological symptoms, including dystonia, ataxia, and peripheral neuropathy. The presentation and severity of these conditions often depend on the heteroplasmic load of mutant mtDNA in different tissues.

Material and methods: As part of the re-analysis of NGS sequencing data from unclarified patients with inherited peripheral neuropathy and HSP, we specifically focused on mutations in known mitochondrial genes. As part of the family segregation analysis, the level of heteroplasmy in the proband was verified by Sanger sequencing. Where possible, samples from different tissues were analysed (peripheral blood, buccal).

Results: Out of a total 800 patients referred for NGS with a diagnosis of CMT or HSP, we clarified 12 patients by mitochondrial gene analysis. Of these, we found a variant in the *MT-ATP6* gene in 11 patients (8 families) and a variant m.8363G > A in the *MT-TK* gene in the heteroplasmic state in one patient diagnosed with CMT. We found 3 variants in the *MT-ATP6* gene in a predominantly homoplasmic state: m.8993T > C (3 patients, 2 families), m.9185T > C (7 patients, 5 families) and in one patient m.9176T > C.

Conclusion: Whole exome and genome sequencing, including mitochondrial genes, has greatly improved the genetic diagnosis of inherited disorders. As mitochondrial gene variants are common in these patients, their inclusion in genetic analysis is crucial for better diagnosis and understanding of these disorders.

C.O.I. Disclosure:

J. Krejcikova: None. **A. Musilova:** None. **P. Lassuthova:** None. **D. Safka Brozkova:** None.

EP12.028 Diagnostic rate of multigene panel testing in a Hungarian inherited neuromuscular disorders patient cohort

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Background: Inherited neuromuscular disorders (INMDs) are genetically and phenotypically heterogenous diseases affecting peripheral nerves, motor neurons, neuromuscular junction or skeletal muscle. Multigene panel testing is the most used methodology for the molecular diagnosis of INMDs.

Material and methods: In 2023, twenty-eight non-related patients with suspected INMD (18 women (64.3%) and 10 men (35.7%), mean age: 53.04 years (29–73 years)) were referred by a neuromuscular specialist to our genetic counselling outpatient clinic. None of the patients had a molecular diagnosis in spite of extensive previous genetic testing. After reviewing patient records single gene testing was ordered in 3 cases, while in 25 cases whole exome sequencing (WES) based virtual gene panel testing was initiated.

Results: Age of onset ranged from 6 to 64 years. Altogether 138 genetic tests (with an average of 4.93 tests/patient) had been ordered previously. Diagnostic rate of WES based multigene panel testing was 64%, while the overall diagnostic rate was 67.9%, resulting in 19 definitive molecular diagnoses in 28 patients. In two families the diagnosis was followed by change in patient management. Between disease presentation and diagnosis on average 22.2 years (ranging from 3–50 years) have passed. In nine-nine cases autosomal dominant and recessive, while in one case X-linked dominant inheritance pattern was shown. Cascade testing was performed in 24 family members.

Conclusion: For INMDs when there are insufficient findings to guide a specific diagnosis, the most powerful genetic testing option is multigene panel testing, which is cost-effective and significantly reduces diagnostic odyssey.

C.O.I. Disclosure:

K. Koczok: None. **E. Kovács:** None. **P. Baksa:** None. **O. Nagy:** None. **I. Balogh:** None. **J. Boczán:** None.

EP12.029 Pathogenic variants found in the Bulgarian population associated with rare neurological and neuromuscular disorders in Roma/Gypsy ethnic groups

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Background: The Roma population is the largest transnational ethnic minority in Europe, with a high prevalence of inherited disorders due to migration patterns and endogamy driven by cultural isolation. Founder variants in Bulgarian Roma groups have been reported since 1996, initially linked to HMSN Lom type and LGMD2C. This study summarizes newly identified pathogenic variants associated with severe atypical neurological phenotypes in Roma/Gypsy ethnic groups in Bulgaria.

Methods: Patients were analyzed using whole-exome sequencing (WES). Identified potentially pathogenic variants were confirmed through Sanger sequencing, and segregation analysis was performed in the families.

Results: Up to now pathogenic variants in several genes were published and identified in Bulgarian Roma patients with atypical neurological presentation. During the course of routine diagnostics two pathogenic variants were detected in *VAMP1* gene and *UFM1* gene.

Conclusion: The identification and characterization of pathogenic variants in specific Roma groups enable precise genetic diagnoses and facilitate targeted screening for heterozygous carriers. These findings contribute to improved clinical management, healthcare education and identification of new cases in the Roma populations and genetic counseling for affected individuals and their families.

C.O.I. Disclosure:

C.I. Stoikova: None. **N. Ivanova:** None. **K. Mihova:** None. **I. Ivanov:** None. **I. Pacheva:** None. **V. Varbanova:** None. **I. Litvinenko:** None. **I. Tournev:** None. **A. Jordanova:** None. **I. Dimova:** None. **R. Kaneva:** None.

EP12.030 Phenotypic and genetic characterization of Tunisian patients with congenital muscular dystrophies

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Background: Congenital muscular dystrophies (CMDs) are a clinically and genetically heterogeneous group of neuromuscular disorders that typically manifest at birth or in early infancy. Their clinical spectrum ranges from mild to severe forms involving the central nervous system. Herein, we present cases of patients with CMDs, including two severe forms with prenatal onset.

Methods: Patients with suspected CMDs were recruited to this study. Molecular analysis was performed using targeted next-generation sequencing (NGS) (clinical exome or panel including 17 genes associated to CMDs).

Results: Six patients from six unrelated families were included in this study. Consanguinity was observed in 4 cases. The age of symptom onset ranged from the prenatal period to 14 months. The most common presenting sign was congenital hypotonia (4/6 cases) revealed by walking difficulties in 2 cases. Prenatal clinical manifestations as major hydrocephalus and corpus callosum and vermian atrophy were observed in two cases. Electroneuromyography revealed a myogenic pattern in all patients. One case exhibited occipital micropolygyria. Death occurred in one case.Molecular analysis identified six distinct pathogenic or likely pathogenic variants in four different genes: one in *COL6A2*, one in *COL6A3*, two in *POMT1*, and two in *LAMA2*, including one novel variant. These findings confirm the diagnosis of congenital muscular dystrophy in all cases, and allow for appropriate genetic counseling.

Conclusion: Our study highlights the crucial role of NGS in diagnosing complex clinical presentations. This approach facilitates molecular confirmation, enhances management strategies, and ensures accurate genetic counseling.

C.O.I. Disclosure:

W. Essalah: None. **Y. Elaribi:** None. **S. Hizem:** None. **A. Ghenimi:** None. **I. Kraoua:** None. **B. Gilbert-Dussardier:** None. **L. Ben Jemaa:** None. **H. Jilani:** None.

EP12.031 The *TDP1*:c.1478A>G variant as the main cause of spinocerebellar ataxia with axonal neuropathy 1 (SCAN1): a possible founder mutation in the Middle East

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Background: The 1478A > G;p.His493Arg variant in the *TDP1* gene, encoding tyrosyl-DNA phosphodiesterase 1, is the main cause of Spinocerebellar ataxia with axonal neuropathy-1 (SCAN1), an extremely rare neurological disorder, with only three reported families worldwide. It is mainly characterized by progressive ataxia and axonal polyneuropathy. In this study, we present the first Iranian, and the fourth SCAN1 family totally, which carries 1478A > G variant in *TDP1*.

Material and methods: Whole-exome sequencing (WES) was conducted on the proband's DNA, who had an initial diagnosis of axonal neuropathy. The WES data were analyzed, and a candidate variant was confirmed through Sanger sequencing. To validate the variant within the family, co-segregation analysis was performed. Finally, a systematic review was carried out.

Results: WES results revealed the known pathogenic *TDP1*:c.1478A>G variant, which was later confirmed in the family. The clinical and paraclinical findings in our case were consistent with those typically documented in the previously identified three SCAN1 families; nonetheless, clinical heterogeneity was conspicuous. Our systematic review elucidated 15 distinct variants across 19 families. Among these families, three were classified as SCAN1.

Conclusion: Given that all documented SCAN1 families harbored *TDP1*:c.1478A>G variant and originated from the Middle East, we propose the notion of genetic homogeneity and the potential existence of a founder mutation in SCAN1 cases within this geographic region. Therefore, it is recommended that for patients from this region who present symptoms of cerebellar ataxia and polyneuropathy prior to the age of 30, the *TDP1*:c.1478A>G variant and SCAN1 be prioritized in diagnostic considerations.

C.O.I. Disclosure:

M. Mohammadi: None. **M. Ravanbod:** None. **A. Ghasemi:** None. **H. Gharebaghian:** None. **S. Nafissi:** None. **A. Alavi:** None.

EP12.032 Novel*HACE1* deep intronic variant affects splicing, causing Spastic Paraplegia - Severe Developmental Delay - Epilepsy Syndrome

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Background: Biallelic mutations in *HACE1* are known to cause Spastic paraplegia - severe developmental delay - epilepsy syndrome. We investigated three siblings of a consanguineous Bedouin family with a similar phenotype, whose causative mutation had eluded standard genetic testing methods.

Material and methods: Whole-genome sequencing (WGS) was employed to investigate non-coding regions, complemented by linkage analysis using Homozygosity-Mapper. In silico splicing predictions were performed using SpliceAI. Molecular verification was carried out through Sanger sequencing to confirm the segregation of the identified variant.

Results: Combining linkage analysis and WGS we identified a novel deep intronic variant in *HACE1* (NM_020771.4: c.535-686A > G) in three siblings presenting with axial hypotonia, global developmental delay, generalized epilepsy, and recurrent fever. In silico splicing analysis using SpliceAI predicted that this variant is likely to introduce a 37-nucleotide cryptic exon, leading to a deleterious frameshift and nonsense-mediated decay (NMD).

Conclusion: This study presents the first reported deep intronic pathogenic variant in *HACE1*, emphasizing the crucial role of intronic elements in disease pathogenesis. It also highlights the utility of WGS in elucidating undiagnosed cases, particularly those related to *HACE1*-associated disorders.

C.O.I. Disclosure:

A. Biller*: None. **T. Poleg*:** None. **N. Hadar:** None. **V. Dolgin:** None. **R. Proskorovski-Ohayon:** None. **O.S. Birk:** None.

EP12.033 Childhood-onset of neutral lipid storage disease with myopathy in a hungarian family: a case report

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Background: Neutral lipid storage disease with myopathy (NLSDM) is a rare genetic disorder caused by impairment in Adipose Triglyceride Lipase (ATGL) activity that leads to an accumulation of neutral lipids within lipid droplets in most patients' tissues. Main clinical symptoms include skeletal myopathy, cardiomyopathy and liver dysfunctions, with an average onset of around 30 years. Here, we describe a 27-year-old Hungarian patient characterized by an unusual childhood onset.

Material and methods: Clinical evaluation, histological analyses of peripheral blood, muscle biopsy, MRI and liver ultrasound were conducted. Genomic DNA, RNA and proteins were extracted from peripheral blood and used for molecular analyses.

Results: In patient, clinical manifestation began at age 6 with weakness to pes planus muscles and elevated CK levels. Thigh muscle MRI and histological analyses of muscle biopsy showed lipid accumulation. Leucocytes, stained with May-Grünwald-Giemsa, revealed lipid vacuoles (Jordans' anomaly). Persistent and progressive myopathy and muscle atrophy in lower limbs were observed. Recently, hepatic steatosis was also detected.

Our patient is a compound heterozygous for c.24G>A and c.798dupC *PNPLA2* mutations. RT-PCR expression and sequencing showed that the mRNA with the frameshift mutation underwent to nonsense-mediated mRNA decay process, while the c.24G>A *PNPLA2* allele was normally expressed. Nevertheless, western blotting analysis failed to detect ATGL protein expression in white blood cells.

Conclusion: This is a very interesting NLSDM case displaying severe *PNPLA2* mutations with no ATGL production and characterized by an unusual clinical presentation consisting in a symmetric myopathy of pes planus muscles in early infancy.

C.O.I. Disclosure:

S. Missaglia: None. **E. Martegani:** None. **V. Fylymonenko:** None. **D. Tavian:** None. **C. Angelini:** None.

EP12.034 Clinical and genetical study of patient from Republic of Bashkortostan (Russian Federation) with novel mutation in *GJB1* gene

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Background: Hereditary motor-sensory neuropathies (HMSN, Charcot-Marie-Tooth disease, CMT) comprise a group of clinically and genetically heterogeneous disorders of the peripheral nervous system. Hereditary motor-sensory neuropathy 1X (HMSN 1X) is caused by mutations in the *GJB1* gene and is the second most common form of HMSN in most populations.

Material and methods: We analyzed 234 unrelated HMSN families from Republic of Bashkortostan (total population is 4.06 million people) for mutations in the *GJB1*gene.

Results: HMSN 1X occurs in 14,7 % of all HMSN cases in the Republic of Bashkortostan. Five out of six *GJBI* missense mutations were previously described: c.65G>A (p.Arg22Gln), c.224G>A (p.Arg75Gln), c.257C>T (p.Thr86Ile), c.259C > G (p.Pro87Ala), c.658C>T (p.Arg220Stop). The c.259C > G (p.Pro87Ala) mutation is major, it occurs in the 72% of families with HMSN 1X. The c.71G>A (p.Trp24Stop) mutation is novel. Proband (male patient) with c.71G>A (p.Trp24Stop) mutation is the only patient in his family (Russian origin), but his mother was not examined. The disease manifested in the proband in his early teenage years and was characterized by severe impairment of the peripheral nerves. The clinical picture includes progressive weakness and muscular atrophy of the distal extremities, tendon areflexia, decreased proprioception, sensitive ataxia, bilateral pes equinovarus deformity. Median motor conduction velocity (MCV) was 23–32 m/s, the M-amplitude was 0,2/0,3 mV.

Conclusion: The study found that HMSN 1X accounts for 14.7% of all HMSN cases in Bashkortostan, with a novel mutation c.71G>A (p.Trp24Stop) identified in one patient. To clarify the origin of this mutation, examination of the proband's mother is recommended.

C.O.I. Disclosure:

E.V. Saifullina: None. **R.V. Magzhanov:** None. **A.N. Gabitova:** None. **R.R. Enikeeva:** None. **I.M. Khidiyatova:** None. **D.R. Saifullina:** None.

EP12.035 Investigation of smoke and genetic risk factors on Multiple Sclerosis severity score

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Background: Multiple Sclerosis (MS) is a complex demyelinating, clinically heterogeneous disease of the Central Nervous System.

More than 200 genetic loci are known to be involved in its susceptibility. However, genetic and environmental disease modifier factors concurring with a more severe disease course are still elusive. Several epidemiological studies and clinical trials have confirmed an association between smoking and an increased risk of developing MS. We aimed to investigate the correlation between genetic factors, smoke habits and severity of the disease, assessed as MSSS (Multiple Sclerosis Severity Score).

Material and methods: We calculated a Polygenic Risk Score (PRS) using the 201 non-HLA SNPs kwon to be associated with MS susceptibility and correlated it with MSSS score with an ANCOVA test using smoking status as covariate in 768 patients. Smoking status was assessed with a validated questionnaire.

Results: The mean MSSS was higher in smokers compared to non smokers, consistently with what has been reported in the literature, although the difference was not statistically significant. No correlation was observed between the PRS and the MSSS status.

Conclusion: Our results suggest that smoking may be an aggravating factor in the progression of MS. However, this finding need confirmation on a bigger sample set.

C.O.I. Disclosure:

M. Thavamani: None. **N. Barizzone:** None. **N. Pomella:** None. **M. Leone:** None. **S. D'Alfonso:** None.

EP12.036 Analysis of genetic variants of uncertain significance (vus) in complex diseases

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Background: Variants of uncertain significance (VUSs) are genetic variants that cannot be classified as benign or pathological due to limited scientific or clinical data. This study aimed to manually reinterpret the VUS detected in our ALS cohort and assess their functional impact using clinical, molecular and population databases and bioinformatics tools. To complete the analysis, we also studied the 3D structure of the mutated proteins.

Material and methods: In a group of 49 ALS patients and 5 with other neurological diseases from our database, we evaluated the VUS genetic data reported by different NGS panels. We used clinical databases, bioinformatic tools (like PolyPhen-2, SIFT, MutationTaster, I-Mutant2.0, PROVEAN, MUpro, SNP&GO and AlphaMissense), and 3D analysis on ChimeraX.

Results: A total of 55 VUS were found in 44 of the 54 patients who underwent genetic testing. These VUS consisted of 44 missense, 3 intronic, 1 frameshift, 1 InDel, 1 3'UTR and 1 inframe variation. In particular, at least 7 out of 8 bioinformatics tools agreed on an in silico pathogenic prediction for 13 of these mutations, underlining their potential pathogenic role.

Conclusion: These findings highlight the importance of detailed characterization of VUSs to improve understanding of the genetic basis of the disease and to refine diagnostic interpretation. Functional studies are needed to validate these findings and to support a more accurate classification of VUS.

C.O.I. Disclosure:

In silico pathogenic prediction of mutations

GENE	Coding	Protein	GENE	Coding	Protein
ALS2 NM_020919.4	c.1037A>G	p.Asn346Ser	SETX NM_015046.7	c.3052G>T	p.Asp1018Tyr
ALS2 NM_020919.4	**c.529G>C**	**p.Gly177Arg**	SETX NM_015046.7	c.2479A>G	p.Lys827Glu
ALS2 NM_020919.4	**c.1595G>C**	**p.Gly532Ala**	SETX NM_015046.7	c.245G>A	p.Gly82Glu
AP4E1 NM_007347.5	c.2903A>T	p.Lys968Met	SETX NM_015046.7	c.2096A>G	p.Glu699Gly
ATL1 NM_015915.5	**c.1295A>C**	**p.Tyr432Ser**	SETX NM_015046.7	c.4612 C > T	p.Arg1538Trp
ATP13A2 NM_022089.4	**c.1498C>T**	**p.Arg500Cys**	SETX NM_015046.7	c.3878C>T	p.Pro1293Leu
ATP13A2 NM_022089.4	**c.2617G>A**	**p.Val873Met**	SETX NM_015046.7	c.245G>A	p.Gly82Glu
BICD2 NM_001003800.2	c.81G>T	p.Lys27Asn	SETX NM_015046.7	**c.3878C>T**	**p.Pro1293Leu**
DDHD2 NM_015214.3	c.574del	p.Arg192GlufsTer3	SETX NM_015046.7	c.245G>A	p.Gly82Glu
DHTKD1 NM_018706.7	c.1043T>C	p. Leu348Pro	SETX NM_015046.7	c.6085A>G	p.Lys2029Glu
DNAJB2 NM_006736.6	**c.544C>T**	**p.Arg182Cys**	SPG11 NM_025137.4	c.2636C>T	p.Ser879Phe
ErbB4 NM_005235.3	c.371G>A	p.Arg124Lys	SPG11 NM_025137.4	c.3047C>G	p. Pro1016Arg
FIG4 NM_014845.6	**c.1334 G > A**	**p.Gly445Asp**	SPG11 NM_025137.4	c.3122_3124del	p.Arg1041del
FUS NM_004960.4	c.1293-5 C > T	/	SPG11 NM_025137.4	c.4085T>C	p.Leu1362Pro
HNRNPA1 NM_031157.4	c.508 G > A	p.Val170Met	SPG11 NM_025137.4	c. 359T > G	p. Leu120Trp
KIF1A NM_001244008.2	c.5044 T > C	p.Tyr682His	SPG7 NM_003119.4	c.2219A>G	p.Tyr740Cys
KIF1A NM_001244008.2	c.2467C>T	p.Pro823Ser	SQSTM1 NM_003900.5	c.1316 C > T	p.Pro439Leu
KIF5A NM_004984.4	c.1958A>G	p.Lys653Arg	SQSTM1 NM_003900.5	c.86C>G	p.Pro29Arg
KIF5A NM_004984.4	**c.170 C > T**	**p.Thr57Met**	SQSTM1 NM_003900.5	**c.1001G>A**	**p.Gly334Glu**
KIF5A NM_004984.4	c.2920 T > G	p.Ser974Ala	SQSTM1 NM_003900.5	c.1316 C > T	p.Pro439Leu
MATR3 NM_018834.6	**c.2410A>G**	**p.Lys804Glu**	TAF15 NM_139215.3	c.1637G>C	p.Ser546Thr
NEFH NM_021076.4	c.370A>T	p.Ser124Cys	TBK1 NM_013254.4	c.1319G>A	p.Arg440Gln
NEK1 NM_001199397.3	c.271C>T	p.Arg91Ter	TBK1 NM_013254.4	c.1718G>A	p.Arg573His
OPTN NM_001008212.2	**c.1698C>A**	**p.Asp566Glu**	TRIP4 NM_135855.2	**c. 1508C > T**	**p.Pro503Leu**

In silico pathogenic prediction of mutations

	SIFT	POLYPHEN-2	SNPs&GO	Mutation taster	MUPRO	AlphaMissense	PROVEAN	I-Mutant 2,0
ALS2 c.1595G>C p.Gly532Ala	Predict Not Tolerated	Probably damaging score of 1.000	DiseaseRI 7	Disease causing	ΔΔG = -0.88444755 (DECREASE stability)	0,982	Deleterious −3.035	Stability Decrease RI 0
ATP13A2 c.2617G>A p.Val873Met	Predict Not Tolerated	Probably damaging score of 1.000	DiseaseRI 6	Disease causing	ΔΔG = -0.70511833 (DECREASE stability)	0,744	Deleterious-2.859	Stability Decrease RI 4
ALS2 c.529G>C p.Gly177Arg	Predict Not Tolerated	Probably damaging score of 1.000	DiseaseRI 6	Disease causing	ΔΔG = 0.010680246 (INCREASE stability)	0,949	Deleterious −3.579	Stability Decrease RI 8
ATP13A2 c.1498C>T p.Arg500Cys	Predict Not Tolerated	Probably damaging score of 1.000	DiseaseRI 7	Disease causing	ΔΔG = -1.4755942 (DECREASE stability)	0,239	Deleterious −5.960	Stability Decrease RI 4
ATL1 c.1295A>C p.Tyr432Ser	Predict Not Tolerated	Probably damagingscore of 0.999	DiseaseRI 4	Disease causing	ΔΔG = -0.8444385 (DECREASE stability)	0,480	Deleterious −5.535	Stability Decrease RI 5
DNAJB2 c.544C>T p.Arg182Cys	Predict Not Tolerated	Probably damaging score of 0.998	DiseaseRI 1	Disease causing	ΔΔG = -0.59214057 (DECREASE stability)	0,430	Deleterious −4.673	Stability Decrease RI 5
FIG4 c.1334 G > A p.Gly445Asp	Predict Not Tolerated	Problably damaging score of 1.000	DiseaseRI 7	Disease causing	ΔΔG = -0.0094938108 (DECREASE stability)	0,994	Deleterious −6.519	Stability Decrease RI 2
KIF5A c.170 C > T p.Thr57Met	Predict Not Tolerated	Probably damaging score of 0.999	DiseaseRI 2	Disease causing	ΔΔG = -1.1820431 (DECREASE stability)	0,207	Deleterious −2.959	Stability Decrease RI 4
MATR3 c.2410A>G p.Lys804Glu	Predict Not Tolerated	Probably damaging score of 0.993	DiseaseRI 1	Disease causing	ΔΔG = -0.93334095 (DECREASE stability)	0,969	Neutral 0.521	Stability Decrease RI 9
OPTN c.1698C>A p.Asp566Glu	Predict Not Tolerated	Probably damaging score of 1.000	DiseaseRI 1	Disease causing	ΔΔG = -0.62596552 (DECREASE stability)	0,992	Deleterious −3.582	Stability Increase RI 4
SETX c.3878C>T p.Pro1293Leu	Predict Not Tolerated	Probably damaging score of 1.000	DiseaseRI 9	Disease causing	ΔΔG = -1.7232299 (DECREASE stability)	0,475	Deleterious −2.925	Stability Decrease RI 7
SQSTM1 c.1001G>A p.Gly334Glu	Predict Not Tolerated	Probably damaging score of 0.999	DiseaseRI 5	Disease causing	ΔΔG = -0.99919128 (DECREASE stability)	0,267	Deleterious −2.783	Stability Decrease RI 2
TRIP4 c.1508C>T p.Pro503Leu	/	Problably damaging score of 1.000	DiseaseRI 2	Disease causing	ΔΔG = -0.027775664 (DECREASE stability)	0,451	Deleterious −8.533	Stability Decrease RI 8

F. Perrone: None. **B. Perrone:** None. **M. Pecoraro:** None. **V. Mosca:** None. **R. Iuliano:** None. **R. Spataro:** None. **F.L. Conforti:** None.

EP12.037 Recurrence risk assessment for SMA in a family showing both silent carriers, *SMN1* to *SMN2* gene conversion event and multiple copies of *SMN1/2Δ7-8* gene

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Background: Spinal Muscular Atrophy (SMA) is a neuromuscular disorder causing muscle weakness and atrophy; it is the second most common severe autosomal recessive disease, caused by pathogenetic alterations of *SMN1* gene. Prognosis is affected by copy number of the paralog *SMN2*, for which the difference is related to two variants in exons 7 and 8. Most cases show homozygous absence of *SMN1* and majority of carriers present a single *SMN1* copy. However, carrier screening and genetic counselling can be tricky due to silent carriers, carrying two copies of *SMN1* on the same chromosome (2 + 0 genotype), *SMN1-SMN2* hybrid genes, and *SMN* variant lacking exons 7 and 8 (*SMN1/2Δ7-8)*.

Material and methods: *SMN1/2* MPLA was performed in a pregnant woman whose brother died with SMA due to *SMN1* homozygous deletion. Screening of her parents and sister was needed to clarify her genotype.

Results: proband's sister was found to carry 3 copies of *SMN1*. Proband's MPLA analysis showed 2 copies of *SMN1* exon 7, one copy of *SMN2* exon 7, 3 copies of *SMN1* exon 8 and one copy of *SMN2* exon 8, suggestive of a silent carrier with a hybrid *SMN* gene due to gene conversion event, and 2 copies of the *SMN1/2Δ7-8* gene. Parent's genotyping confirmed gene conversion in the father and a "2 + 0" genotype in the mother, also showing 4 copies of *SMN1/2Δ7-8* gene.

Conclusion: we presented a peculiar SMA carrier genotyping, which emphasizes the importance of accurate molecular

characterization in risk recurrency assessment and for therapeutic purposes.

C.O.I. Disclosure:

E. Zacchi: None. **P. Rimessi:** None. **M. Fabris:** None. **C. Trabanelli:** None. **A. Percesepe:** None. **V. Uliana:** None.

EP12.038 Clinical, Pathological and Genetic Spectrum of Collagen VI- Related Disorder in Russia

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Background: Collagen VI-Related Dystrophies are the most commonly wide spread Congenital muscular dystrophies (CMDs) in clinical practice. Mutations in the genes encoding collagen VI main chains (*COL6A1, COL6A2, COL6A3* and *COL12A1*), cause Ullrich congenital muscular dystrophy (UCMD), Bethlem myopathy (BM) and myosclerosis myopathy (MM), which cover a wide clinical spectrum of muscle wasting and weakness, joint contractures, distal laxity, and respiratory compromise. A registry of patients with collagen VI is being created in Russia in January 2024 and updated every 6 months.

Material and methods: From January 2024 to January 2025 169 patients, of whom 156 (92,3 %) patients had all registry data completed, including pathogenic variants. We evaluated clinical, sex, age, onset and genetic data of 156(92,3%) patients.

Results: Of the 156 patients 92 (60%) males and 64(40%) females, 123(78.8%) patients were younger than 18 years of age. According parents voice, the debut of first symptoms was at 2 ± 2 years. Pathogenic variants were identified in 72(46,1%) *COL6A1*, 27 (17.3%) *COL6A2*, 44 (28,2%) *COL6A3*, 8 (5,1%) *COL12A1* chromosomes. 25(16,03%) patients with UCMD were non-ambulatory since birth. Currently, 90 (57.6%) patients are able to walk.

Conclusions: mutations in the *COL6A1* gene were detected in the majority of 72(46,1%) patients. The age of onset and severity of clinical manifestations differed even in patients with the same genotype, combined with the absence of contractures and distal hypermobility complicates diagnosis and assessment of prognosis for patients. Maintaining a patient registry opens up new opportunities for patient management and for clinical research.

C.O.I. Disclosure:

D.S. Abramova: None. **S.B. Artemyeva:** None. **S.N. Bardakov:** None. **L.M. Kuzenkova:** None. **E.A. Melnik:** None. **A.V. Monakhova:** None. **A.F. Murtazina:** None. **I.V. Shulyakova:** None. **D.V. Vlodavets:** None. **S.A. Kurbatov:** None.

EP12.039 Alternative splicing events of three rare variants in *CHKB* gene causing Megaconial Congenital Muscular Dystrophy

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Background: *CHKB* gene encodes the Choline/ethanolamine kinase (CHKB) protein, which catalyzes the first step of phosphatidylcholine biosynthesis. Pathogenic *CHKB* variants have been associated with Megaconial congenital muscular dystrophy (MDCMC).

Methods: Whole-exome sequencing (WES) was used to identify the carrier gene(s) in a Spanish female patient with progressive myopathy and severe muscle atrophy, intellectual delay, absence of expressive language development, overweight, and mitochondrial abnormalities. In order to assess the pathogenicity of variants found, expression experiments of RNA were carried out.

Results: WES uncovered three heterozygous *CHKB* variants in the patient, one from the paternal allele and two from de maternal allele (NM_005198.5: c.[581G > A];[843T > C;1031 + 3G > C]). The c.581G>A variant, instead to produce a missense change (p.Arg194Gln), induces an aberrant splicing event resulting in the deletion of exon 4 (*V1* and *V2*: r.448_581del). On the other hand, the other two variants (c.843T>C (p.Phe281=) and c.1031+3G > C splice site variant) induce five alternative splicing events by altering the splice sites of exons 8 and 9 (*V3*: r.928_1031del, *V4*: r.970_1033del, *V5*: r.1026_1033del, *V6*: r.820_1033del and *V7*: r.819_927del). In all cases, the predicted codified proteins are truncated in carboxy-terminus, affecting to important domains of the protein or are likely to be degraded by NMD.

Conclusion: We describe for the first time the pathological mechanism of the c.581G>A variant, and show that c.843T>C (synonymous variant) is involved in deletion of exon 8. Consequently, our findings expand the genetic spectrum associated with *CHKB* variants.

C.O.I. Disclosure:

F.J. Cotrina-Vinagre: None. **M.E. Rodríguez-García:** None. **M. Martín-Cazaña:** None. **A. Cárdenas Del Carre:** None. **M. Morales-Conejo:** None. **E. Martín-Hernández:** None. **F. Martínez-Azorín:** None.

EP12.040 Investigating the molecular mechanisms of 5'UTR variants in the *MYH3* gene in CPSFS1B patients

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Background: The *MYH3* gene plays a critical role in encoding myosin heavy chain proteins, which are essential for proper muscle development and function. Pathogenic variants in this gene have been associated with a spectrum of congenital disorders, including Contractures, Pterygia, and Spondylocarpotarsal Fusion Syndrome-1B (CPSFS1B). Studying pathogenic variants of MYH3 is crucial for understanding the molecular mechanisms underlying these conditions and improving diagnostic accuracy.

Material and methods: Total RNA was isolated from cultured patient fibroblasts using Trizol. Splicing alterations were assessed using RT-PCR followed by targeted next-generation sequencing. The impact of genetic variants on splicing was further investigated using an in vitro luciferase reporter system.

Results: Whole-exome sequencing (WES) of three patients with CPSFS1B identified several pathogenic variants in the *MYH3* gene, including c.-9 + 1G > A in the second donor splice site. Although this variant disrupts the canonical splice site, its functional impact on the gene remained unclear, as the second exon is noncoding. RT-PCR analysis revealed no splicing abnormalities but demonstrated reduced allele-specific expression associated with the splicing variant. Using a luciferase reporter assay, we confirmed decreased transcription and translation linked to this variant. Furthermore, we tested additional variants in the first and second donor sites, as well as the first acceptor site, and observed that all resulted in reduced expression of the *MYH3* gene.

Conclusion: These findings highlight the role of splice site variants, even in noncoding regions, in modulating MYH3 expression, potentially contributing to the CPSFS1B phenotype.

C.O.I. Disclosure:

M. Skoblov: None. **A. Kuzevanova:** None. **V. Tabakov:** None. **V. Zabnenkova:** None. **M. Bulakh:** None. **T. Markova:** None. **A. Filatova:** None.

EP12.041 Implementation of fragment analysis for the detection of *FGF14* GAA-repeat expansions in patients with late-onset ataxia

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Background: The *FGF14* gene encodes the fibroblast growth factor 14, which exhibits high expression in the brain. It interacts with voltage-gated ion channels to modulate neuronal excitability and is crucial for synaptic plasticity and neurogenesis in the hippocampus. Heterozygous variants in the *FGF14* gene are associated with autosomal dominant spinocerebellar ataxia (SCA) 27A and SCA27B. A GAA-repeat expansion within the intron 1 of the *FGF14* gene has been identified as a common cause of SCA27B. Current evidence suggests a pathogenic threshold in repeat length of ≥ 250 uninterrupted GAA-repeats. A GAA-repeat number of 716 has been identified in a French Canadian family.

Material and methods: Usually these large repeat expansions are determined through long-read sequencing, but this analysis is not yet provided on a routine basis by the manufacturer of our choice. We have implemented a fragment analysis and gel electrophoresis protocol, based on the method outlined by Bonnet et al. This genetic analysis method involves fluorescent labeling of DNA fragments, their separation by capillary electrophoresis, and sizing by comparison to an internal standard.

Results: Our protocol was validated using seven patients with and without ataxia and known *FGF14* GAA-repeat numbers. Following the implementation of this method, we analyzed eight Austrian patients with ataxia of unknown genetic etiology, five of whom we diagnosed with SCA27B. The repeat length in these patients with SCA27B ranged from 280 to 650 GAA-repeats.

Conclusion: We adopted and validated a simple, reproducible and fast protocol for detecting GAA-repeat expansions in *FGF14*, associated with late-onset ataxia.

C.O.I. Disclosure:

M. Hagen: None. **A. Zeilner:** None. **R. Praschberger:** None. **E. Indelecato:** None. **S. Boesch:** None. **M. Witsch-Baumgartner:** None. **J. Zschocke:** None.

EP12.042 Cardiac manifestations in female carriers of *DMD* pathogenic variants

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Background: Dystrophinopathies are X-linked disorders caused by pathogenic variants in the *DMD* gene. While Duchenne muscular dystrophy (DMD) predominantly affects males, female carriers can also exhibit symptoms, including neuromuscular weakness and cardiac abnormalities. Two-thirds of male cases are inherited from carrier mothers. Between 2.5–19% of female carriers show symptoms. This study aims to compare cardiac manifestations in symptomatic and asymptomatic carriers of pathogenic *DMD* variants.

Material and methods: A cross-sectional study was conducted on 30 female carriers of pathogenic *DMD* variants, divided into symptomatic (n = 14) and asymptomatic (n = 16) groups. Clinical evaluation included CPK levels, echocardiography, and FEVI assessment. Statistical comparisons were made between groups.

Results: The mean age was 37.57 ± 11.55 years in symptomatic and 37.06 ± 12.40 years in asymptomatic carriers. Symptomatic carriers exhibited significantly higher mean CPK levels (603.58 vs. 155.18, $p < 0.05$). Echocardiographic findings revealed that 57% of symptomatic carriers had cardiac abnormalities, including left ventricular remodeling. Mean LVEF was slightly lower in symptomatic carriers (56.7 ± 4.92 vs. 62.0 ± 3.00). No correlation was observed between specific genetic variants and clinical severity, or influence of X-chromosome inactivation.

Conclusion: Symptomatic DMD carriers have a higher prevalence of cardiac alterations, particularly ventricular remodeling and valvular dysfunction, compared to asymptomatic carriers. These findings highlight the need for routine cardiac screening in female carriers to facilitate early detection and intervention.

C.O.I. Disclosure:

A.F. Perez-Sanchez: None. **S. Godínez-Hernández:** None. **J. Ramírez-Zenteno:** None. **F. Ramiro:** None. **M. Laura Elia:** None. **G. Calvo-Anguiano:** None. **J. Lugo-Trampe:** None. **A. Ordaz-Farías:** None. **B. Garfio-Pacheco:** None. **M. Ibarra-Ramírez:** None.

EP12.043 *NKX2-1*-related disorder associated to balanced structural variants (SVs): novel disease-causing mechanism for benign hereditary chorea

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Background: *NKX2-1*-related disorders range from benign hereditary chorea (BHC) to choreoathetosis, congenital hypothyroidism, and neonatal respiratory distress syndrome. Mostly *NKX2-1* pathogenic variants are within the coding region. A few deletions are also reported involving the whole gene or a noncoding region downstream of *NKX2-1*. We present two cases due to balanced structural variants detected and characterized by optical genome mapping (OGM).

Patients and Results: Patient 1. Male patient 2.5-year-old with abnormal neurodevelopment, hypothyroidism and continuous choreoatetotic movements. Normal exome and array results. Karyotype showed a complex endowment involving chromosomes 7q11.21, 13q14, 14q11.2, 22q13.1. OGM showed a balanced t(7;14) with the breakpoint on chromosome 14 located between the noncoding region downstream of *NKX2-1* and the gene itself. In addition, a balanced t(7;13) interrupting the *AUTS2* gene related to intellectual development disorders was also characterized. **Patient 2**. Female patient 12-year-old with benign chorea and hypothyroidism. Father with similar but milder symptoms. Normal exome, array, and karyotype results. OGM showed an interstitial inversion (~4 Mb) involving the whole *NKX2-1* gene relocated it far away from the noncoding region downstream of *NKX2-1*. Father is a carrier of the same interstitial inversion in mosaic state.

Conclusion: SVs emerge as a new disease-causing mechanism in *NKX2-1*-related disorders when interrupting regulatory elements in this critical interval expecting a similar effect to that of the deletion. Karyotype and OGM are diagnostic techniques to be considered when conventional molecular diagnostic techniques do not allow the characterization of diseases or characteristics with a high suspicion of a genetic basis.

C.O.I. Disclosure:

C. Pérez: None. **E. Lloveras:** None. **B. Méndez:** None. **S. Martín:** None. **D. Gómez:** None. **B. Pérez:** None. **A. Cueto-González:** None. **N. Castells-Sarret:** None. **A. Plaja:** None.

EP12.044 The role of *SMN1/SMN2*copy number and variants in identifying silent carriers and risk assessment

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Background: Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disease and is the primary genetic cause of infant death. More than 95% of SMA patients have a homozygous *SMN1* exon 7 deletion while *SMN2* copy number affects disease severity. Carriers typically have one functional *SMN1* copy (1 + 0) with a carrier risk of 1:35, whereas "silent carriers" have two *SMN1* copies on one chromosome and none on the other one (2 + 0). Recent studies have shown that two *SMN1* variants (c.*3 + 80T > G and c.*211_*212del) increase carrier risk for "silent carriers", regardless of ethnicity, when co-occurring with two *SMN1* copies.

Material and methods: We analyzed 3137 Caucasian individuals from 2022 to 2024 using AmplideX®SMN1/2Kit which quantifies *SMN1/SMN2* copy number and detects *SMN1* and *SMN2* variants. All participants provided informed consent.

Results: We identified 2 SMA patients, 92 carriers, 13 with an *SMN1* hybrid peak, 26 with *SMN1* variants, and 15 with *SMN2* variants. Among the 26 patients with *SMN1* variants, 13 had three *SMN1* copies, 12 had two copies, and 1 had four copies. In particular, we investigated a pregnant woman with a classic SMA carrier and her partner with two *SMN1* copies and *SMN1* variants, reaching a 1/12 residual risk of being a "silent carrier". The genetic analysis of the fetus showed three copies of *SMN1* gene and *SMN1* variants, confirming the male partner's silent carrier status.

Conclusion: Our study underlines the importance to investigate the variants alongside *SMN1* and *SMN2* copy number to define the risk of silent carrier.

C.O.I. Disclosure:

M. Casillo: None. **L. Circelli:** None. **A. Di Carlo:** None. **G. Savarese:** None. **R. Ruggiero:** None. **M. Fantuz:** None. **L. De Falco:** None. **R. Sirica:** None. **C. Ramiro:** None. **E. Evangelista:** None. **R. D'Angelo:** None. **M. Bruno:** None. **R. Castiello:** None. **A. Mori:** None. **F. Del Peschio:** None. **S. De Falco:** None. **M. Ianniello:** None. **N. Petrillo:** None. **P. Sabbatino:** None. **U. Lazzaro:** None. **F. Capone:** None. **S. Marcella:** None. **E. Pescatore:** None. **T. Miraglia:** None. **L. Langella:** None. **T. Suero:** None. **D. Cino:** None. **M. Barbato:** None. **V. Feola:** None. **A. Fico:** None.

EP12.045 The prevalence of spinal muscular atrophy carriers in pregnant women in Serbia

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Background: Spinal Muscular Atrophy (SMA) is a progressive, autosomal recessive neuromuscular disease mainly caused by *SMN1* exon 7 deletion. It is a major genetic cause of infant mortality and morbidity, underscoring the importance of carrier screening in early pregnancy. This study aimed to assess the prevalence of SMA carriers among a cohort of pregnant women and their partners, all with no family history of the disease.

Material and methods: A total of 1025 pregnant women (age range: 23–43 years; gestational weeks: 10–12) and 41 paternal partners were enrolled. Genomic DNA was extracted and screened for copy numbers of *SMN1* exons 7 and 8 using a quantitative PCR method with the *SMN1* gene detection kit (Tianlong) on a Gentier 96E real-time PCR system.

Results: Deletion analysis revealed that 31 individuals exhibited a deletion of both exons 7 and 8, while 6 individuals showed a

deletion of only exon 7. Overall, 37 out of 1066 participants (3.47%) were identified as SMA carriers. One couple was identified in which both partners were SMA carriers and prenatal diagnosis of SMA was advised.

Conclusion: These findings provide insights into the carrier frequency of SMA in Serbia, highlighting the relevance of integrating SMA carrier screening into routine prenatal care. The early identification of carriers facilitates genetic counseling and decision-making, potentially affecting the incidence of SMA through informed reproductive choices. Future studies are warranted to expand upon these findings and further improve screening protocols.

C.O.I. Disclosure:

R. Mandic: None. **M. Jankovic:** None.

EP12.046 School education, learning difficulties and dystrophin isoforms in Duchenne muscular dystrophy

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Introduction: Duchenne muscular dystrophy (DMD) is a rare neuromuscular disorder characterized by progressive muscular weakness. It is known that dystrophin isoforms (Dp) are expressed in other tissues than muscles, e.g. in brain. That could explain the presence of cognitive impairment, intellectual disability (ID) and autistic spectrum disorders (ASD) in DMD.

Materials and methods: The questionnaire regarding school difficulties and KIDSCREEN-10 were filled in by 81 DMD carers, in 2024. DMD boys were divided into 3 groups based on type of Dp disruption (Dp427, Dp140, Dp71). Bioethics Committee approval was given.

Results: 45% of DMD boys attend mainstream school (or special/integration/home/individual/no school), 73% studies at school (or home/online/hybrid/no school), for 54% local community provides transport to school. At school: only 40% attends PE classes, 74% eat meals and 82% drink beverages, 77% use toilet. 59% of DMD students has no teacher-assistant, and 92% no family-assistant. 45,6% presented ID or ASD, 72% learning difficulties and 4,9% psychotic disorders. Only 41,6% remain under constant psychological care (vs 99% had physiotherapy). Analysis of type of Dp vs frequency of ID showed 34,37% vs 51,1% vs 75% for Dp427 vs Dp140 vs Dp71, respectively, and learning difficulties 59,37% vs 80% vs 100%. KIDSCREEN-10 revealed: everyday/few times a week 73,2% of DMD boys feel comfortable, but 40,6% feel pain, 29% feel worry, 60% feel tired.

Conclusion: DMD is chronic and incurable disease. Psychological care is crucial for adequate well-being. Genotype-phenotype correlation has been proven in terms of cognitive impairment and mutation location within DMD gene.

C.O.I. Disclosure:

K. Sledzinska: None. **J. Szymańska:** None. **E. Skrzypek:** None. **J. Bobula:** None. **E. Pilarska:** None. **A. Lemska:** None. **N. Dubaj:** None. **J. Wierzba:** None. **N. Irga-Jaworska:** None.

EP12.047 Deficiency of L-Amino Acid Decarboxylase with Neuromuscular and Epileptic Manifestations: Importance of Diagnosis and Treatment

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Background: L-Amino Acid Decarboxylase (AADC) deficiency is a rare neurometabolic disorder caused by variants in the *DDC* gene, characterized by severe hypotonia, motor delay, and oculogyric crises from childhood. We present a case with neuromuscular symptoms of infantile onset and late-onset epileptic seizures, in which genetic diagnosis helped guide therapeutic management.

Materials and methods: A 44-year-old patient with motor and cognitive developmental delay since childhood. He presented with hypotonia, delayed walking, and learning difficulties. He had a history of chronic diarrhea syndrome with no diagnosis. Currently, he shows progressive muscle weakness, ptosis, uncontrolled movements in the right hand, and epileptic seizures for the past two years. He also reports chronic nasal congestion and sleep disorders. He has a family history of a deceased brother with similar symptoms. Exome sequencing was carried out.

Results: two heterozygous variants in the *DDC* gene were found: c.1357C>T (p.Arg453Cys), likely pathogenic, and c.1041+1G > C, pathogenic.

Conclusion: AADC deficiency affects the synthesis of essential neurotransmitters, leading to severe neurological symptoms. It presents with hypotonia, oculogyric crises, autonomic dysfunction, and seizures. Early diagnosis is crucial for treatment with dopaminergic agonists, MAO inhibitors, vitamin B6, and folinic acid. Diagnostic suspicion is key for proper management and prevention in carrier families.

C.O.I. Disclosure:

M. García De Burgos: None. **M.C. González:** None. **S. Franco Freire:** None. **M. Benito López:** None. **M. Aguilar Castillo:** None. **R. Yahyaoui Macías:** None.

EP12.048 Spectrum of phenotypes and inheritance pattern of *TUBA1A* variants

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Background:*TUBA1A* variants, linked to "Lissencephaly 3" in OMIM, are associated with a spectrum of neurodevelopmental disorders characterized by a broad and overlapping range of brain malformations. Although *TUBA1A*-related tubulinopathy typically follows an autosomal dominant inheritance pattern and primarily arises de novo, parental testing rarely reveals mosaicism, with only one case reported to date. We report eight patients with *TUBA1A* variants underscoring the importance of comprehensive parental testing in genetic diagnostics.

Material and methods: Whole exome sequencing was performed for unrelated patients. The variants were confirmed by next-generation sequencing (NGS) in the index cases, and parental samples were also analyzed using NGS, with deep sequencing employed to detect low-level mosaicism when indicated. Variant interpretation followed standard ACMG guidelines.

Results: Among the eight patients, six harbored de novo variants in *TUBA1A*, one patient inherited the variant from an affected father. Notably, in one case, the mother was identified as germline mosaic (mosaicism ratio: 11%) for the variant. This represents only the second reported case of parental mosaicism for a *TUBA1A* variant. Variable levels of neurodevelopmental delay, brain malformations, and other neurological symptoms were detected in the majority of patients.

Conclusion: Our study provides further evidence of *TUBA1A*-related tubulinopathy and underscores the importance of parental testing. The identification of a mosaic mother contributes a rare but critical data point to the literature, emphasizing the potential for recurrence in families and highlighting the necessity of high-sensitivity methods for detecting low-level mosaicism in clinical practice.

Table: *TUBA1A* (NM_006009.4) variants.

	DOB/ Sex	Clinical findings	Detected variant	Pathogenicity	Inheritance
Patient 1	2021/F	Agenesis of the corpus callosum, microcephaly, developmental, speech and gait delay and ptosis	c.1202A>C p.(Lys401Thr)	LP	The mother is mosaic - 11%
Patient 2	2019/M	Lissencephaly, motor and mental retardation	c.1205G>A p.(Arg402His) rs137853044	P	De novo
Patient 3	2022/F	Corpus callosum hypoplasia, pontocerebellar hypoplasia, ventricular asymmetry and enlargement, seizure, hypotonia, strabismus	c.368G>A p.(Arg123His) rs1555162456	P	De novo
Patient 4	Prenatal	Partial agenesis of the corpus callosum, cerebellar hypoplasia, and ventriculomegaly in obstetric ultrasound (USG)	c.1151T>C p.(Ile384Thr)	LP	De novo
Patient 5	2021/M	Hydrocephalus, asymmetric dilation of the left lateral ventricle and a thin corpus callosum, gait delay and mild dysmorphic features	c.1129A>C p.(Met377Leu)	LP	De novo
Patient 6	2023/F	Agenesis of the corpus callosum and microcephaly	c.26T>C p.(Val9Ala) rs1565627795	LP	De novo
Patient 7	2020/F	Global developmental delay, hypotonia, motor delay, hypermobility of joints, inability to walk, speech delay, and subtle bilateral T2 hyperintense area in peritrigonal area	c.518C>T p.(Pro173Leu) rs1565627304	P	De novo
Patient 8	2019/M	Global developmental delay, mild spasticity and brisk reflexes	c.211G>A p.(Glu71Lys)	LP	The father is heterozygous.

C.O.I. Disclosure:

D. Turkyilmaz: None. **H. Kulak Abay:** None. **F. Alehan:** None. **P. Özbudak:** None. **H. Tolunay:** None. **I. Erol:** None. **R. Akin:** None. **S. Kirmani:** None. **R. Khaılany:** None. **H. Bas:** None.

EP12.049 Whole-genome sequencing-based spinal muscular atrophy carrier screening and disease diagnosis in Taiwan

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Background: The carrier frequency of spinal muscular atrophy (SMA) in East Asians ranges from 1.24% to 3.97%. The most extensive study on Taiwanese pregnant women using multiple ligation-dependent probe amplification (MLPA) reported a carrier frequency of 2.10%. Despite challenges due to structural variation and high sequence similarity between *SMN1* and *SMN2* genes, sequencing-based approaches have been developed in recent years. This study evaluates whole-genome sequencing (WGS) and computational tools in SMA carrier screening and disease diagnosis in Taiwan.

Material and methods: WGS data from 1492 Taiwan Biobank samples were analyzed to identify SMA mutation carriers. *SMN1* and *SMN2* copy numbers were determined using Illumina DRAGEN SMN Caller and validated with MLPA. The same methods were initially applied to two SMA patients to detect copy number variations; however, additional variants were identified by integrating further bioinformatics approaches.

Results: Among 1480 samples, 23 SMA carriers were identified, yielding a carrier frequency of 1.56%. MLPA confirmed the accuracy of *SMN1* and *SMN2* copy number results. Both SMA patients exhibited compound heterozygotes variants, with one *SMN1* copy loss and small variants on the other *SMN1* allele, specifically *SMN1*,c.815A>G and *SMN1*,c.81+2_81+3delTG, respectively.

Conclusion: Combining WGS with advanced bioinformatics tools is a feasible and promising approach for SMA carrier screening and disease diagnosis. This strategy holds great potential for broader implementation in extended carrier screening programs.

Distribution of SMN1 and SMN2 copy number types in our study

N(%)	*SMN1* CN0	CN1	CN2	CN3	CN4	Total
SMN2 CN0	0	0	51(3.45)	9(0.61)	1(0.07)	61(4.12)
CN1	0	2(0.14)	456(30.81)	53(3.58)	1(0.07)	512(34.59)
CN2	0	9(0.61)	818(55.27)	27(1.82)	1(0.07)	855(57.77)
CN3	0	12(0.81)	34(2.30)	6(0.41)	0	52(3.51)
CN4	0	0	0	0	0	0
Total	0	23(1.56)	1359(91.82)	95(6.42)	3(0.20)	1480

SMN Caller predictions and MLPA validation results			
N	**SMA carrier predicted by SMN Caller**	**Non-carrier predicted by SMN Caller**	**Total**
SMA carrier validated by MLPA	23	0	23
Non-carrier validated by MLPA	0	10	10
Total	23	10	33

C.O.I. Disclosure:
L. Lin: None. **P. Chien:** None. **T. Hsiao:** None. **S. Liu:** None. **H. Ye:** None. **P. Chen:** None. **W. Chen:** None. **Y. Chou:** None. **T. Ko:** None. **C. Wu:** None. **H. Chen:** None. **Y. Chien:** None. **J. Hsu:** None.

EP12.050 Homozygous p.Lys416Glu variant in *TRNT1* expands the SIFD syndrome phenotype to include peripheral nervous system involvement

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Background: Biallelic pathogenic variants in the *TRNT1* gene cause sideroblastic anemia with B-cell immunodeficiency, periodic fevers, and developmental delay (SIFD) syndrome. This rare multisystemic disorder has a median survival age of 5.7 years. It demonstrates marked clinical heterogeneity, with specific variants associated with milder phenotypes.

Material and methods: A female patient born to consanguineous parents presented with ataxia and demyelinating polyneuropathy at age 10. She was referred for genetic testing during her follow-up in a neurology outpatient clinic. Trio long-read genome sequencing (lrGS) was performed and analyzed using a gene-agnostic approach.

Results: lrGS identified a homozygous *TRNT1* variant (c.1246A>G, p.Lys416Glu) in the proband, located within a large autozygous region and co-segregating with the disease phenotype in the family. This variant has an allele frequency of 0.00006, with no homozygotes reported in gnomAD v4. It has previously been identified in compound heterozygosity with truncating alleles in SIFD patients and is classified as likely pathogenic according to ACMG criteria. SIFD patients with p.Lys416Glu in compound heterozygosity exhibit milder immunological phenotypes, longer survival and predominant neurological features. Reverse phenotyping of the proband revealed additional features of immune dysregulation and anemia consistent with SIFD spectrum. No other pathogenic variants were identified to explain her peripheral nervous system symptoms.

Conclusions: This is the first reported case of a homozygous p.Lys416Glu variant in *TRNT1*, likely representing a phenotypic expansion of SIFD syndrome to include peripheral neuropathy. Functional studies are required to elucidate the contribution of p.Lys416Glu to the milder SIFD presentation and peripheral nerve pathology.

C.O.I. Disclosure:
A. Candayan: None. **E. Yildiz:** None. **L. Pires Ramos:** None. **A. Jordanova:** None.

EP12.051 *ERLIN1*-Related Hereditary Spastic Paraplegia (SPG62): A Recurrent Variant Expanding Phenotype

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Background: Hereditary spastic paraplegia type 62 (SPG62) is an ultra-rare neurodegenerative disorder with an autosomal recessive inheritance pattern, caused by biallelic *ERLIN1* variants. To date, approximately 20 individuals have been reported. *ERLIN1*-related variants have been implicated in early-onset amyotrophic lateral sclerosis (ALS), further complicating genotype-phenotype correlations. This study aims to expand the clinical spectrum of *ERLIN1*-related SPG62 by reporting two affected siblings with novel phenotypic features.

Material and methods: Two siblings born to consanguineous parents were referred for genetic evaluation due to progressive spastic paraplegia. Whole-exome sequencing identified a homozygous pathogenic variant (c.763C>T, p.Arg255) in *ERLIN1* in both siblings, confirmed by Sanger sequencing. Clinical evaluations included neurological assessments, imaging, neurophysiological studies, and family segregation analysis.

Results: Both siblings exhibited slowly progressive spastic paraplegia, mild intellectual disability, behavioral findings, and hyperacusis, a feature not previously reported in SPG62. The older sibling became wheelchair-dependent in adolescence, while the younger sibling displayed a more rapid disease progression, lost his ambulation in childhood. Neuroimaging and electrophysiological studies were unremarkable. Carrier screening in extended family members identified no affected heterozygous individuals.

Conclusion: This study provides further insights into the phenotypic variability of SPG62, supporting its classification as a distinct spastic paraplegia subtype rather than an ALS-related disorder. The novel symptoms of hyperacusis and behavioral abnormalities warrant further investigation to assess their relevance in *ERLIN1*-related HSP.

C.O.I. Disclosure:
G.S. Ozkose-lyigel: None. **Y. Topcu:** None. **B. Ay:** None. **O. Ozdemir:** None. **O. Akgun Dogan:** None. **O. Hatirnaz Ng:** None. **Y. Alanay:** None.

EP12.052 Patient registry on the way to diagnosis accuracy in cases of skeletal muscle channelopathies

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Background: Hereditary skeletal muscle channelopathies(SMCs) are rare, genetically heterogeneous disorders characterized by episodic skeletal muscle excitability disturbances. Mono- or digenic cases can present with similar phenotypes, complicating diagnosis, DNA sequencing strategies, and identification of causative genes.

Material and methods: Since 2012, a hereditary neuromuscular disease registry has been maintained in Voronezh, Russia, including over 2,200 patients, 310 of whom exhibited SMC symptoms. Genetic testing was performed in 254(81.9%) of 310 patients. All genotyped patients underwent clinical examinations, while 173(68.1%) underwent electromyography(EMG), 115(42.3%) creatine kinase(CK) analysis and 53(20.7%) muscleMRI.

Results: Among the 254 patients (mean age 32.2 ± 16.9 years; 147(57.9%) males), 82(45.8%) had a positive family history. Among 179 probands from unrelated families, causative mutations were detected in *CLCN1*(69, 38.5%), *SCN4A*(19, 10.6%), *DMPK*(52, 29.1%), *ZNF9*(21, 11.7%), *HINT1*(8, 4.5%), *HSPG2*(1, 0.6%), and digenic presentation of these genes (9, 5.0%). Elevated CK levels were found in 62(53.9%), with very high levels (8630 ± 3351) in 4(3.5%). Short exercise test patterns 1 and 2 did not align with Fournier's classification in 16(34.4%) of 44 cases. Needle EMG revealed myogenic patterns in 12(9.7%) of 124 cases with *CLCN1, SCN4A*, or *HINT1* mutations. In 139(93.3%) of 149 cases, gene-specific decrement values in repetitive nerve stimulation at 50Hz were observed. In 21(39,6%) typical muscleMRI pattern were found.

Conclusion: Based on the data from the registry of genotyped patients, we identified clinical, CK, EMG and MRI features that facilitate the identification of SMC forms, determine the sequence of DNA diagnostics, and indicate cases where more than one gene involvement is likely.

C.O.I. Disclosure:

S.A. Kurbatov: None. **V.P. Fedotov:** None. **S.N. Bardakov:** None. **N.M. Galeeva:** None. **P.A. Chausova:** None. **D.S. Druzhinin:** None. **E.S. Druzhinina:** None. **V.A. Kadnikova:** None. **V.M. Kenis:** None. **A.A. Kokorina:** None. **O.V. Kolokolov:** None. **Y.V. Kryukov:** None. **E.A. Melnik:** None. **N.S. Priymak:** None. **N.V. Ryadninskaya:** None. **E.V. Saifullina:** None. **M.V. Savina:** None. **O.A. Shchagina:** None. **D.V. Sidnev:** None. **I.V. Sitkali:** None. **O.A. Tikhonova:** None. **V.Y. Udalova:** None. **A.V. Polyakov:** None.

EP12.053 Identification and characterization of two novel *CASQ1* variants in two families with dominant myopathy

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Background: Calsequestrin-1 (CASQ1) is the most crucial calcium-binding protein in the fast twitch fibers of skeletal muscle, where it facilitates rapid Ca^{2+} release to initiate muscle contraction. To date, six pathogenic *CASQ1* variants, causing vacuolar myopathy with CASQ1 aggregates, have been published. The typical symptoms include muscle weakness, myalgia, exercise intolerance, cramps, and fatigue.

Material and methods: We identified the disease-causing variants in two previously unsolved families with dominant myopathy using exome sequencing. Further characterization of the variants is underway.

Results: In Family 1, three patients in three generations presented with exercise intolerance, cramps, myalgia, and miosis. Additionally, the proband had muscle weakness and her muscle biopsy showed nemaline bodies. In electron microscopy, there were changes in the triads and the SR-feet in all patients. A missense variant in *CASQ1*, p.(Glu89Lys), was found in all patients, whereas the proband also had two likely pathogenic compound heterozygous variants in *NEB*, likely explaining the nemaline bodies and muscle weakness not seen in the other two patients. In Family 2, three patients in two generations presented with progressive muscle weakness. The proband's muscle biopsy showed heavy accumulation of connective tissue and fat. A frameshift variant p.(Gly383Alafs*39) in *CASQ1* was found in all three patients. In silico analyses indicated that the variant extends the protein beyond the wild-type stop codon by 24 residues, which was confirmed by western blotting of patient muscle.

Conclusion: This study expands the spectrum of pathogenic *CASQ1* variants.

C.O.I. Disclosure:

M. Laarne: None. **M. Jokela:** None. **J. Sarparanta:** None. **S. Huovinen:** None. **F. Zhao:** None. **B. Udd:** None. **M. Johari:** None. **J. Laitila:** None. **F. Rostedt:** None. **P. Hackman:** None. **V. Lehtokari:** None. **K. Pelin:** None.

EP12.054 Molecular insights from a large cohort of patients with inherited muscle disorders - the relevance of splicing variants and cDNA studies

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Background: The clinical and genetic heterogeneity of inherited skeletal muscle disorders (ISMD), together with gene size and complexity, makes molecular diagnosis very challenging. As a national reference laboratory with over three decades of genetic testing experience, our ISMD cohort represents in a very comprehensive mutational profile of Portuguese patients.

Material and methods: Retrospective study collecting variant and methodology data from patients with congenital, distal and myofibrilar myopathies, and limb-girdle, congenital, oculopharyngeal and Becker/Duchenne muscular dystrophies.

Results: Pathogenic variants were identified in 1058 patients, distributed among 39 genes. Prevalence of disorders and genes involved were in agreement with other cohorts, although population-specific variants and founder effects were evident. Our results also revealed a significant proportion of patients with at least one variant affecting splicing (15–33%). Most were located in canonical splicesites or splicesite regions, therefore easily detected by single gene/NGS studies. In silent and missense variants that could potentially affect splicing, cDNA studies were crucial in determining their impact. cDNA analysis also disclosed deep intronic and more complex variants in *LAMA2* and *DMD* genes, clarified genotype-phenotype discrepancies and aided prognosis.

Conclusion: Understanding the genetic aetiology of a particular population helps prioritize the analysis to relevant variants (even in NGS) and apply specific methodologies. Our findings highlight the relevance of splicing variants in ISMDs and supports the integration of transcript/transcriptome analysis as an indispensable tool in the diagnostic approach of these disorders: it enables a precise diagnosis, better patient management and contributes towards the development of new therapeutic targets.

C.O.I. Disclosure:

A. Goncalves: None. **E. Vieira:** None. **S. Paulo:** None. **A.B. Rodrigo:** None. **M.E. Oliveira:** None. **R. Santos:** None.

EP12.055 Mitochondria-based anticipation effect. Insights from an Oculopharyngeal Muscular Dystrophy Cohort

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Background: Oculopharyngeal muscular dystrophy (OPMD) (OMIM #164300) is a late-onset, autosomal dominant myopathy caused by a triplet repeat expansion in the first exon of *PABPN1* (polyadenosine [poly(A)] binding protein nuclear 1). This study presents genetic and clinical data from Greek OPMD patients, with a focus on a three-generation matrilineal inheritance case.

Material and methods: A retrospective analysis was performed on 22 patients from 18 Greek families with pathogenic *PABPN1* expansions, including demographic data, pedigrees, and additional biochemical and molecular findings. In three cases, further investigation involved mitochondrial DNA (mtDNA) deletion analysis and electron microscopy of muscle biopsies.

Results: Patients with abnormal *PABPN1* (GCN) expansions across seven genotypes included 7 females (32%) and 15 males (68%). Probands had a mean age of onset of 55.3 ± 7.3 years ($p = 0.025$). Offspring developed OPMD earlier than their parents (mean difference: 4.3 years, $p = 0.025$). Patients with maternally inherited expansions exhibited earlier onset and more severe progression than those with paternally inherited expansions. Muscle biopsies from three unrelated patients with the same expansion revealed variable mitochondrial alterations, most pronounced in the maternally inherited case.

Conclusion: Genotype-phenotype heterogeneity was observed even within the same family. The most severe cases, characterized by rapid progression of muscle weakness, were associated with maternally inherited expansions. The proposed hypothesis suggests that dysregulated mitochondria inherited through the maternal lineage may contribute to intrafamilial heterogeneity, in the context of an idiosyncratic anticipation phenomenon, which is proposed to be termed *mitodrasis*.

C.O.I. Disclosure:

K. Kekou: None. **C. Papadopoulos:** None. **M. Svingou:** None. **M. Chrysanthou-Piterou:** None. **E. Nitsa:** None. **D. Veltra:** None. **N. Marinakis:** None. **F. Tilemis:** None. **D. Parissis:** None. **M. Arnaoutoglou:** None. **M. Moschou:** None. **S. Xirou:** None. **C. Bakirtzis:** None. **G. Tsivgoulis:** None. **G. Papadimas:** None. **P. Makrythanasis:** None. **C. Sofocleous:** None.

EP12.056 MSTO1-related muscular dystrophy syndrome: A case series and description of phenotypes

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Background: MSTO1 is a nuclear DNA gene which encodes misato homolog 1 (MSTO1), a mitochondrial membrane protein involved in maintaining the mitochondrial network and promoting mitochondrial fusion. Pathogenic variants in MSTO1 have been reported as a cause of myopathy and ataxia, with fewer than 40 reported cases to date.

Material and methods: Exome or genome sequencing, muscle biopsy, MRI of the brain.

Results: The patients were 13 to 30 years old at diagnosis. Two patients had hypotonia and global developmental delay since birth, while one had weakness beginning at age 2 (Pt 1) and other at age 5 (Patient 3, sibling of Patient 1). All patients have elevated CK level. Marked cerebellar atrophy was noted in one of 2 patients that underwent brain MRI at age 15. Additionally, Patient 1 has learning disability and two nonfebrile seizures; Patients 2, 3, and 4 have autism. Patients 1,2, and 3 had muscle biopsy which showed chronic myopathic changes and patient 2 also had mild mitochondrial dysfunction. All patients had biallelic mutations in the *MSTO1* gene.

Conclusion: The phenotype of MSTO1-related syndrome includes early-onset muscle weakness, hypotonia with chronic myopathic changes in the muscle biopsy. Cognitive impairment including autism, developmentall delay and learning disability is

present in all our patients. Ataxia may not be present in all patients or be the prominent feature of this syndrome. As genome and exome sequencing becomes more accessible, further characterization of the phenotypic spectrum is likely.

C.O.I. Disclosure:

R. Sharma: None. **L. Schimmenti:** None. **B. Smith:** None. **F.P. Vairo:** None. **R. Dhamija:** None. **D. Selcen:** None.

EP12.057 Validation of a rapid and cheap method for *SMN* gene copy number screening for Spinal Muscular Atrophy

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Background: Spinal muscular atrophy (SMA) is a neuromuscular disorder characterized by loss of motor neurons in the spinal cord. More than 95% of SMA cases arise from homozygous deletion of the telomeric survival motor neuron gene, *SMN1*. Centromeric *SMN2* gene copy number can influence disease severity. The development of therapeutic options and recent approval of genetic drugs for treatment of SMA has driven increased impetus for pre-symptomatic screening and carrier detection of SMA. In this study, we compared three methods: droplet digital polymerase chain reaction (ddPCR), multiplex ligation probe amplification (MLPA) and standard real-time PCR (qPCR).

Material and methods: 100 DNA samples were tested using MLPA P021 probemix (MRC Holland) kits, ddPCR *SMN* Copy Number kits with QX200™ Droplet Digital™ PCR System (Bio-Rad Laboratories) and qPCR assays using standard protocols.

Results: *SMN* copy numbers were mostly concordant between ddPCR and MLPA but discrepancies were observed with qPCR. For *SMN2* determination, inclusion of control samples carrying 0 to 4 copies were critical to resolve ambiguities. ddPCR displayed tight coefficient of variation (CV), as only 9% of *SMN1* samples and 16% of *SMN2* samples had CVs higher than 5%, with the maximum CVs being 15.2% and 10.1%, respectively. Comparing among the three methods, ddPCR has good test reliability with most samples having CVs<5%.

Conclusion: ddPCR is rapid, reliable, cost-effective and amendable for high throughput determination of *SMN1* and *SMN2* copy numbers. This makes it feasible for mass or population-based screening at affordable cost especially where such programmes are not funded nationally.

C.O.I. Disclosure:

P. Lai: None. **C. Liu:** None. **G. Tan:** None.

EP12.058 Nanopore sequencing for diagnosis of Facioscapulohumeral Muscular Dystrophy (FSHD): a rapid and high-resolution approach to genetic and epigenetic variants

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Background: Facioscapulohumeral muscular dystrophy (FSHD) results from epigenetic deregulation of DUX4 gene, associated with the D4Z4 repeat array of 3.3 kilobase units. There are two main types of FSHD: FSHD1, caused by deletions or contractions in the D4Z4 region, reducing the repeat number <10 and leading to DUX4 expression, and FSHD2, which occurs in individuals with normal D4Z4 repeat lengths but epigenetic defects in genes like SMCHD1, LRIF1, TIF1, DNMT3B, and HMGN1, causing loss of DUX4 silencing. While FSHD1 is primarly genetic, caused by repeat deletions, FSHD2 is epigenetically driven. Both forms cause muscle degeneration, with similar manifestations but differing in severity and onset age. A precise diagnosis distinguishing between the variants requires a combination of methods and analyses, making the process both costly and time-consuming.

Material and methods: Using Methylation PRO kit (4bases, RC3230Y-16), we sequenced NA12878 (Coriell) sample on a P2solo (Oxford Nanopore Technology) for 72 hours, applying adaptive sampling to enrich the D4Z4 region and related genes. Methylation analysis was performed directly from sequencing data (5mC), bypassing bisulfite treatment. Data analysis was conducted using the 4eVar platform.

Results: We identified D4Z4 repeats and promoter methylation status with 30X enrichment, covering SMCHD1, LRIF1, TIF1, DNMT3B, and HMGN1 genes for variant calling in a single run and analysis.

Conclusions: Nanopore sequencing offers a fast, high-resolution method for diagnosing FSHD and other complex genetic and epigenetic disorders such as Duchenne muscular dystrophy, myotonic dystrophy, Huntington's disease, and Fragile X-syndrome, providing a diagnostic tool that bypasses the need for traditional techniques.

C.O.I. Disclosure:

G. Mazzucco: A. Employment (full or part-time); Significant; 4bases S.R.L. **S. Casali:** A. Employment (full or part-time); Significant; 4bases S.R.L. **V. Favalli:** A. Employment (full or part-time); Significant; 4bases S.R.L.

EP12.059 Analysis of miRNAs, mRNA targets, and lncRNAs as potential biomarkers for monitoring disease progression in spinal muscular atrophy

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Background: With the advent of new therapies for spinal muscular atrophy (SMA), the need for objective biomarkers to monitor disease progression is increasing. miRNAs, along with their target mRNAs, and interacting lncRNAs are promising candidates due to their regulatory role in muscle function and neurodegeneration. In this study, the expression of four selected miRNAs, ten predicted mRNA targets, and two lncRNA interactors was investigated.

Material and methods: A total of 52 SMA patients were included. RNA was extracted from whole blood, and reverse transcribed. The expression levels of selected RNAs were quantified by RT-qPCR. Expression data at baseline (before treatment) were analyzed for associations with SMA type, ambulatory status, motor and pulmonary function. Longitudinal changes were analyzed to assess treatment-related dynamics of expression.

Results: Expression of miR-206 was significantly higher in SMA types III and IV compared to type II, consistent with its increased levels in ambulatory patients and its positive correlation with motor and lung function. *HDAC4*, which showed an inverse correlation with miR-206, was elevated in non-ambulatory patients and correlated negatively with motor function outcomes. Similarly, miR-133a-3p and miR-133b showed negative correlations with motor and lung function, while LINCMD1 positively correlated with motor function. Several genes, including *PGD*, *G6PD*, *TKT*, *HDAC4*, and *SP1*, were significantly downregulated from baseline to 24 months of treatment, while LINCMD1 expression significantly decreased at 42 months of treatment.

Conclusion: Selected miRNAs, target mRNAs, and lncRNAs influence the progression of SMA and may serve as potential biomarkers for assessing disease severity and treatment monitoring.

C.O.I. Disclosure:

M. Barbo: None. **B. Koritnik:** None. **L. Leonardis:** None. **V. Dolžan:** None. **M. Ravnik Glavač:** None.

EP12.060 The identification of a new mutation in the ATGL LC3-interacting region motif expands the genetic understanding of neutral lipid storage disease with myopathy

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Background: Mutations in the *PNPLA2* gene, encoding adipose triglyceride lipase (ATGL), are associated with neutral lipid storage disease with myopathy (NLSDM). This rare disorder is marked by abnormal triacylglycerols (TAG) accumulation in lipid droplets of many tissues, leading to muscle-related symptoms such as weakness, cramps and progressive myopathy. Here, we report the molecular and clinical characterization of an Italian 42-year-old patient.

Material and methods: *PNPLA2* coding region was analyzed by PCR and sequencing. Protein production was assessed by western blotting, and structural predictions were conducted using I-Tasser software.Skin fibroblasts were cultured with/without oleic acid (OA), and intracellular TAG content was quantified by a colorimetric assay.

Results: A novel *PNPLA2* mutation (c.45_47delCGG) that causes a single in-frame deletion of the 16th amino acid within the ATGL first LC3-interacting region (LIR) motif (p.Gdel16) was identified.-Bioinformatic evaluation predicted significant changes in the mutated protein's structure. Notably, most of these changes affected the patatin domain, which contains both the catalytic dyad and LIR motifs.TAG quantification showed that patient's fibroblasts showed higher TAG levels than control fibroblasts (272%) and those of another patient in whom ATGL mutations only partially abrogated enzymatic activity (44%). As expected, after pulse-chase experiment with OA, TAG degradation was strongly reduced.

Conclusion: Our study indicates that the deletion of the 16th amino acid may disrupt the interaction between ATGL and LC3, potentially impairing lipophagy in addition to lipolysis and causing a more severe TAG accumulation. This underscores the intricate interplay between LD biology and autophagic mechanisms.

C.O.I. Disclosure:

D. Tavian: None. **B. Risi:** None. **L. Moro:** None. **E.M. Pennisi:** None. **A. Padovani:** None. **C. Angelini:** None. **S. Missaglia:** None. **M. Filosto:** None.

EP12.061 The first report of pathogenic variant in the promoter region of *MFN2* associated with axonal CMT

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Background: Charcot-Marie-Tooth disease is the most common hereditary neuropathy, characterised by variable phenotypes and age of onset. CMT is associated with more than 100 causative genes, with CMT2A being the most common axonal type associated with *MFN2* pathogenic variants.

Material and methods: Members of an axonal CMT family were clinically evaluated, and samples were collected. The molecular investigation was performed using Sanger sequencing, and the functional effects of the novel *MFN2* variant were investigated using cell lines. In silico pathogenicity and transcription binding site prediction analysis were performed. Transcription factors RNA expression analysis was achieved using Real-Time PCR.

Results: A novel *MFN2* promoter/5′ UTR c.-301G > A variant was identified in the proband, and family segregation analysis supports the association of this variant with the phenotype. MFN2 expression analysis, using patients and control lymphoblastoid cells, showed reduced mRNA and protein levels in the patient. In addition, overexpression of wild-type MFN2 in Hela cells showed an increase in MFN2 levels, whereas no increase was observed with overexpression of the mutant, thus further supporting an effect of this variant on MFN2 expression. In silico prediction analysis showed potential transcription factor binding sites (TFBSs) that this variant may affect. Expression analysis of these transcription factors revealed dysregulation of the *NF1, TCF3, TFCP2 FOXB1, SP1, and ZBTB6* at the RNA level.

Conclusion: We identified the first pathogenic variant in the *MFN2* promoter/5′ UTR associated with CMT2. This regulatory pathogenic variant alters multiple TFBSs, leading to the dysregulation of several transcription factor RNA expression levels.

C.O.I. Disclosure:

P. Nicolaou: None. **N. Nicolaou:** None. **P. Chairta:** None. **D. Kittou:** None. **K. Kleopa:** None. **K. Christodoulou:** None.

EP12.062 Telomere Shortening in Patients with Spinal Muscular Atrophy and The Impact of Gene Therapy

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Background: Spinal muscular atrophy(SMA) is an autosomal recessive disease that is characterized by progressive muscle weakness and atrophy resulting from degeneration of alpha motor units. It is caused by the loss-of-function of the *SMN1* gene encoding the SMN protein located in Cajal bodies where the telomerase holoenzyme is assembled and might have an important role in telomerase biogenesis by altering the interaction of the proteins. We therefore assessed telomere length in patients with SMA, compared to the healthy controls; and investigated the potential impact of gene therapy (onasemnogene abeparvovec).

Material and methods: Relative telomere length in peripheral blood lymphocytes was evaluated using SensiFAST SYBR Master Mix-No ROX Real-Time PCR Kit (Meridian Bioscience Inc.; Cincinnati, Ohio, USA) in 128 subjects; consisting of 64 SMA patients 19 of whom were able to receive gene therapy, and 64 healthy age and gender-matched controls.

Results: Telomere length in SMA cases was found to be shorter after the newborn period, compared to healthy controls ($p = 0,02$). Moreover, gene therapy appears to successfully prevent telomere attrition ($p = 0,03$). The exact mechanism by which telomere shortening contributes to the SMA pathogenesis remains to be delineated. However, disrupting the repair and regeneration capacity of the motor neurons is one possible scenario.

Conclusion: Spinal Muscular Atrophy is one of the most common neuromuscular diseases of childhood. Telomere attrition might be an exacerbating factor and a potential therapeutic target in SMA cases. Further elucidating the mechanism should pave the way for new studies to be conducted on this particular clinical condition.

C.O.I. Disclosure:

B. Okur Altındaş: None. **M. Yıldırım:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Necmettin Erbakan University Scientific Research Projects Coordination Unit. **K. Çarman:** None. **S. Öktem:** None.

EP12.063 Assessing the Impact of Rare Variant Mutational Burden in Charcot Marie Tooth Disease

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Background: Charcot-Marie-Tooth (CMT) disease is a genetic disorder affecting the peripheral nervous system with varying symptoms. The underlying factors contributing to phenotypic variability and disease progression remain mainly unknown. Recent evidence suggests that CMT and other hereditary neuropathies may not be purely monogenic but instead follow an oligogenic inheritance model involving multiple genes.

Material and methods: To explore possible oligogenic inheritance in CMT cases, we calculated the cumulative mutational burden of rare nonsynonymous variants from exome sequencing (ES) data across known CMT genes in CMT patients ($n = 153$) and non-neurological controls ($n = 564$). We then compared the average mutational burden between solved cases, unsolved cases and controls using Mann-Whitney U test, followed by permutation test of 10000 iterations. Additionally, in cases with available CMTNSv2 scores ($n = 77$), which describes the disease severity and progression, we correlated the mutational burden with CMTNSv2 score to assess the rare variant contribution to CMT phenotypic variability.

Results: The average mutational burden of rare nonsynonymous variants did not differ between solved cases, unsolved cases and controls ($p > 0.05$). The correlation between mutational burden and CMTNSv2 score was not statistically significant (Spearman correlation, $r = 0.032$, $p > 0.05$).

Conclusion: Our findings did not support a significant role for cumulative mutational burden in CMT cases. The lack of difference in average mutational burden in known CMT genes between CMT cases and controls, as well as the absence of correlation with CMTNSv2 score, suggests that oligogenic inheritance may not be a major contributor in this cohort.

C.O.I. Disclosure:

L. Bardina: None. **L. Gailite:** None. **D. Pretkalnina:** None. **V. Kenina:** None. **D. Rots:** None.

EP12.064 Muscle DNA Whole Genome Sequencing identifies mtDNA deletion signatures with diagnostic implications for genetic and acquired myopathies

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Background: Mitochondria-related muscle dysfunction is reported in aging, inclusion body myositis (IBM), genetic myopathies and mitochondrial myopathies. Histological changes (including COX negative fibres and ragged red fibres) and molecular changes (depletion in mtDNA copy number and accumulation of mtDNA deletions) are seen across these conditions and may result in diagnostic uncertainty, particularly in atypical clinical presentations. There is preliminary evidence that mitochondrial disease, IBM and aging may have discriminatory hallmarks in the patterns of mtDNA deletions. However, studies to date may be biased by PCR enrichment and are limited by small numbers of samples and genes studied. The aim was to identify mutational signatures between primary, acquired, and age-related mtDNA deletions.

Material and methods: Genomic DNA was extracted from fresh frozen muscle samples and PCR-free whole genome sequencing undertaken. Mitochondrial DNA reads were extracted and studied using the MitoSALT bioinformatic tool. Results were compared with clinical gold standard sequencing (next generation sequencing on long range PCR-enriched mitochondrial DNA).

Results: An apparent abundance of large deletions was observed with PCR-enriched samples. In addition, discrete differences were demonstrated in deletion patterns between age-related, non-mitochondrial and mitochondrial myopathies including number of deletions seen, deletion break points and degree of mtDNA ablation.

Conclusion: Mitochondrial DNA deletion signatures may offer a new diagnostic tool for undiagnosed myopathies and evidence towards or against pathogenicity in the classification of variants of uncertain significance. Age-related mitochondrial dysfunction can be discriminated from true primary muscle disease by whole genome sequencing and deletional analysis.

C.O.I. Disclosure:

K.B. Oprych: None. **W.L. Macken:** None. **R. Kabiljo:** None. **A. Moylett:** None. **J. Williams:** None. **C. Pizzamiglio:** None. **E. Bugiardini:** None. **E. Erdil:** None. **M. Falabella:** None. **C. Woodward:** None. **Y. Patel:** None. **R. Labrum:** None. **M. Reilly:** None. **J. Polke:** None. **M.G. Hanna:** None. **R.D. Pitceathly:** None.

EP12.065 Challenges to the "reading-frame rule" in Duchenne and Becker muscular dystrophies: molecular insights from in-frame deletions and duplications

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Background: Deletions or duplications in the *DMD* gene are major genetic causes of Duchenne and Becker muscular dystrophies (DMD/BMD). The "reading-frame rule" predicts that in-frame deletions preserving the open reading frame (ORF) result in BMD, while frameshift mutations cause DMD. However, exceptions to this rule have been observed, where patients with in-frame

copy number variations exhibit a severe DMD-like clinical phenotype.

Material and methods: Sixty-five patients with clinically diagnosed DMD and disease onset before five years were studied. In-frame hemizygous deletions (46/65) and duplications (19/65) in *DMD* gene were previously detected using Multiplex Ligation-Dependent Probe Amplification. Whole-genome sequencing was performed for all patients using paired-end sequencing (PE150) on a DNBSEQ-T7 genetic analyzer with PCR-free library preparation.

Results: Four of 46 (8.7%) in-frame *DMD* deletions were identified as potentially disruptive to the ORF, including cases with exonic breakpoints and complex rearrangements. One of these cases, initially classified with a 33-exon deletion in *DMD* gene, was found to have a nonsense variant. Among the 19 in-frame duplications, eight (42%) were complex rearrangements, including one affecting the *FRMPD4* gene, associated with X-linked intellectual disability.

Conclusion: A significant proportion (18.4%) of DMD cases with in-frame *DMD* deletions/duplications were reclassified as pathogenic due to ORF disruption or complex rearrangements. These findings highlight the complexity of genotype-phenotype correlations in DMD/BMD, emphasizing the limitations of the traditional reading-frame rule in predicting clinical outcomes. The results underscore the need for comprehensive genetic diagnostics to precisely characterize molecular defects, improve prognosis, and guide personalized therapeutic strategies including pathogenetic therapies.

C.O.I. Disclosure:

M. Bulakh: None. **E. Zinina:** None. **A. Polyakov:** None. **O. Ryzhkova:** None.

EP13 Multiple Malformation/Anomalies Syndromes

EP13.001 HRAS related Costello Syndrome caused by HRAS variant in child suspected with achondroplasia

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Background: HRAS related Costello syndrome is a rare multiple congenital anomaly syndrome associated with a coarse facies, short stature, distinctive hand posture and appearance, severe feeding difficulty, and failure to thrive. Other features include cardiac anomalies and developmental disability.

Material and methods: Whole genome sequencing - Centogenome Solo was performed. Parental targeted testing is pending.

Results: Autosomal dominant HRAS c.34G>A p.(Gly12Ser) variation was detected. This variant causes an amino acid change from Gly to Ser at position 12 in exon(s) no.2 (of 5). The patient was 5 months old, primarily suspected with achondroplasia or other type of dwarfism. Detailed pediatric developmental assessment showed dysmorphic appearance. The head is dolichocephalic, the forehead is prominent, there is a hypertelorism. The anterior fontanelle is at the level of the surrounding bones, it is open 2×2 cm. The heart's action is rhythmic, clear tones, and a discreet systolic murmur is heard. The abdomen is soft and painless to palpation. The patient is conscious, occasionally in opisthotonos position, there is muscle hypotonia especially of the body axis and pronounced neck laxity. The shoulder girdle is tight and the extremities are somewhat shorter in relation to the body. A certain level of developmental delay is present.

Conclusion: HRAS related Costello syndrome has wide phenotypic spectrum, ranging from a mild to a severe phenotype. The child is monitored in our hospital, has showed signs of huge improvement in physical and cognitive development. Prenatal genetic counseling for future pregnancies is issued once the parental target testing is performed.

C.O.I. Disclosure:

M. Vasilevska: None. **E. Ivanovska:** None. **A. Demerdzieva:** None.

EP13.002 Neurofibromatosis Type 1 in Morocco: Clinical and genetic findings from a case series study and literature review

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Neurofibromatosis Type 1 (NF1) is a complex autosomal dominant disorder caused by germline mutations in the NF1 tumor suppressor gene. Nearly all individuals with NF1 develop dermal neurofibromas and pigmentary lesions, including café-au-lait macules, skinfold freckling, and Lisch nodules. Some individuals may also develop brain tumors (e.g., optic pathway gliomas and glioblastomas), skeletal abnormalities (such as scoliosis, tibial pseudarthrosis, and orbital dysplasia), peripheral nerve tumors (e.g., spinal neurofibromas, plexiform neurofibromas, and malignant peripheral nerve sheath tumors), as well as learning disabilities and social or behavioral challenges, all of which can significantly affect quality of life.In this study, we present the clinical data of a Moroccan series of patients diagnosed with Recklinghausen's disease. Among the 20 families studied, 8 cases were familial forms. All patients had pigmentary lesions, while peripheral nerve tumors were identified in 10 patients. Additionally, a single case of glioma was reported.This work examines the clinical and genetic aspects of Neurofibromatosis Type 1, based on the first series of patients managed at the University Hospital of Marrakech.

C.O.I. Disclosure:

M. Mansouri: None. **G. draiss:** None. **M. Hattaoui:** None. **M. Bouskraoui:** None. **O. Hocar:** None. **A. Said:** None. **S. elkarimi:** None. **N. Aboussair:** None.

EP13.004 When 22q11.2 deletion is not the answer: the challenge of differential diagnosis with other syndromes

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Background: The 22q11.2 deletion syndrome (22q11.2DS) is the most common microdeletion syndrome. However many patients with suggestive clinical features do not exhibit the expected deletion. Due to its high clinical variability and lack of distinct phenotypic markers, the presentations of 22q11.2DS often overlap with those of other conditions.

Material and methods: We investigated twenty-two patients with clinical features indicative of 22q11.2DS whose chromosomal microarray analyses (CMA) failed to detect copy number variants (CNVs) at this locus. Whole exome sequencing (WES) was performed, and variant interpretation followed American College of Medical Genetics (ACMG) guidelines. Genotype-phenotype correlations were established to aid in differential diagnosis.

Results: CMA identified a pathogenic 12-Mb deletion in 7p15.3p14.3 in one patient, associated with Hand-Foot-Genital

syndrome. WES revealed a pathogenic variant in the *CBL* gene in one patient, consistent with a Noonan syndrome-like disorder. Additionally, two unrelated patients with phenotypes suggestive of Rubinstein-Taybi syndrome were found to carry variants of uncertain significance (VUS) in the *CREBBP* gene. Ongoing segregation studies may lead to reclassification of these VUS.

Conclusion: When molecular results do not align with the initial clinical hypothesis, the identification of genetic variants can aid in reverse phenotyping, wherein the genotype data is first delineated followed by the associated phenotype. These approaches enhance differential diagnosis, provide valuable clinical insights, and optimize the diagnostic odyssey.

C.O.I. Disclosure:

H.G. Silveira: None. **G.M. Toccoli:** None. **B.C. Nunes:** None. **P.R. Soares:** None. **T.P. Vieira:** None. **C. Steiner:** None. **V. Gil da Silva Lopes:** None. **B. Favilla:** None. **M. Melaragno:** None.

EP13.005 Identification of SLC12A2 mutation associated with an ultra-rare neurodevelopmental disorder with omphalocele

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Background: Pathogenic mutations in *SLC12A2* gene, encoding a Na-K-Cl cotransporter have been earlier associated with autosomal dominant inherited disorders. Recently, biallelic variants of the gene have also been correlated with an autosomal recessive neurodevelopmental disorder, Kilquist syndrome (OMIM 619080).

Material and methods: The examined eight-months-old girl was born at term, from young, healthy and non-consanguineous parents, with severe hypotonia and dysmorphic features. She underwent reconstructive surgery on the second day of life due to omphalocele detected in the 12th week of gestation. At the time of her genetic evaluation severe feeding difficulties, delayed psychomotor development and bilateral sensorineural deafness were evident. Whereas, based on clinical findings, the primarily performed karyotyping and hearing loss NGS panel sequencing test gave normal results, whole exome sequencing (WES) examination was indicated.

Results: WES analysis identified a novel homozygous c.868G>C (p.Gly290Arg) variant in *SLC12A2* gene. The preliminary classification of the missense variant was variant of uncertain significance according to the classification schemes of the laboratory. Segregation analysis proved that the parents are heterozygous carriers. Taking into account that the variant is in trans position, the classification of the detected variant was modified to be likely pathogenic, following the ACMG guideline. Moreover, the features of *SLC12A2* associated autosomal recessive Kilquist syndrome is in line with the symptoms of our patient, with omphalocele as an additional symptom.

Conclusion: Our results contribute to the further characterisation of Kilquist syndrome's phenotypic spectrum and provide additional evidence to support the not yet completely elucidated biallelic inheritance of the disorder.

C.O.I. Disclosure:

A. Till: None. **R. Szalai:** None. **T. Bujtas:** None. **A. Szabo:** None. **K. Hadzsiev:** None.

EP13.006 A novel pathogenic GLI3 variant in Greig cephalopolysyndactyly syndrome: Genotype-phenotype correlation and clinical implications

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Background: GLI3 encodes a transcription factor functioning both as an activator and repressor in the sonic hedgehog signaling pathway. Pathogenic variants in GLI3 cause several autosomal dominant disorders, such as Pallister-Hall syndrome (PHS) and Greig cephalopolysyndactyly syndrome (GCPS). While both conditions share a common feature of postaxial polydactyly, they exhibit distinct clinical characteristics. The specific type and location of GLI3 mutations may provide insights into phenotype prediction. This case study aims to contribute to this understanding.

Methods and Results: We describe a 2-year-old female with macrocephaly, frontal bossing, hypertelorism, low-set ears, a broad nasal root, accessory polydactyly on the left hand, and mesoaxial polydactyly on both feet. The patient's family history includes several relatives (mother, aunt, and cousins) with varying forms of polydactyly, primarily affecting the feet. Whole exome sequencing using the Illumina NGS platform identified a novel heterozygous pathogenic GLI3 variant (c.4332T>G; p.Tyr1444Ter), predicted to result in a truncated or absent protein. GLI3 haploinsufficiency has been linked to GCPS and is considered pathogenic. The variant was absent in the gnomAD and ClinVar databases, and in silico predictions suggest a deleterious effect on protein function.

Conclusion: This novel GLI3 variant contributes to the expanding list of mutations associated with GCPS and reinforces the genotype-phenotype correlation, especially for variants affecting the first third of the GLI3 gene. Identifying specific pathogenic variants is essential for accurate diagnosis, genetic counseling, and clinical management, given the variable presentations of GCPS. Ongoing family testing will help further clarify the pathogenicity of this variant.

C.O.I. Disclosure:

G. Milanovski: None. **E. Shukarova-Angelovska:** None. **M. Kirijas:** None. **T. Savevska:** None. **D. Trajkov:** None. **T. Brnjarchevska Blazhevska:** None. **A. Petlichkovski:** None.

EP13.007 Variabilidad fenotípica de la variante c.794G>A en PTPN11: informe de dos casos

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Antecedentes: Pathogenic variants in PTPN11 are associated with several rasopathies, mainly Noonan syndrome (NS). The most common characteristics of patients with NS include: heart disease, short stature, developmental delay of variable degree, and characteristic facies with downslating palpebral fissures, ptosis, hypertelorism, epicanthus, and low-set ears. The c.794G>A variant in PTPN11 has been previously reported in SN, but its clinical impact may differ significantly between individuals. We describe two unrelated patients with short stature, in whom this variant was detected, with clinical characteristics that differ from the classic phenotype of rasopathies.

Material y métodos: The clinical history and genetic findings of two patients were analyzed. Whole Exome Sequencing (WES) was performed on genomic DNA, with an Illumina HiSeq 4000 sequencing platform.

Resultados: Patient 1 male with microcephaly, low height and weight; without intellectual disability or associated malformations.

Patient 2, female with developmental delay, low height for family genetic range and ectropion. WES analysis detected heterozygous missense variant in exon 7 of PTPN11 (NM_002834.3, c.794G>A, p.Arg265Gln) in both children.

Conclusión: Both exhibit clinical characteristics that differ from the classic phenotype of rasopathies. In 2019 Ranza et al. report patients with this variant as a clinically distinct phenotype within the Noonan spectrum related to PTPN11. This variability highlights the need for additional studies to understand the mechanisms that regulate its clinical expression.

C.O.I. Disclosure:

C. Martinez: None.

EP13.008 A new pathogenic variant in *WRN* gene identified in a Moroccan patient with Werner syndrome

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Background: Werner syndrome is a rare hereditary disease, with an autosomic recessive transmission, due to pathogenic variants in the WRN gene, also referred to as RECQL2. This syndrome is caracterised by a premature appearance of aging, and several pathologys that are associated with aging, like atherosclerosis, cataracts and neoplasms

Case presentation: It is a 53-year-old patient with a personal history of, dyslipidemia, ischemic heart disease, and no notion of similar cases in the family. The anamnesis found a history of lack of growth since puberty, premature graying and thinning of hair, bilateral cataract at age of 20-year-old, hypothyroidism, and a squeaky voice. In view of the suspicion of a Werner syndrome, a genetic consultation was indicated, then a study of the WRN genes by Next Generation Sequencing (NGS) was requested, in order to confirm the clinical diagnostic, and allow a genetic counseling to the family of the propositus. The genetic analysis of the WRN genes revealed a homozygous missense variant : c.724G>A, confirming the clinical diagnosis of Werner syndrome.

Conclusion: This case report described a newly identified mutation of WRN gene of a patient with clinical phenotype of Werner syndrome.Werner syndrome is a rare autosomal recessive disease It is also known as adult progeria because the symptoms start in early adolescence, It occurs due to the mutation of the WRN gene that inactivates the function of helicase involved in DNA replication, repair, and telomere maintenance

C.O.I. Disclosure:

N. Serbati: None. **F. Outtaleb:** None. **H. Dehbi:** None.

EP13.009 Reciprocal cryptorchidism as the first sign of the Snijders Blok-Campeau syndrome

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Background: Snijders Blok-Campeau syndrome (SBC) is an autosomal dominant neurodevelopmental disorder characterised by speech impairment, distinctive facial features and intellectual disability (mild to severe). Most cases of this disorder arise as a de novo variant with no family history. Prenatal diagnosis is possible if WES or WGS test is performed. We present a clinical case that illustrate an early diagnosis of the SBC syndrome as an incidental finding.

Methods: buccal DNA extraction and WES were performed at Medicover genetics according to the company`s protocols. Routine phenotypic assessment of the patient was performed.

Results: On referral from a urologist a 28-day-old newborn was referred for genetic counselling for bilateral cryptorchidism with suspicion of Klinefelter's syndrome. The phenotype assessment revealed: Bilateral cryptorchidism HP:0008689; Thin vermilion border HP:0000233; Long philtrum HP:0000343; Smooth philtrum HP:0000319; Hypotonia HP:0001252; Epicanthus HP:0000286; Narrow mouth HP:0000160; Bifid uvula HP:0000193; Hypertelorism HP:0000316. Genetic testing ruled out the Klinefelter syndrome while WES revealed heterozygous variant of unknown significance of CHD3 gene NM_001005273.3:c.3709T>C, p.(Phe1237Leu). Mutations in CHD3 gene are associated with developmental delay-speech delay-intellectual disability-abnormalities of vision-facial dysmorphism syndromą (sin. SBC syndrome ORPHA:599082). Both parents and older brother were checked for this variant. No clinically relevant findings were found. Prenatal period was normal. No significant family history was observed.

Conclusion: Snijders Blok-Campeau syndrome is a very rare condition. In this case, the non-specific symptom of cryptorchidism allowed early confirmation of an extremely rare disease that could have taken several years to diagnose. VES is a powerful genetic diagnostic tool for ultra-rare diseases.

C.O.I. Disclosure:

V. Asmoniene: None. **R. Sereikiene:** None.

EP13.010 A natural history of a 20 years-old girl with Shaaf-Yang syndrome

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Background: Shaaf-Yang syndrome (SYS) (OMIM#615547) is a rare neurodevelopmental disorder characterized by delayed psychomotor development, impaired intellectual development, hypotonia, and behavioral abnormalities. Additional features include contractures, feeding difficulties, and variable dysmorphic facial features. SYS was first known as Prader-Willi-like syndrome due to a phenotypic overlap.

Material and methods: We present a natural history of a 20 years-old girl with Shaaf-Yang syndrome. First she was referred to clinical geneticist due to developmental delay, obesity and dysmorphic syndromes at the age of 18-months. Next she was evaluated at the age of 4, 9, 16, 18 and 20 years.

Results: At the age of 16 y.o. the clinical diagnosis of SYS was suspected. Molecular testing was performed and likely-pathogenic mutation c.3089dupT; p.Leu1031Profs*25 in MAGEL gene was detected.

Conclusion: Phenotypic changes during the developmental age will be demonstrated.

C.O.I. Disclosure:

R. Posmyk: None. **B. Stasiewicz-Jarocka:** None. **N. Wawrusiewicz-Kurylonek:** None. **B. Panasiuk:** None.

EP13.011 A natural history of a 8-years-old boy with Cornelia de Lange syndrome due to a balanced RCT. 6,XY,t(5;8)(p13;q31)

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Background: Carriers of balanced reciprocal translocations (RCT) are usually asymptomatic. It is a huge challenge to give in appropriate genetic counselling and estimate risk in prenatal cytogenetic diagnosis in such cases. Here, we present a male patient with typical Cornelia de Lange syndrome (CdLS) due to of 46,XY,t(5;8)(p13;q31).

Material and methods: We present a natural history of a 8-years-old boy with Cornelia de Lange syndrome, who's mother was first referred for non-invasive prenatal testing at 12 weeks of gestation, where elevated NT (4,1 mm) and low CRL (44 mm) were found. Amniocentesis was ordered. Initial prenatal cytogenetic testing (after amniocentesis) revealed RCT- 46,XY,t(5;8)(p13;q31). Karyotypes of both parents were normal (46,XX- mother, 46,XY-Father). Now any internal organ abnormalities were seen during the second prenatal screening except of IUGR (Intrauterine growth retardation).

Results: Pregnancy was not terminated after genetic counselling, because no any evident basis was found. After child's birth, clinical geneticist was asked for the consultation due to IUGR and dysmorphic features. Typical for CdLS dysmorphic features were noticed. Mapping of breakpoints of balanced RCT was performed and disruption of NIPBL gene was confirmed.

Conclusions: High NT (>3 mm), along with IUGR, normal PAPPA protein and b-HCG levels, dysmorphic features (microtetrognathia) in prenatal screening should be suspicious, especially if we find any chromosomal abnormality, even balanced.

C.O.I. Disclosure:

B. Stasiewicz-Jarocka: None. **B. Panasiuk:** None. **N. Wawrusiewicz-Kurylonek:** None. **R. Posmyk:** None.

EP13.012 Chromosomal aberrations in pathogenesis of hydrocephalus - clinical and cytogenetic analysis of 4 patients

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Background: Hydrocephalus (HC) refers to the excessive accumulation of cerebrospinal fluid in the ventricles of the brain, which can result from overproduction of fluid, impaired absorption or circulation. Hydrocephalus occurs with an incidence of 4–8/10,000 of all births and is one of the most common disorders of brain development. A variety of external (viral/bacterial infections) and internal factors (e.g. vascular) can disrupt embryonic development leading to hydrocephalus. However, it is now known that genetic factors play an important role in about 20–40% of hydrocephalus cases. Studies of the genetic basis of hydrocephalus conducted worldwide in recent years have identified more than 100 genes and several signaling pathways associated with hydrocephalus, such as genes of the WNT pathway, dystroglycanopathy, ciliopathy, RAS-MAPK, PI3K-AKT-mTOR as well as chromosomal aberration (dupXp22.33, dup6p25.3, dup7p21.3, dup12q24 and del12q22q23.1).

Patients and methods: We present clinical evaluation of 4 patients HC with the chromosomal aberrations identified in array CGH analysis, aged from 1 to 3 years.

Results: All patients presented congenital HC, motor or psychomotor DD, macrocephaly, delayed/absent speech and dysmorphic features. Congenital heart defect (PS) and optic nerve hypoplasia were found in 1 patient. Array CGH analysis revealed the presence of pathogenic or likely pathogenic aberrations arising de novo: del2q34(214491708_214997351), del5p15.33 (22149_315088), mosaic del9q34.3 (139388124_139412705), dup6q15q16.1 (91391803_94526635), dup22q11.21 (18842715_21457610).

Conclusions: Our patients present with congenital hydrocephalus and rare chromosomal aberrations that were not yet connected with this pathology. We conclude that this may offer an insight into the pathogenesis of hydrocephalus because our genetic understanding of HC is incomplete.

C.O.I. Disclosure:

A. Kutkowska-Kaźmierczak: None. **I. Plaskota:** None. **M. Bartnik-Głaska:** None. **M. Śmieszek:** None. **M. Niemiec:** None. **K. Kowalczyk:** None. **E. Obersztyn:** None. **M. Gos:** None. **B.A. Nowakowska:** None.

EP13.013 Molecular genetics findings from individuals with cleft lip and/or cleft palate tested by a phenotypically focused next generation sequencing panel

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Background: Orofacial clefts (OFCs) are the most common type of congenital craniofacial anomaly with variable worldwide prevalence. Most frequent subclinical forms include cleft lip only, cleft lip and palate, and cleft palate only. OFCs may be syndromic or non-syndromic and represent a group of clinically and genetically heterogeneous disorders. Despite this heterogeneous nature, OFCs display strong familial aggregation, suggesting that heredity plays a major role. Currently there are limited studies on the genetics of orofacial clefts and this study aims to provide additional information.

Material and methods: A large cohort of individuals with OFC-spectrum symptoms were clinically tested at PreventionGenetics, using Next Generation Sequencing technologies. Data analyses were performed using internally developed software. When necessary, the sequence and copy number variants were confirmed by Sanger sequencing, CMA or aCGH. In this retrospective study, positive cases with pathogenic/likely pathogenic SNVs and/or CNVs were identified and reviewed.

Results: Overall, we observed 81 positive cases, 68 with SNV(s) and 13 with CNV(s). Majority had pathogenic variants in genes associated with autosomal dominant conditions, including *IRF6*(10), *COL2A1(7), COL11A1(6), TFAP2A(5), KMT2D(4), CHD7(5), TP63(3), CTNND1(3), GRHL3(3), COL11A2(2), TCOF1(2), FGFR1(2), SATB2(2), EFTUD2(2), CDH1(3), IGF1R(2)*, and one case for each of the following genes *MID1, SF3B4, GLI2, FGFR2, FGF10, FGFR3, WDR11, ARHGAP29, MSX2, PAX3 and TWIST1*. Sixteen cases had a confirmed de novo pathogenic variant.

Conclusion: This study provides additional information and enhances our understanding of molecular pathways and genes involved in orofacial clefting, leading to improved clinical testing and genetic counseling for affected individuals and family members.

C.O.I. Disclosure:

S.Z. Berisha: A. Employment (full or part-time); Significant; PreventionGenetics, a part of Exact Sciences. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; PreventionGenetics, a part of Exact Sciences. **B.K. James:** A. Employment (full or part-time); Significant; PreventionGenetics, a part of Exact Sciences. **M.S. Piazza:** A. Employment (full or part-time); Significant; PreventionGenetics, a part of Exact Sciences. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; PreventionGenetics, a part of Exact Sciences.

EP13.014 Turner Syndrome in a Pediatric Patient with Abdominal Neuroblastoma: A Case Report and Literature Review on the Importance of Genetic Evaluation

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Background: Neuroblastoma is the most common extracranial solid tumor in children. While the majority of cases are sporadic, certain chromosomal disorders have been linked to an increased risk of this malignancy. Turner Syndrome (TS), characterized by complete or partial loss of one X chromosome, has been linked to an increased risk of neuroblastoma, though this association is not definitive: only a few cases have been described in the literature and the biological connection between the two remains unclear.

Material and methods: We performed a comprehensive clinical and genetic assessment using SNParray, karyotyping and FISH and supplemented this with a literature review to better contextualize our findings within the broader clinical landscape.

Results:A five-month-old girl was diagnosed with abdominal indifferentiated stage IV neuroblastoma. No significant clinical concerns had been raised prior to this diagnosis. Mild dysmorphic facial features and slight hypotonia, previously unrecognized, prompted the oncologist to refer the patient for genetic consultation. Genetic testing revealed a mos 45,X/46,X,psu idic(X)(p11.22) karyotype, confirming the diagnosis of TS. To our knowledge, this is the first case of neuroblastoma described in a patient with mosaicism involving chromosome X monosomy and pseudoisodicentric Xq.

Conclusion: Based on our findings and supported by existing literature, we emphasize the importance of a thorough evaluation for subtle dysmorphisms and even mild clinical signs of underlying chromosomal abnormalities in children diagnosed with neuroblastoma. Early identification of such conditions is crucial for optimizing clinical management, providing accurate counseling to affected families and facilitating further investigation into the biological link between TS and neuroblastoma.

C.O.I. Disclosure:

D. Mazzà: None. **A. Custrin:** None. **S. Naviglio:** None. **S. Chinetti:** None. **C. Agrati:** None. **D. Satgé:** None. **M. Rabusin:** None. **A. d'Adamo:** None. **G. Girotto:** None. **P. Gasparini:** None.

EP13.015 Del Xq22 IN A MOSAIC TURNER FEMALE: CLINICAL AND CYTOGENETIC CHARACTERIZATION

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Background: Deletion of the long arm of the X chromosome (del Xq) leads to a broad spectrum of clinical manifestations, which may include short stature, premature ovarian failure, and fertility problems. The phenotype can be relatively benign in large deletions due to skewed X inactivation. However, the presence of 45,X/del Xq mosaicism can complicate the genotype-phenotype correlation.

Material and methods: We report a case of a 16-year-old girl referred to the genetic department of Farhat Hached Hospital in Sousse for primary amenorrhea and gonadal dysgenesis. Karyotyping and Fluorescence in-situ Hybridization (FISH) were performed.

Results: The karyotype revealed 45,X/46,X,del(X)(q22) mosaicism, and FISH showed integrity of the XIST gene along with a subtelomeric deletion on the abnormal X chromosome. The karyotype result is probably explained by a meiotic with post-zygotic loss of the deleted Xq chromosome, leading to the 45,X mosaicism. The patient's phenotype is probably related to both the 45,X status and the Xq deletion. Such as for the gonadal gysgenesis which critical region was mapped between Xq13-q26 and for the primary amenorrhea which critical region was mapped between Xq26.2 and Xq28. However, the patient had a noraml stature and a mild Turner stigmata with absence of pigmented naevi probably due to the low proportion of 45,X mosaicism.

Conclusion: Phenotypes associated with mosaic Xq deletions are heterogeneous. Further investigation into the genetic and molecular basis of Xq deletions is essential for refining critical regions, enhancing genetic counseling, and developing tailored therapeutic strategies.

C.O.I. Disclosure:

A. Benzarti: None. **I. Bayoudh:** None. **S. Dimassi:** None. **J. Romdhan:** None. **N. Gaaloul:** None. **S. Mougou-zerelli:** None.

EP13.016 Phenotypic and Clinical Features of the Case with Biallelic Variant Detected in *INTS1* Gene

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Background: *INTS1* gene encodes a subunit of the Integrator complex 1. The largest integrator unit integrator complex (INT) consisting of 14 subunits is the INTS1 subunit. INT, associated with the C-terminal domain of RNAPolimeraseII, is crucial in transcriptional regulation in eukaryotes and is essential for 3′-end processing of snRNAs. Homozygous or compound heterozygous variants in *INTS1* (OMIM:611345) gene cause Neurodevelopmental disorders with cataracts, poor growth, and dysmorphic facies (OMIM:618571). We present a novel variant in the whole exome sequencing (WES) analysis of a patient with 46, XY sex development disorder and neurodevelopmental delay.

Material and methods: Our proband is a 3-month-old, born spontovaginally at 31 weeks as a twin, from a healthy consanguineous couple, referred to the pediatric genetics clinic due to ambiguous genitalia and dandy walker malformation, hypoplastic cerebellum, thin corpus callosum on the cranial MRI images. In physical examination, proband's weight was 3800 gr, height was 52 cm, and head circumference was 35 cm, had relative macrocephaly, a wide forehead, 3×3 cm anterior fontanelle, deep-set eyes, microphthalmia, hypertelorism, narrow palpebral fissure spaces, upslanding palpebral fissures, sparse eyebrows, broad nasal root, wide nasal wings, hypospadias, blind vagina, retractile testicles.

Results: Karyotype analysis is 46,XY, and in WES analysis, homozygous c. 5594C > T (p.Ala1865Val), VUS variant was detected in the *INST1* gene.

Conclusion: The role of the integrator complex in human development and disease has been discovered recently. In cases where dysmorphic facial findings, neurodevelopmental delay, and

disorders of sex development coexist, *INTS1* gene should be considered a potential cause.

C.O.I. Disclosure:

N. Bilgec: None. **F. Bedel:** None. **B. Eklioglu:** None.

EP13.017 A rare mutation of *PIGN* gene in fetus with multiple congenital anomalies: A case report

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Background: A congenital abnormality is defined as a structural or functional defect that is present at birth and differs from what is considered normal. Cases with two or more major congenital anomalies in different organ systems can be as part of a syndrome or sequence. Recent advances in prenatal screening of the fetus provide diagnosis for the majority of these disorders.

Case presentation: A pregnant woman was referred for prenatal screening with increased nuchal translucency (5.51 mm). Amniocentesis was performed, and fetal karyotyping and whole genome oligo-array CGH were normal. During the follow up of the pregnant woman, multiple congenital anomalies were observed in the anomaly scan sonography, consisting of: corpus callosum agenesis, nonvisible cavum septum pellucidum, increased nuchal fold, hypertelorism, cleft lip and palate, micrognathia, echogenic kidneys and pelviectasis. The fetus was legally aborted. subsequently, clinical examination and sampling were done.

Material and methods: We sequenced the exome using the Illumina platform and Agilent Sure Select V7 library preparation kit.

Results: A homozygous pathogenic variant (c. 2307T > A p. Tyr769Ter) was detected in the *PIGN* gene that can cause multiple congenital anomalies-hypotonia-seizures syndrome type 1 (MCAHS1) in an autosomal recessive pattern of inheritance. This variant was identified via Sanger sequencing in the fetal sample as well as in her parents.

Conclusion: This report is helpful for clinicians and geneticists who evaluate suspected fetuses with positive sonographic soft markers by using appropriate and wide spectrum tests. It will be useful for genetic counseling and the prevention of disease in future pregnancies.

C.O.I. Disclosure:

S. Seyedhassani: None. **L. Najafi:** None. **M. Neshan:** None. **N. Tavakkoli Banizi:** None.

EP13.018 Case Report of DeSanto-Shinawi Syndrome

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Background: DeSanto-Shinawi syndrome (DESSH) is a rare neurodevelopmental disorder caused by a loss-of-function mutation in the *WAC* gene, first identified in 2015. It is characterized by dysmorphic features, intellectual disability, and behavioral abnormalities. With fewer than 30 cases reported worldwide, this case report aims to further elucidate the clinical spectrum, specifically expanding the understanding of its phenotype in relation to heart defects.

Material and methods: The proband, a 5-year-old girl born at full term, presented with developmental delay, constipation, and a heart defect. Clinical examination revealed facial dysmorphic features, including hypertelorism, epicanthus, a thin upper lip, and macrostomia. Additional clinical findings included a small perimembranous ventricular septal defect with an aneurysm, astigmatism, and optic nerve edema. Developmental assessment using the DISC scale confirmed global developmental delay. Chromosomal microarray analysis yielded negative results.

Results: A heterozygous c.1346C>A variant in the *WAC* gene (NM_016628.5) was identified through whole exome sequencing (WES) data analysis. Based on the ACMG 2015 criteria (PVS1 and PM2), we classified it as likely pathogenic. Pathogenic variants in the *WAC* gene cause autosomal dominant DeSanto-Shinawi syndrome (DESSH), which is characterized by distinct facial features, hypotonia, feeding difficulties, constipation, psychomotor developmental delays, intellectual disability, and behavioral disorders. A previously published case also described a DESSH patient carrying the novel c.1346C>A variant in a heterozygous state.

Conclusion: The diagnosis of DeSanto-Shinawi syndrome was genetically confirmed. Although the c.1346C>A variant has not been previously documented in association with heart defects, we believe it may be causative for the patient's symptoms.

C.O.I. Disclosure:

M. Kaušylaitė: None. **R. Traberg:** None. **S. Žėkienė:** None. **R. Ugenskienė:** None.

EP13.019 Delineation of the 8q12 microduplication syndrome

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Background: Microduplications in the region 8q12 have only been described in a few individuals and it remains to be elucidated whether a distinct clinically recognizable phenotype exists. Duplication sizes varied significantly with a smallest region of overlap of 1.6 Mb. Contained genes of interest are *CA8*, *ASPH*, *RAB2B*, *CLVS1*, and *CHD7* with *CHD7* as a potentially essential gene. Clinical features included developmental delay, facial dysmorphic features, congenital heart disease, neonatal hypotonia, Duane syndrome, and short stature.

Material and methods: Conventional and molecular karyotyping were performed on patients' peripheral blood. Detailed clinical features were collected. Published and unpublished individuals were included in this study.

Results: We provide the first comprehensive clinical overview of individuals with 8q12 microduplications including a newly diagnosed seven-month-old female individual, described as floppy infant with developmental delay, short stature, failure to thrive, and facial dysmorphism.

Conclusion: We delineate the clinical phenotype for microduplications in region 8q12 demonstrating a distinct syndromic entity, which is fundamental to elucidate its essential genes in the future.

C.O.I. Disclosure:

K. Poschmann: None. **A. Röpke:** None. **M. Bonaglia:** None. **D. Genevieve:** None. **N. Bramswig:** None.

EP13.020 Uncovering the clinical and genetic spectrum of RASopathies: A series of seven Tunisian patients

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Background: RASopathies are a group of syndromes caused by germline mutations in genes involved in the Ras/MAPK pathway. This signaling pathway is essential for key cellular processes, including proliferation, differentiation, and apoptosis. This study aimed to determine the genetic diagnosis in a cohort of Tunisian patients with various types of RASopathies.

Material and methods: We analyzed the clinical and molecular profiles of 7 Tunisian patients referred to the genetics department of Monji Slim Hospital with a clinical suspicion of RASopathies. Molecular analysis was performed using targeted next-generation sequencing (NGS).

Results: The study included 7 patients from 7 different families, with a sex ratio of 4:3. The mean age of symptom onset was 4 months. All patients exhibited distinctive craniofacial dysmorphic features, while congenital heart defects were observed in 5 cases. Ectodermal abnormalities, including dry skin and sparse hair, were present in 5 patients. One patient hadmultiple café-au-lait spots associated with a neurofibroma. Molecular analysis identified 7 distinct heterozygous pathogenic variants across 6 genes (*SOS1*, *RAF1*, *PTPN11*, *HRAS*, *BRAF*, and *NF1*), including 6 missense mutations and 1 nonsense. These findings confirmed the diagnosis of Noonan syndrome in 4 patients, Costello syndrome in 1 patient, cardio-facio-cutaneous syndrome in 1 patient, and neurofibromatosis type 1 in the remaining patient.

Conclusion: Our study underscores the clinical and genetic complexity of RASopathies, highlighting the diagnostic challenges due to their overlapping features. NGS tests prove to be an invaluable tool, enabling precise diagnosis and guiding appropriate management.

C.O.I. Disclosure:

W. Essalah: None. **Y. Elaribi:** None. **I. Rejeb:** None. **S. Hizem:** None. **I. Selmi:** None. **H. Ouerda:** None. **L. Ben Jemaa:** None. **H. Jilani:** None.

EP13.021 A novel homozygous frameshift variant in TBC1D23 associated with pontocerebellar hypoplasia type 11 in a Turkish patient

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Background: Pontocerebellar hypoplasia type 11 (PCH11, OMIM #617695) is a rare autosomal recessive neurodevelopmental disorder characterized by severe microcephaly, developmental delay, cerebellar hypoplasia, and coarse facial features. Pathogenic variants in *TBC1D23*, encoding a regulator of endosomal trafficking, are implicated in PCH11. We present a Turkish patient with a novel homozygous *TBC1D23* variant, expanding the genotypic and phenotypic spectrum of PCH11.

Material and methods: The patient underwent detailed clinical and neurological evaluations.Clinical exome squencing (CES) targeting 6380 genes was conduced using the Illumina NextSeq platform. The indentified variant was classified according to ACMG guidelines.

Results: The patient was born at 37 weeks of gestation via cesarean section due to oligohydramnios. There was no history of perinatal complications or neonatal intensive care unit admission. The parents were non-consanguineous but originated from the same village, with no family history of similar conditions.She presented with microcephaly, trigonocephaly, downslanting palpebral fissures, neurodevelopmental delay, inability to walk, and coarse motor impairment. Brain MRI showed structural abnormalities suggestive of a neurodevelopmental disorder. Genetic analysis identified a novel homozygous frameshift variant in the *TBC1D23* gene: NM_001199198.3: c.1280dup p.(Ser428-Glufs*18), classified as *likely pathogenic* (ACMG criteria). The variant was absent in population databases (gnomAD, ClinVar etc.) and segregated from heterozygous carrier parents.

Conclusion: This report describes a novel homozygous *TBC1D23* variant in a Turkish patient with PCH11. The case expands the phenotypic spectrum of PCH11, emphasizing the importance of genetic testing in patients with neurodevelopmental delay and microcephaly.

C.O.I. Disclosure:

B.S. Erarslan: None. **N. Sandal Filikci:** None. **S. Öztürk:** None. **M. Ayanoğlu:** None. **Z. Manav Yigit:** None.

EP13.022 Demographic and clinical characteristics of overgrowth syndromes in Lithuanian and Polish cohort

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Background: Overgrowth syndromes (OGS) are a group of rare, heterogeneous disorders characterized by generalized or segmental excessive growth with associated abnormalities. This study is a part of GOSPL project (No. S-LL-21-5 (Lithuania), NCN/1/DA/21/001/1106 (Poland)), aiming to characterize clinical and demographic features of OGS in Lithuanian and Polish populations to facilitate early diagnosis and tailored management.

Material and methods: The research cohort included 146 unrelated, non-consanguineous individuals of Lithuanian and Polish descent, comprising of 55 females (37.6%) and 91 males (62.3%), with mean age of 9.9 ± 9.7 years. Data was collected on pregnancy history, prenatal and postnatal development, anthropometric measurements, asymmetric growth patterns (if present), dysmorphic features, and other clinical characteristics. Statistical analysis was conducted using Excel and RStudio (Fisher's exact test, t-test, Mann-Whitney U test performed).

Results: Based on a questionnaire of clinical features, participants were classified into two groups: generalized overgrowth (GOG, 101/146, 69.2%) and asymmetric overgrowth (AOG, 45/146, 30.8%). Significant differences were observed between groups in number of postnatal developmental delay cases (GOG: 67/101, 66.3% vs. AOG: 13/45, 28.9%; $p < 0.01$), intellectual disability cases (GOG: 56/101, 55.4% vs. AOG: 6/45, 13%; $p < 0.01$), height >+2SD (GOG: 60/101, 56.4% vs. AOG: 15/45, 13.3%; $p < 0.01$), weight > +2SD (GOG: 57/101, 51.5% vs. AOG: 11/45, 24.4%; $p < 0.01$), head circumference >+2SD (GOG: 41/101, 40.6% vs. AOG: 7/45, 15.6%; $p < 0.01$), and presence of morphological anomalies in the mouth region (GOG: 59/101, 58.4% vs. AOG: 15/45, 33.3%; $p < 0.01$).

Conclusion: Detailed understanding of demographic and clinical characteristics of OGS is essential for early diagnosis and improved patient management.

C.O.I. Disclosure:

K. Šiaurytė-Jurgelėnė: None. **A. Matulevičienė:** None. **B. Aleksiūnienė:** None. **K. Baronas:** None. **M. Mozerė:** None. **N. Wawrusiewicz-Kurylonek:** None. **B. Stasiewicz-Jarocka:** None. **B. Panasiuk:** None. **K. Berk:** None. **R. Posmyk:** None. **A. Utkus:** None.

EP13.023 Legius Syndrome: A RASopathy with phenotypic overlap of neurofibromatosis type 1 and Noonan syndrome

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Legius syndrome (LS) is a RASopathy caused by *SPRED1* mutations, affecting the RAS/MAPK pathway. It is often a differential diagnosis of neurofibromatosis type 1 (NF1) due to overlapping features like café-au-lait spots and occasional freckling.We report a 10-year-old Tunisian female, with no parental consanguinity, referred for growth retardation, developmental delay, and multiple café-au-lait macules (CALMs). Clinical examination revealed facial dysmorphism, including a broad forehead, arched eyebrows, downslanting palpebral fissures, a marked philtrum with a Cupid's bow, pectus excavatum, and delayed bone age. Genetic testing using a costum RASopathy panel identified a pathogenic variant SPRED1:c.1149_1152del p.Gly385IlefsTer20, confirming Legius syndrome. This variant, inherited from a healthy mother with only CALMs, is reported in the literature and documented as pathogenic in ClinVar.This case highlights the importance of genetic testing in distinguishing LS from NF1 and Noonan syndrome. Our patient met three major criteria—typical facial dysmorphism, short stature, and pectus excavatum—leading to a definitive Noonan syndrome classification and guiding our genetic testing approach. Additionally, the presence of multiple café-au-lait macules (CALMs) raised initial suspicion for NF1. However, the absence of other hallmark NF1 features, such as neurofibromas, Lisch nodules, and optic pathway gliomas, supported the distinction between these two conditions. The *SPRED1* variant's inheritance from a mildly affected mother highlights it's the high penetrance of CALMs in this syndrome and its variable expressivity, particularly in features like macrocephaly, learning difficulties, developmental delays, and dysmorphism.

C.O.I. Disclosure:

F. Majdoub: None. **Y. Lajmi:** None. **M. Guirat:** None. **S. Guidara:** None. **A. Ziadi:** None. **M. Hachicha:** None. **N. Belghuith:** None. **H. Kamoun:** None. **R. Mrad:** None. **M. Trabelsi:** None. **S. Masmoudi:** None. **L. Kraoua:** None. **I. Ben Ayed:** None.

EP13.024 Cerebro-Oculo-Facio-Skeletal syndrome type 1: clinical and genetic insights from a new case

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Background: Cerebrooculofacioskeletal syndrome type 1 (COFS1) is a severe autosomal recessive neurodegenerative disorder characterized by growth failure, progressive microcephaly, neurological impairment, skeletal anomalies, ophthalmological abnormalities, sensorineural hearing loss, hepatorenal and cardiovascular involvement, and severe photosensitivity. It is caused by alterations in the *ERCC6* gene, disrupting the nucleotide excision repair (NER) pathway, which repairs DNA damage induced by ultraviolet radiation. Establishing an early molecular diagnosis is crucial for targeted management and prevention of complications.

Material and methods: We present the case of a 27-day-old newborn referred for genetic evaluation due to microsomia, microcephaly, congenital anomalies, and dysmorphic features. Family history revealed consanguineous parents. Prenatal findings included bilateral renal pyelectasis and intrauterine growth restriction. Postnatal examinations identified bilateral congenital cataracts, progressive postnatal microcephaly, hypotonia, rocker-bottom feet, and dysmorphic features. The patient later developed blindness, sensorineural hearing loss, proteinuria, and hypertension, requiring multidisciplinary management. Genetic testing included qF-PCR, arrayCGH, and exome sequencing filtered by HPO terms. Parental segregation analysis was performed.

Results: Exome sequencing identified a homozygous missense variant in *ERCC6* (c.2813C>T, p.(Pro938Leu)), classified as a variant of uncertain significance (VUS). Given consanguinity, phenotypic overlap with COFS1, and in silico predictions suggesting pathogenicity, a highly probable diagnosis of COFS1 was established.

Conclusion: This case highlights the importance of early genetic suspicion and precise molecular diagnosis in congenital anomaly cases, enabling preventive strategies and multidisciplinary follow-up. VUS interpretation remains challenging and requires integrating clinical, bioinformatic, and familial segregation data. We report a previously undescribed variant, contributing to the expansion of the COFS1 genotypic spectrum.

C.O.I. Disclosure:

M. Domínguez Jiménez: None. **C. María Dolores Sánchez:** None. **D. Doval Calvo:** None. **A. Serrano Antón:** None. **M. Sánchez-Soler:** None. **P. Gargallo Tatay:** None. **V. López González:** None. **M. Ballesta-Martínez:** None. **E. Guillén Navarro:** None.

EP13.025 A three-generation family with a *KAT6B*-related disorder and variable expressivity: Identification of a novel truncating *KAT6B* variant by Whole-Exome Sequencing

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Background: *KAT6B*-related disorders, including genitopatellar syndrome (GPS) and Say-Barber-Biesecker-Young-Simpson variant of Ohdo syndrome (SBBYSS) exhibit a broad phenotypic spectrum with variable expressivity. Clinical features include global developmental delay, intellectual disability, hypotonia and genital abnormalities. Skeletal anomalies are common and additional features may include congenital heart defects, small bowel malrotation, feeding difficulties, slow growth, cleft palate, hearing

loss and dental abnormalities. We present a case of a 6-year-old patient with short stature, dysmorphic features, global psychomotor delay and autism. His mother exhibits a more severe phenotype, including intellectual disability. The maternal grandmother, diagnosed with 65% disability, showed a similar phenotype to the proband and also had osteoarthritis and a history of learning problems.

Material and methods: Clinical evaluation was performed on the proband and his mother. Whole-Exome Sequencing (WES) was conducted on the proband's DNA. Direct Sanger sequencing was used to analyze the variant in the mother, but the grandmother was unavailable for genetic testing.

Results: A novel truncating variant, c.1054C>T (p.(Arg352Ter)) in *KAT6B*, classified as likely pathogenic, was identified in the exome analysis of the proband. Direct Sanger sequencing confirmed the presence of the same variant in his mother. These findings indicate that both mother and son are affected by a *KAT6B*-related disorder with variable expressivity.

Conclusion: We present a familial case of *KAT6B*-related disorder. To date, only three familial cases have been described in the literature, as most patients harbor de novo *KAT6B* variants. Clinical features vary significantly among affected family members, highlighting a high degree of intrafamilial variability.

C.O.I. Disclosure:

M. Gamundi: None. **A. Faus Pérez:** None. **Y. Vázquez García:** None. **E. Borràs Angosto:** None. **C. Molina Anguita:** None. **B. Mañé Valverde:** None.

EP13.026 A rare and complex case of a male patient with DiGeorge - like phenotype, carrying three different mosaic copy number variants on chromosome 22

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Background: 22q11.2 microdeletions cause various phenotypes, including DiGeorge Syndrome (DGS; OMIM#188400). However, some patients with clinical phenotypes suggestive of DGS, require additional investigation to delineate the exact genetic cause of their symptoms. We present a patient with DGS-like phenotype, carrying a rare combination of mosaic aberrations encompassing the Velocardiofacial/DiGeorge region.

Material and methods: A 17-year old male patient along with his non-affected parents was referred for DGS genetic testing, with developmental delay, hypotonia, joint laxity, hypospadias, intellectual disability and dysmorphic features. Genetic testing included Fluorescence in situ Hybridization (FISH)(DGS-LSI DiGeorge/VCFS Region-VYSIS Inc), karyotype and array-CGH (SurePrint ISCA array V2, Agilent). For confirmation and further investigation, multiplex ligation-dependent probe amplification (MLPA), targeted FISH and trio-based whole-exome sequencing (trio-WES) were used.

Results: Normal results were obtained from DGS-FISH and karyotype. Array-CGH, however, revealed a 6Mb mosaic duplication and two mosaic deletions of 1.7Mb and 1.9Mb. All aberrations were de novo and no balanced rearrangements were detected in the parents. Targeted FISH and MLPA confirmed all aberrations and their mosaic state. No causative single-nucleotide variants were identified by trio-WES, although the structural variants described above were detectable. Thus, the final result for the patient is [arr 22q11.21q12.1 (18035777_25903543)x3[0.6] dn,22q12.1 (27320031_29029992)x1[0.6]dn,22q12.3 (33681958_35630078) x 1[0.6]dn].

Conclusion: We described a patient carrying a causative rare and complex aberration delineated by a combination of different genetic tests that complement each-other in terms of resolution and accuracy. We emphasize the importance of a combined targeted and non-targeted approaches to successfully resolve such rare cases.

C.O.I. Disclosure:

P. Evangelidou: None. **L. Kousoulidou:** None. **A. Theodosiou:** None. **E. Panayiotou:** None. **A. Alexandrou:** None. **V. Christofidou - Anastasiadou:** None. **G. Tanteles:** None. **C. Sismani:** None.

EP13.027 Genetic and Phenotypic Spectrum of MECP2 Duplication Syndrome: Case Studies of Three Male Patients

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Background: MECP2 duplication syndrome is a severe neurodevelopmental disorder affecting predominantly males, characterized by early-onset hypotonia, feeding difficulties, and gastrointestinal issues such as gastroesophageal reflux and constipation. Affected individuals experience delayed psychomotor development, intellectual disability, poor speech development, and progressive spasticity. Seizures and recurrent respiratory infections are also common. This study aims to evaluate the clinical and molecular characteristics of MECP2 duplication syndrome through three male cases.

Material and methods: We present three male patients diagnosed with MECP2 duplication syndrome. Clinical and molecular data were collected from hospital records.

Results: Case 1: A 2-year-old male with hypotonia, epilepsy, and recurrent infections. Born to consanguineous parents, his physical examination revealed relative macrocephaly, micrognathia, and downslanting palpebral fissures. Cranial MRI showed Dandy-Walker malformation. Karyotype analysis was normal.Case 2: A 17-year-old male with global developmental delay, epileptic encephalopathy, and spasticity. Born to non-consanguineous parents, he had progressive spasticity and refractory epilepsy.Case 3: A 9-year-old male with neurodevelopmental delay, epilepsy, and recurrent respiratory infections. He walked at age 4 and had speech delay. All patients' chromosomal microarray revealed a duplication on the Xq28 locus, including the MECP2 gene.

Conclusion: Early-onset hypotonia, progressive spasticity, epilepsy, and recurrent respiratory infections are diagnostic features of MECP2 duplication syndrome. Genetic testing, particularly chromosomal microarray, is crucial for diagnosis.

C.O.I. Disclosure:

D. Ayyildiz emecen: None. **C. Yilmaz uzman:** None. **T. Turkut Tan:** None. **S. Pekuz:** None. **T. Okur:** None. **F. Çelebi Çelik:** None. **N. Harputluoglu:** None. **F. Hazan:** None. **H. Tekgul:** None. **O. Cogulu:** None.

EP13.028 Syndrome of deafness with labyrinthine aplasia, microtia, and microdontia: molecular diagnosis of the first Tunisian case

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Introduction: Deafness with labyrinthine aplasia, microtia, and microdontia (LAMM) is a rare syndromic congenital hearing loss, inherited in an autosomal recessive manner. It is linked to pathogenic variants in the FGF3 gene. We aimed to describe the clinical and genetic features of LAMM syndrome in a Tunisian patient.

Materiel and methods: We report the case of a patient referred for syndromic hearing loss. Molecular analysis was performed using whole exome sequencing. Identified variants were classified according to the ACMG guidelines.

Results: The patient was a two-and-a-half-year-old girl, the only child of a consanguineous couple. She presented with developmental delay, achieving sitting at 10 months and still unable to walk. Clinical examination revealed microtia, microdontia, and mild axial hypotonia. Imaging revealed bilateral labyrinthine aplasia and vermian agenesis with elongated cerebellar peduncles. Ophthalmologic examination showed high myopia and chorioretinal atrophy. WES identified four variants in the FGF3, NID1, ACY1, and OTUD6B genes. The pathogenic variant (NM_005247.4):c.310C>T p.(Arg104Ter) in the FGF3 gene confirmed the diagnosis of LAMM syndrome. The cerebral anomalies observed have not been reported in the LAMM syndrome and may be related to a variant of uncertain significance (NM_002508.3):c.3109T>C p.(Trp1037Arg) in the NID1 gene. The functional impact of this variant remains to be confirmed. The variants in the ACY1 and OTUD6B genes were of interest for genetic counseling.

Conclusion: WES confirmed the rare diagnosis of LAMM syndrome in this patient and provided accurate genetic counseling for the family. However, the NID1 gene variant remains challenging to interpret without functional studies.

C.O.I. Disclosure:

S. Ben Yahia: None. **A. Achour:** None. **M. Ksentini:** None. **F. Maazoul:** None. **A. Zanati:** None. **S. Blibech:** None. **M. Trabelsi:** None. **N. Belguith:** None. **R. Mrad:** None.

EP13.029 Systematic literature review on twins with neural tube defects

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Background: Neural tube defects (NTD) are congenital malformations arising when the neural tube, precursor of the brain and spine, fails to properly close during neurulation. The etiology is multifactorial, with environmental and genetic factors variably contributing. Knowledge about the role of genetics in NTDs is increasing, and may benefit from genetic analyses on twins. Here we report a literature review on the reported cases of monozygotic and dizygotic twins affected by spinal dysraphisms. Case series and reports on twin pregnancies characterized by NTDs were included in our work, dealing with all the main kinds of spinal dysraphisms.

Material and methods: A systematic literature review was performed applying multiple free-text keywords and search strings in order to cover the widest spectrum of relevant terminologies. Through the identification of precise inclusion and exclusion criteria, we selected eligible papers that were critically reviewed. We particularly focused on the collections of case reports, case series and cohort studies providing details on prenatal and post-natal diagnostic, demographic and clinical data about NTDs in twins. All pertaining data were summarized and discussed.

Results: For each of the selected cases we provide detailed descriptions about pregnancy, prenatal diagnosis, clinical features of the affected foetuses, delivery, clinical outcome and genetic data, when available.

Conclusion: The aim of our work is to provide a critical literature revision on twins affected by open or closed spina bifida, leading to expand knowledge about NTD genetic etiology, and hopingly to ameliorate clinical management.

C.O.I. Disclosure:

F. Romano: None. **P. De Marco:** None. **M. Catala:** None. **G. Piatelli:** None. **M. Scala:** None. **V. Capra:** None.

EP13.030 A rare maternally inherited 11p14.1p15.4 duplication in a female teenager with intellectual disability

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Background: Duplications within the short arm of chromosome 11 (11p) cause partial trisomy 11p and classically involve the Beckwith-Wiedemann syndrome (BWS) critical region. We report a female 16-year-old proband with mild intellectual disability, seizures with onset at 3 years, facial dysmorphism, hypoplasia of the external genital, congenital nystagmus, and moderate myopia. Proband's mother has similar phenotype and mild intellectual disability; maternal grandmother and both uncles had varying grade of cognitive impairment, psychoemotional features, and non-specific facial dysmorphism. Chromosomal aberration within 11p but outside the BWS-region was identified in the family.

Material and methods: Conventional GTG-banding postnatal cytogenetic analysis found various chromosomal aberrations, involving chromosome 11 in all affected family members. Shallow sequencing confirmed the duplication of the affected locus in the proband child and her mother.

Results: The conventional cytogenetic analysis revealed karyotype with identical recombinant chromosome 11 in the proband and her mother: 46, XX, rec(11)dup(11)ins(11)(q14.3p15.4p14.1)dmat. The shallow WGS confirmed the presence of a 22.20 Mb duplication at 11p15.4p14.1: sseq[GRCh37]11p15.4p14.1(7163931_29359902)×3 chr11:g.7163931_29359902dup. The conventional cytogenetic analysis of proband's maternal grandmother and the two uncles identified the same insertion ins(11)(q14.3p15.4p14.1) in all samples.

Conclusion: To the best of our knowledge, there are only a few reported cases of hereditary 11p duplications (outside the BWS locus). The described clinical phenotypes are non-specific, usually involving learning and intellectual disabilities, and non-specific facial dysmorphism. This case demonstrates the utility of the shallow sequencing in resolving complicated cytogenetic findings and supports the significance of conventional cytogenetic analysis in revealing the genetic background of syndromic intellectual disability.

C.O.I. Disclosure:

P.A. Nikolova-Hristova: None. **P. Hristova:** None. **E. Betcheva-Krajcir:** None. **L.V. Nikolova-Georgieva:** None. **L. Dimova:** None. **N. Todorov:** None. **T.V. Vasilev:** None. **V. Simov:** None. **T. Vekov:** None.

EP13.031 The role of genetic testing in Neurodevelopmental Disorders: A case study of 16p CNVs

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Background: Neurodevelopmental disorders (NDDs) are early-onset conditions affecting language, behavior, and motor skills, with a prevalence of up to 20% in children. Chromosomal anomalies, such as deletions and duplications, are common contributing factors. Chromosome 16 is particularly relevant due to its genetic complexity and susceptibility to structural rearrangements. Copy number variants (CNVs) in the short arm of chromosome 16 (16p) are associated with NDDs, making genetic analysis essential for accurate diagnosis and management.

Material and methods: We report a case of a four-year-old girl referred for specialized follow-up due to epileptic episodes and global developmental delay. Next-generation sequencing (NGS) targeting epilepsy-related genes showed no pathogenic variants. However, two CNVs on chromosome 16 were identified confirmed by Array-CGH.

Results: Two CNVs on chromosome 16 were identified: a 2,76 Mb deletion in 16p13.11, associated with intellectual disability and epilepsy, and a 5557 Kb duplication in 16p11.2, linked to autism spectrum disorders and cognitive deficits.

The patient exhibited a phenotype potentially resulting from the combined effect of both CNVs, despite their incomplete penetrance and variable expressivity. This rare genetic configuration highlights the complexity of NDD etiology.

Conclusion: This case highlights the importance of genetic studies in NDD diagnosis and management.

The coexistence of a deletion and duplication on chromosome 16 inherited from mildly affected or asymptomatic parents emphasizes the need for segregation studies and genetic counseling.A multidisciplinary approach is crucial to providing personalized care and improving developmental outcomes for NDDs patients.

C.O.I. Disclosure:

M.M. Corzo Corbera: None. **M. del Águila García:** None. **S. García Linares:** None. **A. Poyatos Andújar:** None. **T. de Haro Muñoz:** None. **C. Tapia Córdoba:** None.

EP13.032 A case of restrictive dermopathy type 1 with a novel homozygous variant in *ZMPSTE24* gene

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Background: Restrictive dermopathy (RD) (OMIM: 275210) is a rare, lethal laminopathy caused by homozygous mutations in ZMPSTE24 (RSDM-1) or heterozygous mutations in LMNA (RSDM-2). RSDM1 is characterized by intrauterine growth retardation (IUGR), reduced fetal movement, premature rupture of membranes, translucent rigid skin, dysmorphic features and joint contractures. RSDM1 is a rare disorder caused by biallelic pathogenic variants in ZMPSTE24 gene.We described a premature newborn girl of consanguineous parents who had a novel homozygous variant in the ZMPSTE24 gene in the patient with RSDM1, which enriched the spectrum of ZMPSTE24 variants implicated in restrictive dermopathy syndrome.

Material and methods: After obtaining informed consent from the family, molecular studies were initiated. G-banding karyotyping analysis and next-generation sequencing (NGS) were performed for the patient. NGS was performed with the ROCHE kit and MGI DNBSEQ-400 device. The results were interpreted using Genomize Seq program.

Results: The proband showed clinical features of RSDM1, such as prematurity, IUGR, generalize rigid skin, joint contractures, unique dysmorphic facial features including microretrognathia, low-set dysplastic ears, and small nose. Echocardiography revealed a small secundum ASD. The karyotype of the patient was 46, XX NGS-based genetic testing was identified a likely pathogenic mutation, a frameshift variant in ZMPSTE24 gene (c.209_210del p. Tyr70Serfs*4)

Conclusion: In this review, we identified a novel ZMPSTE24 gene mutation related to restrictive dermopathy in a patient. We aimed to extend the knowledge of the phenotype-genotype correlation of the disease. Our findings contribute to the global pool of data, providing practical insights into the manifestations of restrictive dermopathy.

C.O.I. Disclosure:

N. Cinkara: None. **H. Kina:** None. **B. Hekimoğlu:** None.

EP13.033 Differences in sex development type 3 with BRCA2 pathogenic variant - difficulties in establishing preventive measures and treatment - - compex case of siblings

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Background: Sex reversal disorders are challenging to diagnose due to the complexity of molecular mechanisms affecting sex differentiation. Pathogenic variants in the *NR5A1* gene are associated with various phenotypes, including 46,XY gonadal dysgenesis, male infertility, and, in 46,XX individuals, premature ovarian insufficiency or ovotesticular DSD. Adrenal insufficiency is rare.

Case Presentation:

Sister 1: Ambiguous genitalia were noted at birth. Investigations revealed dysgenetic gonads, a residual uterus, and a 46,XY karyotype. Genetic tests excluded mutations in *SRY*, *SOX9*, and *AR* genes. At age 4, subnormal AMH and inhibin B levels indicated partial gonadal dysgenesis.

Sister 2: At age 15, Sister 2 was referred for primary amenorrhea. Imaging detected an irregular 24×13 mm mass in the right gonad (atrophic testicle), confirmed by MRI. Hormonal analysis suggested hypergonadotropic hypogonadism. Genetic tests revealed *NR5A1* and *CYP21A2* mutations in both sisters, with Sister 2 additionally having a *BRCA2* pathogenic variant, indicating high risk for hereditary breast and ovarian cancer (HBOC).

Conclusion: Mutations in *NR5A1* are responsible for up to 10–15% of non-syndromic 46,XY DSD cases and can lead to sex reversal type 3. These cases highlight the importance of comprehensive genetic testing for accurate diagnosis and treatment. In Sister 2, the *BRCA2* mutation necessitates a tailored therapeutic strategy to manage HBOC risk, including prophylactic monitoring and early intervention.

C.O.I. Disclosure:
E. Preizner-Rzucidlo: None. **D. Skoczylas:** None.

EP13.034 Genetic complexity in WAGR Syndrome. A case report with uncharacterized genetic findings

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Background: WAGR syndrome is a rare genetic disorder characterized by Wilms tumor, aniridia, genitourinary abnormalities, and developmental delay or mental retardation[1]. Its prevalence ranges from 1 in 500,000 to 1 million individuals. We present a case of a 1-year, 3-month-old male diagnosed postnatally with WAGR syndrome. The patient was born at 39 weeks of gestation, weight 2920g, height 50cm.

Methods: Prenatal ultrasound findings suggested congenital adrenal hyperplasia (CAH)[2] due to micropenis, therefore, FISH analysis of the amniotic fluid and postanal karyotyping were performed. Other used methods were genetic counseling, physical examination, and Comparative Genomic Hybridization (CGH).

Results: The diagnosis was established based on clinical findings and genetic testing. FISH analysis revealed 46,XY. Karyotyping revealed a balanced translocation *46,XY,t(1;8)(p22;p21)* the clinical significance of which is unknown, and excluded CAH. Physical examination performed by a pediatrician included findings such as micropenis, hypospadias, unilateral cryptorchidism, glaucoma, swollen eyes, and aniridia[3]. A family member reported aniridia during a clinical geneticist's consultation, raising suspicion of WAGR syndrome. Comparative genomic hybridization (CGH)[4] identified *microdeletions on chromosome 7 (83480903-84722334)* and *chromosome 11 (29593220-33042591)*, confirming the diagnosis. Karyotyping of both parents did not reveal any abnormalities, stating that the translocation is de novo.

Conclusion: While the 3.4mb microdeletion identified on chromosome 11 (29593220-33042591) confirms the diagnosis, the role of 1.2mb microdeletion on chromosome 7 (83480903-84722334) and translocation t(1;8)(p22;p21) remain unknown and leave room for further discussion and research.

C.O.I. Disclosure:
S. Muradyan: None. **A. Aywaz:** None. **N. Kostandyan:** None. **T. Sarkisian:** None. **N. Dzoyan:** None. **L. Barseghyan:** None.

EP13.035 Crouzon Syndrome. Mexican female case with clinical diagnosis in the neonatal stage, the importance of early surgical management, and follow-up at 20 months of age

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Background: Craniosynostosis is a congenital disorder from premature cranial suture ossification, with a prevalence of 1:2,500 live births in Mexico. It's classified as non-syndromic (75%) and syndromic (25%). Crouzon syndrome accounts for 4.8% of cases, with autosomal dominant etiology. Surgery is recommended between 0–6 months. Early diagnosis, timely imaging studies, and surgical intervention are crucial for proper psychomotor development and reducing craniofacial characteristics.

Material and methods: Female 11 days of life. Maternal history: 12th pregnancy (A:9; C:3). Abortion threat at 6 WOG. Cesarean at 38 WOG, OFC: 32.5 cm (+97centile) APGAR 9/9. Initial physical examination: Brachyturricephaly, flat broad frontal, tense fontanel, downstand palpebral fissures, eyes of rising sun, horizontal nystagmus, hypertelorism, depressed nasal bridge, high palate, low-set ears, generalized hypotonia.

Results: Evolution in months: 2m. Brachyturricephaly, narrow biparietal diameter, low-set ears. 4m. Significant craniofacial involvement, psychomotor delay. 7m. Neurosurgery with fronto-orbital advancement and parietal release. 9m. Improvement of head shape, phenotypic characteristics and psychomotor development. The zeizures were resolved. 12m. Continuous improvement in facial features and psychomotor development is sustained while standing, normal somatometry, without neurological alterations. 15m. Babbling, improved bipedal stability. 20m. Actually normal somatometry, speaks monosyllables and start walk.

Pre-surgical studies: transfontanelar USG: ventriculomegaly; Cranial CT: plagiocephaly; 3D-CT: coronal bilaterally closure, lamboid sutures and fontanelle posterior closure, osteolytic areas.

Post-surgical study:3D-CT: anterior fontanel permeable, Chiari malformation, ventriculomegaly, non-oppressive supratentorial hydrocephalus.

Conclusion: Phenotypic characteristics confirm Crouzon Syndrome. Early diagnosis and timely surgery are crucial to prevent neurological issues and improve outcomes. Periodic control and multidisciplinary monitoring are essential.

C.O.I. Disclosure:
N.O. Davalos Rodriguez: None. **F.E. Mendez De Armas:** None. **E.O. Garcia Cambero:** None. **J.R. Ortega Águila:** None. **R. E´Vega Hernández:** None. **M.S. Barrios Guyot:** None. **J.J. Padilla Navarro:** None. **F. Gonzalez Gallo:** None. **L.A. Delgado Rosas:** None. **A.R. Rincon Sanchez:** None.

EP13.036 Epidemiological monitoring of congenital malformations in Yakutia from 2000 to 2023

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Background: Congenital malformations represent a broad group of structural and anatomical defects that arise during intrauterine development and significantly affect the life and health of the child. Monitoring the frequency of congenital malformations in the Republic of Sakha (Yakutia) has been conducted since 2000,

when the Republican Register of Hereditary and Congenital Pathology of the Republican Hospital No. 1 - National Medical Center named after M.E. Nikolaev began its operations.

Material and methods: The data from Medical Genetic Center Register was analyzing by the MedCalc 15.8 program.

Results: The incidence rate of congenital malformations over a 24-year period averaged 26,9 cases per 1000 newborns with a standard deviation of 4,4. It's high indicator In Russia (>20 ‰). At this period, the average annual number of births was 14428 (SD 1708,9). The high frequencies of malformations in last 4 years: 2023, 2022, 2021, 2020 (Table 1). The observed differences are statistically significantly ($p < 0.05$).

Table 1. Congenital malformations prevalence

Years	Incidences with 95% CI
2000	0,02662(0,0239-0,02956)
2001	0,0164(0,01405-0,01845)
2002	0,01728(0,01516-0,01961)
2003	0,0187(0,01652-0,02103)
2004	0,02093(0,01866-0,0234)
2005	0,02627(0,02361-0,02914)
2006	0,0272(0,02451-0,03011)
2007	0,02941(0,02675-0,03226)
2008	0,02669(0,02417-0,0294)
2009	0,02768(0,02516-0,03038)
2010	0,028680,02612-0,03142)
2011	0,02902(0,02647-0,03175)
2012	0,03312(0,03044-0,03597)
2013	0,02748(0,02502-0,03011)
2014	0,02651(0,02412-0,02908)
2015	0,02557(0,02318-0,02815)
2016	0,02931(0,02667-0,03215)
2017	0,03394(0,03095-0,03713)
2018	0,03032(0,02742-0,03345)
2019	0,02973(0,02682-0,03287)
2020	0,02657(0,02385-0,02951)
2021	0,02718(0,02433-0,03027)
2022	0,02997(0,02693-0,03327)
2023	0,03039(0,02724-0,0338)

Conclusion: The Republic of Sakha (Yakutia) is characterized by a high frequency of congenital anomalies, and in recent years there has been an increase in their registration. The prevalence of congenital malformations varies with a periodicity of about 4–5 years, and the observed increasing trend may continue for several years, which is associated with improvements in diagnostic methods.

C.O.I. Disclosure:

A.N. Sleptsov: None. **A.I. Fedorov:** None. **S.A. Petrova:** None. **A.A. Maksimova:** None. **A.L. Sukhomyasova:** None. **N.R. Maksimova:** None.

EP13.037 Oral-facial-digital syndrome and *RAB34*: Uncovering a novel variant in a rare ciliopathy-associated malformations

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Background: Oral-facial-digital syndromes (OFDS) are genetically heterogeneous disorders marked by a wide range of clinical features. Affected individuals often present with cardiac, cerebral, skeletal, and anorectal defects. Bi-allelic variants in the recently identified ciliary gene *RAB34*, which encodes a Rab GTPase involved in ciliary membrane formation, have been implicated in autosomal-recessive OFDS.

Material and methods: This retrospective case report was conducted within our genetic counseling clinic and whole exome sequencing (trio-analysis) was performed.

Results: We report the case of a term newborn, born following an uncomplicated pregnancy, presenting with cleft lip and palate, bilateral duplication of the big toes, and complete aganglionosis of the colon. Despite these malformations, the infant demonstrated age-appropriate development but exhibited failure to thrive. Whole exome sequencing revealed a homozygous deletion (p.Glu153del) in the *RAB34* gene, classified as a variant of uncertain significance (ACMG criteria PM2_supp., PM3_supp., PM4, PP4). This in-frame deletion of a conserved amino acid results in a shortened protein. The phenotypic overlap is striking, suggesting this variant is likely responsible for the clinical features observed.

Conclusion: This case contributes to the growing evidence supporting *RAB34* involvement in OFDS, particularly those with gastrointestinal and skeletal manifestations. Identifying a homozygous variant enhances our understanding of the genetic basis of these syndromes and underscores the critical role of genetic testing in diagnosing complex congenital conditions.

C.O.I. Disclosure:

A. Mayer: None. **S. Stößlein:** None. **M. Gusic:** None. **D. Wahl:** None.

EP13.038 Survival and follow up of live newborns with trisomies 13 and 18 in a spanish tertiary hospital

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Background: Advice on survival and quality of life in cases of children with trisomies 13 and 18 may be complicated, given the complexity of these conditions. The objetive of this study is to review the development of a spanish cohort of trisomic children.

Material and methods: Retrospective study by reviews of clinical histories of live newborn with trisomies 13 or 18 in a tertiary hospital (January 2008-February 2024).

Results: 18 cases were collected, 11 trisomy 18 (8 girls) and 7 trisomy 13 (3 girls). In one case, the parents decided prenatally the aplication of palliative care at delivery room. 3 cases requiered deep resuscitation and 5 didn´t need support after birth (loss of data in 2 cases). Prenatally, one case had confimatory cariotype and 9 cases had ultrasound suspicion. In 10 cases families accepted adequacy of therapeutic effort or non-application of resuscitation maneuvers (7 died during the first 18 days of life, 1 died at the second month of life, 2 lost data). 4 families wanted to apply extraordinary measures (2 children died in the first 2 years and 2 remain alive at 12 and 13 years of age). The median number

of days of survival was 18. Survival rate al one year was 16% and at 5 years 11%.

Conclusion: In our review, no changes in survival data have been observed over time. It is important for families to know the data about survival and development in their environment to make decisions about the management of their children.

C.O.I. Disclosure:

P. Marin-Reina: None. **I. Reig-Talamante:** None. **R. Escrig Fernandez:** None. **A. Gimeno Navarro:** None. **J. Aliaga Vera:** None. **A. Parra Llorca:** None.

EP13.039 A patient with two monogenic disorders: Silver-Russel Sy and Friedreich ataxia

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Background: Silver-Russell syndrome (SRS) is a rare clinically heterogeneous condition, characterized by fetal growth restriction, postnatal growth failure, body asymmetry, protruding forehead and dysmorphic features. The most common mechanism is **hypomethylation at the H19/IGF2-imprinting control region**. Friedreich ataxia (FRDA) is an neurodegenerative disorder presenting with progressive gait and limb ataxia, dysarthria, dysphagia, oculomotor dysfunction, loss of deep tendon reflexes and cardiomyopathy. Here, we report a patient with two monogenic disorders diagnosed over a six-year period.

Material and methods: To diagnose SRS and FRDA, targeted methylation-specific multiplex ligation-dependent probe amplification (TMS-MLPA) and whole-exome sequencing (WES) with FXN gene repeat expansion testing using triplet-primed PCR (TP-PCR) were performed.

Results: A boy born small for gestational age, with disproportionate growth retardation, body asymmetry, leg length discrepancy, neurocranial predominance, protruding forehead, small triangular face with dysmorphic features was referred to genetic examination at age of 7. Hypomethylation at the H19 locus in IC1 confirmed SRS. At the age of 9 fine hand tremor, unsteady gait, and attention disturbances were noticed, with slow progression to ataxic gait, mild dysarthria and hypertrophic cardiomyopathy. WES and FXN TP-PCR revealed a homozygous GAA repeat expansion (>200 and >200 repeats) in the FXN gene, confirming FRDA. No consanguinity was reported. Clinical overlap between SRS and FRDA features was noted.

Conclusion: This case highlights the rare occurrence of two monogenic disorders in one patient. It underscores the importance of considering dual diagnoses in rare diseases, especially when the phenotypes cannot be explained by a single condition.

C.O.I. Disclosure:

J. Jovanovic: None. **O. Miljanovic:** None. **G. Stojanovic:** None. **N. Teran:** None. **A. Hodzic:** None. **B. Peterlin:** None.

EP13.040 Identification of a de novo novel MARK2 variant in a patient with intellectual disability

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Background: Intellectual disability (ID) and autism spectrum disorder (ASD) are both neurodevelopmental conditions, but with differences in phenotypic features. Numerous genes linked to ASD and ID are still under investigation. Microtubule affinity-regulating kinase 2 (MARK2) plays a crucial role in neuronal polarity and dendritic spine formation. According to Zhou et al. in 2022, MARK2 has been linked to an increased risk of ASD, primarily due to de novo variants. Its association with autism was further supported by Gong, Jiayi Li et al. in 2024.

Material and methods: A 38-year old male was referred to our laboratory with coarse facial features, moderate intellectual disability, speech delay, fine and gross motor delay behavioral abnormalities and emotional disturbances (ADHD, aggression, anxiety and psychiatric disorder). NGS SureSelect Exome V8 panel (Agilent) was employed following analysis on Franklin by genoox.

Results: The patient was found heterozygous for a de novo missense variant c.298G>A, p. Glu100Lys in MARK2 gene. The variant lies on the kinase domain of the protein, it is absent from gnomAD and REVEL score of pathogenicity predicts a deleterious effect on the protein.

Conclusion: This study presents a de novo heterozygous MARK2 variant in a patient with intellectual disability and secondary autistic characteristics. To our knowledge, the heterozygous MARK2 variant identified in association with this condition is novel and contributes to the recently reported cohort of cases harboring MARK2 variants. Further studies are required to elucidate the functional effect of this variant at the protein level.

C.O.I. Disclosure:

M. Kanezou: None. **A. Miltiadous:** None. **P. Gerasimou:** None. **A. Elpidoforou:** None. **M. Johnson:** None. **G. Shianiou:** None. **G. Kyprianou:** None. **C. Ioannou:** None. **C. Zartila:** None. **E. Georgiou:** None. **C. Avgousti:** None. **A. Christophi:** None. **J. Chi:** None. **V. Christofidou Anastasiadou:** None. **R. Metaxa:** None. **P. Costeas:** None.

EP13.041 Mosaic Turner syndrome in two patients with concomitant clinical Kabuki syndrome type 1

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Background: Kabuki syndrome (KS) is a rare genetic disorder with a mixture of features: specific facial dysmorphism, mild-to-moderate mental retardation, short stature, dermatoglyphic and skeletal abnormalities. Mosaic Turner syndrome (MTS) shows great variability in phenotypic TS features and overlapping KS signs. There are only a few reported cases of concomitant MTS and KS type 1.

Material and methods: Cytogenetic analysis: G-banding karyotype, whole chromosome X painting plus SRY FISH analysis. Next generation sequencing (NGS) sought to identify germinal clinically significant variants in two patients with both KS and TS clinical aspects.

Results: Both patients had similar presentation suggesting KS: long palpebral fissures, eversion of the lower lateral eyelids, arched eyebrows, strabismus, palate malformations, large prominent ears, brachydactyly, vertebral abnormalities. In first MTS patient cytogenetic analysis showed mos 45,X[24]/46,X,+mar[1].ish der(X)(wcpX+), and NGS a monoallelic missense variant *KMT2D*(NM_003482.4):c.971G>A(p.Arg324Gln) (VUS). The second patient harbour a monoallelic non-frameshift variant:

KMT2D(NM_003482.4):c.11217_11222del (p.Gln3744_Gln3745del) (VUS), and cytogenetics revealed mos 45,X[36]/46,X,+mar[14].ish der(X)(DXZ1 + ,SRY-). Short stature, moderate mental retardation, short wide neck, low hairline at the back of the neck, short metacarpal and metatarsal IV bones, kypho-scoliosis-were TS manifestations in the first case, and short stature, hypertension, small lower jaw, high arched palate, cholesteatoma left ear, short metacarpal and metatarsal IV bones, broad chest, pigmented naevi, horseshoe kidney- in the second one.

Conclusion: Our results expand the molecular spectrum of *KMT2D* variants as causes of Kabuki syndrome type 1, highlighting the role of both clinical and molecular data for more precise and personalized management of patients with admixed rare syndromes.

C.O.I. Disclosure:

S. Schipor: None. **C. Procopiuc:** None. **O. Popa:** None. **A. Muresan:** None. **I. Nedelcu:** None. **D. Manda:** None. **E. Braha:** None.

EP13.042 Duplication of the *SOX3* gene in an *SRY*-negative boy with 46,XX karyotype: a case report and a literature review

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Background: 46,XX testicular disorder/difference of sex development (DSD), also known as XX male syndrome, is a rare condition in which an individual with a 46,XX karyotype develops a male phenotype. In 90% of cases, the syndrome is caused by a translocation of the *SRY* gene, located on the Y chromosome, to the X chromosome or an autosome. The remaining 10% of cases are characterised by the absence of the *SRY* gene. We report an *SRY*-negative 3-year-old boy with normal male secondary characteristics, despite a 46,XX karyotype.

Methods: Exome sequencing, array CGH and quantitative methylation sensitive QF-PCR were performed.

Results: We identified a 723 kb duplication of the Xq27.1q27.2 region, encompassing the *SOX3* gene. An imbalance in the methylation of one X chromosome was observed, with a ratio of 5:1. Exome sequencing was negative for pathogenic variants (SNVs) associated with DSD. Furthermore, the patient's mother was found to be a carrier of the duplication.

Conclusion: The duplication of the *SOX3* gene has been previously reported in 11 *SRY*-negative male patients, 9 of whom exhibited various stages of genital development disorders, including hypospadias, cryptorchidism, and bifid scrotum. In accordance with the present case, only 2 patients with normal male genitalia (albeit infertile due to the absence of the azoospermia factor region) have been reported. The present case report further supports the hypothesis that the overexpression of the *SOX3* gene in the developing gonads is likely to induce testis development, thereby compensating for the loss of *SRY* gene function in 46,XX male patients.

C.O.I. Disclosure:

J. Martinková: None. **H. Řezáčová:** None. **A. Šantavá:** None. **M. Balaščaková:** None. **M. Štolfa:** None. **R. Vodička:** None. **A. Křepelová:** None.

EP13.043 Case report of mosaic 16p11.2p11.1 duplication for patient with severe intellectual disability and treatment resistant epilepsy

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Background: Mosaic 16p duplication is a very rare chromosomal abnormality. Only few cases reported in the medical literature and these cases suggest potential associations with developmental delay, intellectual disability, autism spectrum disorder, and congenital anomalies. We report of adolescent case with mosaic 16p11.2p11.1 duplication with supernumerary chromosome.

Methods: Our patient is 17 years old female. She was born from the 1st uncomplicated pregnancy and delivery. Abnormal skin pigmentation and developmental delay was noticed during the 1st year of life. Epileptic seizures started at 3 years old with resistance for treatment. The patient presented with severe intellectual disability, microcephaly and scoliosis. Brain MRI showed non-specific changes in white matter and suspected cerebral cortex atrophy. The patient was recently diagnosed with deep venous thrombosis.

Result: The patient was diagnosed with Hypomelanosis of Ito at initial genetic consultation in childhood. After follow up consultations karyotype and molecular karyotype from peripheral blood was performed and was normal, 46,XX. Whole exome sequencing was negative. Karyotype from skin fibroblasts showed mosaic female karyotype with marker chromosome (77%): mos47,XX,+mar[23]/46,XX[7]. Chromosomal microarray from cultured fibroblasts confirmed mosaic heterozygous 5,1 Mb duplication of 16p11.2p11.1 chromosome region.

Conclusions: Mosaic 16p11.2p11.1 duplication explained patient's phenotype and finished diagnostic odyssey. Mosaic chromosomal anomalies are always a diagnostic challenge.

C.O.I. Disclosure:

R. Traberg: None. **Z. Zemeckiene:** None. **J. Laurynaitiene:** None. **R. Ugenskiene:** None.

EP13.044 A case of distal chromosome 14q trisomy syndrome due to familial translocation

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Background: Distal trisomy 14q is a rare syndrome characterized by variable clinical features, including growth retardation, low birth weight, hypotonia, developmental delay, intellectual disability, microcephaly, facial dysmorphism. Here, we present a case of a 3-year-old girl who was referred to genetic counseling due to development delay. The mother's pregnancy was uneventful and the girl was born at 38 weeks of gestation with a low birth weight.

She had mild dysmorphic features and microcephaly. The patient followed by a cardiologist due to a patent foramen ovale.

Material and methods: Molecular karyotyping based on SNP-array (Illumina) was done for the patient. A G-banded karyotype analysis of peripheral blood lymphocytes was performed for the patient's parents to characterize possible chromosomal rearrangements and inheritance patterns.

Results: Two pathogenic variants were detected in the patient: 14,8 Mb size terminal deletion of the long arm of chromosome X (Xq27.2-q28), and 46,7 Mb size terminal duplication of the long arm of chromosome 14 (14q23.1-q32.33).The deletion of Xq27.2q28 has no specific syndrome reported, whereas the duplication of chromosome 14q results in distal trisomy 14q syndrome (ORPHA:1705), whose characteristic features match the patient's phenotype. However, the patient's features may change over time due to the large deleted region of chromosome X. The karyotype of the patient's father showed no structural aberrations, whereas mother had balanced translocation between chromosomes X and 14, along with pericentric inversion of chromosome 9: 46,X,t(X;14)(q27;q23),inv(9)(p11q13).

Conclusion: The reported patient inherited derivative chromosome with unbalanced translocation from her mother resulting in distal trisomy 14q syndrome.

C.O.I. Disclosure:

Z. Zemeckiene: None. **R. Traberg:** None. **D. Radzeviciene:** None. **R. Ugenskiene:** None.

EP13.045 Expanding the Phenotypic Spectrum of *NR2F2*-Associated Disorders: Clinical and Molecular Insights from Two Novel Cases

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Background: Heterozygous pathogenic variants in *NR2F2* gene result in a recently characterized multiple congenital anomalies and neurodevelopmental delay syndrome with pre and postnatal manifestations. Clinical features include intrauterine growth restriction (IUGR), increased nucal translucency, congenital heart defects (CHD), dysmorphic features, neurodevelopmental delay/intelectual disability (NDD/ID), hypotonia, feeding difficulties, renal, genital and skeletal anomalies, hearing loss, strabismus, asplenia and vascular malformations. The molecular mechanism leading to disease is *NR2F2* loss of function mostly due to single nucleotide variants (SNVs) but also copy number variants (CNVs).

Material and methods: Two patients with de novo truncating variants in NR2F2 were characterized to further enhance our understanding of the clinical and molecular aspects of this disdorder. Exome sequencing was conducted with identification of disease-causing SNVs.

Results: Patient 1: 21 year-old female. IUGR, coarctation of aorta, strabismus, dismorphic features, feeding difficulties, NDD, DI, occipital aplasia cutis, short stature with limb length discrepancy, 5th toenail dysplasia and short 3–5 left metatarsi. Whole exome analysis revealed an heterozygous likely pathogenic variant in *NR2F2* (NM_021005.4:c.443_450del;p.(Ala148GlufsTer158)). Patient 2: 3 month-old baby boy. IUGR, diaphragmatic hernia, atrial septum defect, tube feeding and dismorphic features. Exome sequencing uncovered an heterozygous likely pathogenic variant in *NR2F2* (NM_021005.4:c.527_533del;p.(Ser176CysfsTer78))

Conclusion: The identification of novel likely pathogenic NR2F2 variants has expanded the phenotypic spectrum of NR2F2-associated disorders, including features such as aplasia cutis. We suggest that NR2F2-associated disorders be considered in the differential diagnosis when aplasia cutis (HP:0001057) is observed. Ongoing data collection from new patients will further refine the phenotype of NR2F2-associated disorders.

C.O.I. Disclosure:

P.D. Jolin García: None. **B. Masotto:** None. **C. Trujillano-Lidon:** None. **A. Lasa-Aranzasti:** None. **A. Cueto-González:** None. **M. Valenzuela-Palafoll:** None. **P. Muñoz-Cabello:** None. **M. Torres-Lopez:** None. **A. Herencia-Ropero:** None. **M. Costa-Roger:** None. **E. García-Arumi:** None. **E. Tizzano:** None.

EP13.046 Co-occurrence of two rare genetic conditions: First reported case of 49,XXXXY and X-linked Cornelia de Lange syndrome; diagnostic challenges, phenotypic overlap, and potential mechanism

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49,XXXXY is a rare sex chromosome aneuploidy with an estimated incidence of 1 in 85,000–100,000 newborn males. Individuals with this syndrome exhibit variable clinical manifestations, typically including intellectual deficits, developmental delay, hypogonadism, and distinctive facial features such as ocular hypertelorism, epicanthic folds, a flat nasal bridge, prognathism, folded-over ears, and a short neck. Unlike patients with Klinefelter syndrome, they are often shorter in stature.

Cornelia de Lange syndrome (CdLS) is an unrelated rare disorder with an incidence of 1 in 10,000–30,000 live births, affecting both sexes. CdLS shares overlapping features with 49,XXXXY, including intellectual deficits and hypogonadism. However, it also presents with unique facial characteristics, such as synophrys, thick or highly arched eyebrows, low-set ears, upturned nasal tips, long eyelashes, and microcephaly. CdLS is a clinically heterogeneous condition, with severe cases involving congenital malformations including limb anomalies, and milder cases showing only subtle facial dysmorphism. Both syndromes may also involve cardiac and renal anomalies.

We report the first documented concurrence of 49,XXXXY and X-linked CdLS, emphasizing the challenges in diagnosis and the phenotypic overlap between these two rare syndromes, and propose a theoretical mechanism for the co-occurrence.

C.O.I. Disclosure:

S.H. Al Rashdi: None. **L. Ekstrom:** None. **A. Collins:** None. **H. L. Schulz:** None. **A. Dinwiddie:** None. **C. Samanaga-Sprouse:** None. **E. Moser:** None. **A. J Green:** None.

EP13.047 Challenging genetic diagnosis in siblings with severe primordial dwarfism, osteopenia, craniofacial dysmorphism, neurodevelopmental delay and muscle wasting

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Background: Three siblings in a consanguineous Pakistani family are afflicted with a neurodevelopmental and neuromuscular disease and skeletal anomalies characterized by microcephaly, short stature, osteopenia, thin and lean physique, delayed developmental milestones, and in the limbs, fragile long bones and dysplastic joints. IQ, vision, hearing and speech are normal. Two other siblings have short stature and low weight. One has congenital deafness and the other congenital scoliosis.

Material and methods: The putative disease locus for the syndrome was not identified by linkage analysis using SNP data. Then, we analyzed the exome data individually to detect rare and novel variants and performed Sanger sequencing to validate familial segregation.

Results: We found 6 very rare homozygous mutations in genes *CENPE, POC1A, PCNT, TMEM38B* and *TMEM43* already related to 4 recessive and 1 dominant disease. Patients were homozygous for different combinations of those variants. In deaf-mute sib, a *GJB2* homozygous mutation is found. Sibling with severe scoliosis and *POC5* p.Ala404Val, known to predispose to scoliosis, has also mutations in proteins *HOOK2* and *ESR1* known to interact with POC5, indicating that those proteins are possible partners for POC5 in manifesting scoliosis.

Conclusion: Such formidable genetic heterogeneity in siblings sharing very similar phenotypes and different combinations of mutations demonstrate an unusual difficulty in establishing genetic diagnosis. Geneticist should always keep in mind locus heterogeneity and caution for seemingly "novel syndromes". *HOOK2* and *ESR1* arise as possible partners for POC5 in manifesting scoliosis.

C.O.I. Disclosure:

E. Önel: None. **S. Mumtaz:** None. **R.M. Shabbir:** None. **M.U. Raza:** None. **S. Malik:** None. **A. Tolun:** None.

Candidate variants and relevant clinical findings in family members

Gene	*CENPE*	*POC1A*	*PCNT*	*TMEM38B*	*TMEM43*	*POC5*
Variant	c.2906C>T (p.Ala969Val)	c.719C>T (p.Ser240Leu)	c.9572G>A(p.Arg3191His) c.2033A>G (p.Lys678Arg)	c.662T>C (p.Ile221Thr)	c.601G>A (p.Asp201Asn)	c.1211C>T (p.Ala404Val)
SAS frequency	0.00003405	0.0002635	0.00001098 / 0.005413	0.0002471	0.00002196	0.004622
Highest frequency	0.00003405	0.0002635 SAS	0.00003199 Remaining/ 0.007635 Finnish	0.003444 Ashkenazi Jewish	0.002875 East Asian	0.01204 European
CADD score	23.1	24.8	25.6 / 14.2	22.0	20.7	11.5
Locus	4q24	3p21.2	21q22.3	9q31.2	3p25.1	5q13.5
rs ID	rs754690682	rs749870224	rs767844895/ rs149623054	rs201882609	rs138182276	rs146984380
Disorder	Primary microcephaly	Short, facial dysmorphism, onychodysplasia, hypotrichosis	Microcephalic osteodysplastic primordial dwarfism	Osteogenesis imperfecta	Emery-Dreifuss muscular dystrophy 7	Isolated scoliosis
Inheritance	AR	AR	AR	AR	AD	AD, reduced penetrance
401 (Severe syndrome)	Hom	Hom	Het, het	Hom	Hom	Het
402 (Severe syndrome)	Het	Het	Hom, hom	Hom	Hom	None
403 (Severe syndrome)	Het	None	Het, het	Het	None	Het
404 (Deaf-Mute)	Het	None	Hom, hom	None	Het	Het
405 (Scoliosis)	None	None	Hom, hom	Het	Hom	Het
406 (Unaffected sib)	Het	Het	Het, het	Het	None	Het
305 (Unaffected mother)	Het	Het	Het, het	Het	Het	Het
306*(Unaffected father)	Het	Het	Het, het	Het	Het	None

* Deduced genotype from the SNP haplotypes and variant alleles of sibs.

EP13.048 The first report of an Iranian patient with Zaki syndrome

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Background: The *WLS* gene plays a crucial role in the secretion of Wnt proteins, which are associated with various vital biological processes. Recent studies have identified biallelic variants in *WLS* among twelve patients diagnosed with Zaki syndrome (ZS), across diverse regions including the UAE, Egypt, the USA, Brazil, India, and China. ZS is classified as an autosomal recessive disorder and presents with a range of phenotypes such as microcephaly, facial dysmorphism, alopecia, syndactyly, microphthalmia, dental

irregularities, skeletal dysplasia, and Dandy-Walker malformation. This report describes the first case of an Iranian patient with ZS.

Methods: A 14-year-old Iranian girl, born to a consanguineous marriage, exhibiting clinical features consistent with ZS underwent whole exome sequencing (WES), and the identified variant was confirmed through Sanger sequencing, demonstrating co-segregation within the family.

Results: The patient presented with microcephaly, blindness, a cystic lesion in the brain, repetitive movements, difficulty walking, dysmorphic face, clinodactyly, dental anomalies, a flat occiput, seizures, and premature puberty. WES analysis uncovered a homozygous missense mutation in exon 12 of the *WLS*, NM_024911.7: c.1433A>G, p.(Tyr487Cys), which was classified as likely pathogenic.

Conclusion: This case represents the thirteenth case of ZS, contributing to the expanding understanding of the phenotypic manifestations associated with *WLS* mutations. The findings underscore the necessity for comprehensive clinical assessments to accurately characterize the phenotypic spectrum linked to *WLS* variants. Nonetheless, further research involving additional patients and functional studies is essential to deepen the understanding of genotype-phenotype relationships and the intricate role of *WLS* in developmental processes.

C.O.I. Disclosure:

Z. Sadr: None. **N. Ehtesham:** None. **M. Yavari:** None. **M. Mazaheri:** None.

EP13.049 Clinical and genetic investigation of two new cases of the rare 10q22.3q23.2 microdeletion syndrome

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Background/Objectives: 10q deletions [del(10)(q22.3q23.2)] have been characterized as a rare syndrome associated with craniofacial dysmorphisms and intellectual developmental disorders. The presence of low-copy repeats (LCRs) in this region can explain recurrent genomic changes.

Material and methods: Two patients were referred to the Laboratory of Medical Genetics. A two-month-old male with micrognathia, arachnodactyly, long palpebral fissures, skeletal abnormalities, overlapping toe on the right feet and feeding difficulties, and an eighteen-year-old female with intellectual developmental disorder and skeletal abnormalities including short stature. Following clinical evaluation, both conventional karyotype and Whole Exome Sequencing (WES) were ordered.

Results: Conventional karyotype in both patients revealed the 10q deletion [del(10)(q22.3q23.2)]. WES confirmed the 7.6Mb heterozygous pathogenic deletion (Chr10:81,468,455-89,118,188del).

Conclusion: Conventional karyotype and WES confirmed the genetic diagnosis. Parental karyotype is necessary in order to investigate if one of the parents carries an interstitial translocation, but unfortunately parents were not available. Parent testing through conventional karyotype remains an important tool for diagnosis and accurate calculation of recurrence risk and its implications in genetic counseling.

C.O.I. Disclosure:

N. Selenti: None. **D. Veltra:** None. **F. Tilemis:** None. **K. Kosma:** None. **A. Kampouraki:** None. **C. Sofocleous:** None. **P. Makrythanasis:** None.

EP13.050 Novel PBX1 variant identified by whole exome sequencing in a pediatric patient with stage 2 chronic kidney disease, gout and mild intellectual disability

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Background: We present a case of a 16-year-old girl with chronic kidney disease stage 2, gout, arterial hypertension, proteinuria, obesity and mild intellectual disability. The patient complains of unconfirmed hearing impairment. The imaging studies revealed chronic changes in renal parenchyma and malrotation of the left kidney.

Material and methods: Exome sequencing was preformed due to the complex clinical picture.

Results: Whole Exome analysis identified a heterozygous splice donor variant PBX1 c.510+1G > T, classified as likely pathogenic. This variant has not been reported in medical literature and typically leads to a loss of protein function, which is an established disease mechanism for this gene. PBX1 encodes a nuclear protein which is a part of the PBX homeobox transcription factors and regulates target genes involved in embryogenesis. The role of the gene product as a critical regulator in mesenchymal function during renal morphogenesis has been confirmed in recent years.

Conclusion: Heterozygous variants of PBX1 have been linked to Congenital anomalies of kidneys and urinary tract syndrome with or without hearing loss, abnormal ears or developmental delay (CAKUTHED). Disease caused by PBX1 variants is inherited in an autosomal dominant manner. Any offspring of the patient is at 50% risk of inheriting the variant and of being affected. Our case adds to the understanding of genotype-phenotype correlations of PBX1 variants and suggests the importance of genetic counselling and proper genetic testing of patients with undiagnosed chronic kidney disease.

C.O.I. Disclosure:

T. Sokolova: None. **H. Ivanov:** None. **P. Markova:** None. **V. Stoyanova:** None.

EP13.051 Why *TP63* ectodermal dysplasia should be considered an inborn error of immunity

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Background: The *TP63* gene encodes a transcription factor crucial for the development and maintenance of epithelial tissues. *TP63* is involved in thymic epithelial development, affecting T-cell maturation and proliferation. *TP63*-related conditions represent a class of overlapping phenotypes that share a common spectrum

of features: variable forms of ectodermal dysplasia (ED: nail dysplasia, hypohidrosis, tooth abnormalities, sparse hair), cleft lip/palate, hypopigmented skin, split-hand/foot malformation/syndactyly. We aim to find arguments that *TP63* -ED reflects the characteristics of an inborn error of immunity (IEI) due to its genetic basis, clinical manifestations, and the impact on immune function.

Material and methods: Clinical and genetic investigations were performed in a child with diagnostic suspicion of IEI and ED. Genetic analysis used Ectodermal Dysplasia with or without Tooth Agenesis Gene Panel.

Results: Our patient presented with alopecia of the scalp, eyebrows, and eyelashes, complete cleft palate, hypoplasia and dystrophy of the nails on both hands and feet, multiple scars from bacterial and fungal infections, glandular hypospadias, a history of recurrent infections during infancy, decreased switched memory B-cells and marginal zone B cells. A de novo heterozygous pathogenic variant NM_003722.4:c.1727T>C (p.Ile576Thr) in exon 13 of *TP63* was identified. Previous reports have documented T cell lymphopenia, altered T cell subsets, and increased susceptibility to infections in individuals with *TP63*-related ED. Compared to previously published cases, our patient demonstrated persistent B-cell lymphopenia.

Conclusion: IEI often present with multisystem involvement. The phenotypic presentation of ED overlaps with immune dysfunction, suggesting that both systems are affected by the underlying genetic defect.

C.O.I. Disclosure:

C.L. Pantea: None. **M. Bataneant:** None. **A. Chirita-Emandi:** None.

EP13.052 Clinical manifestations of mosaic interstitial duplication 16p12.3p11.2 as an additional derivative chromosome

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Background: Partial duplication of 16p is a rare aberration and is characterized by a variable phenotype depending on the chromosomal breakpoints. Phenotypic manifestations of mosaic dup 16p12.3p11.2 as an additional derivative chromosome are of clinical interest.

Material and methods: The case from own practice, clinical-genialogical, cytogenetic, molecular-genetic examination methods.

Results: The 2-year-old boy was under observation from 2 months after birth with ventricular septal defect with aneurysm, patent foramen ovale, additional left superior vena cava with dilated coronary sinus; initial pulmonary hypertension, heart failure grades I-III; bilateral pyelectasis; severe deformity of the gallbladder, chronic constipation; epilepsy, West syndrome, attention deficit syndrome, delayed speech and statokinetic development. The proband was born at 40 weeks of gestation with normal anthropometric parameters. Apgar score is 8/9. The proband has three healthy half-siblings. Phenotype: dolichocephaly, high forehead, high hairline, prominent frontal tubercles, deep-set eyes, concomitant strabismus, sparse eyebrows and eyelashes, smoothed long filter, short upper lip, short neck, valgus deformity of the knee joints and feet. Cytogenetic study results: Karyotype (GTG) mos 47,XY,+mar[15]/46,XY[78]. NGS revealed the presence of mosaicism of genes on the short arm of chromosome 16. Array-CGH: mosaic interstitial duplication of the short arm of chromosome 16p: arr[GRCh37] 16p12.3p11.2 (20337901_31874832) x 2 ~ 3. Size 11.54 Mb. It was suggested that the marker chromosome is a derivative of chromosome 16.

Conclusion: The identified abnormality includes the duplicated region 16p12.3p11.2, covers the area of microduplication syndrome 16p11.2p12.2 and proximal microduplication syndrome 16p11.2, which can manifest as multiple defects, dysmorphic facial features, epilepsy, mental disorders, which correlates with phenotypic manifestations in the proband.

C.O.I. Disclosure:

I. Lastivka: None. **V. Antsupova:** None. **O. Polodiyenko:** None. **L. Sheiko:** None. **L. Brisevac:** None. **I. Ushko:** None. **V. Davydiuk:** None.

EP13.053 Expanding the Clinical Phenotype of 12p13.33 Microdeletion Syndrome: A New Case Report

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Background: The 12p13.33 microdeletion syndrome is a very rare disorder of the distal end of the short arm of chromosome 12 and exhibits variable phenotype comprising apraxia of speech, which is a specific language disorder, developmental delay, behavioral problems and mild/absent dysmorphic features. The aim of the study is the presentation of a new 12p13.33del case and the enrichment of the phenotypic/genotypic correlations of the syndrome.

Material and methods: A male 12-year-old patient with absence of speech, moderate cognitive deficit, motor delay, generalized hypotonia and history of epileptic seizures, cryptorchidism and ventricular septal defect is presented. On clinical examination, the patient demonstrated maxillary prognathism with lip closure inability and drooling, teeth anomalies, sunken eyes, strabismus and low-set ears. The first-tier genetic testing was chromosomal microarrays (CMA) (Agilent Technologies 8X60K G3 ISCA V2 microarrays)

Results: CMA revealed a de novo ~2,6 megabase deletion within the 12p13.33 chromosomal region (chr12:230,421_2,873,676), encompassing 15 genes enlisted in the OMIM database, among which the genes *WNK1* (*605232), *CACNA2D4* (*608171), and *CACNA1C* (*114205) are of known clinical significance.

Conclusion: Approximately 30 cases of 12p13.33del syndrome have been described in the literature so far, presenting with a variable neurodevelopmental phenotype, possibly depending on the deletion size or undefined epigenetic effects. Mild dysmorphic features were observed in 60% of the cases, as also described in our patient. The detailed clinical documentation and subsequent genetic analysis of new cases will contribute to the comprehension of the clinical phenotypes and the guidance of the patients and their families.

C.O.I. Disclosure:

S. Psoni: None. **E. Manolakos:** None. **E. Tsoutsou:** None. **H. Fryssira:** None.

EP13.054 Setleis syndrome phenotype in a patient with 1p36 microduplication

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Background: Setleis syndrome (SS), also known as Focal Facial Dermal Dysplasia Type III (FFDD3, OMIM #227260) is a rare ectodermal dysplasia characterized by distinctive facial dysmorphisms, intellectual and growth delay. The Setleis syndrome's aetiology remains unclear: while recessive mutations in the TWIST2 gene have been implicated, Copy Number Variations (CNVs), particularly microduplications in the 1p36.23 - p36.21 cytobands have also been reported. We present the case of a 1-year-old child, referred to our department for language and growth delay, as well as facial dysmorphisms features characteristic of SS. The child's mother had previously been diagnosed with a 1p36.23p36.22 microduplication, detected during a prior pregnancy.

Material and methods: Peripheral blood samples were obtained from the proband. After Genomic DNA extraction, clinical exome sequencing (CES), along with SNP array (SNPa) analysis, was performed.

Results: The proband is the second child of non-consanguineous parents. Clinical examination revealed scar-like bitemporal lesions (also present in his mother), axial hypotonia, impaired upright postural control and language delay. CES analysis excluded the presence of homozygous deleterious variants in TWIST2 gene; SNPa analysis identified a microduplication: arr[GRCh37]1p36.23p36.22(8910347-12070292)x3, identical to the one observed in his mother.

Conclusion: SNPa analysis confirmed that the proband inherited the maternal CNV, which overlaps with the dosage sensitive region associated with SS. The presence of shared facial features between mother and son may be explained by the phenotypic heterogeneity associated with this condition. These findings support the role of 1p36 microduplications in SS and highlight the need for further investigation into the genotype-phenotype correlation.

C.O.I. Disclosure:

Y. Krylovska: None. **S. Lattarulo:** None. **S. Martino:** None. **M. Piglionica:** None. **M. Stasi:** None. **E. Meneleo:** None. **M. Iacoviello:** None. **O. Tabaku:** None. **C. Simone:** None. **N. Resta:** None. **N. Bukvic:** None.

EP13.055 Stapes dysplasia and lateral semicircular canal malformation in Cornelia de Lange syndrome

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Background: Cornelia de Lange syndrome (CdLS) is a rare multisystem developmental disorder. More than 80% of patients have conductive and/or sensorineural hearing loss, but due to the difficulty in managing these complex patients, audiological evaluations are not always performed and the range of ear imaging abnormalities is poorly documented. We therefore sought to investigate ear malformations in CdLS patients with hearing loss.

Methods: We performed a retrospective chart review, including all patients followed in the clinical genetics department of Lille University Hospital with molecular diagnosis of CdLS and documented hearing loss. When available, ear imaging was reviewed by the expert radiologists.

Results: We identified sixteen patients with a molecular diagnosis of CdLS (11 NIPBL, 2 SMC3, 2 SMC1A, 1 HDAC8). Nine of these sixteen patients (7 NIPBL, 1 SMC3, 1 HDAC8) presented with hearing loss. Ear MR/CT scans were available in only four of the nine cases (2 NIPBL, 1 SMC3, and 1 HDAC8). All four patients had stapes dysplasia, and three of them also had lateral semicircular canal malformation.

Conclusions: We found recurrent ear malformations in the four CdLS patients with hearing loss and available ears imaging. To our knowledge, stapes dysplasia has not been described previously in CdLS. Further descriptions of larger cohorts of patients are needed to allow a better understanding of the structural ear abnormalities and the hearing impairment in CdLS.

C.O.I. Disclosure:

S. Costantini: None. **C. Vincent-Delorme:** None. **L. Bellengier:** None. **R. Caumes:** None. **A. Dieux:** None. **T. Smol:** None. **F. Petit:** None. **F. Dubrulle:** None.

EP13.056 Mosaicism in CHARGE syndrome: severe phenotypic expression and genotype-phenotype correlation challenges

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Background: CHARGE syndrome is a rare congenital disorder caused by pathogenic variants in the *CHD7* gene, leading to multisystemic damage. We report a case of mosaic CHARGE syndrome, expanding the phenotypic spectrum and highlighting challenges in genotype-phenotype correlation.

Material and methods: A male infant, born at 39 weeks with intrauterine growth restriction, presented with craniofacial dysmorphism, microtia, microphthalmia, cranial nerve deficits, congenital heart defects, and sensorineural hearing loss. His clinical course included neurological impairment, developmental delay, respiratory insufficiency requiring tracheostomy, and oropharyngeal dysphagia requiring gastrostomy tube placement.

Results: Whole exome sequencing identified a mosaic *CHD7* variant, NM_017780.4(*CHD7*):c.6850C>T, leading to NP_060250.2:p.(Arg2284Ter), detected in 31.96% of sequencing reads. Parental Sanger sequencing confirmed a de novo origin. The variant introduces a premature termination codon in a gene where pathogenicity is due to loss of function (PVS1), is absent in population databases (PM2_supporting), de novo in CHARGE patients (PS2), and associated with CHARGE symptoms in multiple publications (PS4). Despite the low allelic burden, the phenotype was severe.

Conclusion: Mosaic *CHD7* variants are rare, with phenotypic manifestations ranging from unaffected individuals to severe disease, as observed in our case. This case highlights the complexity and unpredictability of mosaic CHARGE syndrome, emphasizing the limitations of predicting phenotype based solely on allelic burden from blood samples, which remains unreliable even in low-level mosaicism. Further investigation of other

affected embryonic layers may reveal a higher allelic burden, improving the accuracy of phenotype prediction.

C.O.I. Disclosure:

L. Kievišiene: None. **M. Lartsuliani:** None. **E. Stoukutė:** None. **R. Jonikas:** None. **R. Traberg:** None. **R. Ugenskienė:** None.

EP13.057 A novel GJB2 mutation in a 1-year-old Iranian child with Keratitis-Ichthyosis-Deafness (KID) syndrome

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Objective: Keratitis-Ichthyosis-Deafness (KID) syndrome is a rare genetic disorder characterized by skin abnormalities, hearing loss, and ocular complications. The condition is primarily caused by mutations in the GJB2 gene, which encodes the connexin 26 protein. The objective of this study is to report a novel mutation in the GJB2 gene associated with KID syndrome in a pediatric patient.

Materials and methods: We examined a 1-year-old Iranian child presenting with the classic symptoms of KID syndrome, including keratitis, ichthyosis, and profound hearing loss. Genetic analysis was conducted using whole exome sequencing, and the identified mutation was confirmed by Sanger sequencing. Bioinformatics tools were employed to evaluate the mutation's pathogenicity, and in silico protein modeling was used to predict its structural and functional effects.

Results: A rare heterozygous missense variant in the GJB2 gene was identified in the affected child. Protein modeling indicated that this mutation disrupts the connexin 26 protein's ability to form functional gap junction channels, potentially impairing intercellular communication and contributing to the observed phenotype.

Conclusion: This study reports a novel mutation in the GJB2 gene associated with KID syndrome in a pediatric patient. These findings enhance our understanding of the genetic underpinnings of KID syndrome and may contribute to improved diagnostic and therapeutic approaches for this condition.

C.O.I. Disclosure:

R. Aryan: None. **D. Farhud:** None.

EP13.058 Dual diagnosis in exome diagnostics: Twin girls with Emanuel syndrome and mosaic paternal UPD 11p15.5

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Background: "Exome first" diagnostics often is requested for newborns with multiple malformations in intensive care, in this case prematurely born twin girls with VSD, an ASD II and a double aortic arch (twin 1) and a left diaphragmatic hernia, a hypoplastic aortic arch and an ASD II (twin 2).

Material and methods: Quattro exome diagnostics was performed for the twin girls and their parents.

Results: Two copy number gains in 11q23.3-q25 (approximately 17.6 Mb) and 22q11.1-q11.21 (approx. 4.0 Mb) in both twins raised the suspicion of Emanuel syndrome (MIM # 609029). Cytogenetics confirmed the presence of an additional derivative translocation chromosome 47,XX,+der(22)t(11;22)(q23.3;q11.2) corresponding to Emanuel syndrome in twin 2 as well as the corresponding balanced translocation in the mother. Additionally, an unequal parental allele distribution (approx. 2/3 paternal and 1/3 maternal) with normal copy number in approx. 18.4 Mb in 11p15.5 was detected. Methylation-sensitive MLPA showed hypermethylation of the paternally methylated IC1 and hypomethylation of the maternally methylated IC2 in mosaic form, i.e. mosaic paternal uniparental disomy, which is detected in approximately 20% of patients with Beckwith-Wiedemann syndrome (MIM # 130650).

Conclusion: The easily detected Emanuel syndrome was considered causative. The less obvious diagnosis Beckwith-Wiedemann syndrome could affect the clinical picture, e.g. regarding growth, and be associated with an increased risk of neoplasia. This highlights the potential importance of dual diagnoses even in cases with convincing genotype-phenotype correlation for the first, obvious diagnosis.

C.O.I. Disclosure:

S. Peters: None. **J. Trautmann:** None. **E. Engels:** None. **J. Maric-Biresev:** None. **N. Ortega:** None. **S. Stankovic:** None. **C. Prell:** None. **T. Dreschbach:** None. **L. Schröder:** None. **E. Mangold:** None. **M. Kreiß:** None. **K. Cremer:** None. **C. Perne:** None. **H. Lesmann:** None. **M. Esther:** None. **H. Hundertmark:** None. **P. Incadona:** None. **S. Heilmann-Heimbach:** None. **T. Eggermann:** None. **T. Bender:** None. **A. Müller:** None. **H. Engels:** None.

EP13.059 Three cases with novel variants in exon 4 of the SOX10 gene: Phenotypic heterogeneity

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Background: The SOX10 gene encodes the SRY-BOX10 transcription factor and is a member of the SOX transcription factor family.It plays a significant role in neural crest development and is implicated in various diseases. Previous studies have shown that SOX10 heterozygous loss of function mutations can lead to Waardenburg syndrome(WS) Type 2E,4C and PCWH(Peripheral demyelinating neuropathy, Central dysmyelination, Waardenburg syndrome, Hirschsprung disease)[1].The main clinical features in patients with WS are congenital sensorineural hearing loss, dystopia canthorum and pigment disturbances of hair, skin and iris[2].SOX10-related WS may show variable phenotype. Genotype-phenotype correlation depending on the variant type and position is very important in determining the clinical diagnosis of SOX10-related allelic diseases.

Material and methods: Three patients underwent clinical exome sequencing using the QIAseq Actionable Exome Kit and QIAseq FX® after routine diagnostic tests.

Results: The heterozygous variants in SOX10(NM_006941.4) identified in our three patients are as follows: c.698-1G > A, c.1258_1264dup, c.762_772delAGACCCGAAGC. Variants and clinical characteristics of patients are listed in Table 1.

Conclusion: Further research is needed to understand the full spectrum of SOX10-related disorders and improve patient care. Literature reports that truncating-frameshift variants in the last exon typically lead to the PCWH phenotype, which is also observed in two of our patients[4].In the other patient with a splicing variant, although the current findings suggest it to be Type 2E, the patient's follow-up is important in terms of the potential development of different phenotypic features.

Table 1: Clinical Characteristics of Patients

	Patient 1(c.698-1G > A)	Patient 2(c.1258_1264dup)	Patient 3(c.762_772delAGACCCGAAGC)
Variant region	Intron 4	Exon 4	Exon 4
Variant type	Splice site	Frameshift	Frameshift
Age at clinical presentation	3 years	27 years	2 years 8 months
Chromosome analysis and microarray	Normal	Normal	Normal
Sanger confirmation	Not confirmed	Not confirmed	Confirmed(de novo)
Postnatal history	Hypotonic infant	History of surgery due to aganglionic megacolon at 3 months of age	Normal
Clinical features			
/Eyes	Oculomotor apraxia	Nistagmus/ Heterochromia iridis	Lens heterochromia
/Anosmia-Hypogonadism	No	No	No
/Ears	No	Bilateral hearing loss/Temporal MRI: bilateral superior and posterior semicircular cabal aplasia, bilateral vestibule mild enlargement, bilateral cochlea hypoplasia	Bilateral hearing loss
/Cardiovascular	Patent foramen ovale	No	No
/Gastrointestinal	No	Aganglionic megacolon	No
/Neurological	Developmental delay/MRI:Thin corpus callosum/Left olfactory bulb hypoplasia	Developmental delay/Ataxia	No
/Skin-hair	Cafe-au-lait spots	No	No
/Skeletal	No	Bilateral clinodactyly and thenar atrophy of the hands	No
/Phenotype(MIM phenotype number)	WS Type2E (# 611584)	PCWH (# 609136)	PCWH (# 609136)

C.O.I. Disclosure:

A.C. Sagdiç: None. **M. Altan:** None. **B. Cavdarli:** None. **C.N. Semerci Gündüz:** None.

EP13.060 Missense Variation in *TRRAP* gene in two Brazilian females with microcephaly, orofacial cleft, distinct facial phenotype, acral and renal anomalies, and global development delay: confirmation of a multisystemic syndrome

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Background: The *TRRAP* gene plays an important role in transcriptional activity, DNA damage repair, and chromatin remodeling. Heterozygous variations in *TRRAP* are the cause of developmental delay with or without dysmorphic facies and autism, in association with other anomalies.

Patients and methods: Description of clinical and molecular findings of two Brazilian females with syndromic orofacial cleft. Exome sequencing was performed using NOVAseq equipment (Illumina). Exome data analysis was performed using Franklin Genoox platform, and prioritized variants were classified according to the ACMG guidelines.

Results: Case 1 was born in 1995 by a non-consanguineous couple. She had growth deficiency, microcephaly, blepharophimosis, upslanting palpebral fissures, epicanthus, smooth philtrum, cleft lip and palate, micrognathia, low-set ears, brachyclinocamptodactyly, kidney abnormalities, developmental and language delay, learning difficulties, and moderate intellectual disability. Case 2 was born in 2000 by a non-consanguineous couple. She had microcephaly, blepharophimosis, upslanting palpebral fissures, Robin sequence, broad and smooth philtrum, low-set ears, brachyclinocamptodactyly, heart defect, kidney abnormalities, genital abnormalities, developmental and language delay, learning difficulties, severe intellectual disability, and behavior disorder. Karyotype, array-CGH, low-pass genome sequencing, and Sanger sequencing of *PATCH1, SHH, GLI2, SIX3* and *GAS1* gene were normal. Exome sequencing revealed heterozygous missense variation in the *TRRAP* gene in both patients: NM_001375524.1:c.3475G>A (p.Gly1159Arg), in Case 1, and NM_001375524.1:c.3091A>T (p.Ile1031Phe), in Case 2.

Conclusion: Both cases have a similar phenotype, with variable degrees of intellectual disability. The *TRRAP* gene mutations affected the residues 1031 and 1159, confirming the more severe phenotype of this recognizable multisystemic syndrome.

C.O.I. Disclosure:

R.M. Zechi-Ceide: None. **S. Vendramini-Pittoli:** None. **L.A. Virmond:** None. **V.C. Segarra:** None. **H.R. Serigatto:** None. **F.M. Jehee:** None. **A.V. Krepischi:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; FAPESP 13/08028-1; 2023/09879-7. **C. Rosenberg:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; FAPESP 13/08028-1; 2023/09879-7. **N.M. Kokitsu-Nakata:** None.

EP13.061 A novel pathogenic frameshift variant in MID1 gene causing MID1-related Opitz G/BBB syndrome in four affected family members

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Background: *MID1*-related Opitz G/BBB (*MID1*-OS) is an X-linked syndrome characterized by hypertelorism and/or telecanthus,

hypospadias, laryngotracheoesophageal defects, and cleft lip and/or palate. Other malformations include mild intellectual disability, congenital heart defects, anal anomalies, and midline brain defects. Female heterozygotes usually manifest isolated hypertelorism. In a family with more than one affected individual, the mother of an affected male is an obligate heterozygote. Wide clinical variability occurs even among members of the same family.

Material and methods: Clinical exome sequencing was performed in a 27-year-old male proband with hypertelorism, telecanthus, hypospadias, laryngeal cleft, patent ductus arteriosus, supraumbilical hernia, epilepsy, torticollis, astigmatism and hyperopia, and his mother with hypertelorism. DNA libraries were prepared using Illumina's TruSightOne kit and sequenced on the NextSeq550 platform. Variants were interpreted using ClinVar, HGMD, and LOVD databases. Sanger sequencing was performed in the proband, the aunt's daughter with hypertelorism, and the aunt's son with hypertelorism, bilateral cleft lip/palate, hypospadias, and epilepsy.

Results: In two male probands molecular testing revealed a novel hemizygous, and in females heterozygous, frameshift variant c.1642del p.(Ser548Valfs) in *MID1* gene. According to the ACMG/AMP classification, the variant is annotated as pathogenic (PVS1, PM2, PP4).

Conclusion: Males with novel *MID1*-variant developed the main clinical features of *MID1*-OS, while females expressed only hypertelorism. The novel maternally inherited *MID1* variant is considered responsible for the clinical presentation of the four affected family members.

C.O.I. Disclosure:

I. Sansovic: None. **A. Bobinec:** None. **M. Miklos:** None. **K. Vulin:** None. **L. Odak:** None.

EP13.062 Tuberous Sclerosis and Type 2 Diabetes: Key Considerations

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Background: Tuberous sclerosis complex (TSC) is a tumor suppressor syndrome of neurocutaneous origin caused by mutations in the *TSC1* and *TSC2* genes. It represents a monogenic condition with autosomal dominant inheritance. It is characterized by the presence of cutaneous lesions, epilepsy and hamartomas that can develop in multiple organs

Material and methods: The authors present a 36-year-old female who has been under the care of the Regional Center of Medical Genetics in Bihor County since 2021, with diagnosis of TSC. Her medical history reveals epilepsy diagnosed at 8 months of age, followed by a diagnosis of TSC at 18 years. In 2013, she was diagnosed with type 2 diabetes mellitus (T2DM). The clinical examination revealed growth delay, obesity, facial angiofibromas, sebaceous adenomas, depigmented macules, papillomatous tumors on the thorax and neck, periungual fibromas on the lower limbs, and seizures. Biochemical investigations showed elevated glycemia and glycated hemoglobin levels. Brain MRI shows bilateral subependymal hamartomatous nodules and cortical/subcortical tubers in the frontal, temporal, and occipital regions

Results: Molecular analysis confirmed a pathogenic variant in the *TSC1* gene, exon 13 (c.1270A>T, p.Arg424*). The patient's current treatment regimen includes Metformin, Gliclazide, and Semaglutide for diabetes, alongside Carbamazepine and Clonazepam for epilepsy. Notably, Metformin appears to have beneficial effects not only on glycemic control but also on tumor progression and seizure frequency, making it a promising therapeutic option in such complex cases

Conclusion: This case underscores the rare coexistence of TSC and T2DM while highlighting the therapeutic potential of Metformin in addressing both metabolic and neurological complications.

C.O.I. Disclosure:

A. Jurca: None. **A. Trandafir:** None. **S. Jurca:** None. **C. Petchesi:** None. **C. Jurca:** None. **A. Jurca:** None.

EP13.063 Expanding the phenotypic spectrum of*DOHH*-related disorders with: cardiomyopathy, hypoparathyroidism and mild neurodevelopmental delay

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Background: Deoxyhypusine hydroxylase *DOHH* encodes an enzyme involved in post-translational modifications of the eukaryotic translation initiation factor eIF5A1. Pathogenic variants in *DOHH* have recently been described, associated with severe neurodevelopmental delays and congenital heart diseases, in five patients (ziegler et al.). We present a patient with a homozygous pathogenic *DOHH* variant associated with cardiomyopathy, endocrine dysfunction, and mild developmental delay.

Case: A 10-year-old boy, first child of consanguineous parents, was born at term with normal birth metrics and adequate postnatal adaptation. During pregnancy, increased nuchal translucency was observed, and fetal echocardiography noted a mild pericardial effusion with apparent thickening of the right ventricular anterior wall. Antenatal karyotype was 46, XY.Postnatal assessment identified left ventricular non-compaction cardiomyopathy with a restrictive apical ventricular septal defect and hypoparathyroidism. Follow-up revealed delays in motor and cognitive milestones. Initial genetic testing by array CGH and Noonan syndrome and related disorders panel test, was inconclusive. Trio whole-exome sequencing (WES) identified an inherited homozygous c.455C>T (p.Pro152-Leu) variant in *DOHH*. Interestingly, this variant has previously been reported in a compound heterozygous state in a patient with an increased nuchal translucency and severe neurodevelopmental impairment (1). In our case, the clinical presentation includes hypoparathyroidism, cardiomyopathy, and mild neurodevelopmental delay without microcephaly, suggesting phenotypic variability. Comparison with published cases will be discussed.

Conclusion: This case expands the phenotypic spectrum of the recently described *DOHH*-related disorder, suggesting a possible association with cardiomyopathy and endocrine abnormalities, along with milder neurodevelopmental outcome than in previously published cases.

C.O.I. Disclosure:

E. Daire: None. **S. Dirani:** None. **K. Braun:** None. **S. Delmas Lanta:** None. **H. Cave:** None. **A. Bonnard:** None. **B. Demeer:** None.

EP13.064 Recurrent 17q23 deletion including TBX4 gene. Further delineation of a new emerging syndrome

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Introduction: Recurrent 17q23.1q23.2 deletions, including TBX4 gene have been reported in few cases with clinical heterogeneity and disease severity. In the few reported cases, the overlapping clinical features include hand and foot anomalies, heart defects, microcephaly, postnatal growth retardation, musculoskeletal abnormalities, and hearing loss. Herein, we describe a newborn with neonatal pulmonary hypertension and respiratory distress syndrome in whom molecular analyses revealed a heterozygous recurrent deletion on 17q23.1q23.2.

Patient and Results: Female patient, born at 39 weeks' gestation, who presented cyanosis, hypoxemia (SatO2 min 65% despite O2 in nasal cannula), and tachypnea from birth, with pulmonary hypertension and peripheral blood eosinophilia. Array analysis (CytoScan 750K, ThermoFisher) was performed and detected a 2,26 Mb deletion in the region 17q23.1q23.3, arr[GRCh38] 17q23.1q23.2(59998800_62261730)x1, including TBX4 gene. Evaluation at 7 months of age, the patient presented weight percentile <P1, height percentile on P8 and head circumference on P1. Other clinical characteristics detected include mild hypertelorism with epicanthus, slight tendency to adduct the thumbs, slight axial hypoptonia and suspected hypoacusia.

Comments: - Lung disease manifesting with pulmonary hypertension at birth, often associated with microcephaly, postnatal growth retardation, and hearing loss are the main common clinical features in the recurrent 17q23.1q23.2 deletion. -The identification of common clinical features suggests that microdeletions at 17q23.1q23.2 constitute a novel syndrome. -Array is still an easy and quickly clinical diagnostic test for newborns with abnormal features.

C.O.I. Disclosure:

E. Lloveras: None. **A. Canellas:** None. **I. Espejo:** None. **M. Barcos:** None. **A. Girós:** None. **R. Tena:** None. **L. Puig:** None. **J. Mendoza:** None. **C. Pérez:** None.

EP13.065 Maternal uniparental heterodisomy UPhD(20) detected by SNP microarray

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Background: UPhD(20)mat has been reported infrequently. It is characterized by severe feeding difficulties, failure to thrive, and intrauterine/postnatal growth retardation. The most common molecular mechanisms that can lead to heterodisomy include trisomic rescue, and some reported cases show evidence of mosaic trisomy 20 and, a more severe phenotype.

Patients and Results:

Patient 1. 40-year-old pregnant with growth restriction and sulcation malformation. Array analysis detected an absence of heterozygosity (AOH) of 27,5 Mb at 20p11.23q13.33 and no CNV. Gestation was terminated.

Patient 2. 1-year-old boy with feeding difficulties, premature, weight at p3, skin spots, cardiopathy and developmental delay. Array analysis shows an AOH of 13Mb at 20p13p12.1 and 16Mb at 20q13.13q13.33 and no CNV.

Patient 3. Newborn with bilateral cryptorchidism, peculiar phenotype, clubfoot, axial hypotonia, sucking difficulties and brain abnormalities. Array analysis shows an AOH of 4.8 Mb at 20p12.3p12.2 and 12.7 Mb at 20q11.1q12 and an interstitial copy number gain of 15.7 Mb at 20p12.2p11.1 region suggesting the presence of a supernumerary marker chromosome 20. Not confirmed by karyotype. A trio analysis was performed in the three patients, and the presence of an UPhD20mat was confirmed.

Conclusion:

- Long contiguous stretches of homozygosity detected by SNP array is suggestive of UPhD and could be confirmed performing an analysis trio with the same technique.
- The presence of a partial copy number gain or mosaic trisomy involving chromosome 20, increases the severity of the phenotype.
- Genetic counselling of prenatal UPhD20mat is a challenge, even with no copy number gain detected.

C.O.I. Disclosure:

A. Canellas: None. **E. Lloveras:** None. **C. Rivera:** None. **I. Espejo:** None. **M. Barcos:** None. **A. Girós:** None. **R. Tena:** None. **C. Alves:** None. **M. Reis-Lima:** None. **M. Augé:** None. **L. Linares:** None. **C. Pérez:** None.

EP13.066 An unusual case of Bardet- Biedl Syndrome with blended phenotype

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Background: Bardet- Biedl syndrome(BBS) cause of genetic obesity, having multi system involvement. It has autosomal recessive inheritance. It has very characteristic presenting features like obesity, polydactyly, hypogonadism, retinitis pigmentosa, developmental delay, seizures, multiple endocrine and dental abnormalities. Here we report an unusual case of Bardet - Biedel syndrome with blended phenotype.

Material and methods: A 4 year old male child was admitted to pediatric ward with intractable seizures. The child was found to have developmental delay and his mother was earlier operated for cleft palate. During his examination, child was obese, having post axial polydactyly, micropenis, cleft palate, lip pits, dental abnormalities and hypertension. All these features were not pointing to a single syndrome. Some features were of BBS and few features of Vander Woude Syndrome(VWS). On whole exome sequencing, two variants were found. One was homozygous variant in intron 11 of *TTC8*:c.1049+2_1049+4del (5'Splice variant) and another one was heterozygous variant in exon 9 of *GRHL3*:c.1172G>A. WES report explains the blended phenotype found in our index child.

Results: BBS has clinical and genetic heterogeneity. But a complete clinical evaluation and suspicion for other co-existing disorders too helps in making a complete diagnosis which further help in management and prenatal genetic counselling.

Conclusion: With availability of WES, multiple genetic variants are identified with relative ease. A thorough clinical evaluation and database search can give valuable clues to the diagnosis.

C.O.I. Disclosure:

S. Jindal: None. **I. Panigrahi:** None.

EP13.067 A case of Short Rib Thoracic Dysplasia with a *novel* homozygous *IFT140* variant in the setting of complete uniparental isodisomy (UPiD) of chromosome 16

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Background: Short-rib thoracic dysplasia (SRTD) is a genetically diverse group of autosomal recessive skeletal ciliopathies characterized by narrow thorax, short tubular bones, cone-shaped epiphyses, retinal dystrophy, renal and hepatic anomalies.

Material and methods: We present a 16-year-old boy, the first child born from non-consanguineous healthy parents, diagnosed in infancy with sagittal craniosynostosis, near total visual impairment, mild neurodevelopmental delay and dysmorphic features including craniofacial asymmetry, high forehead, bitemporal narrowing, upslanting palpebral fissures, narrow chest and brachydactyly of toes and fingers. Upon a follow-up evaluation by age 10, thrombocytopenia, elevated transaminases, and splenomegaly were noted, with subsequent tru-cut liver biopsy establishing the diagnosis of congenital hepatic fibrosis. Chromosomal microarray and whole-exome sequencing (WES) were subsequently performed for further evaluation.

Results: WES revealed a *novel* homozygous missense variant of uncertain significance (NM_014714.4:c.2885C>T, p.Ala962Val) in the *IFT140* gene associated with SRTD-9 and regions of homozygosity on the entire chromosome 16 was detected through autozygosity mapping using the WES data and SNP probes from chromosomal microarray, indicating complete uniparental isodisomy (UPiD) of chromosome 16. Sanger sequencing confirmed a paternally inherited homozygous variant in the proband and wild-type alleles in the mother. Identification of a paternal, but not maternal, carrier of the variant allele was consistent with paternal UPiD of chromosome 16.

Conclusion: We reported a rare case of complete uniparental isodisomy unveiling a potentially deleterious variant inherited from a carrier parent that resulted in ciliopathy. This case highlights the importance of considering all inheritance patterns, including UPD, when interpreting chromosomal microarray and WES data.

C.O.I. Disclosure:

S.T. Ozden: None. **S. Akbas:** None. **T. Kalayci:** None. **A.D. Aslanger:** None. **B. Karaman:** None. **Z.O. Uyguner:** None. **V. Karaman:** None. **Z. Onal:** None. **E. Turkmen:** None.

EP13.068 Co-occurrence of two rare diseases: Emery-Dreifuss muscular dystrophy 4 and Alazami syndromes in a patient with novel LARP7 variant

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Background: Alazami syndrome (ALAZS, OMIM #615071) is a rare autosomal recessive disorder caused by pathogenic biallelic variants in *LARP7* gene. Clinically, it is characterized by growth retardation, intellectual disability, dysmorphic facial features. Congenital anomalies and skeletal anomalies have also been reported. Mutations in *SYNE1* gene have been associated with autosomal recessive 8 spinocerebellar ataxia and myogenic arthrogryposis, autosomal dominant Emery-Dreifuss muscular dystrophy 4 (EDMD). EDMD4 is characterized by muscular weakness, atrophy, early joint contractures, and cardiac abnormalities. Here, we present a case with a novel homozygous variant in *LARP7* and a heterozygous variant in *SYNE1* gene.

Material and methods: Our patient is a 3-year-old male who was referred because of neuromotor retardation, dysmorphic facial features, and limb deformities. He is the fourth child of consanguineous parents and was born at 38 gestation weeks. He had decreased fetal movement in the prenatal period. Birth weight and height were normal. Brain-spinal MRI, abdominal ultrasound, and EKO were normal. He had elevated creatine kinase(1465 UI/l). On physical examination, he had growth retardation, microcephaly, muscle weakness, contractures upper extremity, malar hypoplasia, broad nose, wide mouth.

Results: Whole-exome sequencing analysis displayed a likely pathogenic homozygous novel variant of c.475_478dup (p.Lys160Ilefs*2) in *LARP7* gene and also a pathogenic heterozygous variant of c.15586C>T (p.Arg5196*) in *SYNE1* gene.

Conclusion: ALAZS and EDMD4 are very rare diseases. Here we present a case in which these two rare syndromes are seen together aimed to broaden the phenotypic spectrum of these syndromes. Moreover, we emphasize the importance of WES in resolving complex phenotypes.

C.O.I. Disclosure:

N. Ozturk: None. **A. Gurler:** None.

EP13.069 Pathogenic variant PRMT7 (c.820C>T), as a cause of autosomal recessive short stature, brachydactyly, intellectual developmental disability, and seizures (SBIDDS) - the probable 16th case of SBIDDS in a 27-year-old patient of Ukrainian origin

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Background: SBIDDS is a rare autosomal recessive genetic disorder caused by Arginine Methyltransferase 7 (PRMT7) disfunction. Apart from the most prominent manifestations (Short stature, Brachydactyly, Intellectual Developmental Disability and Seizures; SBIDDS) it's characterized by craniofacial dysmorphism and obesity. According to ORPHANET, less than 15 cases of SBIDDS have been reported in the literature up to date.

Material and methods: 27-year-old patient of Ukrainian origin, was born from the fourth pregnancy, after two pregnancies losses and healthy sister. There were no cases of neurodevelopmental or skeletal disorders in familial history. The patient was presented with absent speech, profound intellectual disability, generalized seizures, microcephaly, craniofacial anomalies, disproportionate short stature with short trunk, thoracic kyphoscoliosis, brachydactyly, but there wasn't obesity. Clinical Genetic Testing, CentoArray, WES (CENTOGENE) was done.

Results: A homozygous pathogenic variant of PRMT7 gene (c.820C>T) (p.(Arg274*) in exon 9 of PRMT7 gene was identified. It is classified as pathogenic both upon ClinVar and CENTOGENE's guidelines. Carrying of biallelic pathogenic PRMT7 variants now is consistent with a genetic diagnosis of autosomal recessive short stature, brachydactyly, intellectual developmental disability, and seizures; SBIDDS (OMIM: 617157). Although the patient's parents deny any consanguinity, the homozygous state of rare gene variant PRMT7(c.820C>T) (p.(Arg274*) in their son requires heterozygous carrier testing for other family members.

Conclusion: Genetic testing confirm the SBIDDS as a primary cause of neurodevelopmental delay and multiple skeletal

anomalies in Ukrainian patient, that could be a 16th diagnosed case of this ultimately rare disease.

C.O.I. Disclosure:

K. Bakum: None. **H. Akopyan:** None. **N. Helner:** None.

EP13.070 Identification of a pathogenic SHOC2 variant in a patient with Noonan-like syndrome with loose anagen hair 1

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Background: Noonan-like syndrome with loose anagen hair 1 (NSLH1) is a rare autosomal dominant disorder that shares phenotypic similarities with Noonan syndrome. Establishing a differential diagnosis is crucial for accurate genetic counseling and clinical management. Our patient was initially referred with a suspected Noonan syndrome diagnosis, but genetic analysis identified a pathogenic SHOC2 variant, confirming NSLH1.

Material and methods: A 16-year-old male presented with asymmetric hypertrophic cardiomyopathy, partial growth hormone deficiency, dysmorphic features, and suspected Noonan syndrome. Genetic analysis was conducted using next-generation sequencing (NGS) with a custom RASopathy-associated gene panel. The sequencing library was prepared using the SureSelect QXT reagent kit (Agilent Technologies) and sequenced on a MiSeq platform (Illumina). DNA sequences were aligned to the GRCh38 reference genome, and variant analysis was performed using Agilent SureCall 4.2.2.3. Variants were evaluated using OMIM, ClinVar, HGMD, and dbSNP databases, covering all coding regions and exon-intron boundaries (±25 bp).

Results: NGS identified a pathogenic missense variant in exon 2 of SHOC2: NM_007373.4(SHOC2):c.4A>G (p.Ser2Gly). The variant is recorded in ClinVar (ID 6821), has a population frequency of 0.007% (gnomAD), and is reported in NSLH1 patients. Experimental studies confirm its impact on SHOC2 function (PMID: 19684605, 22606262), supporting its pathogenic classification.

Conclusion: This case underscores the importance of precise genetic testing in suspected Noonan syndrome cases. Identifying the SHOC2 c.4A>G (p.Ser2Gly) variant confirmed NSLH1, guiding clinical management and genetic counseling. Our findings highlight the role of SHOC2 in RASopathies and the utility of NGS in differential diagnosis.

C.O.I. Disclosure:

K. Crkvenac Gornik: None. **A. Juras:** None. **J. Skorput:** None. **N. Krnić:** None. **S. Huljev Frkovic:** None.

EP13.071 Expanding the phenotypic spectrum of NCAPD3-related condensinopathies; a novel variant associated with skeletal anomalies, and autoinflammatory disease

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Background/Objectives: Condensin complexes are essential for chromosome condensation and segregation during mitosis. NCAPD3, a component of the condensin II complex, has been associated with primary microcephaly, growth retardation, and mild intellectual disability due to chromatin decatenation failure. To date only three patients with biallelic NCAPD3 variants have been reported. Here, we present a novel NCAPD3 variant in a patient with a broader phenotype, including skeletal abnormalities, severe growth deficiency and autoinflammatory disease.

Material and methods: Whole exome sequencing (WES) was performed on a 5-year-10-month-old female patient with intrauterine growth restriction (IUGR), short stature (−5,12 SD), microcephaly (−4,87 SD), inflammatory bowel disease, duplicated thumbs, aplasia of terminal phalanx of fifth finger hypoplastic nails, onychodystrophy, preaxial polydactyly of the feet, pes planus, and prominent heels.

Results: A homozygous c.196A>C (p.Thr66Pro) variant in NCAPD3 (NM_015261.2) was identified. Parental segregation analysis confirmed the heterozygous status of the variant in the parents via Sanger sequencing.

Conclusion: This case broadens the phenotypic spectrum of NCAPD3-related condensinopathies, highlighting the presence of previously unreported skeletal anomalies, and autoinflammatory disease. While previously described NCAPD3 mutations predominantly resulted in microcephaly, our patient presents additional skeletal anomalies such as polydactyly, phenotypic features more commonly linked to NCAPG2-related disorders. Given their shared role in condensin II function, it is plausible that NCAPD3 and NCAPG2 mutations might lead to overlapping phenotypes in some cases. However further investigations are needed to confirm this hypothesis and to elucidate the genotype-phenotype correlations within condensinopathies.

C.O.I. Disclosure:

G. Memiş: None. **T. Kalayci:** None. **A. Dogru:** None. **A.D. Aslanger:** None. **A.E. Bayrak:** None. **Z.O. Uyguner:** None.

EP13.072 New case of missense variant in ZBTB47 broadening the clinical spectrum (of this novel neurodevelopmental disease)

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Background: Zinc finger protein transcription factors (ZNFs) constitute the largest family of transcription factors in eukaryotes, playing a crucial role in regulating cell development and differentiation. More than 80 ZNFs have been implicated in neurodevelopmental disorders. In 2023, Ward *et al*. reported five unrelated patients harboring de novo missense variations, presenting with global developmental delay, intellectual disability, seizures, gait abnormalities, and, in some cases, abnormal movements. To date, no additional cases have been reported.

Material and methods: Trio whole-genome sequencing was carried out for a 4-year-old girl who presents moderate developmental delay and epilepsy.

Results: Genome sequencing identified a heterozygous de novo pathogenic variant in *ZBTB47* (NM_145166.4: c.1429G>A, p.(Gly477Lys)), which has been previously reported. No other pathogenic variants were detected.The propositus was born after an uncomplicated pregnancy. At one month of age, she underwent surgery for pyloric stenosis. At 11 months, she experienced a febrile status epilepticus, followed by recurrent febrile and afebrile generalized seizures, requiring Levetiracetam treatment. Her developmental progress slowed, with independent walking

achieved at 20 months and language delay noted. Clinically, she exhibits joint hyperlaxity, genu recurvatum, amyotrophy, left strabismus, recurrent infections and heteroaggressivity. She also presents special facial features with a prominent forehead, full cheeks, deepset eyes, tented mouth, short philtrum, thin upper lip, which could be recognizable.

Conclusion: This new case confirms the implication of *ZBTB27* in neurodevelopmental disorder and expands the phenotypic spectrum with description of pyloric stenosis. An international collaboration is in progress to gather additional clinical cases with *ZBTB47* pathogenic variants and continue this work.

C.O.I. Disclosure:

E. schaefer: None. **S. baer:** None. **C. Schluth Bolard:** None. **A. gouronc:** None.

EP13.073 A Novel *SALL1* Frameshift Mutation in a Turkish Family with Mild Renal Involvement and Features of Townes-Brocks Syndrome

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Background/Objectives: Townes-Brocks syndrome (TBS, OMIM #107480) is a rare autosomal dominant disorder characterized by anorectal malformations, dysplastic ears, and thumb anomalies. Renal involvement, although variable, is a key feature and may lead to chronic kidney disease (CKD). Mutations in *SALL1*, a transcriptional repressor gene, cause TBS primarily through haploinsufficiency or dominant-negative mechanisms. We describe a novel *SALL1* frameshift mutation in a Turkish family with mild renal involvement and familial history of anorectal anomalies.

Methods: A 15-year-old male presented with mild CKD, pollakiuria, nocturia, magnesium deficiency, and a history of anal dilation. Family history revealed similar anal and renal manifestations in the father and two siblings. Whole-exome sequencing (WES) identified a novel heterozygous *SALL1* c.2260dup (p.Ser754-LysfsTer51) frameshift mutation, confirmed by Sanger sequencing and segregation analysis. An additional *PKD2* c.1549-3del intronic variant was identified, but its clinical relevance remains uncertain.

Results: Renal ultrasound and DMSA scintigraphy were normal, yet persistent mild renal dysfunction led to CKD diagnosis. Sanger sequencing confirmed the mutation's segregation in the father and two affected siblings. Bioinformatics analysis suggested a truncated protein with loss of zinc-finger domains, supporting haploinsufficiency as the pathogenic mechanism.

Conclusion: This case expands the mutational spectrum of *SALL1* and highlights the variable renal phenotype in TBS. Genetic testing and early nephrological follow-up are crucial for proper management. Further studies are needed to assess genotype-phenotype correlations in mild renal phenotypes of TBS.

C.O.I. Disclosure:

N. Altunoglu: None. **L. Yılmaz:** None. **A. Kiraz:** None. **M. Dündar:** None.

EP13.074 Application of whole exome sequencing in a pediatric patient with cardiomyopathy and neurodevelopmental disorder

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Background: Cardiomyopathies are a genetically heterogeneous group of disorders that often present in childhood with significant morbidity and mortality. These conditions can occur as isolated cardiac abnormalities or as part of broader syndromic presentations. Advances in genetic testing, including whole exome sequencing, have revolutionized the diagnostic approach, enabling the identification of underlying molecular mechanisms in many cases.

Material and methods: This report presents the case of a 5.5-year-old girl with dilated cardiomyopathy who required mitral valve surgery at the age of 2.5 years. Postoperative complications included ischemic stroke and epilepsy, followed by moderate intellectual disability and limited speech development. Whole exome sequencing was performed.

Results: A likely pathogenic, previously unreported variant c.749C>T p.(Ser250Phe) in the *ACTN2* gene was identified. Pathogenic variants located in the same exon (8/21) have been previously associated with dilated cardiomyopathy. Additionally, a novel pathogenic variant c.125del p.(Lys42Argfs23) in the *PHIP* gene was detected. The phenotype of Chung-Jansen syndrome, caused by pathogenic *PHIP* variants, aligns with the patient's symptoms initially attributed to postoperative complications. A well-known, breast cancer-related pathogenic variant c.1690C>T p.(Gln564*) in the *BARD1* gene was also reported as a secondary finding.

Conclusion: This case highlights the critical role of high-throughput molecular testing in diagnosing pediatric patients with complex phenotypes, including cardiomyopathy and neurodevelopmental disorders. The findings emphasize the importance of integrating genetic, clinical, and family data to improve diagnostic accuracy and informed patient care. Further functional studies and long-term follow-up are essential to clarify the pathogenicity and clinical implications of the novel variants.

C.O.I. Disclosure:

T. Kałużewski: None. **A. Kosińska:** None. **&. Kępczyński:** None. **M. Orczykowski:** None. **B. Kałużewski:** None. **A. Gach:** None.

EP13.075 Unique rare diseases co-occurrence: association of TBX5 and FLNA genes causative variants in a girl with multiple congenital anomalies

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Background: TBX5 gene haploinsufficiency causes Holt-Oram syndrome characterized by upper limb anomalies, congenital heart defects and rhythm disturbances. FLNA gene displays a wide spectrum of phenotypes: loss-of-function (LOF) mutations result in X-linked dominant (XLD) periventricular nodular heterotopia type 1 (PVNH1) which may also associate cardiovascular defects and thrombocytopenia; gain-of-function variants drive various skeletal and connective tissue disorders named otopalatodigital spectrum disorders (OPDS).

Material and methods: We describe the case of a 15-year-old girl presenting daily focal to bilateral tonic-clonic seizures unresponsive despite different antiepileptic drugs (AEDs) regimens, and who also associates multiple cardiac defects, scoliosis, right triphalangeal thumb and thrombocytopenia. EEGs and ECGs show bilateral epileptic discharges and arrhythmias respectively. Brain MRI revealed bilateral periventricular nodular heterotopias. Due to the complex phenotype a whole exome sequencing (WES) was performed.

Results: Two heterozygous variants were detected by WES: NM_181486.4:c.710G>A, p.Arg237Gln in the TBX5 gene, previously reported as pathogenic, and NM_001110556.2:c.6734dup, p.Gly2247Trpfs*6 in the FLNA gene which has never been

reported before. The latter variant is predicted to result in a LOF effect consistent with the FLNA XLD PVNH1 phenotype thus it was classified as likely pathogenic.

Conclusion: We describe a unique association of a novel FLNA likely pathogenic variant and a known pathogenic mutation in the TBX5 gene. Patient's cardiac anomalies could be explained by either one or both of the two genes disruption. Thrombocytopenia and arrhythmias are known side effects of some AEDs, therefore caution should be made in choosing the right antiepileptic treatment in order to prevent symptoms progression.

C.O.I. Disclosure:

T. Barbarii: None. **A. Dica:** None. **F.M. Nedelea:** None.

EP13.076 A case report : simultaneous *JAG1-associated* Alagille syndrome and *NEXN*-associated cardiomyopathy predisposition

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Background: Alagille Syndrome is a rare, multisystem, autosomal dominant disorder with prevalence 1/70 000 livebirths. The onset is typically in childhood and the clinical manifestation is variable; however, six major groups of symptoms typically are present: cholestasis with or without jaundice; cardiovascular, skeletal, renal, and eye abnormalities; specific facial phenotype. A variety of cardiovascular malformations have been described in affected patients, including Fallot tetralogy, pulmonary and aortic stenosis, coarctation of the aorta, ventricular septal defects. The proband is 1-y.o. boy with cholestasis, transitory pulmonary stenosis, renal dysplasia, facial dysmorphism. The family history shows 4 paternal siblings (2 F and 2 M) that have died before the age of 5 years, due to unspecified heart condition. The father has had a congenital pulmonary stenosis and unilateral nephrectomy.

Material and methods: Next generation sequencing (NGS) based analysis for SNVs and CNVs detection, and Sanger sequencing for variant confirmation were used.

Results: A heterozygous pathogenic nonsense *JAG1* variant confirmed the diagnosis Alagille syndrome in the proband and paternal inheritance. In addition, a heterozygous likely pathogenic frameshift *NEXN* variant, described in patients with autosomal dominant dilated or hypertrophic cardiomyopathy, was found.

Conclusion: The simultaneous detection of PV/LPV in two genes, both associated with hereditary cardiac pathology predisposition, requires family adapted genetic counselling and monitoring recommendations. Using of broader testing methods (not strictly single-gene or targeted panel method) enables detection of additional genetic variations with clinical significance, when personal or family history are not fully explained by one condition.

C.O.I. Disclosure:

T. Vasilev: None. **E. Betcheva-Krajcir:** None. **P. Nikolova-Hristova:** None. **N. Todorov:** None. **L.V. Nikolova-Georgieva:** None. **L. Dimova:** None. **M. Gaydarova:** None. **T. Vekov:** None.

EP13.077 Reproductive consequences of the presence of t(8;22): a clinical family case of supernumerary der(22)t(8;22) syndrome

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Background: Supernumerary der(22)t(8;22) syndrome (OMIM:613700) is a rare genetic pathology caused by an additional derivative chromosome 22, which consisting of a part of the long arm of chromosome 22 and of a part of the long arm of chromosome 8. The phenotype is characterized by extremity anomalies, mild dysmorphia and intellectual impairments. We are present a family case in which all children are carriers of a chromosomal rearrangement due to mother`s t(8;22)(q24.1;q11.22).

Material and methods: Proband was born from the III pregnancy. At the 5 months motor development was delayed, followed by growth retardation, weight loss and bilateral cryptorchidism which was operated on. At 5, the child was taking growth hormone, which gave a positive result. Now the patient is 14, he does not have dysmorphic features, but have mild intellectual disability and dysarthria. Clinical, standard karyotyping and fluorescence in situ hybridisation (FISH) methods were used.

Results: Chromosomal analysis was performed on the whole family. A balanced t(8;22)(q24.1;q11.22) was found in the mother, as well as in the older brother and sister of the proband. The father's karyotype is normal. The third child inherited an additional der(22)t(8;22): 47,XY,+der(22)t(8;22)(q24.1;q11.22)dmat. FISH analysis of derivative chromosome 22 showed the presence of locus 22q11.21 (HIRA) and 8qtel telomeres and the absence of BCR (22q11) and ARSA.

Conclusion: Carriers of balanced translocations have the high risk of miscarriages and chromosomal abnormalities in the offspring. Genetic counselling is important to choose the best family planning options, including prenatal and preimplantation genetic testing.

C.O.I. Disclosure:

V. Kurakova: None. **O. Zhurakhovska:** None. **O. Miakushko:** None. **V. Galagan:** None. **M. Tsygankova:** None. **O. Baronova:** None. **D. Mykhailova:** None.

EP13.078 Epileptic spasms and RNA analysis in a new case of Kabuki Syndrome type 2

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Background: Kabuki Syndrome (KS) is a rare genetic syndrome due to pathogenic variants in the autosomal dominant *KMT2D* gene or in the X-linked dominant *KDM6A* gene. Among other clinical features and symptoms, epilepsy is often reported in KS but it is not fully discussed, especially in patients harboring variants in *KDM6A*. Here we present a 19-year-old girl showing severe intellectual disability, speech delay, facial dysmorphisms and drug-resistant epilepsy, mostly characterized by epileptic spasms arisen at the age of 3 years. The diagnosis of epilepsy was established when nocturnal and diurnal clusters of spasms occurred, either spontaneously or when induced by skin touch.

Material and methods: Having received parental written informed consent, we performed whole-exome sequencing (WES) on the family trio. Sanger sequencing was then applied to confirm results. Total RNA was purified from blood and it was reversely transcribed using the 1st Strand cDNA Synthesis Kit.

Results: WES showed a novel de novo variant in KDM6A, c.3549-3C > G (NM_021140.4) predicted to alter the splicing process at 3′UTR of exon 25 and a prematurely truncated protein. The consequences of the variant on splicing were confirmed by RT-PCR using flanking primers.

Conclusion: Epileptic spasms have very rarely been described in KS, exclusively in cases harboring pathogenic variants in KMT2D. This is the first report that describes spasms in association with the *KDM6A* gene and delves into the precise seizure semiology. Our data further confirm WES trio as a first-line approach in diagnostic studies of childhood idiopathic epilepsies, intellectual disability and dysmorphic features.

C.O.I. Disclosure:

F. Privitera: None. **C. Meossi:** None. **E. Bartolini:** None. **F. Santorelli:** None.

EP13.079 *SHOX* gene copy number changes in Czech patient´s group with idiopathic short stature

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Background: *SHOX* gene (Xp22.33/Yp11.32) copy number changes are the most common cause of idiopathic short stature (ISS) and associated diseases such as Léri-Weill dyschondrosteosis (LWD), Madelung deformity (MD) or Langer mesomelic dysplasia (LMD). Regarding *SHOX* gene copy number changes, 70–80 % are deletions, 2–6 % are partial deletions and 20–25 % are point mutations of *SHOX* gene. Duplications have also been published in connection with ISS and LWD. Changes in the non-coding *SHOX* gene regulatory regions can also result in reduced expression of *SHOX* gene, incorrect mRNA splicing or folding of peptide chains.

Material and methods: In 2017–2025, 101 patients with ISS were tested using SALSA MLPA probemix P018 SHOX and subsequent *SHOX* gene coding region sequencing analysis.

Results: 4 out of 101 patients carried deletion of the entire coding region and 2 patients carried partial deletion (exons 2- 6) of *SHOX* gene. Duplication of SHOX - area - down (non-coding regulatory regions) was detected in 7 patients. 2 out of these 7 patients carried a missense variant of unknown clinical significance in exon 3. Pathogenic frameshift variant was detected in 1 patient.

Conclusion: Copy number changes in highly conserved non-coding elements may have the same effect as copy number changes in *SHOX* gene itself. Similar duplication cases have been reported, both in completely asymptomatic individuals as well in patients with variable phenotypic manifestations such as above-mentioned ISS or LWD. Further investigations are required to establish the significance of duplication of both the entire *SHOX* gene and its adjacent regulatory regions.

C.O.I. Disclosure:

I. Urbanovska: None. **M. Smolikova:** None. **B. Kubova:** None. **J. Simova:** None. **D. Konvalinka:** None. **L. Schreiberova:** None. **J. Zmolikova:** None. **N. Dvorackova:** None. **D. Cerna:** None. **J. Zvolska:** None. **M. Uvirova:** None.

EP13.080 Warsaw Breakage Syndrome in a Colombian patient with a novel variant in the DDX11 gene: a case report

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Background: Warsaw Breakage Syndrome ((WBS) is a very rare type of cohesinopathy caused by biallelic loss-of-function variants in the *DDX11* gene. It was reported for the first time in 2010 and currently, 24 cases have been reported. Affected probands usually present intra and extra-uterine developmental delay, sensorineural hearing loss, facial dysmorphism, intellectual disability and microcephaly.

Case report / Material and methods: The proband is a 14-year-old female born from non-consanguineous parents but originating from an isolated region in eastern Antioquia (Colombia). We describe a patient with intra and extra-developmental delay, intellectual disability, dwarfism, sensorineural deafness, microcephaly, exophthalmos, facial gestalt, intestinal malrotation, talipes equinovarus, bilateral clinodactyly, metopic synostosis, bilateral hypoplastic cochlea and patent ductus arteriosus (PDA). Exome sequencing, with subsequent validation by Sanger sequencing, was performed for the proband and her parents through Illumina HiSeq 4000. The novel variant c.792+1G > A was detected in the *DDX11* gene of the patient in homozygosis state and was classified as Probably Pathogenic criteries ACMG.

Discussion: WBS is a largely unknown disease to date, so data about patients with this condition is needed to elucidate its clinical spectrum. This is the first WBS case that reports intestinal involvement and the second case reporting craniosynostosis. Furthermore, we describe a novel variant, c.792+1G > A, affects the donor splice site in intron 7 and it is expected to disrupt.

Conclusion: This case contributes to the phenotypical and mutational spectrum of this complex and very rare disease. To the best of our knowledge, this is the first WBS case reported in Colombia.

C.O.I. Disclosure:

L.V. Carvajal Del Castillo: None. **S. Giraldo:** None. **H. Pachajoa:** None.

EP13.081 The First Non-De Novo Au-Kline Patient with a Novel HNRNPK Variant from Turkey

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Background: Au-Kline Syndrome (AKS) is a rare autosomal dominant disorder caused by pathogenic variants in the *HNRNPK* gene. At least 75 cases have been reported, all identified as de novo mutations. Here, we present the first documented case of an inherited (non-de novo) Au-Kline Syndrome patient.

Methods: Single-gene sequencing (SGS) was performed using next-generation sequencing (Illumina NextSeq 500).

Results: The patient was a 6-month-old female referred to our clinic for hypotonia and facial dysmorphism.The patient was born to non-consanguineous parents at 38 weeks of gestation via normal spontaneous vaginal delivery (NSVD). Echocardiography revealed a coronary arterioventricular fistula. She achieved head control at 5.5 months. Weight and head circumference was below the 3rd percentile. The patient exhibited dolichocephaly, a long face, downslanting palpebral fissures, ptosis, a wide nasal bridge, an open and downturned mouth, and a midline crease on the tongue. Face2Gene suggested Au-Kline Syndrome as a potential diagnosis. *HNRNPK* single-gene sequencing identified a novel heterozygous c.452T>G (p.Ile151Ser) variant. This variant is not reported in existing databases and was classified as likely

pathogenic according to ACMG criteria. Segregation analysis revealed that the patient's mother also carried the same variant. Sequencing was repeated with separate samples from both parents, yielding consistent results. The mother exhibited a milder phenotype.

Conclusion: This is the first report of Au-Kline Syndrome inherited from a parent rather than arising as a de novo mutation. While AKS is typically described as a de novo disorder, mild or subclinical phenotypes in parents may obscure its recognition, complicating diagnosis in some cases.

C.O.I. Disclosure:

A. Ersoy Karayumak: None. **O. Hanoglu:** None. **M. Daglar:** None. **I. Akbeyaz:** None. **D. Turkdogan:** None. **B. Geckinli:** None.

EP13.082 A novel case of*CTC1* related CRMCC syndrome with bilateral radial defects and kidney hypodysplasia and ectopy

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Background: *CTC1* encodes for a protein crucial for telomeres maintenance. It is responsible for a recessive telomeropathy characterised by cerebro-retinal microangiopathy with calcifications and cysts (CRMCC,#612199), leukoencephalopathy, intrauterine growth retardation (IUGR), osteopenia, ectodermal changes, and/or anemia. Bone-marrow failure, gastrointestinal bleeding and portal hypertension are potentially life-threatening complications. Patients tipically carry compound heterozygous, with one allele encoding a frameshift or nonsense mutant and the other a missense mutant.

Material and methods: Here we report a 6-year-old boy with a compound heterozygosity in *CTC1*: the c.150G>A(p.Trp50Ter),and the not-yet-reported c.2385G>A(p.Lys795Lys), predicted to alter the splicing, revealed by trio-WES. DEB test and SNP Array were normal. He had IUGR, absent left thumb and hypoplastic right thumb,left radioulnar sinostosis, ectopic,hypoplastic kidney, bilateral cryptorchidism, Coats retinopathy responding to Ranibizumab, global developmental delay,feeding problems, poikiloderma, fine sparse hair, iron-deficiency anemia.The last brain-MRI detected as stable a small sphenoidal cyst. We made a narrative review of the literature.

Results: CRMCC-key feature is the Coats retinopathy accompained by neurological and radiological signs that are almost always present. We expand the phentoype reporting a case with kidney abnormalities and bilateral radial defects. In literature any patient carried homozioug truncating mutations,suggesting it would be lethal in utero. Only 1, with few extracranial manifestations, carried the homozygous in-frame deletion c.3586_3606del (p.Leu1196_Arg1202del).

Conclusions: We first report kidney and severe radial defects highlighting how the CRMCC-phenotype overlaps with secondary telomeropathies due to DNA repair defects leading telomere maintenance machinery impairment (i.e. Fanconi anemia). Such parallelisms offer further insights for a bed-to-bench side researches.

C.O.I. Disclosure:

M. Colonnese: None. **F. De Prisco:** None. **M. Iorio:** None. **A. Torella:** None. **G. Piluso:** None. **A. Grandone:** None. **C. Picardi:** None. **M. Esposito:** None. **(. Telethon Undiagnosed Diseases Program:** None. **S. Perrotta:** None. **V. Nigro:** None. **C. Santoro:** None.

EP13.083 Characterization of a novel human phenotype with cortical malformations and congenital anomalies linked to *PHF3*

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Background: PHF3 (PHD Finger Protein 3) is a transcriptional regulator involved in neuronal gene expression. *PHF3* loss has been linked to increased gene expression and impaired neuronal differentiation. No human phenotype has been associated with *PHF3* to date. Here, we report two fetuses from consanguineous parents presenting with cortical malformations and multiple congenital anomalies, carrying a novel homozygous *PHF3* variant.

Materials and methods: Prenatal and postmortem clinical examinations were performed, followed by whole-exome sequencing (WES) to identify potential disease-causing variants.

Results: The first pregnancy of the family was referred at 24 gestational weeks due to abnormal ultrasound findings, including anhydramnios, cystic hygroma, short and thick corpus callosum, interhemispheric cysts, vermian hypoplasia, anechoic urinoma, renal dysplasia, tetralogy of Fallot, short tubular bones, and narrow thorax. Postmortem examination revealed facial dysmorphism, including prominent forehead, hypertelorism, bilateral epicanthal folds, depressed nasal root, short nose, short columella, long philtrum, thin lips with downturned corners, and low-set ears. In the second pregnancy, ultrasound findings showed similar cortical malformations, along with cystic hygroma and postaxial polydactyly. Postmortem examination confirmed a consistent dysmorphic profile. WES identified a homozygous missense variant [c.5483G>A, p.(Gly1828Asp)] in both fetuses, with Sanger sequencing confirming heterozygosity in the parents.

Conclusion: This study presents the first evidence of a human phenotype associated with *PHF3*. Given its critical role in neuronal function and development, we propose that this variant underlies a novel disorder, warranting further research.

C.O.I. Disclosure:

T. Kalayci: None. **T. Sarac Sivrikoz:** None. **U. Altunoglu:** None. **B. Karaman:** None.

EP13.084 Genomic autopsy to identify causes of intrauterine fetal death with fetal abnormalities by using preserved fetal tissues

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Introduction: Intrauterine fetal death (IUFD) is a sudden and traumatic event with often unknown causes, leading to significant

anxiety for the family. Thus, clarifying the cause of the IUFD is indispensable for future pregnancies. Recently, genomic autopsy has emerged as a powerful tool for investigating the underlying genetic causes of IUFD, especially in cases of fetal abnormalities. Cases: Case 1 was a female fetus diagnosed with polyhydramnios at 22 weeks of gestation. She showed persistent bladder enlargement on fetal ultrasonography. IUFD occurred at 31 weeks, and the fetus weighed 1980g with no apparent structural abnormalities. Case 2 was a male fetus diagnosed with oligohydramnios at 22 weeks. He showed bilateral renal hypoplasia and a ventricular septal defect on fetal ultrasonography. Fetal MRI revealed renal and pulmonary hypoplasia. IUFD occurred at 35 weeks, and the fetus weighed 1190g with a cleft lip and palate. In both cases, the umbilical cords were preserved for future genetic testing. Subsequently, genetic counseling for the parents and genetic testing using DNA extracted from the umbilical cords were conducted. Case 1 was diagnosed with Type 5 Bartter syndrome by comprehensive genetic analysis for hereditary kidney diseases. Case 2 was diagnosed with 22q11.2 deletion syndrome by chromosomal microarray analysis. The families accepted the diagnosis positively. The genetic testing results helped them understand the disease and assess the genetic risks for future pregnancies.

Conclusions: In cases of IUFD with fetal abnormalities, genomic autopsy using fetal tissue provides valuable information for family and future family planning.

C.O.I. Disclosure:

M. Nagasaka: None. **Y. Yotsumoto:** None. **N. Morisada:** None. **K. Nozu:** None. **T. Tamaoki:** None.

EP13.085 Chromatin assembly factor subunit *CHAF1A* as a monogenic cause for Oculo-Auriculo-Vertebral Spectrum

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Oculo-auriculo-vertebral spectrum (OAVS and encompassing Goldenhar syndrome, hemifacial microsomia, and facio-auriculo-vertebral spectrum), includes an abnormal development of the 1st and 2nd branchial arches and remains loosely defined with no consensual minimum clinical diagnostic criteria (MIM#164210, 620444). Despite arguments against a monogenic condition, a few genes have been involved in a minority of cases, the most robust being Chr14q22.3 duplications including *OTX2* and variants in *SF3B2* and *FOXI3*. Via exome or genome sequencing through Genematcher, we report heterozygous, presumably loss-of function variants in the *CHAF1A* gene in 8 individuals, including 3 members of the same family. Four cases fulfill stringent diagnostic criteria for OAVS, including asymmetric ear dysplasia, preauricular tags, mandibular asymmetry +/- vertebral malformations. Two patients also presented with kidney malformations. The nature of the variants (splice variant in canonical sites, nonsense or frameshift variants) predicted loss-of-function in a gene highly intolerant to loss-of-function (pLI = 1, LOEUF = 0.05) and were absent from all databases tested, including gnomAD v4. *CHAF1A* encodes a subunit of CAF-1 (chromatin assembly factor-1), a heterotrimeric protein complex responsible for the deposition of newly synthesized histones H3-H4 onto the newly synthetized DNA strand during replication. As with *OTX2* and *FOXI3* (transcription factors; regulation of gene expression) and *SF3B2* (component of the spliceosome; mRNA maturation), the identification of LoF variants in *CHAF1A* is consistent with the hypothesis of early developmental genes dysregulation driving OAVS and other associations recently lumped under the acronym Recurrent Constellations of Embryonic Malformations (RCEM).

C.O.I. Disclosure:

J. Amiel: None. **C. Neiva-Vaz:** None. **J. de Oliveira:** None. **N. Martínez-Gil:** None. **A. Lasa-Aranzasti:** None. **B. Campos:** None. **I.M. Lakeman:** None. **E. Nibbeling:** None. **R. Stoeva:** None. **P. Jayakar:** None. **T. Dabir:** None. **H. Elloumi:** None. **S. Hanein:** None. **A. Picard:** None. **P. Blanc:** None. **V. Pingault:** None.

EP13.086 Chromodomain Helicase DNA-Binding Domain 8 (CHD8) related Neurodevelopmental Disorder with Overgrowth: A case report of five patients and associated neuroradiological findings

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Background: Chromodomain Helicase DNA-Binding Domain 8 (CHD8)-Related Neurodevelopmental Disorder with Overgrowth is a rare disorder, with approximately 120 cases reported to date.

Material and methods: We report five cases that presented to our hospitals with intellectual disability, autism spectrum disorder and overgrowth and one related person. We performed a systematic review on neuroradiological findings in people with CHD8 mutations and people with submicroscopic abnormalities of the chromosomal region 14q11.2 that includes the CHD8 gene.

Results: Patient 1 carried a heterozygous pathogenic frameshift variant: c.3638del, p.Gly1213Aspfs*40. Patient 2 harbors a heterozygous pathogenic missense variant in CHD8: c.5488C>T, p.Arg1830Cys). In Patient 3 and 4 (mother and daughter) a heterozygous frameshift variant c.3343del, p.Ala1115Leufs*39 was identified, classified as variant of unknown significance (class 3). In Patient 5 we detected a splice variant in CDH8: c.2486+1G > A, p.?

(class 4) and patient 6 and 7 showed two stop gain variants. Patient 6: c.1540G>T,p.Glu514* (class5) and patient 7: c.1690C>T, p.Arg564* (class 5) Magnetic resonance imaging (MRI) revealed mild bilateral white matter T2 hyperintensities in patient1, Patient 3 and 4 showed a slight asymmetric hypoplasia of the left cerebellar lobule (VIII). Patient 5 and 6 show low lying cerebellar tonsils and enlarged Virchow robin spaces with patient 5 also showing macrocephaly. In the literature neuroradiological findings were highly variable, ranging from supratentorial white matter abnormalities to cerebellar findings and ventriculomegaly.

Conclusion: CHD8-Related Neurodevelopmental Disorder with overgrowth can lead to various supra- and infratentorial neuroradiological findings.

C.O.I. Disclosure:

K. Grundmann-Hauser: None. **L. Zerweck:** None. **K. Christiane:** None. **U. Grasshoff:** None. **A. Tzschach:** None. **T. Haack:** None. **A. Bevot:** None. **T. Marco:** None. **P. Pretzel:** None. **T. Hauser:** None. **M. Kehrer:** None. **A. Dufke:** None. **M. Schuelke:** None. **U. Ernemann:** None. **B. Bender:** None.

EP13.087 Characterization of molecular cytogenetics and clinical features of a unique and complex chromosome 8 rearrangement in two cases

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Background: Complex chromosome 8 rearrangement disorder is a rare chromosomal abnormality.In this report, we examine and compare phenotypic characteristics, sizes, and the breakpoints of complex rearranged chromosome 8 in two cases.

Phenotypes and Material & Methods: Both cases (named case#1 & case#2) share common phenotypic characteristics of dysmorphic features, severe developmental delay and brain malformations. However, case#2 presented severe congenital heart defects (CHD). GTG banding and aCGH analyses used.

Results: karyotype results of the index cases revealed a rearranged chromosome 8. Further analyses using (aCGH) identified:• A terminal deletion in the short arm (8p23.3p23.1),• An interstitial duplication in the sub-telomeric region of 8p (8p23.1p11.11)• A terminal duplication on the long arm of chromosome 8.The breakpoints of the duplicated segment of 8q in case#1 is (dup 8q23.3q24.3) of 28.6 Mbs while in case#2 is (dup 8q21.3q24.3) of 75 Mbs. These results are consistent with 8p inverted duplication/deletion syndrome. However, our cases showed a unique copy gain of 8q terminal that may cause additional clinical features.

Conclusion: Differences in the 8q copy gain breakpoints may explain the clinical variability. While 8p inverted duplication/deletion syndrome is linked to CHD when the *GATA4* gene is deleted, *GATA4* was intact in both cases. Case#2 has unique 8q gain included *ZFPM2*- also named as friend of GATA family (*FOG2*) - a regulator of heart morphogenesis and development. It has been reported that overexpression of *ZFPM2* (FOG2) gene leads to downregulation of *GATA4* gene function. This may explain the severe congenital heart defects in case#2.

C.O.I. Disclosure:

M.O. Alsulami: None. **H. Elghezal:** None.

EP13.088 Syndrome of inverted duplication/deletion of the short arm of chromosome 8 in Ukrainian child with congenital anomalies

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Background: Syndrome of inverted duplication/deletion of 8p (ORPHA:96092) is rare chromosomal pathology, associated with congenital defects of central nervous system, heart defects, sensorineural deafness, skeletal anomalies, dysmorphic facial features. The cause is non-allelic homologous recombination during maternal meiosis.

Material and methods: Child aged 2,5 months has been directed for genetic counselling with multiple congenital defects (MCD). Agenesia of the corpus callosum has been detected prenatally in term of 19–20th week. Phenotype: failure to thrive, hypertrichosis, deformed external ears, muscle hypotonia. Instrumental methods: brain MRI - hypoplasia of the corpus callosum, echocardiography - accessory left superior vena cava. Standard chromosome analysis, subtelomeric FISH, chromosomal microarray (CMA) have been used for diagnosis confirmation. CMA has been carried out on the Afymetrix platform using CytoScan750 Suite.

Results: Standard karyotyping identified additional material of unknown origin, attached to the short arm of the chromosome 8, de novo. Standard karyotype result - 46,XX,add(8)(p23). Using FISH first signal of subtelomeric locus of 8p on the normal chromosome and the absence of the second signal on the derivative chromosome 8 have been detected. FISH result - ish 8p23.3 (AFM197XG65-). Using CMA 3 copies of the locus 8p23.1p11.1 (31,8 Mb, 249 genes) and 1 copy of locus 8p23.3p23.1 (6,9 Mb, 47 genes) have been identified. CMA confirmed inverted duplication/deletion of 8p syndrome.

Conclusion: Using molecular-cytogenetic analysis (CMA) allows to clarify the diagnosis of combined chromosomal pathology, determine the prognosis of the disease, the symptomatic treatment plan and genetic risks for children with MCD syndrome and cytogenetically identified additional chromosomal material.

C.O.I. Disclosure:

V.O. Galagan: None. **T. Ivanova:** None. **N. Pozhar:** None. **V. Kurakova:** None. **O. Zhurakhovska:** None. **M. Tsyhankova:** None. **V. Fisun:** None. **O. Baronova:** None. **O. Miakushko:** None.

EP13.089 The emerging role of mitochondrial dysfunction in a cohort of patients with Rasopathy

Maria Anna SIANO[1], *Federica Gentiluomo*[1], *Antonia Assunto*[2], *Vittoria D'Esposito*[3], *Serena Cabaro*[3], *Marianna Caterino*[4], *Fortunata Carbone*[5], *Mariateresa Falco*[1], *Antonio Guerriero*[1], *Federica D'Agostino*[1], *Benedetta Beatrice*[1], *Margherita Ruoppolo*[4], *Giuseppe Matarese*[5], *Daniele De Brasi*[6], *Pietro Formisano*[3], *Daniela Melis*[1]

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Background: Recent studies suggest a link between the RAS-MAPK pathway, mitochondrial function, and bioenergetics, though the mechanisms remain unclear.

Patients and methods: In this study, 72 patients (mean age 8.7 years) with RASopathies were enrolled, alongside a control group. Eleven patients were further analyzed for mitochondrial dysfunction, including clinical assessments, biochemical tests, and gene expression analyses. Investigation of the main serum amino acids and acylcarnitines was conducted by tandem mass spectrometric. Seven patients and controls were studied to investigate the impact of RAS/MAPK pathway mutations on T-cell metabolism, using real-time oxygen consumption rate (OCR) measurements. We also analyzed a panel of specific inflammatory cytokines.

Results: BMI values were significantly lower ($p < 0.01$) in patients than in controls. Fasting blood glucose levels were significantly lower in patients compared to controls ($p = 0.0036$). The analysis of plasma acylcarnitines demonstrated a significant reduction ($p < 0.05$) of C4, C6, C8, C10, C14, C4DC, C5DC, C16:OH, C12:1, C14:2, C16:1 in patients. Conversely, C0/C16 + C18 levels were significantly increased in patients ($p = 0.0017$). A significant reduction ($p < 0.05$) of Alanine, Valine, Methionine, Aspartate, Glycine, Ornitine and Citrulline was recorded in patients. Gene expression analysis showed lower levels of *CPTA1, NRF1, and AKT3* ($p < 0.05$) in patients. Oxygen consumption rate (OCR) measurements indicated impaired glycolysis, suggesting altered bioenergetics and metabolism in T lymphocytes of patients. All tested patients showed an increase in inflammatory cytokines.

Conclusion: Overall, the findings suggest that mitochondrial dysfunction is responsible for the altered energy metabolism in patients with RASopathy and contribute to T-cell impairments, potentially leading to autoimmunity.

C.O.I. Disclosure:

M. Siano: None. **F. Gentiluomo:** None. **A. Assunto:** None. **V. D'Esposito:** None. **S. Cabaro:** None. **M. Caterino:** None. **F. Carbone:** None. **M. Falco:** None. **A. Guerriero:** None. **F. D'Agostino:** None. **B. Beatrice:** None. **M. Ruoppolo:** None. **G. Matarese:** None. **D. De Brasi:** None. **P. Formisano:** None. **D. Melis:** None.

EP13.090 The *WDR44*-related ciliopathy: identification of three novel variants and delineation of the clinical phenotype

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Background: A small proportion of ciliopathies has been linked with ciliary membrane assembly dysfunctions, and defective PI3K-Akt signaling. Specifically, this pathway regulates ciliogenesis initiation via phosphorylation of WDR44, a member of the large WD40-repeat (WDR) family, and its interaction with RAB11. X-linked variants in the *WDR44* gene have been associated to a rare multiple congenital anomalies disorder caused by primary cilium dysfunction. Due the rarity of this condition, however, its clinical picture and the spectrum of the *WDR44* variants are not fully elucidated.

Materials and methods: genomic DNA from 3 individuals with a ciliopathy was analyzed by WES. Functional validations of the identified *WDR44* variants were carried out by using in-silico analyses, cDNA validations, and confocal microscopy-based studies of *cilia* of patient-derived fibroblasts.

Results: three novel *WDR44* variants were identified in this study. A stop gain variant (p.Glu9Ter) was observed in a male subject with the most severe phenotype. A missense variant (p.Ser665Asn) affecting the bladed β-propeller structure of the WDR domain was found in a subject with a milder phenotype. Finally, a female with a consistent phenotype has been shown to carry a splice site variant (c.1533+1G > A), which had been proven to be the result of a skewed X-inactivation. Functional assessments demonstrated that these *WDR44* variants resulted in defective primary *cilia* formation and morphology.

Conclusion: we report novel molecular/clinical findings of the *WDR44*-related ciliopathy. Functional validations provided evidence that loss-of-function variants impairing WDR44-dependent intracellular membrane trafficking are the major cause of this novel X-linked ciliopathy. **Grants:** Italian_Ministry_of_Health (RCR-2022-23682289).

C.O.I. Disclosure:

M. Niceta: None. **E. Flex:** None. **A. Gazzin:** None. **C. Mancini:** None. **M. Parrino:** None. **M. Carvetta:** None. **S. Cecchetti:** None. **G. Carpentieri:** None. **P. Rump:** None. **C. Morando:** None. **A. Accogli:** None. **A. Baban:** None. **M. Priolo:** None. **M. Digilio:** None. **A. Mussa:** None. **M. Tartaglia:** None.

EP13.091 Genotype-phenotype mapping in Myhre syndrome: a UK study

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Background: Myhre syndrome (MS) is a multisystem progressive disorder caused by gain of function variants in the *SMAD4* gene. Two recurrent variants in *SMAD4*, p.Ile500 and p.Arg496, have been reported in nearly all patients. We discuss the genotypic and phenotypic spectrum Of *SMAD4* variants in a previously unreported cohort of UK MS patients.

Material and methods: Patients with a molecular confirmation of MS identified from DECIPHER, 100,000 genome project and NHS Genomic Medicine Service are included. Genotype and phenotype details are collected via clinicians involved in their care and Myhre syndrome foundation UK.

Results: Thirty-three patients with a molecular confirmation of MS have been identified of which 20/32(62.5%) are males. Age range is between 8 months to 14 years. The variant is de novo in all cases. Commonly recurring variants p.Arg496Cys and p.Ile500-Val were found in 14/32(43.8%) and 16/32(50%) respectively. Rare pathogenic variants p.Ile500Thr and p.Pro130Leu were seen in one

patient each. Preliminary results showed facial dysmorphism-21/32(65.6%),developmental delay-20/32(62.5%),growth insufficiency-16/32(50%),cardiac anomalies-10/32(31.3%),musculoskeletal abnormalities-16/32(50%),endocrinological abnormalities-4/32(12.5%) and genitourinary abnormalities-3/32(9.4%). Hearing impairment and neurobehavioral issues were observed in 8/32(25%) each; sleep abnormalities were noted in 3/32(9.4%). Skin abnormalities and recurrent infections were seen in 2/32(6.3%) each. An in-depth phenotype analysis will be presented, with additional genotypic data from the literature.

Conclusion: Myhre syndrome is associated with high morbidity and although most cases are caused by 2 recurrent variants, other rarer variants may cause disease. The presence of recurrent variants makes it an interesting candidate for developing targeted molecular therapies.

C.O.I. Disclosure:

K. Gunawardena: None. **T. Sazonova:** None. **B. Banos-Pinero:** None. **U. Kini:** None.

EP13.092 Distinctly Unique Karyotypes in Variant Turner Syndrome:A retrospective study of 35 cases

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Background: Turner Syndrome (TS) is caused by a missing sex chromosome. It is characterized by the total or partial loss of the second sex chromosome, either X or Y. Conventional karyotyping is the gold standard for TS diagnosis. While the most common presentations in regular TS are short stature and primary amenorrhea, mosaic cases have broad spectrum of phenotypes. We report genotype-phenotype correlations of patients with different clinical indications and very rare variants, reported very few times in the literature.

Material and methods: The study included 35 patients who were admitted to our centre with commonly short stature, primary amenorrhea, and unexpected phenotypes such as dysmorphism, infertility, and hypospadias. Conventional G banding technique using Trypsin-Giemsa was applied for karyotype analysis. At least 100 cells were analyzed in mosaic cases.

Results: In nine cases, the diagnosis was confirmed as regular TS. Of 20 numerical TS variants, 5 of them had male phenotypes. The remaining 6 cases were rare structural variants of TS (Table 1).

Conclusion: We present the genotype-phenotype correlations of TS and variant TS. Variant forms of TS may present with a range of distinct clinical manifestations. Mosaic TS in male patients is an exceptionally rare occurrence in the literature. In cases of variant TS, structural or numerical chromosomal rearrangements serve as important predictors of the clinical phenotype, thereby enabling personalized genetic counseling tailored to the specific case.

Rare structural variants of Turner syndrome

Age	Clinical Indication	Karyotype
15	Primary Amenorrhea	46,X,i(X)(q10)[70]
5 m	Turner Syndrome	45,X[46]/46,X,r(X)(p?;q?) [54]
12	Short Stature	45,X[28]/46,X,r(X)(p22;q26)[22]
7	Short Stature	mos 45,X[36]/46,i(X)(q10)[57]/idic(X)(q22)[7]
11	Facial Dysmorphism	45,X [14]/ 47,X,i(X)(q10),i(X)(q10)[4] / 46,X,i(X)(q10)[102]
43	Turner Syndrome	45,X[31]/46,X,idic(X)(q10)[8]/46,XX[1]

C.O.I. Disclosure:

A. Kalayci: None. **C. Cinar:** None. **D. Bingol Aydin:** None. **B. Turk:** None. **A. Kaykioglu:** None. **D. Agirbasli:** None.

EP13.093 Investigating genetic variants underlying Pierre Robin Sequence in Ghanaian Orofacial cleft cohorts

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Background: Pierre Robin Sequence (PRS), characterized by micrognathia, glossoptosis, and airway obstruction, presents significant clinical and genetic complexity, particularly within African populations where genetic aetiology remains understudied. This study examines the genetic architecture of PRS in a Ghanaian cohort with orofacial clefts (OFCs), exploring genetic variants and environmental contributions to its manifestation and inheritance.

Material and methods: Whole exome sequencing (WES) was performed at 100X on DNA samples from 18 Ghanaian families. Quality control checks on sequencing reads and their alignment to GRCh38, and variant calling (including single nucleotide variants [SNVs] and small insertions/deletions), were conducted using Sentieon v202112.01. Variants were annotated using Ensembl VEP. Variants with a minor allele frequency (MAF) < 0.01 were prioritized and further evaluated using 12 in silico variant effect predictors embedded in dbNSFP and VarElect. Clinical assessments, medical record reviews, and pedigree analysis aided in identifying syndromic PRS and inheritance patterns within the study cohort.

Results: Two novel genetic variants were identified: a missense variant (p.Asn2204Asp) in the *MED13L* gene and a frameshift variant (p.Met293AsnfsTer11) in *SATB2*. Additionally, a stop-gain variant (p.Arg344Ter) was detected in *COL2A1*. The presence of pathogenic variants in *COL2A1*, *SATB2*, and *MED13L* among multiple unrelated probands suggests that they may significantly contribute to PRS in the study population.

Conclusion: Despite the potential involvement of various syndromes in the aetiology of PRS, Stickler Syndrome (*COL2A1, SATB2*) is likely a major contributor. These findings may inform genetic counselling and interventions tailored to populations with analogous genetic backgrounds.

C.O.I. Disclosure:

C. Asamoah: None. **G.O. Mensah:** None. **B. Tsri:** None. **E. Tackie:** None. **E. Acheampong:** None. **S. Obiri-Yeboah:** None. **A. Butali:** None. **P. Donkor:** None. **L. Gowans:** None.

EP13.094 'Identification of a Novel LOXL3 nonsense variant in a patient with Stickler-like phenotype'

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Background: LOXL3 is a gene essential for crosslinking of collagen and elastin and plays a crucial role in connective tissue development. To date, this gene has been associated with autosomal recessive early onset myopia. Its involvement in connective tissue disorders has also been investigated the past years in association to Stickler syndrome phenotype. Specifically to our knowledge, pathogenic homozygous variants have been identified in two unrelated families (father-son, two siblings) and two single cases, all presenting with Stickler syndrome or Stickler syndrome-like phenotype.

Materials and methods: A 2-year old female patient of Syrian origin was referred to our laboratory with severe myopia, soft skin with hyperlaxity, joint hypermobility, motor delay, congenital hypotonia, prominent forehead, low set fleshy ears, micrognathia, curvature of the spine and suspected connective tissue disorder, including Stickler syndrome as differential diagnosis. Parents are consanguineous.

Results: A homozygous likely pathogenic variant c.184C>T, p.Arg62* was identified in LOXL3 gene. The variant is nonsense, predicted to result in a truncated product that undergoes nonsense mediated decay and has not been described before in literature. The variant is rare, observed in 0.002% in gnomAD and is absent from CyprusGenome population dataset. Parental testing revealed that the parents are carriers of the LOXL3 variant.

Conclusion: To our knowledge, this is the fifth proband harbouring a LOXL3 homozygous variant presenting with Stickler phenotype. This study adds to the limited cases reported to date with LOXL3 gene as a cause of Stickler syndrome and strengthens the association of LOXL3 gene with this disorder.

C.O.I. Disclosure:

A. Miltiadous: None. **M. Kanezou:** None. **P. Gerasimou:** None. **G. Shianiou:** None. **C. Zartila:** None. **C. Ioannou:** None. **A. Elpidoforou:** None. **E. Georgiou:** None. **C. Avgousti:** None. **R. Metaxa:** None. **P. Costeas:** None. **V. Christofidou Anastasiadou:** None.

EP13.095 Truncated*SPAG9*as a novel candidate gene for a new syndrome: Coarse facial features, albinism, cataract and developmental delay (CACD syndrome)

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Background: Sperm-associated antigen 9 (SPAG9) is a member of cancer-testis antigen, having characteristics of a scaffold protein, which is involved in the c-Jun N-terminal kinase JNK signaling pathway, suggesting its key involvement in different physiological processes, such as survival, apoptosis, tumorigenesis, and cell proliferation.

Material and methods: We identified two families (A and B) having multisystem features like coarse facial features, albinism, cataracts, skeletal abnormalities, and developmental delay. Whole genome sequencing (WGS) in families A and B revealed a homozygous frameshift variant (c.903del; p.Phe301Leufs*2) in the SPAG9 gene. Sanger sequencing of both families revealed perfect segregation of the identified variant in all family members. 3D protein modeling revealed substantial changes in the protein's secondary structure. Furthermore, RT-qPCR revealed a substantial reduction of SPAG9 gene expression at the mRNA level in the affected individuals of both families, thus supporting the pathogenic nature of the identified variant.

Results: For the first time in the literature, biallelic SPAG9 gene variation was linked to multisystem-exhibiting features like coarse facial features, albinism, cataracts, skeletal abnormalities, and developmental delay.

Conclusion: This data supports the notion that SPAG9 plays an important role in a multisystemic disorder in humans.

C.O.I. Disclosure:

M. Alfadhel: None. **M. Umair:** None. **A.M. Alfaidi:** None.

EP13.096 Single nucleotide variants and copy number variants in patients with syndromic orofacial clefts using next generation sequencing: Experience from tertiary care hospital

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Background: Orofacial cleft (OFC) is a common congenital defect resulting from the incomplete fusion of the lip and/or palate. When accompanied by involvement of other organ systems, it is classified as syndromic OFC. Although there have been sporadic reports of syndromic OFC, large-scale data remains limited. We present the phenotypic characteristics and causative variants identified through NGS in north Indian cohort.

Material and methods: Patients with syndromic OFC who tested negative for common 22q11 microdeletion were enrolled. After detailed phenotyping, Whole Exome Sequencing (WES) was performed using Illumina platform.

Results: The common phenotypic abnormalities included microcephaly (62%), eye abnormalities (48%), cardiovascular defects (40%). After genetic testing, variants were identified in 72% (18/25) cases, with six novel variants, not previously reported in literature. WES revealed single nucleotide variants (SNV) in 15 cases and copy number variants (CNV) in 3 cases. These variants were seen in the following genes: *EFTUD2* (n = 2), *CHD7, TGFBR2, RUNX2, GPC3, TP63, SATB2, TFAP2A, CTNND1, GNAI3, FLNA, COL11A1, COL2A1, NIPBL* (n = 2), *FOXP1, and SATB2*. Appropriate genotype-phenotype correlations, and reverse phenotyping was performed where necessary. Genetic counselling and prenatal testing for subsequent pregnancies was offered.

Conclusion: Syndromic OFC traditionally have been known to be associated with chromosomal abnormalities. NGS offers a significant advantage, enabling the detection of both SNVs and CNVs using advanced pipelines and comprehensive databases, thus increasing diagnostic yield significantly. Accurate diagnosis allows for personalized care, tailored genetic counselling, anticipation of potential health risks, hence provides support to families of children with OFC and associated abnormalities.

C.O.I. Disclosure:

S. Gupta: None. **I. Panigrahi:** None. **A. Kaur:** None. **S. Dhiman:** None.

EP13.097 Whole genome reanalysis detected a novel biallelic variant in ZSCAN10with phenotypic expansion of otofacial neurodevelopmental syndrome

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Background: Zinc Finger- and Scan Domain-containing Protein 10 (ZSCAN10) is a 14-zinc finger transcription factor (ZF-TF) of the C2H2 ZF-TF subfamily with an N-terminal SCAN domain. ZSCAN10 deficiency is a newly characterized, clinically recognizable syndrome causing a form of neurodevelopmental disorder with characteristic otofacial malformations. To date, a total of seven patients have been described in medical literature with a spectrum of clinical presentations.

Material and methods: Clinical whole genome sequencing (WGS) reanalysis was performed in an 8-year-old boy with clinical presentations of hemifacial microstomia, unilateral hearing loss, bilateral ear anomalies, and congenital facial nerve palsy as the initial WES and WGS were negative.

Results: A thorough reanalysis performed on the WGS resulted in the annotation of a novel biallelic truncating variant in a gene where LOF is a known mechanism of disease (ZSCAN10: NM_032805.3: c.598_602del:p.Pro200LysfsTer48). Parents segregation results revealed carrier-ship status.

Conclusion: Our study highlights the importance of reanalysis of WGS in cases with negative initial results. Moreover, it underscores the significance of reporting new cases in recently described syndromes for further delineation of the spectrum of the disease. Additionally, the presence of congenital facial palsy expands the previously described phenotype associated with this genetic disease.

C.O.I. Disclosure:

A. Alfalah: None. **H. Naji:** None. **S. Maddirevula:** None. **Z. Al-Hassnan:** None.

EP13.098 Clinical and molecular characterization of CHARGE and CHARGE-like syndromes

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Background: CHARGE syndrome is a rare autosomal dominant genetic disorder characterized by various congenital anomalies and dysmorphic features. It is caused by pathogenic variants in the *CHD7* gene, which plays a crucial role in embryogenesis.

Material and methods: Fifteen patients suspected of CHARGE and CHARGE-like syndromes were recruited over 2.5 years in the Genetic Clinic after obtaining informed consent from the parent or caretaker. Ethical clearance was obtained from PGIMER, Chandigarh, and the study was funded by ICMR, New Delhi. For each of the 15 cases, 2–3 ml of EDTA blood was collected for DNA extraction. Multiplex ligation-dependent probe amplification (MLPA) or Chromosomal microarray (CMA) and Whole exome sequencing (WES), were performed according to the manufacturer's protocols. The results were analyzed, validated, and classified as per the guidelines.

Results: The male-to-female ratio was 7:8. Clinical presentations included facial asymmetry, cleft lip, cleft palate, square facies, radial club hand, dysplastic ears, ocular coloboma, hypocalcemia, and limb deformities. Genetic variants in the *CHD7* gene were identified in a heterozygous form in four patients. Additionally, variants in *KMT2B, SPEN, POGZ, MAP3K7, SEMA3*, and *USP9X* genes were detected in six patients. In the remaining cases, WES, CMA, or MLPA yielded inconclusive results, requiring further molecular investigations.

Conclusion: CHARGE-like presentations are observed in many children with multiple congenital malformations, including cleft lip, cleft palate, and cardiac defects. Further research is needed to identify additional genes contributing to this syndrome or similar clinical phenotypes.

C.O.I. Disclosure:

R. Sharma: None. **S. Gupta:** None. **K. Kaur:** None. **I. Panigrahi:** None.

EP14 Cytogenetics, Genome Variation and Architecture

EP14.001 Sex ratio (SR) in epidemiology of human chromosome abnormalities: An underappreciated tool for recognition and examination of pathological processes, and risk prediction

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Background:

Material and methods:

Results:

Conclusion: While SR in the general population is considered paramount genetic, medical, and social essence, the association of its deviations with chromosome abnormalities is still apparently underappreciated. This author's studies demonstrated SR may be considered an effective tool for recognition and examination of pathologic processes, and risks prediction. Male predominance in patients with trisomy 21 (T21) raised the assumption of non homologous meiotic co-orientation of X chromosome and chromosome 21 in spermatogenesis. That was subsequently supported by SR-based analysis of offspring of carriers of non-contributing rearrangements, dup(21q), and gonadal mosaicism for T21. Female predominance, found among carriers of rearrangements with breakpoints in the pericentromeric regions, was explained by sex-specific instability of pericentromeric regions. SR-based studies suggested a low-level mosaicism for a normal cell line in homologous Robertsonian translocation carriers (which would alter their reproductive options); indicated that gender affects clinical suspicion of Down syndrome (which may attenuate the feasibility of Down syndrome monitoring as a sentinel phenotype); and suggested that a paternal rearrangement may affect maternal chromosomes' segregation after fertilization. Female predominance among newborns with non homologous Robertsonian translocations was discovered and explained by the mechanism of sex-specific correction of the initial translocation trisomy through loss of the maternal chromosome. Male shortage among carriers of reciprocal translocation appeared to be not due to their sterility but the factors inherent in oogenesis. Recent SR-based studies discovered female predominance among carriers of either diploid/trisomic mosaicism or segmental mosaicism along with carrier gender's impact on clinical manifestations.

C.O.I. Disclosure:

N.V. Kovaleva: None.

EP14.002 A population-based cohort retrospective cytogenetic study from Romania

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Background: This study provides a comprehensive retrospective overview of the cytogenetics department of a Romanian laboratory, focusing on cases registered in 2024. The study examines the conventional karyotype analysis results of patients referred for infertility or suspected chromosomal anomalies.

Material and methods: A total of 1,818 samples were analysed, including 1,787 peripheral blood samples and 31 amniotic fluid samples. Conventional karyotype analysis was performed in order to identify chromosomal abnormalities and genetic disorders, with patient referrals originating from various clinical indications. Karyotype was obtained from peripheral blood lymphocyte cultures or amniotic fluid cultures, and cytogenetic analysis was performed according to standard protocols by Giemsa (G) banding.

Results: Abnormal postnatal karyotypes were detected in 44 patients from our 1,787 (2.5%) and abnormal prenatal karyotypes were detected in 3 patients out of our 31 patients (9.7%). Overall numerical chromosomal anomalies (37 cases) were more frequent than structural anomalies (10 cases). Among the numerical chromosomal anomalies, 16 patients presented sex chromosome aneuploidies and 11 patients presented trisomy 21.

Conclusion: This retrospective cohort study highlights the critical role of cytogenetic analysis in the diagnosis of infertility and chromosomal anomalies in Romania. The results highlight the need for continued genetic testing and counseling to better understand the genetic causes of reproductive health issues and chromosomal disorder management. These findings suggest that karyotyping should still be part of the routine investigation for selected patients diagnosed with infertility or suspected chromosomal anomalies.

C.O.I. Disclosure:

I. Sabau: None. **R. Maria:** None. **A. Curici:** None. **I. Florea:** None. **M. Mitroi:** None.

EP14.003 A case report of rare XXY/XX mosaicism in a male newborn

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Background: Klinefelter syndrome (KS) is the most common aneuploidy of gonosomal chromosomes caused by meiotic nondisjunction of either maternal or paternal sex chromosomes during gametogenesis. Most patients with KS have a 47,XXY genotype. Mosaic forms are most often present with two cell lines, 47,XXY/46XY. Mosaicism 46,XX/47,XXY is an extremely rare disorder of sex development with different phenotypical outcomes. In the literature, some of these mosaics have been described with the Klinefelter phenotype.

Material and metod: A case of a rare gonosomal aneuploidy disorder with mosaic 47,XXY/46,XX in a one-month-old boy with hypospadias. Cytogenetic analysis of peripheral blood performed on 100 metaphases by GTG banding with a resolution of 400–550 bp in the haploid set. Analysis of Y chromosome microdeletions, based on multiplex PCR, was performed for loci: SRY, ZFX/Y and two loci each in three deletion regions: AZFa, AZFb and AZFc.

Results: Cytogenetic analysis revealed the presence of two cell lines: a normal female 46,XX cell line in 86% and an aneuploid 47,XXY cell line in 14% of the examined cells. Karyotyping was performed on 100 metaphases. PCR based Y microdeletion analysis successfully identified SRY and ZFX/Y loci for the presence of the Y chromosome. Also, the specified deletion regions were not detected.

Conclusion: Early recognition of clinical symptoms and genetic diagnosis are very important to improve clinical treatment and quality of life in people with gonosomal chromosome imbalance. The earlier the condition is diagnosed and medical treatment started, it can help reduce the effects of the genomic imbalance.

C.O.I. Disclosure:

S. Teofilov: None. **O. Mlljanovic:** None. **S. Perovic:** None. **T. Ostojic:** None. **M. Bulatovic:** None. **J. Jovanovic:** None.

EP14.004 Prenatal detection of 45,X/46,X,idic(Y)(q11.22) karyotype

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Background: While NIPT can be an early screening tool with high accuracy for common chromosomal disorders, like trisomy 13, 18 i 21, it can also be used to detect sex chromosome abnormalities, such as monosomy X. Monosomy X refers to a condition where there is only one X chromosome, which is characteristic of Turner syndrome. However, NIPT can only indicate a potential risk or suspicion of monosomy X, it does not provide a definitive diagnosis. Karyotyping through amniocentesis or CVS offers a definitive diagnosis and can uncover structural chromosomal anomalies that NIPT might not detect.

Material and methods: A 44-year-old pregnant woman was tested with an NIPT test for the most common aneuploidies. The test result indicated a high risk for monosomy X.

Results: The multiplex QF-PCR test showed a peak ratio of 2:1 for markers AMXY, DXYS218, DXYS267, and was positive for SRY, indicating XXY trisomy. In an amniotic cell culture, the karyotype revealed two cell lines: 45,X[16]/46,X,+mar[3]. Further FISH analysis identified the marker chromosome as idic(Y)(q11.22)(DXYS130 + , SHOX + , SRY + , DYZ3 + , DYZ1-,DXYS224-, DYZ3 + , SRY + , SHOX + , DXYS130+), which was present in 8.5% of the cells. The monosomy X cell line was found in 91.5% of the cells. The final karyotype was mos 45,X[16]/46,X,idic(Y)(q11.22)[3].

Conclusion: This case emphasizes that combining multiple methods in genetic testing is important because it increases the accuracy, sensitivity, and comprehensiveness of the diagnostic process. This allows for better genetic counseling, as parents can make more informed decisions regarding the pregnancy, possible interventions, or further diagnostic testing if required.

C.O.I. Disclosure:

A. Pokupec Bilic: None. **I. Tonkovic Djurisevic:** None. **K. Baotic:** None. **I. Bilic:** None. **S. Huljev Frkovic:** None.

EP14.005 Can living in close proximity to a pollution source trigger genotoxic effects and arrayCGH alterations in children?

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Background: Genotoxic insults affecting a gametic cell during the preconception period may result in DNA mutation in the prospective fetus.

ArrayCGH and Next Generation Sequencing (NGS) are powerful methodologies for detecting genomic mutations and structural rearrangements. With growing concerns about environmental pollution and the rapid advancement of genetic research, a critical question emerges: does residing near a pollution source increase the risk of DNA damage in pediatric patients?

Patients and methods: In this study, a total of 64 patients affected by developmental disorders and other medical conditions were enrolled. Thirty-three patients came from regions with high environmental pollution (the Sarno river land, one of the most polluted river in Europe), while 28 lived at least 30km away. ArrayCGH was performed in each patient and parents.

Results: Array CGH showed pathologic and pathogenetic results in 7/36 patientsliving in areas with high pollution (19.44%). It is noteworthy that ACGH showed patologia results in 2/28 patients living far away from polluted areas (7.14%).

Conclusions: The results obtained in the present study suggest an association between environmental elements and chromosomal alterations. These data do not represent a pathogenetic correlation but deserve a possible validation through studies on larger populations.

C.O.I. Disclosure:

G. Franzese: None. **M. Siano:** None. **R. Genesio:** None. **F. Moccia:** None. **F. Ambrosanio:** None. **B. Lombardo:** None. **M. Di Iorio:** None. **S. Sestito:** None. **D. Concolino:** None. **V. Panettiere:** None. **M. Chianese:** None. **D. Melis:** None.

EP14.007 Lenticulostriate vasculopathy in newborns: whole genome sequencing data analysis

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Lenticulostriate vasculopathy (LSV) refers to hyperechogenic vessels detected in thalami and basal ganglia neonates using cranial ultrasound. Increased awareness of LSV has led to its association with various fetal and neonatal diseases. The main risk factors for LSV include congenital infections and hypoxic-ischemic conditions; however, its precise aetiology remains unknown. The aim of this study was to analyze WGS data from newborns diagnosed with LSV to investigate potential genetic associations. We analysed WGS variation data from six newborns diagnosed with LSV and compared it to data from 19 control newborns. The variants were annotated using ANNOVAR with GRCh37 (hg19), RefSeqGene, gnomAD, SIFT, dbSNP151, CADD, and gerp + +gt2. A custom Bash script was developed to count variant frequencies and perform comparisons between groups. Variants exclusive to the case group were selected for further analysis. In total, 3,469 variants were identified and analysed. The clinical relevance and pathogenicity were assessed using ClinVar and OMIM. We identified a nonsynonymous exonic variant located in *WNK1* gene (NM_213655.5: c.2219T>C p.(Leu740Pro)) that may be associated with LSV. This variant is associated with pseudohypoaldosteronism type 2C and hereditary sensory and autonomic neuropathy type 2A. Pseudohypoaldosteronism can increase blood pressure, resulting in damaged or stiff blood vessels. However, the variant is currently classified as a variant of uncertain significance due to insufficient evidence for a definitive role association with these conditions. Further investigation of a potential association between the *WNK1* variant and LSV may offer new insights into the etiopathogenesis of LSV. However, more comprehensive genetic studies are necessary.

C.O.I. Disclosure:

A. Urnikyte: None. **S. Dauengauer - Kirlienė:** None. **D. Pranauskas:** None.

EP14.008 Various CNV findings in a patient with recurrent Hodgkin's lymphoma, lung adenocarcinoma in samples of different origin using SNP array

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Background: We present a case study of cancer duplicity associated with germinal partial deletion of *ATM* gene with different expressions in peripheral blood and buccal smear respectively.

Material and methods: A 74-year-old female with recurrent Hodgkin's lymphoma diagnosed at the age of 48, duplicated by lung adenocarcinoma at the age of 63. Her oncologic family history is positive. She underwent repeated courses of chemo/actinotherapy and right-sided pneumonectomy. DNA from peripheral blood was tested by NGS-based oncopanel for variants of 226 cancer-susceptibility and candidate predisposition genes (PMID: 29649263) using in-house bioinformatic pipeline. Peripheral blood and buccal swab were tested by Illumina GSAV3_CYTO array analyzed by NxClinical 6.2.

Results: NGS-based oncopanel has revealed no significant finding. A mosaic deletion (80%) was detected in the 11q14.1q23.2 locus (30.6Mb) containing more than 200 genes (including *ATM* and *SDHD*) by peripheral blood array. Buccal cell testing revealed deletion affecting exons 2–44 of *ATM* gene (96.1 Kb, 66% of the gene).

Conclusion: A germline deletion in the *ATM* gene, classified as pathogenic, could be the etiological factor of cancer duplicity. The large mosaic deletion 11q14.1q23.2 detected in peripheral blood probably arose from exogenous exposure and masked the germline mutation. The results of dual array testing helped to elucidate diagnosis and are the basis for ongoing family segregation study.

C.O.I. Disclosure:

V. Brezna: None. **L. Haban:** None. **M. Koudova:** None. **D. Stejskal:** None. **J. Horák:** None. **E. Racochova:** None. **M. Urbanova:** None.

EP14.009 Expanding the clinical spectrum of mosaic trisomy 14: a case report of phenotypic manifestations and diagnostic insights

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Background: Mosaic trisomy 14 is a rare chromosomal abnormality associated with congenital anomalies, growth and developmental delay, dysmorphic craniofacial features, and skin abnormalities. We report a 6-year-old female with mosaic trisomy 14 presenting with diverse phenotypic manifestations.

Material and methods: The patient underwent clinical evaluation, neurodevelopmental assessment, whole-exome sequencing (WES), and cytogenetic analysis via G-banded karyotyping from a peripheral blood sample to confirm chromosomal abnormalities and assess potential chromosomal rearrangements.

Results: The 6-year-old female patient exhibited intrauterine growth restriction, feeding difficulties, fine and gross motor delays (walking delay, difficulty holding pen, and writing challenges), speech delay (verbal responses with limited comprehension), and intellectual disability. Craniofacial dysmorphism included ocular asymmetry, hypertelorism, flattened nasal bridge, short philtrum, thin lips, downslanting palpebral fissures, an asymmetric mouth, and large pinnae. Additional features included myopia, abnormal dentition, short stature, pes planus, and widespread hypomelanosis resembling Hypomelanosis of Ito. Copy number variation analysis by WES revealed an extra chromosome 14 at a 30% level. Cytogenetic analysis confirmed the trisomy at a much lower level (1 in 40 cells). FISH analyses to accurately determine the mosaicism level, along with investigations to detect potential UPD/methylation defects, are ongoing.

Conclusion: This case expands the clinical spectrum of mosaic trisomy 14, emphasizing the importance of comprehensive cytogenetic and molecular analyses for an accurate diagnosis. The presence of pigmentary skin abnormalities and developmental impairments suggests a correlation between chromosomal mosaicism and systemic manifestations. A limitation of this study is that all genetic tests to clarify the mechanism remain incomplete.

C.O.I. Disclosure:

O. Sahin: None. **H. Abbasova:** None. **S. Karauzum:** None. **G. Ceylaner:** None.

EP14.010 Copy number variation of the Williams-Beuren syndrome critical region

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Background: The characteristic size of a heterozygous deletion or duplication of chromosomal region 7q11.23 is approximately 1.5 Mb, and the affected genes represent the critical region of Williams-Beuren syndrome (WBSCR). The most significant gene in this region, ELN, encodes the protein elastin and copy number variations (CNVs) in this gene are associated with cardiovascular disorders. Chromosome 7q11.23 contains highly homologous sequences, which can lead to recurrent copy number variations (CNVs), resulting in either a microdeletion (Williams-Beuren syndrome) or a microduplication (7q11.23 duplication syndrome).

Methods and Results: Here we described cases of small and larger deletions or duplications in 7q11.23 detected by molecular karyotyping (Array-CGH), MLPA (Multiplex Ligation Probe Amplification), or FISH (fluorescence in situ hybridization), along with the clinical presentation of the patients.

Array-CGH and the MLPA methods were performed on DNA samples extracted from the patients' peripheral blood, following standard protocols. The Array-CGH method utilized SurePrint G3 Human CGH Microarray 8×60 K chips (Agilent Technologies), while the MLPA method used the SALSA MLPA Probemix P245 Microdeletion Syndrpmes-1 (MRC Holland). The FISH method was used on cell cultures using a locus-specific probe for the 7q11.23 region.

Conclusion: The advantages and limitations of each method in detecting CNVs in the WBSCR are useful for determining the exact size of deletions and duplications in the 7q11.23 region, identifying the affected genes, understanding the mechanism of origin, and determining the inheritance of the detected changes, which is essential for genetic counseling of patients.

C.O.I. Disclosure:

I. Tonkovic Djurisevic: None. **A. Pokupec Bilic:** None. **K. Baotic:** None. **S. Huljev Frkovic:** None.

EP14.011 Satellited chromosome 1 - Insights from cytogenetic and FISH analysis

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Background: Satellite regions of chromosomes are small, distinct segments of chromosomal material implicated in RNA synthesis. They are typically located on acrocentric chromosomes, but due to translocation, they can also be found on non-acrocentric chromosomes. On the other hand, satellited non-acrocentric chromosomes arise from translocation between sub- or telomeric regions of non-acrocentric chromosome and the short arm of acrocentric chromosome. These structural changes may or may not lead to phenotypic abnormalities. Determining the origin of satellite regions on non-acrocentric chromosomes presents a significant challenge because these regions are not detectable using available methods.

Material and methods: We report the case of a 41-year-old man referred after genetic counseling due to sterility, with an initial karyotype of 46,XY,der(1). FISH analysis was performed for identification of additional material at the terminal part of the short arm of chromosome 1.

Results: The analysis confirmed normal position of the subtelomeric 1p (RH120573) probe. Acro-P-Arm (NOR) probe revealed that material at the terminal part is additional satellite region, and D15Z1 probe confirmed that it originated from acrocentric chromosome 15, likely due to an unbalanced translocation between the terminal part of chromosome 1 and the III satellite region of chromosome 15.

Conclusion: This case highlights the crucial role of classical cytogenetic and FISH analysis in identifying chromosomal abnormalities that may go unnoticed by other molecular techniques.

C.O.I. Disclosure:

K. Baotic: None. **A. Pokupec Bilic:** None. **I. Tonkovic Djurisevic:** None. **S. Huljev Frkovic:** None.

EP14.012 Genetic insights into 1q21.1 deletions in two pediatric patients: A comparative analysis of contiguous gene loss

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Background: The 1q21.1 deletion syndrome is well described chromosomal disorder with highly variable phenotype. Phenotypic differences are due to the variable length and genes involved in the deleted region on chromosome 1q21. In this study, we present two cases with 1q21.1 deletion highlighting the role of contiguous gene syndrome.

Material and methods: We preformed microarray comparative genomic hybridization (CGH) in two newborns. Detailed phenotyping was performed in both.

Results: Both newborns came from uneventful pregnancy from young and unrelated healthy parents. The first patient had hypotonia, microcephalia, moderate thrombocytopenia and minor dysmorphism without limb malformations. The other baby had typical signs of TAR syndrome comprising microcephaly, forearm aplasia and severe thrombocytopenia. Heart evaluation revealed minor anomalies in both. Microarray CGH results for patient 1 showed two copy number changes of the 1q21 region:arr[hg38] 1q21.1(143, 893,564-144, 498,488)x1 and arr[hg38] 1q21.1(145, 470,345-146, 185,744)x1. The deletion in patient 2was with the following coordinates: arr[hg38] 1q21.1(143, 893,564-144, 922,195) x1. The deletions in both patients overlap considerably, however they comprise variable number of genes.

Conclusion: The 1q21.1 deletion is referred to have substantial clinical variability, due to the difference in number of the deleted genes, modifier genes, as well as the condition of the remaining allele. The importance of genotype-phenotype correlation studies are needed for improving our understanding of this specific 1q21.1 deletion syndrome, and, if needed to as well as to separate as several different entities.

C.O.I. Disclosure:

S. Veleska: None. **E. Sukarova-Angelovska:** None. **G. Ilieva:** None. **E. Shuperliska:** None.

EP14.013 "The Importance of Genetic Investigations in the Diagnosis of Neurodevelopmental Disorders: The Complementary Role of Cytogenetic, Molecular, and Next Generation Sequencing Investigations."

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Background: The growing recognition that neurodevelopmental disorders (NDDs) share common genetic variants has prompted genotype-phenotype correlation studies in pediatric NDD cohorts. Chromosomal microarray (CGH) is now established as a first-tier diagnostic tool for such patients. However, the genetic etiology in some cases remains unknown. For these patients, next-generation sequencing (NGS)-based tests hold significant potential for achieving a diagnosis. This study aims to explore the use of copy number variations (CNVs) identified by array-CGH and NGS to demonstrate the diagnostic value of combining these two methods.

Patients: A total of 183 patients with NDDs were enrolled in the study. All 183 patients underwent Array-CGH testing, and 72 underwent NGS (53 Clinical Exomes and 19 Gene Panels).

Results: Data obtained on 156/183 Array-CGH tests showed in 12.18% (19/156) pathogenic CNVs. Of the 29/72 NGS tests 42.10% (8/19) clinical exome and 20% (2/10) gene panels were pathogenic. Therefore, the diagnostic yield for NGS was 34.48% (10/29).

Conclusion: The described results recorded a higher diagnostic detection rate of NGS than arrayCGH. It is noteworthy that the combined diagnostic yield was over 45%, emphasizing the importance of their complementary use.

C.O.I. Disclosure:

F. Ambrosanio: None. **D. Melis:** None. **M. Falco:** None. **S. Sestito:** None. **C. Daniela:** None. **R. Russo:** None. **G. Esposito:** None. **B. Lombardo:** None. **A. Weisz:** None. **R. Genesio:** None. **E. D'acunto:** None. **F. Gentiluomo:** None. **V. Panettiere:** None.

EP14.014 Unbalanced translocation involving chromosome 11 two cases with different phenotypes

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Background: Chromosome 11 spans about 135 million DNA bp. Copy number variations in chromosome 11 result in various known syndromes. Many cases of structural abnormalities involving chromosome 11 have been reported in the literature with widely different/overlapping phenotypes. Here we describe two patients with different phenotypes found with unbalanced translocation involving chromosome 11.

Material and methods: Case 1. 2-month-old with dysmorphic features; narrow forehead, synophrys, hypertelorism, low-seated ears, and retrognathia. The patient has a history of hypoglycemia, hypothyroidism, and cholestasis. Case 2: 4-month-old with failure to thrive, microcephaly, dysmorphic features with a broad forehead, retro- and micrognathia, high arched palate, bilateral preauricular tag and pits, downslanting palpebral fissures, and prolonged NICU admission due to prematurity and Hirschsprung disease.

Results: Cytogenetic and molecular testing was performed for both cases; case 1 showed terminal heterozygous pathogenic 3.6 MB deletion in chromosome 8 (8p23.3p23.3) and terminal heterozygous pathogenic 4.3 MB duplication in Chromosome 11 (11p15.5p15.4). While Case 2 cytogenetics testing revealed a male karyotype with an extra derivative chromosome 22, followed by the detection of a duplication of 22q10-22q11 and duplication of 11q23-qter on a derivative chromosome 22 [der(22)].

Conclusion: Here we present two cases with a very rare chromosomal aberration, both involving chromosome 11; however, they present with different phenotypes given the involvement of two different chromosomes in the unbalanced translocation. Case 1 shows an overlapping phenotype that could be explained by BWS or SRS depending on the parental origin of the duplication. While case 2 is phenotypically and cytogenetically fitting with Emanuel syndrome.

C.O.I. Disclosure:

O.A. Alshehri: None. **M. Albalwi:** None. **N. Alsaleh:** None.

EP14.015 Chromosomal microarray analysis in a large cohort of patients from eastern region of Türkiye

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Background: Copy Number Variants (CNVs) are structural variations in DNA involving gains or losses of genetic material, ranging from hundreds to several million base pairs. Chromosome microarray analysis (CMA) effectively identifies and characterizes CNVs and is recommended as the primary diagnostic test for patients with neurodevelopmental disorders (NDDs). This study aims to determine the overall diagnostic rate of CMA and to provide detailed data on the CNVs identified in our cohort.

Material and methods: In this study, the clinical and genetic findings of 467 patients who underwent CMA testing for various clinical indications between 2016 and 2024 were retrospectively collected. The Affymetrix Cytoscan Optima (350K) and Agilent 8×60K microarray platforms were used in 80% and 20% of the patients, respectively.

Results: Out of the 467 cases, 263 (56.3%) were male and 204 (43.7%) were female, with ages ranging from 0 to 40 years (mean age: 4.7 years). The most common clinical indications were NDDs (72.3%). Multiple congenital anomalies, dysmorphism, and autism spectrum disorders were reported in 30.1%, 28.9%, and 6.4% of the patients, respectively. CNVs were detected in 111 (23.7%) patients, of which 36 (32.4%) were classified as VUS and 75 (67.6%) as likely pathogenic/pathogenic. Ten patients (2.1%) had CNVs affecting two different chromosomes, while four patients (0.8%) had CNVs affecting different loci on the same chromosome. Velocardiofacial syndrome and Williams-Beuren syndrome were detected in six and three patients, respectively.

Conclusion: Our findings highlight the effectiveness of CMA testing in a large cohort, reinforcing its role in diagnosing NDDs.

C.O.I. Disclosure:

C. Alavanda: None. **H.Y. Yalçın:** None. **S. Demir:** None.

EP14.016 Clinical utility of Multiplex Ligation-dependent Probe Amplification (MLPA) in hematologic malignancies: CLL, MM and MDS

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Background: In the context of hematologic diseases, Multiplex Ligation-dependent Probe Amplification (MLPA) analysis can provide valuable insights into the underlying genetic abnormalities, thus having an important prognostic value.

Material and methods: Over the period of one year, we applied MLPA analysis to patients with chronic lymphocytic leukemia (CLL), multiple myeloma (MM) and myelodysplastic syndrome (MDS), using disease-specific MLPA panels to assess multiple markers in relevant chromosomal regions. Data analysis was conducted with Coffalyser.Net software.

Results: A total of 136 patients were included: 30 with CLL, 44 with MM and 62 with MDS. The mean age was 66.6 years, with a male-to-female ratio of 1:0.78. The highest rate of pathological findings was observed in CLL patients (63%), predominantly deletions in the 13q region (56.6%). MDS patients showed a 39% pathology rate, with deletions most frequently located in the 5q region (12.9%). In MM patients, 27% had detectable abnormalities, primarily deletions or duplications in chromosome 1 (20.4%).

Conclusion: Our findings confirm the frequent occurrence of specific genetic abnormalities in these hematologic diseases. MLPA analysis proves to be a valuable prognostic tool, facilitating accurate diagnosis, risk stratification and treatment planning.

C.O.I. Disclosure:

R. Valeva: None. **M. Levkova:** None. **D. Yahya:** None. **M. Hachmeriyan:** None.

EP14.017 Copy number variants and gene expression data reveals distinct diagnostic markers for distinguishing diffuse large B-cell lymphoma from Burkitt lymphoma

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Background: Overlapping features between diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma (BL) are problematic to diagnose. The aim of this study was to explore copy number variations (CNVs) and microarray data to better classify DLBCL and BL, while also corroborating with cytogenetic abnormalities (RCAs) previously identified.

Material and methods: Cases were retrieved from the SKY-M FISH & CGH database to evaluate CNVs. When evaluating gene expression data, we collected a total of 44 cases from the Gene Expression Omnibus database. Cases were analyzed using the R package. Differentially expressed genes for both entities were then uploaded to the STRING databases to determine pathways. To further characterize the gene expression of both tumor types, a gene set enrichment analysis was performed using the GSEA software package. Finally, a list of CNVs and gene expression data that was positively correlated.

Results: We identified unique CNVs and identified several differentially expressed genes, notably *CDK5R1*, involved in a cell cycle progression pathway with *CCND2* and *STAT3*. Other genes included *SAMSN1*, *FNBP1*, and *ELK3* were specific for DLBCL. In contrast, a series of genes were differentially expressed in BL, notably *MYC*, *ID3, TCF3, SOX11* and *CSE1L*. We also correlated RCAs and CNVs with gene expression profiles. Moreover, we outlined a total of 41 diagnostic markers that may be useful in distinguishing between the two entities

Conclusion: In brief, our results show unique RCAs, CNVs, molecular profiles, as well as a series of immuno-phenotypic markers that may be used to better define BL from DLBCL.

C.O.I. Disclosure:

R. Garcia: None. **P. Koduru:** None.

EP14.018 Rare 9q21.33q22.33 deletion due to an unbalanced transmission of a maternal chromosomal insertion

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Background: Insertions are rare balanced chromosomal rearrangements with an increased risk of imbalances for the offspring.Our aim was to report a case of Gorlin syndrome caused by interstitial deletion of the long arm of chromosome 9 resulting from unbalanced transmission of an insertion: ins(3;9))(p12;q21.3q22.3).

Material and methods: We report a case of a two year old girl who was was referred to the department of Genetics at Charles

Nicolle hospital in Tunis for dysmorphic features and psychomotor retardation.

Results: Physical examination showed macrocephaly and dysmorphic features. Blood karyotype showed shortening of the long arm of chromosome 9. CGH array study showed a 14.6 Mb interstitial deletion at 9q21.33q22.33. Maternal karyotype showed an insertion of the 9q21.33q22.33 region on the short arm of chromosome 3: ins(3;9)(p12;q21.3q22.3). Our patient therefore carried a derivative of chromosome 9 resulting from unbalanced transmission of the maternal insertion. This 9q deletion carried around 80 coding genes, including the PTCH1 gene, responsible for Gorlin syndrome. Genetic counseling was provided to the parents, and a blood karyotype was performed on our patient's sister, who carried the maternal insertion. Our patient underwent multidisciplinary management, which revealed a neuroblastoma of the right kidney with amplification of the Nmyc gene at the age of 3.5 years.

Conclusion: This observation demonstrates the importance of cytogenetic techniques in the diagnosis of chromosomal insertions responsible of abnormal phenotypes. Indeed, confirmation of Gorlin syndrome enabled us to set up appropriate management for this patient and precise genetic counseling for her parents.

C.O.I. Disclosure:

H. Bellil: None. **S. Ben Yahia:** None. **R. Meddeb:** None. **F. Fedhila:** None. **F. Maazoul:** None. **E. Launay:** None. **M. Belaud-Rotureau:** None. **R. Mrad:** None. **L. Kraoua:** None.

EP14.019 Optical genome mapping identifies a homozygous deletion in the non-coding region of the *SCN9A*gene in Individuals with congenital insensitivity to pain

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We are reporting an index patient with complete insensitivity to pain and a history of painless fractures, joint hypermobility, and behavioural problems. The index patient descends from a family with notable cases among his maternal relatives, including his aunt, a mother's first cousin, both of whom suffer from congenital insensitivity to pain. The patient had normal results for prior genetic testing: fragile-X, chromosomal micro-array, and exome sequencing. Optical genome mapping detected a homozygous deletion affecting the noncoding 5' area and the first non-coding exon of the *SCN9A* gene in all affected family members, compatible with recessive disease transmission. Pathogenic homozygous loss of function variants in the *SCN9A* gene are associated with impaired pain sensation in humans. Optical genome mapping can thus detect pathogenic structural variants in patients without molecular etiology by standard diagnostic procedures and seems to be a more accessible diagnostic tool than short read or long read whole genome sequencing.

C.O.I. Disclosure:

A. Boughalem: None. **V. Ciorna-Monferrato:** None. **D. Trost:** None.

EP14.020 The potential importance of mitochondrial variants on the molecular processes underlying biological ageing

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Background: Mitochondria are central to the processes of biological ageing due to their role in cellular energy production and the regulation of metabolic pathways. Specific variants in mitochondrial DNA (mtDNA) and nuclear-encoded mitochondrial genes have been linked to longevity and protective effects against age-related diseases. This study aimed to explore the relationship between mitochondrial variants and their potential protective impact on ageing in a cohort of 40 Lithuanian clean-up workers of the Chornobyl nuclear disaster (LCWC). Despite their significant exposure to ionising radiation, these individuals exhibit relatively healthy ageing, offering a unique opportunity to investigate the potential role of mitochondrial variants in ageing and disease resistance.

Material and methods: mtDNA and nuclear-encoded mitochondrial gene variation were extracted from whole genome short-read sequencing data. mtDNA and exonic nuclear variant allele frequencies from the LCWC cohort were compared to non-Finnish European population frequency data in the gnomAD database. Statistical analysis was performed using the χ^2 and Fisher's exact tests. Statistically significant results were considered at $p \leq 0.05$ for mtDNA and $p \leq 3\times10^{-5}$ for nuclear variants.

Results: Six mtDNA variants (m.146T > C, m.204T > C, m.477T > C, m.2623A > G, m.3244G > A, m.16086T > C) and ten nonsynonymous nuclear genome variants in *COQ6*, *METTL4*, *MFF*, *RHOT2*, and *TIMM23* genes were more prevalent in the LCWC cohort compared to the non-Finnish European population dataset.

Conclusion: Identified mitochondrial and nuclear variants could possibly have a protective effect in the LCWC cohort by affecting mitochondrial function; additional studies are needed to support this hypothesis.

C.O.I. Disclosure:

K. Žukaitė: None. **G. Žukauskaitė:** None. **I. Domarkienė:** None. **K. Samaitė:** None. **T. Rančelis:** None. **L. Ambrozaitytė:** None.

EP14.021 Detection of parthenogenetic chimerism using comprehensive STR analysis

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Background: Parthenogenetic chimerism is an exceptionally rare genetic phenomenon where an individual possesses two genetically distinct cell lines derived from one egg with endoduplicated genome (parthenogenetic nucleus division) occurring just before fertilization with two spermatozoa, commonly detected as a presence of both 46,XX and 46,XY karyotypes within the same individual. Due to its rarity and complexity, parthenogenetic chimerism is often underdiagnosed. STR analysis proved as a valuable tool for detecting such cases.

Methods: An 11-years old proband, with a negative family history of genetic disorders and normal female genitalia, was referred for MPS analysis due to suspicion of aortopathies and collagenopathies. Previous cytogenetic analysis revealed a 46, XX/46, XY karyotype (57:43 ratio). STR analysis was performed using Argus X-12 (QIAGEN) and PowerPlex® Fusion 6C (Promega) kits on DNA from peripheral blood and buccal swab samples, including parental samples for comparison.

Results: All X-STR loci showed the single maternal X chromosome. Of the 23 autosomal STR loci analysed, 18 informative showed only a single maternal allele transferred, with no loci exhibiting more than three alleles in proband's samples. Estimated major/minor genotype ratio was 59:41 in peripheral blood and 89:11 in the buccal swab. STR genotypes ruled out blood microchimerism due to vanished twin and strongly support XX/XY parthenogenetic chimera composed of two different paternal and a single duplicated maternal genome.

Conclusion: As SNP analysis cannot classify chimerism, STR profiling provided a comprehensive assessment, highlighting the diagnostic complexity of chimerism and the need for further research on its pathogenesis and clinical implications.

C.O.I. Disclosure:

M. Baldovic: None. **K. Durisova:** None. **S. Vogelova:** None. **G. Blandova:** None. **G. Krasnanska:** None. **V. Elias:** None. **M. Konecny:** None.

EP14.022 Blood cDNA analysis significantly improves clinical interpretation and reclassification of spliceogenic variants of unknown significance

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Background: Next-generation sequencing (NGS) is now widely used in clinical genetic diagnosis, carrier screening, and cancer risk assessment. Despite its success, NGS presents challenges in terms of variant classification, particularly for variants of unknown significance (VUS). VUS typically include missense variants, in-frame indels, and intronic variants, with some having the potential to alter canonical RNA splicing. However, detecting and evaluating non-canonical spliceogenic variants remains difficult due to limited understanding of the 'splicing code'. Experimental validation is essential for confirming splicing alterations. This study aims to functionally characterize potential splice-altering variants through cDNA analysis to evaluate their impact on splicing.

Material and methods: RNA was extracted from peripheral whole blood from 10 individuals with likely spliceogenic variants. Reverse transcription polymerase chain reaction and Sanger sequencing were performed to evaluate their potential splicing effect.

Results: We confirmed aberrant splicing events in all individuals. Six variants resulted in exon skipping, three activate alternative splice sites and one caused a complex intron retention event. Variants were reclassified based on their splicing impact, with five being upgraded from VUS to likely pathogenic (LP) and three from LP to pathogenic.

Conclusion: Our findings highlight the importance of incorporating RNA analysis into clinical practice for accurate variant classification. This approach enhances diagnostic precision by confirming the functional impact of spliceogenic variants, improving clinical decision-making for genetic disorders.

C.O.I. Disclosure:

P. Trilla: None. **I. Madrigal:** None. **A. Sanchez:** None. **M. Potrony:** None. **M. Jodar:** None. **L. Rodriguez-Revenga:** None. **M.I. Alvarez-Mora:** None.

EP14.023 Characterization of an trpinvdupdel rearrangement on chromosome 4 in a fetus using Bionano® Optical Genome Mapping technology

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We present the case of a fetus with an intrauterine growth restriction (IUGR) at 32 weeks gestation, referred for non-visualization of the testes. SNP-array analysis of amniotic fluid sample identified an interstitial gain of 30.15 Mb on the long arm of chromosome 4 (4q31.3q35.1), adjacent to a terminal heterozygous loss of 7.2 Mb on chromosome 4 (4q35.1qter). The fetal karyotype revealed an elongated chromosome 4 with a homogeneous appearance of the long arm, suggesting an inverted duplication of the distal region. Optical Genome Mapping (OGM) was performed on cultured amniotic fluid. DNA was extracted, labeled with the Bionano® kit, and scanned using the Saphyr™ system. Data were analyzed using Bionano Access v1.7 software with CNV and SV algorithms. The CNV algorithm identified a complex rearrangement, including a 1.04 Mb triplication (4q31.3q31.3), followed by a 29.14 Mb duplication (4q32.1q35.1), and a terminal deletion (4q35.1 to 4qter). SV analysis clarified the structural organization of this rearrangement by confirming an inverted duplication and revealing that the triplicated copy is inserted at the distal 4q end of the rearrangement. FISH analysis validated the structural complexity of the chromosomal rearrangement. In conclusion, OGM provided a more precise characterization of the rearrangement by identifying an additional copy number gain (trp) in the 4q31.3 region that was not identified by the SNP-array analysis. It also clearly determined the inversion, confirming the inversion-duplication-deletion mechanism. This case highlights the utility of OGM in prenatal diagnostics by offering rapid characterization of complex rearrangements like trpinvdupdel, rarely reported, and their breakpoints.

C.O.I. Disclosure:

E. Benbakir: None. **S. Bernard:** None. **V. Jauffret:** None. **O. Pichon:** None. **S. Bezieau:** None. **M. Doco-Fenzy:** None.

EP14.024 Quantitative analysis of pseudogene-associated errors during germline variant calling

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Background: Processed pseudogenes (PPs) represent a class of pseudogenes, which is created by reverse transcription of mRNA and subsequent integration of the resulting cDNA into the genome. PPs pose a significant challenge for the genome analysis due to their high sequence similarity to the parent genes and their frequent absence from the reference genome build. Therefore at the level of individual genome analysis presence of PPs may lead to the erroneous variant calls in the parent gene. We set put to quantitatively estimate the rate of PPs-associated errors.

Material and methods: Due to the absence of intronic sequences PPs can be detected in the whole genome sequencing data (WGS) as a chain of intronic deletions in a parent gene. We analyzed WGS data of a cohort of healthy individuals (n = 13307) for presence of non-reference PPs. We also followed clinical guidelines to identify pathogenic SNPs in our cohort.

Results: Our analysis revealed that all pathogenic SNPs identified in genes with PPs originated from PPs rather than parent genes. We evaluated several variant calling pipelines, finding that DeepVariant outperformed Dragen and HaplotypeCaller in managing PP-related errors. We also demonstrated that the addition of PPs into the reference genome helps calling pipelines to avoid erroneous calls.

Conclusion: Overall, we demonstrated that non-reference PPs are responsible for the identification of false positive pathogenic calls in 4.9% of our cohort, highlighting the importance of pipeline and reference choice.

C.O.I. Disclosure:

A. Podvalnyi: None. **A. Kopernik:** None. **M. Woroncow:** None. **M. Sayganova:** None. **A. Deviatkin:** None. **P. Volchkov:** None. **E. Albert:** None.

EP14.025 Monosomy 18p: about five cases

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Background: Monosomy 18p is a rare genetic disorder caused by a deletion of the short arm of chromosome 18, with an estimated incidence of 1:50,000 live births. It is characterized by facial dysmorphism, intellectual disability, growth retardation, and cardiac, renal and cerebral malformations.

Material and methods: We conducted a retrospective study on patients diagnosed with 18p monosomy in our department from 2016 to 2024. Chromosome analysis was performed on peripheral blood lymphocytes, complemented by FISH or CGH array in some cases.

Results: Five cases were identified, including two diagnosed prenatally. The male-to-female ratio was 1:4. In the three postnatal cases, the age at first consultation ranged from 2 days to 3.5 months, with neonatal hypotonia as the main reason. Prenatal diagnoses were based on abnormal maternal serum markers. Clinical examination showed growth retardation and facial dysmorphism in all postnatal cases, including slanting palpebral fissures (2/3), prominent forehead(2/3), small mouth (2/3), retrognathia (2/3), anteverted nostrils(2/3), and pectus excavatum(2/3). Karyotyping confirmed an 18p deletion in all cases. FISH analysis further confirmed the deletion and showed telomere loss in four cases. One patient had an intact telomere, confirmed by comparative genomic hybridization, which identified an interstitial deletion at 18p11.31p11.21. Parental karyotyping(available for 4/5 cases) was normal. One patient presented with situs inversus, polysplenia, and heart defects, while the others had no malformations. The two fetuses had normal ultrasound.

Conclusion: Monosomy 18p is a rare chromosomal disorder. Its association with intellectual disability highlights the importance of genetic counseling even in the absence of malformations.

C.O.I. Disclosure:

M. Kamoun: None. **L. Kraoua:** None. **H. Bellil:** None. **R. Mrad:** None. **I. Ouertani:** None.

EP15 Genomics of rare diseases

EP15.001 A de novo mutation causing a rare Baraitser Winter syndrome: The first case in Northern Cyprus

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Background: Baraitser-Winter syndrome (BRWS; MIM #243310) is a rare multisystem developmental disorder characterized by intellectual disability ranging from mild to severe and distinctive craniofacial features, including metopic ridging or trigonocephaly, bilateral ptosis, and hypertelorism. Additional hallmarks of the syndrome include cortical malformations such as pachygyria or lissencephaly, as well as ocular colobomata. Other associated features comprise moderate short stature, joint contractures, congenital cardiac anomalies, and genitourinary malformations. BRWS is attributed to missense mutations in the cytoplasmic beta- and gamma-actin genes, ACTB and ACTG1.

Material and methods: A patient presented at the genetics clinic with short stature as the primary concern. Clinical evaluation revealed dysmorphic facial features, including a long philtrum, beaked nose, single eyebrow, and hypotelorism.

Results: Genetic analysis identified a de novo heterozygous c.353A>T (p.K118M) variant in the ACTB gene (MIM #102630). Family segregation analysis confirmed the pathogenicity of this variant as de novo. Notably, this case represents the first diagnosed instance of BRWS in Northern Cyprus.

Conclusion: This study highlights the uncertainty surrounding the genotype-phenotype correlation in BRWS and underscores the complexity of this severe genetic disorder. The findings contribute to the growing body of evidence on BRWS, providing valuable insights that may aid in refining clinical and molecular understanding of the syndrome.

C.O.I. Disclosure:

B. Koyutourk: None. **D. Deryali:** None. **A. Turgay:** None. **M.C. Ergoren:** None.

EP15.002 THE PATTERN OF SILVER-RUSSELL SYNDROME IN PEDIATRICS

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Довідка: Silver-Russell syndrome (SRS) -is an orphan disease characterized by intrauterine and postnatal growth retardation. Children have special phenotypes, difficulties in feeding, a threat of insulin resistance, hypoglycemia, and accelerated sexual development.

Methods: Clinical examination, blood tests, biochemistry, genetic tests, polymer chain reactions, and ultrasound examination have been carried out.

Results: The child, 5 years old, had a special phenotype: asymmetry of the body with a relative increase in head circumference, a small triangular face with a protruding forehead, a narrow chin, a small jaw, and curvature of the 5th finger. From birth, insufficient body weight gain was observed, which was explained by physiological vomiting and lactase deficiency. For the sake of differential diagnosis,a study was conducted for the presence of Gaucher's disease(the result was negative). The results of immunologic tests: denied the presence of celiac disease. Clinical diagnosis was established according to the Netchine-Harbison scoring system (NH-CSS), which consists of 6 clinical criteria of NH-CSS. Gene sequencing revealed changes in the variant of un certain significance SLK25A15, the genetic significance of which is unknown, which did not allow to confirm the diagnosis. Invitae Chromosomal Microarray Analysis of genome research on microdeletions (duplications) revealed, with a normal female genomic profile, uniparental dysfunction with two areas of homozygosity (GRCh370) in chromosome 20: 20q13-p12.3(1-6690101; 6.69Mb and 20q13.32- q13.33(57394420-63025520); 5.63 Mb. This change is characterized as a SRS variant.

Conclusions: the child has a genetically confirmed diagnosis of SRS and congenital lactose intolerance. Somatotropic hormone was prescribed at a dose of 0.6 mg/day continuously.

C.O.I. Disclosure:

V.S. Hlohus: None. **M.Y. Iskiv:** None. **N.M. Gromnatska:** None. **N.A. Petritsa:** None.

EP15.003 Enhanced DNA extraction from dried blood spot cards for low-input nanopore whole genome sequencing

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Dried Blood Spot (DBS) cards are widely used for the long-term preservation of human blood samples, effectively preserving nucleic acids for downstream molecular applications, including clinical diagnostics. These cards provide a simple and efficient method for blood sample collection while ensuring safe storage through integrated pathogen inactivation. These features are particularly valuable in newborn screening programs, which test for genetic conditions such as sickle cell anemia and cystic fibrosis using traditionally complex and time-intensive diagnostic methods.

Here, we highlight the advantages of long-read nanopore sequencing for DBS-based analyses to detect genetic disorders that may be overlooked otherwise. We present an optimised protocol for comprehensive variant identification from DBS using Whole Genome Sequencing (WGS), the PromethION™ device, and EPI2ME™ analysis platform. Our data from multiple donors showcases the improvements for seamless end-to-end processing, spanning sample, sequencing and analysis utilising the EPI2ME Human Variation workflow. This approach achieves sequencing and analytical performance comparable to WGS from whole blood extractions with reduced DNA input from DBS, demonstrating its effectiveness for high-quality genomic exploration.

This highlights the benefits of nanopore sequencing in conjunction with DBS and the potential to influence population scale genetic and epigenetic screening.

C.O.I. Disclosure:

H. Greenfield: A. Employment (full or part-time); Significant; Oxford Nanopore Technologies plc. E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Oxford Nanopore Technologies plc. **J. Gehring:** A. Employment (full or part-time); Significant; Oxford Nanopore Technologies plc. E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Oxford Nanopore Technologies plc. **P. Rescheneder:** A. Employment (full or part-time); Significant; Oxford Nanopore Technologies plc. F. Consultant/Advisory Board; Significant; Oxford Nanopore Technologies plc. **P. James:** A. Employment (full or part-time); Significant; Oxford Nanopore Technologies plc. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Oxford Nanopore Technologies plc. **B. Leland:** A. Employment (full or part-time); Significant; Oxford Nanopore Technologies plc. F. Consultant/Advisory Board; Significant; Oxford Nanopore Technologies plc. **S. Juul:** A. Employment (full or part-time); Significant; Oxford Nanopore Technologies plc. E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Oxford Nanopore Technologies plc.

EP15.004 X-linked sideroblastic anaemia associated with beta thalassemia trait presenting with a peculiar chronic anaemia phenotype in a 47-years-old women

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Background: Chronic anaemias represent a clinical challenge and are frequently associated with a prolonged diagnostic odyssey. We present the case of a 47-year-old woman affected by progressive severe anaemia and progressive splenomegaly since the age of 30. She was initially diagnosed with intermediate thalassemia due to an imbalance in alfa/beta globin chain synthesis (1.83), although only a single beta-globin gene mutation (HBB: c.93-21G > A) was identified. The serious clinical evolution was not consistent with the molecular data and further investigations were considered mandatory.

Materials and methods: Whole Genome Sequencing (WGS) was performed in the hypothesis of a germinal or somatic polygenic molecular pathogenesis.

Results: WGS revealed the c.1273C>A (p.Pro425Thr) maternally inherited pathogenic variant in the ALAS2 gene. Complete opposite skewed X inactivation has been observed in the blood of the proband and her healthy mother, explaining the observed contrasting phenotypes. A bone marrow biopsy confirmed the diagnosis showing the presence of 31% ringed sideroblasts in the proband. The ALAS2 variant is located within the dimerization interface region of the protein, likely impairing dimer stability and enzymatic activity, mapping in the proximity to the Vitamin B6 (VB6) binding site. Administration of high doses of VB6, an approved therapy for X-linked sideroblastic anaemia, has been attempted without any clinical benefit and the patient currently undergoes recurrent blood transfusions.

Conclusion: Complex acquired clinical phenotype, such as chronic anaemias, can be adequately diagnosed with comprehensive genomic analysis as they may pave the way for personalized treatment strategies, improved follow-up strategies and specific therapeutic options.

C.O.I. Disclosure:

F. Pintus: None. **N. Giordano:** None. **C.M. Gaglioti:** None. **I. Motta:** None. **A. Brusco:** None. **D.F. Giachino:** None. **G. Ferrero:** None. **G. Mandrile:** None.

EP15.005 Computational analysis of a familial CHD7 mutation

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CHARGE association is an autosomal dominant syndrome characterized by ocular Coloboma, Heart disease, choanal Atresia, Retarded growth and/or developmental delay, Genito-urinary defects and Ear anomalies. This condition's etiology is usually

correlated to CHD7 aploinsufficiency. The phenotype can be heterogeneous but three features show statistically significant correlation with CHD7 variants: cardiovascular malformations, eye anomalies and facial asymmetry. Although almost all CHARGE cases are sporadic, we report a caucasian family with a 2 years-old child affected by atrioventricular canal defect, cleft lip and palate, unilateral microphthalmia and optic nerve coloboma, mild motor delay and speech delay. Trio exome sequencing revealed a missense CHD7 mutation (NM_017780.4: c.2986G>T / p.Gly996Cys) inherited by unaffected mother. Molecular family studies identified the same variant also in the unaffected grandmother and in the maternal uncle, only showing cleft lip and palate. Variant studies using Missense 3D web tool showed a change between buried and exposed state of the target residue, FoldX analysis of the free energy revealed a destabilizing effect of this variant and in-silico prediction tools confirmed the pathogenic role of the p.Gly996Cys, suggesting a significant functional impact on the protein. This report highlights an extreme intrafamilial phenotypic variability associated with a likely pathogenic CHD7 missense mutation and underscores the importance of considering inherited variants in the molecular analysis of patients suspected of having CHARGE syndrome. Additionally, it emphasizes that a detailed family history can be crucial to identify affected cases that do not meet the classical diagnostic criteria for CHARGE syndrome.

C.O.I. Disclosure:

C. Cesario: None. **A. Mussa:** None. **A. Zonta:** None. **E. Pisaneschi:** None. **V. Daniela:** None. **K. Bejo:** None. **T. Mazza:** None. **A. Novelli:** None.

EP15.006 TTN gene mutation in a large pedigree in a family with arrhythmogenic cardiomyopathy

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Background: Titin (TTN), a sarcomeric protein, is a part of myofibrillar structure of the contractile apparatus of the cardiac muscle. Several variants in the TTN gene have been associated in patients with diverse types of cardiomyopathies. There are several forms to classify cardiomyopathies, they can be genetic/acquired/ mixed or familial-genetic/non-familial-non-genetic or hypertrophic/dilated/ restrictive/arrhythmogenic right ventricular. As an autosomal disease, they present genetic heterogeneity, clinical heterogeneity, variability in expression and incomplete penetrance.

Material and methods: In the present study, we analyzed through sequencing of exome and Sanger confirmation a family with twenty one members in three generations, seven of them with a TTN gene variant and of these, three of them had cardiac symptomatology.

Conclusion: The mutation c.59748C>G was detected in exon 27 and introduces a cysteine instead of serine at position 1937, a crucial region in the TTN protein.

C.O.I. Disclosure:

L. Escobedo-Jaimes: None. **H. Urueta-Cuellar:** None. **O. Messina-Baas:** None. **S. Cuevas-Covarrubias:** None.

EP15.007 A novel NLRP3 variant in a family with clinical and laboratory findings suggestive of Cryopyrin-Associated Periodic Syndrome

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Background: Cryopyrin-associated periodic syndromes (CAPS) are rare, autosomal dominant (AD) autoinflammatory disorders associated to NLRP3 gene. CAPS common clinical features include urticaria-like skin eruptions, edema, fever, and deafness, often accompanied by elevated inflammatory markers. In addition, elevated serum amyloid A (SAA) levels are also frequently associated with CAPS.

Material and methods: The proband, showing lower limb edema and urticaria-like eruptions, was investigated through WES. Variants discovered were classified according to ACMG recommendations and verified through Sanger sequencing. Familial segregation analysis was performed to examine genotype-phenotype associations. Clinical and laboratory assessments of family members included SAA.

Results: We assessed heterozygosity for the NLRP3 p.Cys998Ser variant, with a MAF of 0.009% in the gnomAD database. According to ACMG guidelines, it is classified as a variant of uncertain significance (VUS). However, in silico tools indicated discordant scores related to pathogenicity and cut-off optimization for PP3 and BP4 scores is ongoing. The mother, maternal grandmother and the proband's siblings carried the same genetic variant and exhibited high SAA levels, deafness and skin manifestation. This pattern supports an AD inheritance consistent with a CAPS-related disorder.

Conclusion: We identified a novel NLRP3 variant in a family with features suggestive of CAPS. Such a VUS deserves a more detailed study in order to improve the stratification of patients which could benefit from IL-1β; inhibition. Long-term follow-up are warranted to determine the pathogenicity and full phenotypic consequences of this variant and guide personalized management.

C.O.I. Disclosure:

G.P. Palladino: None. **L. Mercuri:** None. **M.P. Patrizio:** None. **S. De Summa:** None. **R. Santacroce:** None. **M. Margaglione:** None.

EP15.008 Mosaic duplication in 11p15: news about Beckwith-Wiedemann syndrome

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Background: Two growth disturbance syndromes, Beckwith-Wiedemann (BWS) and Silver-Russell (SRS), occur by dysregulation of two clusters of imprinted genes at the 11p15 region. Cytogenetic rearrangements are found in 3% of cases. 11p15 duplications have been rarely reported. We present the first mosaic 11p15 duplication associated with a BWS phenotype. Case report: The proband had the clinical diagnosis of BWS at birth, thirty years ago. Clinical findings were hemihyperplasia and helical pits. In adulthood, a genetic and reproductive assessment was requested. Reviewing her medical history, as newborn she underwent surgery for an "adrenal mass". Pathology analysis showed cytomegaly of the adrenal cortex. With this pathognomonic finding a complete genetic study was performed.

Results: The methylation analysis of the 11p15 region was carried out in samples from blood and saliva with MS-MLPA (Me030. MRC Holland). Results were non-conclusive. Sequence

analysis of *CDKN1C* gene was normal. To investigate somatic mosaicism, we recovered a paraffin block with the affected adrenal gland. MS-MLPA revealed hypomethylation of the centromeric imprinting center (IC2) and hypermethylation of the telomeric imprinting center (IC1) due to an interstitial duplication of approximately 1Mb within the 11p15 region. This finding, observed in mosaic (between 60–90%), was confirmed in a second DNA sample from the same tissue and with CGH-array.

Conclusion: The mosaic duplication in 11p15 associated with a BWS phenotype, represents a great challenge for diagnosis (availability of samples, interpretation) and genetic counselling (quantification of the risk to offspring, presumably low but difficult to estimate and the technical limitations for PGD).

C.O.I. Disclosure:

V. Garcia-Solaesa: None. **M. Artigas-Lopez:** None. **A. Perez-Juana Del Casal:** None. **S. Ciria Abad:** None. **M. Martinez-Rey:** None. **C. Garcia-Vicente:** None. **G. De Lima Piña:** None.

EP15.009 Case Report: An 8 Years Old Boy with Danon Disease, and muscle weakness

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Background: Danon disease is a rare X-linked lysosomal storage disorder caused by mutations in the *LAMP2* gene, which encodes lysosome-associated membrane protein 2 (LAMP-2). This protein is essential for autophagy. Hypertrophic cardiomyopathy, skeletal myopathy, and intellectual disability are the primary clinical presentations of this disease. Males are predominantly affected, while females may also carry it and have less severe symptoms. **Case presentation:** An 8-years-old boy was referred to our center that was born of a non-consanguineous marriage. He suffers from mild developmental delays and muscle weakness in the Pectoralis major, trapezius, shoulder & pelvic girdle muscles & positive Gowers sign. Also, signs of mild cognitive impairment include lack of sufficient concentration and having more trouble coming up with words than other people of the same age have been reported.

Material and methods: The family history & clinical examination was performed. WES was done using the Illumina platform and Agilent SureSelect V7 library preparation kit.

Results: A hemizygous variant (c.864+3_864+6del Splice junction loss) in the *LAMP2* gene standing as Pathogenic was detected in the case. Confirmation of this mutation and evaluation of the unaffected parents were done by Sanger sequencing method. The parents were normal.

Conclusion: Considering that psychoneurological symptoms associated with muscle weakness, the wide spectrum of diagnosis such as muscular disorder & neurological disorders are possible therefore WES could be considered. Genetic counseling for this couple and other relatives is highly recommended as the first line of prevention and leading to effective prenatal diagnosis.

C.O.I. Disclosure:

N. Tavakkoli Banizi: None. **S. Seyedhassani:** None. **S. Dashti:** None. **A. Danafar:** None.

EP15.010 Case-based diagnostic approach for syndromic aortic dilatation

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Background: Syndromic aortic dilatation is defined as aortic dilatation accompanied by intellectual disability or developmental delay (DD). Identifying genetic causes is crucial for diagnosis, management, and counseling.

Methods: We present two cases with intellectual disability/DD, dysmorphic features, and aortic dilatation, diagnosed using chromosomal microarray analysis (CMA) and whole-exome sequencing (WES).

Case 1: A 17-year-old female with ascending aortic enlargement (Z score: 5.1) had normal motor development but cognitive impairment. Physical examination revealed macrocephaly, hypertelorism, drooping nasal tip, and a short neck. CMA confirmed 7q11.23 duplication syndrome.

Case 2: A 16-year-old male with a history of ventricular septal defect (VSD) surgery had aortic root (Z score: 2.4) and ascending aortic (Z score: 2.9) enlargement. His parents were second-degree cousins. He exhibited cognitive impairment and dysmorphic features, including enophthalmos, synophrys, prognathism, and arachnodactyly. CMA was normal. WES revealed a homozygous TMEM94 variant (c.4003C>T), associated with intellectualdisability and cardiac defects.

Conclusion: In cases of aortic dilatation accompanied by developmental delay, a continuous gene deletion syndrome should be considered first, making CMA the preferred initial genetic test over a connective tissue panel. If CMA results are negative in syndromic cases, WES should be pursued to identify ultra-rare monogenic diseases associated with aortic dilatation. However, if WES includes CNV analysis, it can be considered a first-tier genetic test. A precise genetic diagnosis enables targeted risk assessment, surveillance strategies, and individualized management, emphasizing the need for a structured diagnostic approach and long-term follow-up.

C.O.I. Disclosure:

E. Eker: None. **H. Mutlu:** None.

EP15.011 A large deletion, including entire *SF3B4*, identified in a patient with clinical diagnosis of Nager Syndrome

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Background: Acrofacial dysostosis 1, also known as Nager syndrome (AFD1, OMIM #154400), is characterized by craniofacial malformations showing micrognathia with cleft palate and lip, down-slanted palpebral fissures and abnormal ears, and pre-axial limb defects such as hypoplasia or absence of the thumb. Hearing loss is often present. More than 40 loss-of-function variants of the *SF3B4* (splicing factor 3B, subunit 4) gene have been reported in AFD1 patients to date. Almost all of these variants are SNVs or small indels. We report here a patient with clinical diagnosis of Nager Syndrome, with a deletion of the entire *SF3B4* gene.

Patient: The patient was a 5-year and 9-month-old boy born to non-consanguineous parents. He has distinctive facial features with down-slanting palpebral fissures, a significantly small mandibular with retrognathia, low-set ears, and deafness. He also shows thumb hypoplasia, right elbow joint contracture, and failure to thrive. His development was mildly delayed.

Method: After obtaining written informed consent from the patient's parents, we performed trio-based whole-exome sequencing analysis using genomic DNA extracted from peripheral blood. The sequence reads were aligned to GRCh38 and annotated using CompStor NOVOS and Insight (OmniTier). The called variants were

narrowed down based on allele frequency and the assumed modes of inheritance. Copy number variants were detected using tools we developed.

Results and Discussion: A de novo deletion of the entire *SF3B4* gene was identified. To our knowledge, this is the third case of AFD1 patients. Our results strongly support haploinsufficiency is the major cause of this disease.

C.O.I. Disclosure:

K. Yanagi: None. **M. Yamamori:** None. **S. Mizuno:** None. **T. Kaname:** None.

EP15.012 Combination of 1p36 deletion/duplication and 9p24.3 duplication in an Ukrainian patient with congenital malformations

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Background: Chromosomal rearrangements involving 1p36 deletion/duplication and 9p duplication are rare and linked to developmental delay, intellectual disability, and craniofacial abnormalities. 1p36 deletion syndrome is associated with hypotonia, growth deficiency, and congenital anomalies, while 1p36 duplication may present with mild intellectual disability, metopic craniosynostosis, and dysmorphic features. 9p duplication syndrome is commonly associated with skeletal abnormalities, short stature, and motor and cognitive delays. We describe a Ukrainian patient with a combined 1p36 deletion and duplication and 9p24.3 duplication.

Materials and methods: Karyotyping peripheral blood lymphocyte culture was performed using GTG banding method. MLPA was conducted on DNA extracted from leukocytes using P245 and P036 probe sets to confirm structural rearrangements.

Results: The 7-year-old male patient exhibited craniosynostosis, frontal bossing, partial synophrys, craniofacial dysmorphism, hirsutism, microbrachycephaly, low-set ears, short philtrum, and dental anomalies. Neurologically, he had hydrocephalus, corpus callosum thinning, valgus foot deformity, and impaired gait. He displayed limited speech comprehension, selective eating habits, frequent respiratory infections, and sleep disturbances, with a diagnosis of autism spectrum disorder (ASD).

Cytogenetic analysis revealed 46,XY,add(1)(p36.3), indicating an additional segment on 1p36.3. MLPA detected a heterozygous duplication of *GNB1* and *GABRD* in 1p36, a heterozygous deletion of *TNFRSF4* in 1p36, and a heterozygous duplication of *DMRT1* in 9p24.3, confirming 9p involvement.

Conclusions: Comparison with 1p36 deletion syndrome revealed overlapping craniofacial dysmorphism, hypotonia, and intellectual disability. Distal 1p36 trisomy is associated with mild intellectual disability, developmental delay, and craniofacial abnormalities. Features of 9p duplication syndrome, including skeletal abnormalities and cognitive delay, were also present.

C.O.I. Disclosure:

H. Bezkorovaina: None. **M. Dushar:** None. **O. Tyshchenko:** None. **N. Huleyuk:** None. **H. Makukh:** None.

EP15.013 A rare association of two genetic disorders: aldosterone synthase deficiency and XYY syndrome

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Background: Aldosterone synthase deficiency (ASD) is a rare cause of salt-wasting syndrome in infants. It is characterized by hyponatremia, hyperkalemia, increased plasma renin activity and elevated aldosterone concentrations. Patients may experience failure to thrive, vomiting, severe dehydration. Mutations in the *CYP11B2* gene encoding Aldosterone synthase are responsible for the occurrence of ASD. The inheritance is autosomal recessive. XYY syndrome is caused by an aneuploidy and is characterized by a variable neurodevelopmental phenotype, with features including developmental delays, cognitive impairments, and an increased risk for mental health conditions. We present a case with these different types of molecular and chromosomal disorders.

Material and methods: The patient was clinically diagnosed in neonatal period with salt-wasting syndrome. Adrenogenital syndrome was ruled out. The patient received treatment with fludrocortisone with good outcome. Suspicion of ASD was raised and molecular testing was performed at the age of 4 years.

Results: Molecular testing in a panel of 401 genes involved in renal diseases detected two different pathogenic variants in *CYP11B2* gene: c.554C>T (p.Thr185Ile) and c.788T>A (p.Ile263Asn). This compound heterozygous status is concordant with the patient phenotype. The patient does not come from a consanguineous family or geographically isolated region. GTG karyotyping shows the presence of an extra Y chromosome.

Conclusion: The association of a molecular disorder and a chromosomal one isn't common. Genetic testing is important for the diagnosis of ASD which has clinical and paraclinical signs that overlap with other salt-losing tubulopathy/other diseases. It is also important for the appropriate genetic counseling.

C.O.I. Disclosure:

L. Caba: None. **L. Leustean:** None. **C. Rusu:** None. **M. Gramescu:** None. **V. Martiniuc:** None. **I. Iordache:** None. **A. Florea:** None. **M. Panzaru:** None. **E. Gorduza:** None.

EP15.014 The first case of homozygous microdeletion involving *EMC10* gene: expanding mutational spectrum in patients with neurodevelopmental disorder with dysmorphisms and variable seizures (NEDDFAS)

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Background: *EMC10* (MIM *614545) encodes for a subunit of the endoplasmic reticulum (ER) membrane protein complex, responsible for the post-translational insertion of membrane proteins into the ER. Biallelic loss of function variants cause Neurodevelopmental disorder with dysmorphisms and variable seizures (NEDDFAS; #619264) inherited in an autosomal recessive manner. We describe a patient with developmental delay, seizures, scoliosis and nephrocalcinosis, carrier of a homozygous microdeletion involving the *EMC10* gene within a small run of homozygosity (ROH) on chromosome 19. As far as we know, this is the first case of microdeletion involving *EMC10*.

Material and methods: CNVs (Copy Number Variations) and ROH analysis was performed on the proband and his parents using the SNP-array platform Illumina CytoSNP-850K and BlueFuse Multi Software.

Results: SNP-array analysis disclosed a homozygous microdeletion at 19q13.33 region, of about 33 kb (from 50,449,796 bp to 50,482,835 bp; GRCh38/hg38), and including part of *EMC10* gene and two more OMIM genes, *MYBPC2* and *GARIN5A*. The microdeletion mapped within a 3.4 Mb ROH and was inherited from the heterozygous non-affected parents.

Conclusion: To date, 30 individuals have been described with pathogenic biallelic frameshift, stop gained and splicing variants in *EMC10* and functional studies have demonstrated *EMC10* expression is substantially reduced in affected individuals. No intragenic microdeletions/microduplications or larger deletions of whole *EMC10* gene have been reported. Our findings confirm *EMC10* loss-of-function as pathogenic mechanism and expand its mutational spectrum. Moreover, this study supports the importance of CNVs analysis in order to establish the diagnosis of NEDDFAS and improves genotype-phenotype correlation.

C.O.I. Disclosure:

M. Genovesi: None. **S. Loddo:** None. **V. Orlando:** None. **S. Genovese:** None. **S. Di Tommaso:** None. **G. Catino:** None. **C. Calacci:** None. **M. Digilio:** None. **A. Novelli:** None. **R. Capolino:** None. **V. Alesi:** None.

EP15.015 Non-compacted myocardium and ovarian teratoma in patient with a novel *BMP2* variant: expanding clinical insights

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Background: ***B****MP2* haploinsufficient variants are causative of a syndrome characterized by short stature, skeletal anomalies, and congenital heart disease. Recently, a cohort of 18 BMP2 cases have been reported further delineating clinical phenotype variability of the condition[1]. BMP2 has been reported to modify tumor cell behavior through a change in motility and adherence in ovarian cancer but its role on carcinogenesis still needs to be elucidated[2,3]. To date non-compacted myocardium, epidermal nevi and ovarian teratoma have not been reported as part of the BMP2-spectrum.

Material and methods: A 24-year-old female was assessed at Fondazione Policlinico Universitario Agostino Gemelli for ovarian teratoma, short stature, skeletal dysplasia, myocardial non-compaction epidermal nevus and a sternal keloid. Clinical exome sequencing (CES) was performed at our hospital using capture-based Next Generation Sequencing (NGS) Clinical Exome Solution® v3 kit (SOPHiA Genetics). Additionally, an SNP-array analysis was performed to detect CNV and regions of homozygosity.

Results: An heterozygous c.127dup (p.Ser43fs) variant of *BMP2* gene was identified in the proband and in her mother, who exhibited a milder phenotype. SNP-array analysis revealed a homozygous region in 2q32.1q32.2, lacking disease-associated genes.

Conclusion: We report a likely pathogenic BMP2 variant in a patient with short stature, non-compaction cardiomyopathy, epidermal nevus, and ovarian teratoma, expanding the BMP2 phenotype. While BMP2's role in skeletal and cardiac anomalies is characterized, its link to ovarian teratoma remains unclear. Further research is needed to assess BMP2's potential role in tumorigenesis.

C.O.I. Disclosure:

C. Modafferi: None. **V. Trevisan:** None. **P. Concolino:** None. **D. Orteschi:** None. **G. Zampino:** None. **E. Lucci Cordisco:** None. **A. Minucci:** None. **C. Leoni:** None.

EP15.016 Rare ophthalmic manifestations in adult patients with hunter syndrome: personalized therapy

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Hunter syndrome (HS, mucopolysaccharidosis type II) is a rare disorder characterized by glycosaminoglycan (GAG) accumulation, leading to multisystem pathology. While corneal clouding is the most recognized ophthalmological manifestation, we present previously unreported ocular complications in adult patients. We examined two adult siblings with HS who exhibited progressive hyperopia. Hypothesizly hyperopia progression results from GAG accumulation in the sclera, leading to a gradual reduction in eyeball size and increases the distance between the focal point and the retina. Additionally, scleral shrinkage displaces intraocular structures manifestes in retinal folding observed via optical coherence tomography. Retinal folding near the optic disc may mimic or exacerbate chronic optic disc edema (pseudo-papilledema) and, in some cases, contribute to optic nerve atrophy due to lamina cribrosa narrowing.

GAG deposition in the retinal pigment epithelium leads to progressive photoreceptor loss, presenting as bone spicule-like changes, which reduce dark adaptation and peripheral visual field constriction. Moreover, vitreous body compaction, combined with physiological lens thickening, creates anatomical predispositions for angle-closure glaucoma. Elevated intraocular pressure, if untreated, results in irreversible optic nerve damage, necessitating early YAG laser peripheral iridotomy. For advanced cases, lens extraction with high-power intraocular lens (IOL) implantation is required, but the small anterior chamber and extreme hyperopia pose significant surgical challenges. Custom-made IOLs are essential for proper refractive correction, as standard lenses do not meet these patients' needs. This case underscores the need for personalized ophthalmic management in HS to prevent vision loss.

C.O.I. Disclosure:

A. Kononov: None. **N. Kononova:** None. **S. Rashidova:** None. **V. Larionova:** None.

EP15.017 Non-coding genetic cause of BPES: PISRT1 in a polish family case study

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Blepharophimosis syndrome (BPES) is a rare autosomal dominant disorder primarily associated with pathogenic variants in the FOXL2 gene. This case study explores a Polish family comprising a 10-year-old boy and girl, their father, and grandmother, all

presenting with BPES characteristics. Genetic analysis revealed a heterozygous deletion of the PISRT1 gene, a long non-coding RNA upstream of FOXL2, in the proband. Sanger sequencing of the FOXL2 gene in the sister showed no pathogenic variants, suggesting the PISRT1 deletion as the causative factor. This finding highlights the significance of non-coding regulatory elements in BPES etiology. The variability in phenotypic expression within the family indicates potential variable expressivity or incomplete penetrance. This study underscores the necessity of comprehensive genetic testing, including copy number variation analysis, to detect pathogenic deletions beyond coding regions. These insights have important implications for genetic counseling and the management of BPES patients.

C.O.I. Disclosure:

E. Preizner-Rzucidlo: None. **K. Możdżeń:** None.

EP15.018 In silico elucidation of a rare missense variant in the calcium-binding domain of RET associated with famial variable penetrating Hirschsprung disease

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Background: The *RET* gene encodes a receptor tyrosine kinase critical for diverse cellular mechanisms, including neuronal navigation, cell migration, and intestinal organogenesis via different signaling pathways. Variations within this protein disrupt its structural stability and functional conformation, contributing to Hirschsprung's disease (HSCR). This study investigates the novel D230H variation, located in the calcium-binding site of the cadherin-like domain 2 (CLD2), which was identified in a three generation familial HSCR case. In the pedigree, some female carriers developed HSCR, while an asymptomatic mother and grandmother remained unaffected, indicating incomplete penetrance. This research provides the first insights into the mechanistic implications of D230H in HSCR pathogenesis, offering a novel perspective on RET dysfunction.

Material and methods: DNA samples from affected family members were analyzed through whole-exome sequencing, and variants were validated using Sanger sequencing. The D230H variation was modeled in the RET CLD2 domain, and molecular dynamics simulations were performed to assess its effects on calcium binding and protein stability. Pathogenicity predictions were conducted using bioinformatic tools.

Results: Structural modeling and functional analyses predict an increased binding pocket volume and altered protein conformation, supporting the potential pathogenicity of this variation and suggesting a potential mechanistic link to HSCR. Pathogenicity prediction tools classified the variation as likely pathogenic, and to our knowledge, this specific variation has not been previously reported in the literature.

Conclusion: Our findings highlight the critical role of the calcium-binding site in RET function, offering novel insights into the pathogenesis of HSCR.

C.O.I. Disclosure:

E.N. Cakinberk: None. **A.A. Shukur:** None. **E. Acar:** None. **E. Eker:** None. **D. Duman:** None. **H. Mutlu:** None.

EP15.019 Familial Occurrence of Congenital Cataracts and Identification of a Novel Homozygous *COLL11A1* Variant in Three Siblings Born to Consanguineous Parents

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Background: Congenital cataract is a major cause of pediatric blindness, often with a genetic basis. We report three siblings from consanguineous parents, all diagnosed with bilateral congenital cataracts. The eldest, a 12-year-old boy, also presented with hearing loss, reduced vertebral body height, and following cataract surgery, developed bilateral retinal detachment. The second, a 10-year-old girl, exhibited bilateral conductive hearing loss and developed right-sided retinal detachment after cataract surgery. The youngest, a seven-year-old boy, had isolated bilateral congenital cataracts. All three exhibited a long philtrum, flat midface, and mild kyphoscoliosis. Their maternal aunt and her consanguineous husband have three daughters with congenital cataracts. All parents were unaffected.

Material and methods: Whole exome sequencing (WES) was performed on the parents and affected siblings to identify potential pathogenic variants. In silico tools and gnomAD database analysis were used to assess the variant's pathogenicity and frequency.

Results: WES identified a novel homozygous c.4944C>G (p.Cys1648Trp) variant in the *COL11A1* gene in all siblings. This variant, located in the COLFI domain, is predicted to be pathogenic and did not exist in the gnomAD database. Clinical features suggest Marshall-Stickler syndrome, typically autosomal dominant, but homozygosity in the siblings indicates a rare genetic mechanism. The maternal aunt and uncle are heterozygous for the variant, and analysis of their affected daughters is ongoing.

Conclusion: This study highlights a novel homozygous *COL11A1* variant associated with Marshall-Stickler syndrome and underscores the importance of genetic testing for hereditary ocular conditions especially in countries with a high rate of consangineous marriages.

C.O.I. Disclosure:

K. Secgen: None. **H. Ozdemir:** None. **K. Cilesiz:** None. **S. Ozdek:** None. **I. Okur:** None. **F. Ezgu:** None. **H. Atalay:** None.

EP15.020 A new chapter in the *NEK8* story: Neonatal Cholestasis and Severe Liver Dysfunction

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Background: Progressive familial intrahepatic cholestasis (PFIC) is a heterogeneous group of rare autosomal recessive disorders characterized by cholestasis, pruritus, jaundice, hepatomegaly and growth failure. About one-third of patients remain genetically undiagnosed, and new genetic causes of PFIC are still being discovered. In recent years, the *NEK8* gene has been associated with a complex syndrome that includes cholestasis as one of its clinical manifestations.

Material and methods: The patient is a child initially affected by severe cholestasis, who subsequently developed cardiac and renal involvement, which are key features in patients with *NEK8* mutations. Genomic DNA was isolated from peripheral blood and

underwent WES and bioinformatic analysis to identify disease-causing mutations. Sanger sequencing was performed to confirm mutations and investigate segregation within the family. Identified mutations were further analyzed through kinase assay to assess their pathogenic effect.

Results: Exome analysis revealed two novel compound heterozygous missense variants (c.G328A;p.V110M (rs886566274) and c.G380A;p.R127Q (rs2034347673)) in the kinase domain of the *NEK8* gene (NM_178170). Both variants are rare in the general population and predicted to be damaging by in silico prediction tools. Protein expression analysis revealed no difference between the levels of the wild-type and the mutated proteins. Kinase assay showed drastically reduced kinase activity of the mutated protein compared to the wild-type one.

Conclusion: These results could explain the PFIC phenotype and the extrahepatic involvement of heart and kidneys in our patient. This study expands the phenotypic spectrum associated with *NEK8* mutations, but further studies are needed to fully understand the genotype-phenotype correlation in this context.

C.O.I. Disclosure:

I. Ziccardi: None. **M. Di Stazio:** None. **A. Bianco:** None. **G. Indolfi:** None. **F. Giambuzzi:** None. **F. Kharrat:** None. **R. Moura:** None. **A. d'Adamo:** None.

EP15.021 Mythical genes in neurodevelopmental delay - three Bulgarian cases

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Background: Developmental delay can present in a diverse range of phenotypes. These clinical manifestations can be caused by genetic variants in a large number of genes, many of which are not yet known as disease-causing or are not yet connected to a specific phenotype. We report three patients with potential novel genetic disorders or with potential novel inheritance model.

Material and methods: In all three cases WES was performed for the target patient, and in one of the cases for the healthy mother and sibling as well. After analysis, potential causative genetic variants were confirmed and segregated in the families via Sanger sequencing. Novel genes were submitted in the Gene-Matcher platform.

Results: Case 1 is a boy with autistic behaviour with a de novo missense variant in the *KCTD2* gene, which has not been associated with disease yet. Case 2 is a girl with intellectual and speech delay, who carries two missense variants in the *HECTD4* gene - one de novo and one paternally inherited, where only autosomal-recessive inheritance has been reported so far. Case 3 is a girl with psychomotor delay, carrying an assumed de novo frameshift variant in the *HDLBP* gene, which has not been associated with disease yet.

Conclusion: The genetic reason for the clinical presentation of the reported cases was potentially found via WES. More research is needed to confirm the relation between these genes and phenotypes. Understanding the genetic etiology of neurodevelopmental disorders is crucial for the medico-genetic care of patients.

C.O.I. Disclosure:

M. Sleptsova: None. **S. Atemin:** None. **Z. Pavlova:** None. **T. Todorov:** None. **V. Mitev:** None. **A. Todorova:** None.

EP15.022 Siblings with Coffin-Siris Syndrome - Germline Mosaicism in the mother

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Background: A girl born in 2012 to healthy parents exhibits delayed motor development, intellectual disability, epilepsy, dysphagia, dysmorphic features, hypertrichosis, hypoplastic fifth fingers, and muscular weakness. The patient's younger brother, born in 2022, presents with similar symptoms. The parents are seeking a cause for the siblings' condition.

Methods: Trio whole-genome sequencing (WGS) was performed on the patient born in 2012. The younger brother was analyzed using droplet digital PCR.

Results: Trio-WGS revealed a pathogenic (class 5) 4.7 kb heterozygous deletion on 6q25.3 in the patient. This deletion encompasses a significant portion of the *ARID1B* gene and is diagnostic for Coffin-Siris Syndrome (OMIM #135900). Neither parent was found to carry the *ARID1B* deletion in their blood samples. The patients remaining *ARID1B* contained several hemizygous SNPs, which the mother did not carry, and the father was heterozygous for hence the deletion has occurred on the maternally inherited chromosome. The younger brother was found to carry the identical *ARID1B* deletion identified in his sister.

Conclusion: Both siblings have identical pathogenic *ARID1B* deletion diagnostic for autosomal dominant Coffin-Siris Syndrome. Although the deletion is not detected in the mother's blood sample it is on the maternally inherited chromosome, and the recurrence in the siblings demonstrates maternal germline mosaicism for this variant. This case highlights the importance of considering the well-documented phenomenon of gonadal mosaicism in a parent when a seemingly de novo variant is identified. Awareness of this phenomenon is essential as it can significantly impact recurrence risk.

C.O.I. Disclosure:

K. Karrman: None.

EP15.023 Sequence analysis of exons of the *CASQ2* gene in families with a patient with Catecholaminergic Polymorphic Ventricular Tachycardia Hypertension referred to Rajaie cardiovascular institute

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Background: CPVT is a rare inherited disorder characterized by abnormal heart rhythms (arrhythmias). It can lead to cardiac arrest and sudden death. One known cause of CPVT is mutations in the *CASQ2* gene, which account for 1–2% of all CPVT cases. This study aims to perform sequence analysis of exons 1 to 11 of the *CASQ2* gene in ten families with members affected by CPVT, referred to the Rajaie Cardiovascular Institute.

Material and methods: From patients referred to the Rajaie Cardiovascular Institute between 2023 and 2024, ten individuals diagnosed with CPVT by a cardiologist were selected for this study. Informed consent was obtained from all participants. Family pedigrees were constructed, and blood samples were collected. Genomic DNA was extracted and amplified using PCR, with successful amplification confirmed by gel electrophoresis. The amplified DNA samples were then sent for Sanger sequencing. If a pathogenic variant was identified in a patient, additional blood samples were collected from their family members for segregation analysis. Identified variants were analyzed using bioinformatics tools to predict their pathogenicity and potential role in disease causation.

Results: By examining all exons of *CASQ2* gene, homozygous (c.98G>A) and heterozygous (c.265_266insTA) variants were identified, in exon 1 and exon 2, respectively. Segregation analysis of family members suggests a hereditary pattern for these variants within the respective families and their possible role in the observed clinical phenotypes.

Conclusion: Investigating the CASQ2 gene enhances the understanding of CPVT pathology and may pave the way for improved preventive and therapeutic strategies.

C.O.I. Disclosure:

S. Farokhinia: None. **I. Salahshourifar:** None. **S. Kalayinia:** None.

EP15.024 STARD9 and CDK5RAP2 - novel candidate genes for oligogenic 46,XY complete gonadal dysgenesis

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Background: Disruption of genetic program that controls early embryonic testicular development can lead to 46,XY gonadal dysgenesis (46,XY GD), characterized by absence of testicular tissue and presence of female genitalia (46,XY CGD) or underdeveloped testes and ambiguous genitalia. The genetic background of 46,XY GD is solved only in around 50% of patients. Recent studies support the concept that some 46,XY GD cases may result from oligogenic origin.In this study, we investigate a 46,XY CGD patient in whom WES did not reveal any variants in known 46,XY GD genes that could explain the phenotype, aiming to identify novel candidate genes for this condition.

Material and methods: WES was performed on DNA of the 46,XY CGD patient and her parents. Bioinformatic analysis and simulation of mutant proteins were conducted.

Results: WES revealed variants in two genes encoding interacting proteins: steroidogenic acute regulatory-related lipid transfer domain 9 (STARD9) and CDK5 regulatory subunit-associated protein 2 (CDK5RAP2). Molecular simulation revealed that mutations in both genes can affect some regulatory patterns responsible for the PPI interactions.

Conclusion: Cooperation of identified variants may contribute to the 46,XY CGD phenotype development. Based on the results of this study, prior evidence on the expression patterns of STARD9 and CDK5RAP2 in developing gonads as well as their known cellular functions, we propose that these genes should be considered candidate genes for 46,XY CGD, potentially acting in an oligogenic mode of inheritance and can be included for DSD genes panel.

C.O.I. Disclosure:

L. Livshits: None. **D. Sirokha:** None. **A. Rayevsky:** None. **M. Khalangote:** None. **A. Gorodna:** None. **J. Jaruzelska:** None. **K. Kusz-Zamelczyk:** None. **S. Nef:** None.

EP15.027 Bainbridge Ropers Syndrome: Clinical and Genetic Evaluation of Three Patients

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Background: Bainbridge-Ropers Syndrome (BRPS) is an ultra-rare neurodevelopmental disorder caused by de novo pathogenic variants in the *ASXL3* gene, which is located on chromosome 18q12.1. This gene is crucial for chromatin remodeling and regulating gene expression. BRPS is characterized by intellectual disability, developmental delays, and distinct facial features. The disorder is inherited in an autosomal dominant pattern and has been described in fewer than 100 cases worldwide. We aimed to evaluate the clinical and genetic characteristics of three patients diagnosed with BRPS.

Material and methods: Three patients with a history of developmental delay, facial dysmorphism, and hypotonia were included in the study. After evaluation of clinical and family histories, probands were screened via next-generation sequencing (NGS). Segregations were performed via Sanger Sequencing.

Results: Genetic analyses revealed c.3039+1G > A, c.3025del (p.Val1009Phefs*16), c.4120_4123dup (p.Ala1375Aspfs*7) variants in the *ASXL3* (NM:030632.3) gene. The c.3039+1G > A and c.4120_4123dup variants have been reported as pathogenic in the Clinvar database, whereas the c.3025del p.Val1009Phefs*16 variant has not been previously reported in the literature and was evaluated as likely pathogenic according to ACMG criteria (PVS1, PM2). As a result of the segregations, it was determined that the variants detected were de novo.

Conclusion: Clinical findings in BRPS vary considerably between patients. Since very few patients have been reported, it is not possible to establish a genotype-phenotype correlation. We contributed to the literature with three patients and one novel variant. Defining the molecular etiology of BRDS is important for appropriate genetic counseling.

C.O.I. Disclosure:

S. Demir: None. **M. Soğukpınar:** None. **C. Alavanda:** None.

EP15.028 A rare association of Cockayne syndrome and Mitochondrial complex 1 deficiency: Contigious gene deletion of *ERCC8* and *NDUFAF2* genes

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Background: Cockayne syndrome is characterized by growth retardation, a characteristic appearance described as "cachectic dwarfism," progressive microcephaly, white matter demyelination in the brain, pigmentary retinopathy and/or cataracts, peripheral neuropathy, and dental anomalies. The syndrome shows variability in severity and age of onset, and molecular diagnosis is made by the presence of biallelic pathogenic variants in *ERCC6* and *ERCC8* genes. The coexistence of Cockayne syndrome and mitochondrial disorders has been reported. Here, we present a patient diagnosed with contiguous deletions of *ERCC8* and *NDUFAF2* genes.

Material and methods: The patient is a 4-month-old female who initially presented with congenital cataracts, hypotonia and microcephaly. She is the firstborn child of consanguineous parents and was born at term. During follow-up, the patient developed nausea, vomiting, Kussmaul breathing, lethargy, metabolic acidosis, and lactic acidosis. The patient's CT showed signs of cerebellar atrophy. ARRAY-CGH was performed for etiology. The patient developed mitochondrial disease decompensation and died at the age of 9 months despite supportive treatment.

Results: ARRAY-CGH analysis displayed a homozygous ~ 185kb microdeletion of chromosome 5q12.1. The deletion encompassed

both the ERCC8 (12/12 exons) and NDUFAF2 (1/4 exon) genes, which are responsible for Cockayne syndrome (CS) and mitochondrial complex I deficiency, nuclear type 10, respectively. Parents were found to be heterozygous carriers of the deletion.

Conclusion: Contiguous gene deletions create challenges in establishing genotype-phenotype correlations. Here, we wanted to draw attention to the co-occurrence of Cockayne syndrome and Mitochondrial complex 1 deficiency, nuclear type 10, which are both extremely rare conditions with high morbidity and mortality.

C.O.I. Disclosure:

G. Nacak: None. **G. Öner:** None. **B. Nur:** None. **S. Yakut Uzuner:** None. **A. Manguoğlu:** None. **E. Mıhçı:** None.

EP15.029 Utility of WGS in clinical practice. A new avenue for patient diagnosis, treatment, and reproductive risk management - a case report

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Background: Integrating whole genome sequencing (WGS) into routine clinical practice marks a pivotal advance in modern medicine. It facilitates precise diagnoses and personalized treatment and also promotes a more preventive approach.

Material and methods: We describe a 27-year-old female with myoclonic dystonia who underwent WGS for genetic diagnosis. She received genetic counseling beforehand and chose to have incidental findings reported.

Results: WGS identified the likely pathogenic c.546_547del variant in the SGCE gene, confirming the diagnosis of autosomal dominant Myoclonus-Dystonia. Incidental findings reported included the likely pathogenic deletion in MSH6 exon 5 (linked to Lynch syndrome type 5), four heterozygous pathogenic/likely pathogenic variants in genes associated with autosomal recessive disorders, and several pharmacogenomic associations. After testing, the patient received detailed counseling. The result clarified her diagnosis and treatment plan, while evaluating her offspring's risk of inheriting the SGCE mutation and developing the condition. She was also counseled regarding the risk of MSH6-related cancers, her children's risk of inheriting the MSH6 variant, and the reproductive options available, such as preimplantation genetic testing. Also, she was informed of the importance of testing her immediate family for the identified variants in the SGCE and MSH6 genes. Additionally, testing her reproductive partner for mutations in the genes for which she is a carrier and reviewing pharmacogenomic findings before implementing specific treatments were advised.

Conclusion: WGS provided extensive, actionable data that supports more personalized management, including treatment, enhanced cancer surveillance and informed family planning for this patient.

C.O.I. Disclosure:

A.T. Neacsu: A. Employment (full or part-time); Significant; Helixomic SRL. **A. Rad:** A. Employment (full or part-time); Significant; Arcensus GmbH. **N. Ameziane:** A. Employment (full or part-time); Significant; Arcensus GmbH. **G.E. Oprea:** A. Employment (full or part-time); Significant; Arcensus GmbH.

EP15.030 Rare unbalanced chromosomal rearrangement involving chromosomes 4 and 14

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Background: Blepharocheilodontic (BCD) syndrome is a rare autosomal dominant disorder characterized by lower eyelid ectropion, upper eyelid distichiasis, euryblepharon, bilateral cleft lip and palate, and conical teeth (1). Larger deletions on chromosome 4p16.3 beyond the critical Wolf-Hirschhorn syndrome (WHS) region (~1.7 Mb) may result in additional congenital anomalies. Partial duplication of chromosome 14q32 has been associated with facial dysmorphism and a predisposition to myeloproliferative neoplasms.

Material and methods: We report a case of an unbalanced chromosomal rearrangement involving partial monosomy of 4p and trisomy of 14q, diagnosed using whole-exome sequencing (WES) and chromosomal microarray (CMA).

Results: A 6-month-old female presented with dysmorphic features, including bifrontal narrowing, hypertelorism, lagophthalmia, euryblepharon, lower eyelid ectropion, prominent nasal bridge, low-set ears, cleft palate, V-shaped mouth, and micrognathia. Cardiac anomalies included autism spectrum disorder and ventricular septal defect. NGS-based CNV analysis revealed a 5.9 Mb terminal loss at 4p16.3p16.2 and a 12.9 Mb terminal gain at 14q32.12q32.33, confirmed by CMA. These findings suggest BCD syndrome with additional phenotypic contributions from 14q duplication.

Conclusion: Our patient lacks the classical WHS phenotype, likely due to the larger 4p deletion. The associated 14q duplication may further contribute to the observed dysmorphism. We previously reported a boy with classical BCD without detectable genetic alterations, underscoring the complexity of this disorder (2).

C.O.I. Disclosure:

Y.I. Hakami: None. **F. Ababneh:** None.

EP15.031 Novel EXT1 gene loss of function mutations in Tunisian patients with hereditary multiple exostoses

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Background: Hereditary Multiple Exostoses (HME) is an inherited autosomal dominant skeletal disease characterized by the development of multiple benign osteocartilaginous growths and is most commonly caused by *EXT1* mutations. This study aimed to identify the underlying genetic defects of HME in six Tunisian families (HME1- HME6) by *EXT1* gene mutation screening.

Material and methods: *EXT1* gene was analyzed using PCR-sequencing analysis of the coding regions and exon-intron boundaries. Bioinformatic tools assessed the in-Silico analysis of the mutation functional effect and the predicted 3D structural modeling of the EXT1 protein.

Results: *EXT1* gene sequencing revealed three novel pathogenic variants (NM_000127.3:c.1806dup, NM_000127.3:c.784del, and NM_000127.3:c.951del) in unrelated Tunisian probands, leading to a premature translation termination. furthermore, a previously described nonsense mutation NM_000127.3:c.482T>G was identified, leading to a premature codon stop and truncated EXT1 protein expression (NP_000118.2:p.L161*). The functional mutation prediction tools revealed that the newly identified mutations may affect the EXT1 protein function and lead to the disease occurrence. Similarly, the structure modeling analysis revealed that the identified *EXT1* mutations disturbed the predicted 3D structure and led to the protein's function loss. By screening the coding regions of the *EXT1* gene, we identified 11 SNP-type variations, including 5 exonic SNPs, 4 intronic variants, and 2 variants in the 3'UTR region. The prediction of

the functional impact of identified exonic SNP variants did not show any effects on the protein function.

Conclusion: Our findings expand the HME-causing variants spectrum and provide further evidence for *EXT1* gene involvement in the HME pathogenesis.

C.O.I. Disclosure:

S. Sfar: None. **M. Lahimer:** None. **E. Kerkeni:** None. **L. Krawa:** None. **A. Kenani:** None. **K. Monastiri:** None.

EP15.032 Gray matter heterotopia in a cohort of children with autism spectrum disorders

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Background: Gray matter heterotopia (GMH) is a neuronal migration (NM) defect that may lead to various neurodevelopmental phenotypes, including autism spectrum disorders (ASDs). ASDs are complex neurodevelopmental conditions with incompletely defined pathophysiological mechanisms. A link between abnormal NM and ASD has been recently proposed based on genetics and brain anatomical changes (1,2). Aim: to assess the genetic defects in a small ASDs pediatric group presenting with GMH.

Material and methods: Four children were diagnosed with ASD and GMH from a larger ASD group (305); array-CGH performed with 4 × 180K SurePrint G3 Human CGH microarray kit (Agilent Technologies); libraries obtained with Ampliseq Exome Panel and sequenced on NextSeq 550 system (Illumina).

Results: Array-CGH analysis revealed two variants of undetermined significance (VUS) in two patients involving RBFOX1 and IMMP2L genes. Defects in RBFOX1 were shown to alter the expression of genes involved in cortical malformations as well as ASD. WES detected one pathogenic variant in OFD1 gene explaining the phenotype (3). Two patients harbored VUS in two candidate genes for NDD, PTPRZ1 and CASZ1.

Conclusion: Our study found several new or rare genetic variants in candidate genes for NDD and in genes known to be relevant for ASD and/or GMH. Our data adds to the spectrum of genetic variants reported in children with ASD presenting with GMH. Acknowledgment: Regional Center of Medical Genetics Dolj, Emergency County Hospital Craiova for technical support/Illumina NextSeq550 access.

C.O.I. Disclosure:

S. Papuc: None. **M. Budisteanu:** None. **A. Erbescu:** None. **A. Glangher:** None. **M. Dobre:** None. **A. Dica:** None. **L. Mateescu:** None. **C. Iliescu:** None. **A. Arghir:** None.

EP15.033 Five patients with the c.-32-13 t-g mutation in a compound heterozygous state in the *gaa* gene with late-onset Pompe disease

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A comprehensive analysis of clinical and laboratory data was conducted in five patients (two women, three men) with late-onset Pompe disease (LOPD) carrying the c.-32-13T > G mutation in the first intron of the *GAA* gene in a compound heterozygous state. Patient Data:

- Patient 1: 47 years old female, disease onset at 15 years. Moderate hyperCKemia (232 U/L), α-glucosidase activity 0.37 μmol/L/h, *GAA* mutations: c.-32-13T > G, c.307T>G.
- Patient 2: 71 years old female, diagnosed at 69 years. CK level 136 U/L, α-glucosidase activity 0.56 μmol/L/h, *GAA* mutations: c.-32-13T > G, c.307T>G.
- Patient 3: 35 years old male, disease onset at 24 years. α-glucosidase activity 0.09 μmol/L/h, *GAA* mutations: c.-32-13T > G, c.2104G>T.
- Patient 4: 47 years old male, disease onset at 40 years. α-glucosidase activity 0.54 μmol/L/h, *GAA* mutations: c.-32-13T > G, c.2662G>T.
- Patient 5: 48 years old male, disease onset at 35 years. α-glucosidase activity 0.96 μmol/L/h, *GAA* mutations: c.-32-13T > G, c.2238G>A.

The disease onset ranged from 15 to 69 years. Lower enzyme activity correlated with an earlier disease onset (0.09 μmol/L/h - 28 years, 0.56 μmol/L/h - 60 years). Elevated CK levels were not present in all cases (normal CK in Female 2). Clinical Manifestations:

The initial symptoms in all cases were exercise-induced fatigue and gait disturbances. Early-stage signs included falls and muscle weakness. All patients developed lower limb weakness, while females also exhibited weakness in the shoulders, arms, and back. The Gower's sign was positive in all cases. All patients were prescribed avalglucosidase alfa therapy.

C.O.I. Disclosure:

V. Larionova: None. **Z. Muruzheva:** None. **S. Rashidova:** None. **I. Zueva:** None.

EP15.034 Ryanodin receptor related disorders

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Background: The ryanodine receptor (RyR1) is the main sarcoplasmic reticulum Ca^{2+} channel in skeletal muscle and acts as a connecting link between electrical stimulation and Ca^{2+}-dependent muscle contraction. *RYR1* is expressed predominantly in fast and slow-twitch skeletal muscle and also in the esophagus and in cerebellar Purkinje cells in the brain. *RYR1* mutations cause disorders such as malignant hyperthermia, central core disease, congenital fibre type disproportion and centronuclear myopathy. *RYR2* is the predominant isoform in cardiac muscle and brain. Catecholaminergic polymorphic ventricular tachycardia (CPVT) is the most common cardiac ryanodinopathy. Also *RYR2* exon-3 deletion syndrome and the calcium release deficiency syndrome are caused by mutations in *RYR2*. *RYR3* is differentially expressed in the brain. *RYR3* variants are associated with risk of hypertension, diabetes, Alzheimer disease and epilepsy.

Material and methods: Variants were amplified by PCR using standard PCR primers and sequenced using the ABI Prism BigDye Terminator cycle sequencing protocols. Droplet digital polymerase chain reaction (ddPCR) was used for detection and quantification of nucleic acids without using standard curves, based on water-oil emulsion droplet technology and expression analysis.

Results: Here we describe several RYR mutations and correlated them with the disease. We also validated large deletion in ryanodine receptor 1 (RYR1) using droplet digital PCR

Conclusion: We aim to collect as many RYR mutations as possible to build a comprehensive picture of the disease phenotypes in these genes.

C.O.I. Disclosure:
M. Marttila: None.

EP15.035 Genetic investigation of the ubiquitin-protein ligase E3A gene asputative target in Angelman syndrome

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Background: Angelman syndrome is a neurogenetic disease resulting in disorders of motor development and intellectual deficit. It is mainly characterized by severe mental retardation, delayed motor skills, microcephaly and absence of speech. It is due to multiple genetic abnormalities comprising deletion 15q11q13 chromosome, uniparental disomy, imprinting center defect and a mutation in the *UBE3A* gene. Genetic diagnosis of this syndrome is complex and requires the use of cytogenetic and molecular biology techniques.

Methods: We studied 67 patients, clinically suspected to have the Angelman syndrome, we realize different analysis such as FISH, MS-MLPA, microsatellite analysis and *UBE3A* sequencing.

Results: We found, respectively, 11 patients with 15q11q13 deletion, two paternal isodisomy but none mutation was found in *UBE3A* gene, except for polymorphisms whose pathogenic effect has been studied in the concerned patients.

Conclusion: The exome sequencing was realized in selected consanguineous families and show the presence of mutations in new genes who were never previously described in Angelman syndrome and could be responsible for the Angelman syndrome-like syndromes in our patients.

C.O.I. Disclosure:
W. Manoubi: None.

EP15.036 A remarkable case of systematic coverage bias in genome sequencing: Steadily changing sequencing coverage from centromeres to telomeres across all chromosomes

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Background: As part of our national genome program for patients with widespread and incurable cancer, a pancreatic cancer patient exhibited a remarkable sequencing coverage pattern in a blood sample. We observed a steadily increasing sequencing coverage from centromeres to telomeres, a pattern consistently present across all chromosomes. This change in coverage did not follow the expected pattern for copy number alterations such as deletions or duplications. Interestingly, this characteristic coverage pattern was only observed in the blood sample but not in the tumor. To investigate the underlying cause, we performed additional genomic analyses.

Material and methods: DNA was extracted from both tumor and blood samples. Genome sequencing was performed using the Illumina NovaSeq 6000 with the Illumina DNA PCR-Free Prep protocol. Long-read genome sequencing was conducted on an Oxford Nanopore PromethION (R10.4.1 flow cell) using the Ligation Sequencing Kit V14. Additionally, chromosomal microarray analysis was performed using the Affymetrix Cytoscan HD GeneChip Array.

Results: The characteristic coverage pattern initially observed in genome sequencing was consistently reproduced using both additional genome sequencing and chromosomal microarray analysis. All methods revealed a steadily increasing copy number estimate, with the lowest copy number near centromeres and the highest at telomeres across all chromosomes.Surprisingly, B-allele frequency (BAF) analysis did not indicate any copy number gains or losses, further questioning the nature of this phenomenon.

Conclusion: The characteristic coverage pattern in the blood sample was reproducible across multiple genomic methods. While we suspect the cause to be methodological, our additional analyses did not identify the exact root cause.

C.O.I. Disclosure:
M.J. Larsen: None. **E. Lester:** None. **Q. Hao:** None. **B. Larsen:** None. **H.M. Nielsen:** None. **B.S. Kristiansen:** None.

EP15.037 Exploring the genetic intersection of hearing loss and male infertility in a UAE family

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Background: Hearing loss and male infertility are conditions with both genetic and non-genetic origins. Although some syndromes like HIIMS and DIS link these conditions through specific genetic alterations, the genetic diversity and mechanisms behind these associations remain not fully understood.

Material and methods: We utilized whole exome sequencing to investigate a consanguineous Emirati family affected by autosomal recessive hearing loss and male infertility. The study included detailed family ascertainment, clinical assessments, and genetic screenings. Additionally, the expression of the relevant gene was analyzed in mouse models to elucidate its role in auditory and reproductive functions.

Results: The genetic analysis revealed a novel homozygous variant in a gene previously implicated in ciliary functions but not directly connected to hearing or fertility in the existing literature. No mutations in known deafness genes were detected alongside this variant. Expression studies in the mouse model showed localization of the gene product in specific cellular structures within the auditory system, suggesting a functional role that was previously unreported in humans.

Conclusion: Our findings suggest a potential novel genetic link between hearing loss and male infertility, expanding the understanding of the genetic architecture underlying these conditions. The study underscores the complexity of genetic interactions in syndromic presentations and supports the need for further genetic and functional analyses to confirm these findings and their implications for genetic counseling and personalized medicine approaches.

C.O.I. Disclosure:
A. Tlili: None. **A. Kaddoura:** None.

EP15.038 A Rare Molecular Mechanism in Silver-Russell Syndrome: HMGA2 Gene Variants

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Background / Objective: Silver-Russell Syndrome (SRS) is a genetically diverse condition characterized by low birth weight, macrocephaly, a broad forehead, and postnatal growth retardation, often accompanied by body asymmetry. It is typically caused

by chromosomal abnormalities such as methylation disorders, uniparental disomy, or deletions/duplications/translocations on chromosomes 11 and 7. Rarely, pathogenic variants in the *CDKN1C, IGF2, PLAG1, and HMGA2* genes have been linked to SRS.

Method: This study presents two cases diagnosed with SRS due to pathogenic variants in the *HMGA2* gene.

Results: Case 1 is a 14-year-old girl born to non-consanguineous parents. She presented with growth retardation and a family history of short stature. Physical examination revealed a body weight of −5.1 SDS, height of −4.3 SDS, and a head circumference of −4.4 SDS, along with a broad forehead and clinodactyly. Chromosome analysis was 46, XX, and whole exome sequencing identified a heterozygous c.99delG (p.Lys34fs) variant in the *HMGA2* gene, which was also present in her father. Case 2 is an 18-year-old female with short stature and growth hormone deficiency. She was born at term with normal karyotype and microarray results. Whole exome sequencing revealed a heterozygous c.30delG (p.Gln10Hisfs*156) variant in the *HMGA2* gene. Both patients' clinical features were consistent with SRS.

Conclusion: For patients with clinical signs of SRS but negative routine genetic tests, rare single-gene disorders, including *HMGA2* variants, should be considered.

C.O.I. Disclosure:

Z.B. Yılmaz: None. **E. Isik:** None. **E. Avci Durmusalioglu:** None. **T. Turkut Tan:** None. **Y. Dogan:** None. **D. Özalp Kizilay:** None. **T. Atik:** None. **O. Cogulu:** None.

EP15.039 Autosomal recessive developmental and epileptic encephalopathy 71 (AR-DEE71)/ Glutaminase deficiency syndrome caused by two different de novo intronic mutations of GLS gene, identified through rapid whole genome sequencing (rWGS)

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Proband was a 6-week-old Female, born at 38+ weeks by elective C-section for IUGR to a 27 yo, G2P1 mother of Bengali descent without consanguinity. APGARs: 6&6 at 1&5 minutes. Birth weight: 2nd%ile, length 5th%ile, and HC 12th%ile for age. She had **severe hypotonia with hyperreflexia, poor suck/swallow, hypoventilation with frequent, severe apneic episodes, and minor dysmorphism**: narrow bifrontal diameter, mildly posteriorly rotated ears, over-folded upper helices, broad nasal bridge, micro-retrognathia, hypoplastic nipples, a deep sacral dimple, clenched hands, and left partial simian crease.

Diagnostic Workup: EEG: burst suppression pattern without seizures. **MRI:** mild ventriculomegaly, punctate left periventricular stroke. **NBS:** normal. **IEM screening** (plasma, urine, CSF): Normal except for high plasma glutamine (1317 umol/L. N = 356–857), and mild elevations of alanine and proline (probably secondary to hyperammonemia or lactic acidemia). **Methylation studies** for PWS/AS, **CMA**, and **poly A** expansion for PHOX2B, and **rWES** +Mitochondrial genome seq/del were **negative**. The **rWGS Trio** showed Two different de novo **intronic** mutations in GLS gene in **trans** (verified by **Long Reads sequencing):** c.736-1 G > C:p.? in intron 4; Likely Pathogenic Variant **(VLP)**, and c.605+3 A > G:p.? in intron 3; Variant of uncertain significant **(VUS)**.

Conclusion: Combination of hypotonia, respiratory insufficiency/failure, and burst suppression in the setting of an elevated plasma glutamine is compatible with **AR-DEE71** in this patient due to compound heterozygosity for GLS gene mutations. This is the first report of **two** different **de novo intronic mutations, one on each GLS allele** leading to **AR-DEE71/Glutaminase deficiency**, emphasizing the diagnostic yield of **rWGS** in critically ill infants.

C.O.I. Disclosure:

M. Hajianpour: None. **K. Tauber:** None. **J. Pinheiro:** None. **B. Whitbeck:** None. **P. Das:** None. **J.M. Devaney:** A. Employment (full or part-time); Significant; GeneDx, LLC. **J. Pugh:** None.

EP15.040 Novel *ALAD* gene variant and somatic mosaicism in the first Russian case of ALAD-deficiency porphyria

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Background: ADP is the rarest form of acute hepatic porphyria, caused by ALAD deficiency, with only 12 cases reported globally. It is characterized by the accumulation of ALA, leading to neurovisceral symptoms. We report the first case of ADP in Russia with late-onset acute porphyria symptoms, characteristic laboratory findings, and unique genetic variants.

Material and methods: A 47-year-old Russian woman experienced recurrent abdominal pain, tachycardia, hypertension, and neuropathy onset at age 38. Biochemical tests revealed elevated urinary PBG and ALA levels. WES was performed on DNA isolated from blood and skin fibroblasts. Segregation analysis was performed using Sanger sequencing on biomaterial from available relatives.

Results: WES identified two variants in the *ALAD* (NM_000031.6) gene in blood-derived DNA: a novel heterozygous variant c.299dup, p.(Ala101SerfsTer3), and a mosaic (19% VAF) c.415G>A, p.(Gly139Arg). Only the c.299dup variant was detected in fibroblast-derived DNA. Neither variant was detected in the proband's mother, sister, or daughter. The paternally inherited c.415G>A variant has recently been reported in a patient with neonatal ADP.

Conclusion: The case of ADP we described is notable for its late manifestations. The fact that the c.415G>A variant is found in blood cells but not in fibroblasts indicates that it is in a state of somatic mosaicism, which has not been previously described for this disease. Although ADP is classified as a hepatic porphyria, emerging evidence, including our case, suggests a major role of erythroid cells in the pathogenesis of the disease. However, limited and conflicting data due to its rarity preclude definitive conclusions.

C.O.I. Disclosure:

T. Vizerov: None. **N. Demina:** None. **R. Ponomarev:** None. **E. Lukina:** None. **O. Pshenichnikova:** None. **D. Selivanova:** None. **V. Tabakov:** None. **O. Ryzhkova:** None.

EP15.041 Clinical presentation of a new *De-Novo HCN2* variant associated with childhood epilepsy

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Background: Epilepsy is a complex trait mediated by abnormal and uncontrolled signalling of neurons this condition is characterised by elevated genetic and phenotypic heterogeneity.

We present a case of an 11-years-old boy with tonic-clonic seizures, sialorrhea and EEG showing focal alteration during sleep-awake transition.

Material and methods: The proband was tested with clinical exome using next-generation sequencing on a NextSeq 550dx with DNA Prep for Enrichment and TruSight One 2.0 Expanded by Illumina. Bioinformatic analysis and variant filtering were performed using DRAGEN Enrichment 4.2.4 and Geneyx analysis software.

Results: *HCN2* codes for a sodium-potassium channel and the variant interests cyclic Nucleotide Binding Domain (cNBD). The cNBD plays a key role in the regulation of gating processes; without the binding of cyclic nucleotide monophosphate as cAMP and cGMP the channel remains in a closed conformation, a condition that could lead to an alteration of the membrane potential and creates a condition of neuron hyperexcitability. The variant was classified as Variant of Unknown Significance (VUS); but, based on the familial segregation revealing *de-novo* origin and genotype-phenotype correlation, the variant was reclassified as Likely Pathogenic (LP) (PM2 supporting PS2 strong PM4 moderate). There are very few variants identified in this domain, and only one of them has been proposed as LP.

Conclusion: This case is an example of the necessity for new functional studies for VUS to better understand new pathophysiological disease models.

C.O.I. Disclosure:

N. Callegari: None. **A. Bassani:** None. **A. Cianflone:** None. **V. Ferri:** None. **A. Filannino:** None. **E. Meroni:** None. **F. Orsini:** None. **G. Porta:** None. **G. Micheloni:** None. **R. Righi:** None.

EP15.042 A sole case of the FGF23 gene mutation (p.Thr68Ala) associated with multiple severe vascular aneurysms and a hyperphosphatemic variant of tumoral calcinosis - a case report

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Introduction. Tumoral calcinosis is an extremely rare genetic disease caused by mutations in three genes: GALNT3, FGF23, and KL, which disrupt phosphorus metabolism. The hallmark of this condition is the formation of tumors in soft tissues around the joints. Other phenotypic features are dental involvement, brain and vascular calcifications. The case presents for the first time to the scientific community the c.202A>G (p.Thr68Ala) mutation of the FGF23 gene associated with a hyperphosphatemic variant of tumoral calcinosis and multiple severe vascular aneurysms.

Case presentation. A female underwent multiple surgeries for tumor formations in soft tissues that appeared at the age of 12 months. On this occurrence, the patient was found to have hyperphosphatemia, low phosphate clearance, increased tubular reabsorption with normal levels of calcium, vitamin D3, and parathyroid hormone, and no effect of treatment with sevelamer hydrochloride and a low-phosphate diet. At the age of 39, imaging studies due to edema and a pulsating formation in the neck revealed multiple vascular aneurysms with thrombosis, for which she received operative and interventional treatment. In this connection, and because of the established phosphorus metabolism disturbance, a genetic disease was suspected. Sequence analysis and deletion/duplication testing of 358 genes revealed she is homozygous for a variant of the c.202A>G (p.Thr68Ala) of the FGF23 gene.

Conclusion. The established mutation is not present in population databases. This case is the first in the world to demonstrate the role of this type of FGF23 mutation in developing a hyperphosphatemic variant of tumoral calcinosis with aggressive vascular aneurysm formation.

C.O.I. Disclosure:

N.G. Ivanova: None.

EP15.043 SNPs in genes involved in sweet taste perception and intake (SweetG) are associated with Primary Ciliary Dyskinesia (PCD) and related phenotypes

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Background: PCD is a congenital motile ciliopathy caused by impaired mucociliary clearance, characterized by recurrent respiratory infections affecting both the upper and lower airways. Many genes involved in taste perception pathways are expressed in extra oral tissues and have recently emerged as regulators of airway immune responses. The aims of this study were to determine if SNPs in SweetG are more/less frequent in PCD patients, and verify their association with PCD clinical characteristics.

Material and methods: A list of seven SNPs in six SweetG have been tested for differences in allele frequency between PCD patients and gnomAD v4.1.0 using binomial test. Logistic and linear regression models have been performed to study the association between SNPs and PCD patients' clinical features.

Results: A cohort of 34 PCD patients (6–69y, 56% female) was included in the study. The minor allele of rs5415 (*SLC2A4* gene) was less frequent in PCD patients than gnomAD ($p < 0.05$). As regard PCD patients' clinical features, we found that rs5415 was associated with a greater presence of chronic rhinosinusitis ($p < 0.05$). In addition, rs7534618 (*TAS1R2* gene) was associated with the presence of situs inversus, and rs17457384 (*GABRB2* gene) was associated with a decreased respiratory function (FEV_1).

Conclusion: This study shows association between SweetG and specific PCD clinical features. The significance of these findings is not yet fully understood; however, this represents a promising area of research to enhance our understanding of PCD and elucidate the genetic basis of respiratory infections associated with this disease.

C.O.I. Disclosure:

R. Ruberto: None. **G. Piatti:** None. **S. Camarda:** None. **G. Nardone:** None. **A. Pecori:** None. **A. Santin:** None. **P. Tesolin:** None. **E. Tassin:** None. **B. Spedicati:** None. **G. Girotto:** None. **M. Concas:** None.

EP15.044 Utility of whole exome sequencing in the early diagnosis of atypicalphenotypes of rare genetic diseases in Tunisia

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Background: Genetic diseases (GDs) pose a significant public health problem in Tunisia, with over 60% of 589 identified conditions being autosomal recessive, often linked to consanguinity. Notably, atypical forms of rare GDs are frequently observed in Tunisian families, further complicating accurate diagnosis and management. Furthermore, 40% of these pathologies lack defined molecular etiology.

Material and methods: In this study, we used whole exome sequencing (WES) to investigate atypical forms of cutis laxa (CL) (6 patients) and hypohidrotic ectodermal dysplasia (HED) (2 patients). In addition, atopic dermatitis (AD), in which both genetic and environmental factors play vital complementary roles, was also included (1 patient with severe form).

Results: A new founder mutation in the *ATP6V0A2* gene was detected in CL families. CNV analysis allowed us to detect a *COL5A1* duplication in a CL patient with severe phenotype, suggesting a comorbidity in this family. Missense mutations were identified in the *EDA* and *LRP6* loci responsible for HED. Interestingly, for the first time, a mutation in the *HRNR* gene was identified as the underlying cause of AD. As a result, prenatal diagnosis of CL has been introduced at the Charles Nicolle Hospital and genetic counselling is offered to families. Identified mutations have been included in PREMEDIT, a Tunisian platform designed to revolutionize healthcare decision-making for rare diseases.

Conclusion: Our study provides evidence that WES enable high diagnostic yield and clinical utility in patients with atypical disease forms in Tunisia. We believe it is an effective approach to enable appropriate counselling and personalized management.

C.O.I. Disclosure:

N. Mezzi: None. **I. Abdallah:** None. **E. Bouteraa:** None. **D. Ismail:** None. **R. Mrad:** None. **M. Trabelsi:** None. **L. Romdhane:** None.

EP15.045 TUBGCP2-related cortical malformation spectrum disorder: A case report and literature review

Sonia Lomuscio[1,2], *Valentina Pinna*[2,3], *Carmen Cassadonte*[1,2], *Amalia A. F. Ungaro*[1,2], *Laura Tirrito*[1,2], *Francesca Di Lorenzo*[2], *Daniele Ferretti*[3], *Gerarda Mastrogiorgio*[2], *Maria Grazia Di Gregorio*[2], *Antonio Novelli*[3,4], *Alessia Micalizzi, PHD*[2,3]

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Background: *TUBGCP2* gene (OMIM *617817) encodes a multimer of the gamma-tubulin ring complex, which is predominantly involved in the organisation of the neuron cytoskeleton. Biallelic pathogenic variants have been associated to a rare clinical condition characterized by microcephaly, pachygyria and other cerebral anomalies, hypotonia, global developmental delay, intellectual disability, visual impairment, dysmorphic features with or without seizures (OMIM *618737). The *TUBGCP2*-related phenotype manifests with infantile onset, poor life expectancy and variable expressivity.

Material and methods: We report the case of a 20 month-old baby girl presenting with microcephaly, pachygyria, metopic suture overlap and facial dysmorphisms (narrow and sloping forehead, hypertelorism, long and smooth philtrum). Genetic tests were performed on blood-derived DNA of the patient and her parents.

Results: Next-generation sequencing (NGS)-based trio testing identified two novel biallelic variants in *TUBGCP2* gene (NM_006659.4): a maternal frameshift variant, c.1879dup [p.(Ser627PhfsTer74)] and a paternal splice-site region variant, c.1723-3A > G, classified, respectively, as likely pathogenic (LP) and variant of uncertain significance (VUS), according to ACMG criteria.

Conclusion: To date, only 7 patients are described in literature overall. Genotyping our patient let us to map out a specialistic follow-up. Supposing a causative role of c.1723-3A > G (VUS), due to the coexistence of c.1879dup (LP) in trans and to the match of our patient's phenotype with the *TUBGCP2*-related one, it is possible to estimate recurrence risks for the parental couple. However, functional studies are needed to better define reproductive risks and to increase knowledge of this ultra-rare condition.

C.O.I. Disclosure:

S. Lomuscio: None. **V. Pinna:** None. **C. Cassadonte:** None. **A.A. Ungaro:** None. **L. Tirrito:** None. **F. Di Lorenzo:** None. **D. Ferretti:** None. **G. Mastrogiorgio:** None. **M.G. Di Gregorio:** None. **A. Novelli:** None. **A. Micalizzi:** None.

EP15.046 A Rare Case Report of Diencephalic-Mesencephalic Junction Dysplasia Syndrome

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Background: Diencephalic-mesencephalic junction dysplasia syndrome is a rare, non-syndromic cerebral malformation characterized by severe intellectual disability, progressive microcephaly, axial hypotonia, spastic quadriparesis, seizures, and facial dysmorphism. Associated features may include talipes equinovarus, non-obstructive cardiomyopathy, persistent hyperplastic primary vitreous, obstructive hydrocephalus, and autistic traits. This study aimed to identify the genetic cause of the condition in a male proband with progressive microcephaly detected from the 28th week of gestation.

Material and methods: Genetic counseling was conducted, and family, medical, and paraclinical histories were reviewed. Whole exome sequencing (WES) was performed using the Illumina platform and Agilent SureSelect V7 library preparation kit. Sanger sequencing was used to validate the findings.

Results: WES identified a pathogenic homozygous mutation (c.451C>T; p.Arg151Ter) in the *PCDH12* gene and two variants of uncertain significance: c.2649T>G (p.His883Gln) in GNPTAB and c.682C>G (p.Leu228Val) in CIT. The parents were heterozygous carriers of all three variants. Further clinical and molecular analyses confirmed the *PCDH12* mutation as the causative variant. This mutation reduces transcript expression by 84%.

Conclusion: This case demonstrates that diencephalic-mesencephalic junction dysplasia syndrome can be attributed to a homozygous mutation in *PCDH12*. Although typically identified postnatally, this case was detected prenatally at 28 weeks. Differential diagnoses, including metabolic diseases and toxoplasmosis, were excluded. Genetic counseling and prenatal diagnostic strategies are critical for effective prevention in affected families.

C.O.I. Disclosure:

F. Fallah Mehrjardi: None. **S. Seyed Hasani:** None.

EP15.047 The application of Whole Exome Sequencing in the diagnosis of rare genetic disorders, a study from a northern Italian center

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Background: Next-generation sequencing techniques, particularly whole-exome sequencing (WES), are primarily used to diagnose patients affected by rare diseases that cannot be clinically classified, often within the framework of research projects. Over the years, the diagnostic capability of these methodologies has increased from 30% to the current 65–70% of cases, with a potential for further informational gain achievable through whole-genome sequencing (WGS). However, this entails greater complexity in interpreting the results, increased costs, and the use of additional techniques, such as epigenetic profiling (methylome) or other functional approaches.

Material and methods: In this project we analysed 33 of patients suffering from a rare or undiagnosed disease using WES. The healthy parents were preferentially included in the analysis (trio analysis). WES was performed at the Synlab genetics Laboratory, Italy, using Twist comprehensive exome on Element AVITI™ System

Results: Depending on the indication, 30–65% of cases could be solved by WES. The highest rates of solved cases were found in syndromic and neurodevelopment disorders. In 5 cases we found new candidate genes for which functional studies are in progress.

Conclusion: These findings underscore the effectiveness of WES in detecting genetic variants and unraveling the molecular basis of genetic diseases, ultimately facilitating early diagnosis in affected children

C.O.I. Disclosure:

A. Novelli: None. **F. Cro':** None. **N. Chiarelli:** None. **G. Ruggeri:** None. **G. Mellon Franchini:** None. **R. Miragliotta:** None. **E. Agolini:** None. **D. Cocciadiferro:** None. **M. Ferrari:** None.

EP15.048 Comprehensive analysis in patients with Darier and Hailey-Hailey diseases identify novel pathogenic molecular variants

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Background: Darier's disease (DC), or Darier-White's disease, also known as follicular dyskeratosis, is a keratinization disorder that affects the skin, mucous membranes, and nails. This disease is due to variants in the ATP2A2 gene, which encodes the Ca2+ ATPase isoform 2 of the endoplasmic reticulum that is expressed in the skin and brain. Hailey-Hailey disease (HHD) is a bullous genodermatosis, also called benign familial chronic pemphigus (PCFB). This disease is due to variants in the ATP2C1 gene, which causes a dysfunction in calcium channels, resulting in a keratinocyte adhesion defect, causing intraepidermal acantholysis. The aim of the present study is to describe clinical and molecular characteristics of patients with Darier and Hailey-Hailey diseases.

Material and methods: Exome sequencing and Sanger analyses in six patients with diagnosis of Darier's disease and in 11 patients Hailey-Hailey disease were performed.

Results: In Darier's disease, three patients corresponded to single cases and three to family cases. In Hailey-Hailey disease, 10 patients corresponded to family cases and one to a single case.

Conclusions: Four different variants in the ATP2A2 gene were identified in the Darier patients, 3 of which have not been previously reported: c.2860-3C > G, c.1167T> and c.2212_2214del. Regarding the 4 variants identified in the ATP2C1 gene in the Hailey-Hailey patients, three were novel c.436_443del, c.1553del, and c.2246T>C. We present new pathological variants in patients with Darier and Hailey-Hailey diseases, which enriches the knowledge of the mutational spectrum in these diseases.

C.O.I. Disclosure:

T. Barragan-Barrientos: None. **J. Cazarin-Barrientos:** None. **M.R. Rivera-Vega:** None. **S. Cuevas-Covarrubias:** None.

EP15.050 Albinism in the Russian population: Genotype-Phenotype Correlations and Comparative Analysis with European Cohorts

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Background: Albinism is a heterogeneous group of hereditary diseases, associated with partial or complete defects of melanin. The aim of this study was to investigate the cohort of patients from Russia with clinical forms of albinism, characterize pathogenic variants in albinism-associated genes, and analyze the correlation between clinical picture and genotype.

Material and methods: The cohort of albinism patients was analyzed, they underwent comprehensive clinical examinations. Molecular genetic diagnostics were performed using Sanger sequencing, MLPA and next-generation sequencing (NGS). Statistical analyses were applied to compare allele frequencies and genotype distributions with European cohorts. For frequent variants, additional population and functional studies were performed. Clinical-genetic correlations were assessed to explore genotype-phenotype relationships.

Results: The study included 196 patients from the Russian Federation with primary clinical diagnosis «Albinism». As a result of genetic diagnosis, isolated and syndromic forms were identified. The overall genetic spectrum and allele frequencies were comparable to European cohorts, with no statistically significant differences in general trends. However, specific genetic variants exhibited significantly higher frequencies in the Russian population. The functional and population studies for some of them confirmed the pathogenicity and unique distribution. Clinical-genetic correlations revealed new insights into the association between genotypic and phenotypic manifestations.

Conclusion: The genetic and clinical characteristics of albinism in the Russian population largely align with European data. However, there are population-specific features that demand further investigation. These results expand our understanding of the genetic landscape of albinism and provide a basis for more precise diagnostic and therapeutic approaches in the Russian population.

C.O.I. Disclosure:

S. Ionova: None. **A. Marakhonov:** None. **V. Kadyshev:** None. **A. Stepanova:** None. **O. Schagina:** None. **T. Vasilyeva:** None. **N. Sukhanova:** None. **R. Zinchenko:** None.

EP15.052 Molecular genetics in patients with Tuberous sclerosis complex reveals a novel *TSC1*-mutation with neonatal onset

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Background/Objectives: Tuberous sclerosis complex (TSC) is a rare genetic disorder of autosomal-dominant inheritance. Mutations on either of the two genes, *TSC1* and *TSC2*, encoding for the proteins hamartin and tuberin, respectively, play a role and result in hamartomas involving many organs, like the brain, heart, kidneys, skin, lungs, and liver. Herein, we report the results from the molecular testing of Bulgarian TSC patients using whole exome sequencing (WES).

Methods: Seven unrelated patients presenting the typical clinical symptoms of TSC were refered to our genetic department after full clinical examinations. Blood samples were collected from the probands, and genomic DNA was subjected to WES on NovaSeq6000 platform. Variants in TSC-associated genes were extracted from the WES data and filtered using systematic bioinformatics analysis and the ACMG criteria. Candidate variants were verified by Sanger sequencing.

Results: We detected 2 potentially-pathogenic variants: one novel frame-shift change, *TSC1*-c.1703delG,p.(Gly568Glufs*61) in one patient, and the well-known mutation *TSC2*-c.1832G>A, p.(Arg611Gln) found in two probands.The patient carrying the novel *TSC1*-c.1703delG deletion was a 4-months-old baby who presented with the typical facial angiofibromas and hypopigmented skin lesions, but also had multiple rhabdomyomas of heart, dextrocardia, and congenital pulmonary venolobar anomalies such as scimitar syndrome. Further analyses of the negative 4 patients are ongoing.

Conclusion: When TSC is diagnosed in the perinatal or neonatal period, it is associated with high incidence of morbidity and mortality. Our results add novel findings to the genetic heterogeneity and pathogenesis of TSC in terms of the very early onset of this severe disorder.

C.O.I. Disclosure:

K. Mihova: None. **K. Kamenarova:** None. **M. Georgiev:** None. **D. Kachakova-Yordanova:** None. **O. Beltcheva:** None. **T. Delchev:** None. **G. Zlatanova:** None. **D. Stamatov:** None. **I. Dimova:** None. **R. Kaneva:** None.

EP15.053 Syndromes with abnormal pigmentation associated with additional anomalies

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Background: Hypopigmentation is observed in many genodermatoses and also in specific rare syndromes, sometimes in relation to somatic mosaicism.

Material and methods: The children with pigmentation anomalies and segmental overgrowth, or eye and brain abnormalities or developmental delay seen in last 5 years in a speciality clinic are included in this report. Molecular testing was performed with informed consent and standard ethical guidelines. Variant classification was as per ACMG guidelines using in-silico tools and available population databases.

Results: Five children 1month to 11years age were diagnosed with tuberous sclerosis and had *TSC1* or *TSC2* variants on whole exome sequencing, One 7 year child with facial asymmetry, hypopigmented lesions, alopecia, and coloboma had somatic pathogenic KRAS variant and was diagnosed with oculoectodermal syndrome. Another 10 year child with asymmetric overgrowth, hyperpigmented and hypopigmented lesions and hemimegalencephaly showed a pathogenic *AKT1* variant. An 11 year female presented with developmental delay, right sided hemihypertrophy and hypopigmented lesions over right forearm. Exome sequencing from skin biopsy revealed a pathogenic *MTOR* gene variant

Conclusion: The patterns of hypopigmentation, presence or absence of hemihypertrophy, ocular and brain MRI findings and additional anomalies like rhabdomyoma help in making an accurate diagnosis. Molecular testing on blood or skin biopsy as indicated gives a confirmatory diagnosis. Many of these disorders have predisposition to tumors and need appropriate genetic counseling, monitoring, targeted therapy and follow up.

C.O.I. Disclosure:

I. Panigrahi: None. **S. Gupta:** None. **A. Kaur:** None.

EP15.054 DNA methylation profiling in Wilson Disease patients from an isolated community

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Wilson disease (WD) is a rare autosomal recessive disorder caused by ATP7B gene mutations, leading to copper accumulation in the body. While ATP7B mutations define WD, they do not account for its wide clinical variability, suggesting a role for environmental and epigenetic factors in disease pathogenesis. Growing evidence from mouse models and patient studies suggests that DNA methylation changes influence WD onset, progression, and phenotypic manifestations by interacting with ATP7B mutations and epigenetic modifications. High-throughput genome-wide assays have expanded the role of DNA methylation analysis in diagnosing developmental disorders, including WD. Previous work from our research group, in an isolated Romanian community with an exceptionally high WD prevalence, revealed the presence of mutations in seven WD patients from five apparently unrelated families spanning three generations. Direct sequencing of the ATP7B gene revealed that each patient was either homozygous or compound heterozygous for nine SNV: two mutations (p.His1069Gln, p.Met769His-fs) and seven polymorphism (p.Ser406Ala; p.Val456Leu; p.Lys832Arg, p.Arg952Lys; c.2866-13G > C; p.Val1140Ala; c.3903+6C > T). This pilot study examines the genome-wide methylation profile from the blood of WD patients. Using direct read-out of DNA methylation from nanopore sequencing data, we find out 14 significant differentially hypermethylated and hypomethylated regions in our samples. This unique population structure provides valuable settings for studying the genetic and epigenetic factors associated with WD, potentially revealing valuable insights into genotype-phenotype correlations on disease manifestation. This study identified further disease-relevant epigenetic signatures in WD. These results pave the way for further research into epigenetic-based diagnostic tools and treatment strategies for WD.

C.O.I. Disclosure:

I.G. Basaraba: None. **L.C. Bohiltea:** None. **F. Raicu:** None.

EP15.055 Complex rearrangement in *NF1* leads to abnormal transcript with pseudo-exonic antisense sequences

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Background: This case report is about an adult female patient with a typical familial neurofibromatosis type 1 (NF1) phenotype, presenting with multiple café-au-lait spots, freckling, Lisch nodules and multiple cutaneous and subcutaneous neurofibromas. Initial molecular analysis on blood sample using NGS and MLPA technologies identified a duplication of exons 37 to 57 of *NF1* (NM_000267.3). Sequencing of RNA did not confirm the tandem nature of the duplication in *NF1*.

Material and methods: Whole-genome sequencing (WGS) was conducted to define the nature of the duplication. We then applied Sanger sequencing on RNA sample to study the impact of the rearrangement.

Results: WGS revealed a tandem inverted duplication of exons 37 to 57 coupled with a simple duplication of part of intron 57. RNA sequencing with targeted primers showed that this duplication resulted in the formation of aberrant transcripts including pseudo-exonic regions in antisense of *NF1*, together with the *EVI2A* gene naturally present in antisense in intron 36 of *NF1*.

Conclusion: WGS and RNA sequencing allowed us to confirm the nature of a complex rearrangement in the *NF1* gene and its functional consequences on the *NF1* transcript.

C.O.I. Disclosure:

L. Pacot: None. **F. Hamza:** None. **A. Coustier:** None. **T. Maillard:** None. **S. Audebert-bellanger:** None. **D. Vidaud:** None.

EP15.056 Thalassemia syndromes in Serbia:the importance of genetic (re)analysis

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Background: Thalassemia syndromes are heterogeneous group of hereditary anemias characterized by defects in the synthesis of haemoglobin (Hb) polypeptide chains. These disorders comprise thalassemias and thalassemic haemoglobin variants, predominantly caused by mutations in α- and β-globin genes. Clinical manifestations of thalassemia syndromes range from asymptomatic in thalassemia minor to severe anemia in thalassemia major cases.

Material and methods: The study included 269 patients from 196 unrelated families presented with hematological parameters indicative of thalassemia syndromes. Detection of α- and β-globin gene mutations was performed using PCR, direct sequencing, and MLPA analysis.

Results: Three Hb variants and fifteen different β-thalassemia mutations, including six mutations previously not reported in Serbian population, were detected. Hb variant Lepore BW, caused by 7.4 kb deletion, was the most common cause of thalassemia (26,9%), followed by HBB:c.316-106C > G, HBB:c.118C>T and HBB:c.93-21G > A mutations which were detected in more than 50% of all affected β-globin alleles. Using MLPA, genetic variants were identified in seven families affected by α-thalassemia, including an HbH patient, who had previously remained unresolved.

Conclusion: The results of this study revealed that most thalassemia patients carry point variants, however, higher diagnostic rates were only achieved by combination of techniques detecting large deletions as well. Thus, diagnosis obtained for α-thalassemia affected families emphasise the importance of reanalysis of unresolved cases leading to improvement of their treatment and counselling.

Results presented in this study will update Serbian national mutation database and contribute to future implementation of routine clinical reanalysis considering new scientific discoveries, updated databases and/or refined analytical methods.

C.O.I. Disclosure:

M. Ugrin: None. **J. Komazec:** None. **K. Klaassen:** None. **A. Skakic:** None. **M. Andjelkovic:** None. **V. Spasovski:** None. **N. Stevanovic:** None. **M. Parezanovic:** None. **S. Stankovic:** None. **N. Jocic:** None. **S. Pavlovic:** None. **M. Stojiljkovic:** None.

EP15.057 Exome Sequencing Identifies a Likely Pathogenic *TGFB2* Variant in a Multigenerational Family with a Disorder Overlapping Marfan and Loeys-Dietz Syndromes

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Background: Vascular pathologies, including aortic dilatation and aneurysms, are critical health concerns due to their association with life-threatening complications. Thoracic aortic aneurysms are often linked to mutations in genes involved in extracellular matrix maintenance, vascular smooth muscle cell function and TGF-β signaling pathways. This study examines a multigenerational family with a history of vascular anomalies and connective tissue disorders to explore the genetic causes of their condition.

Material and methods: We performed clinical evaluations and clinical exome sequencing on the proband and other family members, including his deceased father. Family segregation studies were performed to investigate the genotype-phenotype correlation.

Results: A novel heterozygous likely pathogenic *TGFB2* variant (c.1159C>T, p.Gln387Ter) was identified in the proband and segregated in all affected family members, including the deceased father. Additional segregating variants of uncertain significance (VoUS) were identified in *KCNQ1* gene (paternal: c.514G>A, p.Val172Met; c.877C>T, p.Arg293Cys).

Conclusion: This study highlights the pathogenic role of a novel *TGFB2* variant in aortic pathology within a multigenerational family. The variant is associated with thoracic aortic dilatation, scoliosis and marfanoid features, consistent with Loeys-Dietz syndrome 4 and aortic aneurysm predisposition. *TGFB2* gene encodes an essential protein for maintaining the structural integrity of vascular walls. Moreover, VoUS in *KCNQ1* gene, implicated in cardiac ion channel function, were identified and may contribute to overlapping phenotypes, complicating the clinical interpretation. These findings emphasize the challenges of interpreting additional VoUS in the context of complex hereditary conditions and the importance of integrating genetic and clinical data for accurate diagnosis and management of vascular syndromes.

C.O.I. Disclosure:

L. Mercuri: None. **G. Palladino:** None. **D. Traversa:** None. **M. Patrizio:** None. **M. Curcetti:** None. **R. Santacroce:** None. **M. Margaglione:** None.

EP15.058 Description of two patients with Prader-Willi syndrome due to a very rare mosaic imprinting defect

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Background: Imprinting defects (ID) commonly cause imprinting diseases, but their proportion varies greatly between the different diseases, as does the occurrence of mosaic IDs. In Prader-Willi syndrome (PWS) only ~1% have an ID. So far, only three patients with a mosaic ID have been reported, two without clinical data. In Angelman syndrome, the disease reciprocal to PWS, ~3% have an ID of which ~40% are mosaic.

Material and methods: MS-MLPA chr15 and multi-locus, MSA chr15.

Results: We describe two patients with PWS due to a mosaic ID with clinical descriptions. Mosaic hypermethylation on chr15 was detected by MS-MLPA, UPD15 and multi-locus imprinting disturbance were excluded. Patient_1 was born by C-section in the 38th WG with low/normal measurements. He showed muscular hypotonia and feeding difficulties requiring partial tube feeding for 33d. Development was normal. At ~1y hyperphagia and weight gain started, which was uncontrollable by diet. He attended primary school, then transitioned to a special needs school, later graduated from regular secondary school, obtained a driver's license, and completed an apprenticeship. At last examination (30y), measurements for height and HC were normal while weight was high (BMI 44), even after gastric band surgery. Patient_2 was born in the 41th WG (after ICSI) with normal measurements. Postnatally, muscular hypotonia was noted, tube feeding was not necessary. Motor milestones were timely. At 4y development was normal.

Conclusion: These patients add considerably to our knowledge of the clinical course and occurrence of very rare mosaic IDs causing PWS. This should be considered in diagnostics.

C.O.I. Disclosure:

J. Beygo: None. **D. Kanber:** None. **S. Ueberberg:** None. **C. Lich:** None. **F.J. Kaiser:** None. **A. Kuechler:** None.

EP15.059 The role of exome sequencing in advancing the diagnosis and treatment of children with rare diseases

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Background: The advancement of diagnosing rare diseases (RD) has become a focus in healthcare, leading to a phenomenon known as the "diagnostic odyssey." In Indonesia, limited physician awareness and access to advanced genetic testing complicate the diagnostic process. Exome sequencing (ES) has emerged as a groundbreaking tool for addressing this challenge by enabling the identification of gene mutations, improving diagnostic accuracy, and shortening the time to diagnosis. With the implementation of precision medicine, personalized therapy can be achieved, resulting in better health outcomes.

Material and methods: ES was conducted on DNA extracted from the peripheral blood samples of nine patients suspected of having rare genetic disorders. Using the Illumina NextSeq platform along with the TruSeq Exome Kit, raw data were processed and aligned to the GRCh37 and GRCh38 reference genomes. Variant calling and annotation were performed following Illumina's bioinformatics pipeline, with pathogenicity classification in accordance with ACMG guidelines. Phenotype data were integrated to support diagnosis and management decisions.

Results: The results revealed significant genetic findings in all patients: three pathogenic variants, one likely pathogenic variant, and five variants of uncertain significance (VUS). Six patients received a revised diagnosis after ES, while others had their initial diagnosis confirmed. Five patients showed clinical improvement following therapeutic changes based on the results of ES.

Conclusion: These findings underscore the transformative potential of ES in enhancing RD diagnostics, particularly in resource-limited settings, and demonstrate how precision medicine can facilitate more accurate diagnoses and personalized patient care.

C.O.I. Disclosure:

K. Iskandar: None. **N.A. Pramono:** None. **I.E. Santosa:** None. **G.R. Wardhana:** None. **S.S. Nurhidayah:** None. **P. Adhityo:** None. **T.I. Hikmawan:** None. **Gunadi:** None.

EP15.060 Rare variants identified in Slovenian cohort of patients with erythrocytosis

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Background: Hereditary erythrocytosis (ECYT) is a rare haematological disorder characterised by an increased red blood cells mass that can lead to thrombotic complications. Until now, genetic variants in nine genes were considered causative. The study objective was to identify rare variants with clinical effects in a national cohort of erythrocytosis patients.

Material and methods: A three-step diagnostic algorithm for non-clonal erythrocytosis was developed to thoroughly screen patients with idiopathic erythrocytosis. Patients were referred for genetic testing using targeted NGS with genes related to erythropoiesis or iron metabolism. The identified variants were localised in the protein structure and characterised by functional tests.

Results: In 65 patients, including two families, NGS confirmed the pathogenic *EPAS1* variant in one patient responsible for ECYT4 and identified several variants of unknown significance (VUS) in 6 patients, including one family (Table 1). In silico structural analysis showed that both *EGLN1* variants are localised in active site, indicating reduced protein activity. In vitro functional assays revealed reduced protein levels for both variants, and cycloheximide chase assays showed significantly reduced stability for p.(Pro358Ser). The luciferase reporter assay did not confirm the effects of the variants on EGLN1 activity.

Conclusion: The genetic cause of erythrocytosis was confirmed in one patient and new rare VUS were identified. The low diagnosis rate suggests that additional genes contribute to the development of the disease. *EGLN1* VUS have an effect on protein expression, further tests will clarify their contribution to the ECYT development.

Table 1: Rare variants identified in Slovenian patients with erythrocytosis

Gene	DNA change	Protein change	No.	Classification (ACMG/AMP)
EPAS1	c.1609G>A	p.(Gly537Arg)	1	Pathogenic (PS1, PS3, PS4, PM1, PM2, PM5, PP1_STR, PP4)
EGLN1	c.1124A>G	p.(Glu375Gly)	1	VUS (PM1_SUP, PM2, PP3, PP4)
EGLN1	c.1072C>T	p.(Pro358Ser)	2	VUS (PM2, PP3, PP4)
EPAS1	c.2120A>C	p.(Lys707Thr)	1	VUS (PM2)
JAK2	c.1767C>A	p.(Asn589Lys)	1	VUS (PM2)
SH2B3	c.901G>A	p.(Glu301Lys)	1	VUS (PP3)

C.O.I. Disclosure:

N. Debeljak: None. **S. Anžej Doma:** None. **I. Preložnik Zupan:** None. **T. Pajic:** None. **A. Maver:** None. **R. Količ:** None. **A. Vuga:** None.

EP15.061 Detection of three novel Loss-of-Function variants in *SUPT5H*; rare cause of beta-thalassemia trait

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Beta (β) thalassemia is an inherited disorder that occurs following mutations or deletions in the β globin (*HBB*) gene. Rarely, it is caused by variants in genes coding for erythroid transcriptional factors or trans-acting factors. Here, we report three novel variants of *SUPT5H* revealed by next generation sequencing. This, gene has been progressively acknowledged as a mimicker of β thalassemia trait in two independent individuals and one family. These individuals have the same features, including hypochromic microcytic indices, increased Hb A_2 levels, without mutations in the β globin gene. The three novel *SUPT5H* variants identified in this study (c.1168_1169del, c.2688del and c.307+1G > A) are frameshift variants leading to a premature stop codon or an intronic variant predicted to alter the splice site consensus sequence by in silico software. All three variants are characterized as Loss-of-function variants either by generating a truncated protein or haplo-insufficiency due to nonsense-mediated decay. These findings confirm the general observation that most variants in *SUPT5H* associated with a β thalassemia trait phenotype are Loss-of-Function variants. This gene should be considered as a potential target gene in the genetic diagnosis of any unsolved cases of increased HbA_2 and unexplained inconsistency of phenotype and genotype of β thalassemia intermedia.

C.O.I. Disclosure:

C.L. Harteveld: None. **N. Fatma Fairuz Nik Mohd Hasan:** None. **A. Achour:** None. **A. van Gammeren:** None. **J. van der Leeuw:** None. **H. Ceelie:** None. **D. Stieber:** None. **F. Baas:** None. **T. Koopmann:** None.

EP15.062 End-to-end interpretable Machine Learning pipeline for genetic risk prediction in Primary Biliary Cholangitis

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Background: Machine Learning (ML) applied to genetic data offers significant promise in improving the prediction and understanding of rare autoimmune liver diseases, such as Primary Biliary Cholangitis (PBC). This study aims to evaluate the feasibility and accuracy of using an ML-based model for predicting PBC risk using genetic data.

Material and methods: Genome-wide significant variants from the second international meta-analysis of PBC genome-wide association studies were analyzed. The analysis focused on quality-controlled genomic data from two Italian cohorts, including 13 million Single-Nucleotide Polymorphisms (SNPs) per individual. Cohort-1 included 1345 individuals (33% PBC cases), and Cohort-2 included 834 individuals (31% PBC cases). Feature selection was performed in two stages using the Rulex platform (www.rulex.ai): intra-chromosome feature selection based on correlation with the target variable, followed by an advanced feature selection to combine results and eliminate redundancy. A Logic Learning Machine (LLM) was used to generate interpretable "if-then" predictive rules.

Results: Feature selection reduced the dataset to 165 relevant SNPs, enabling the derivation of 150 predictive rules. The model achieved an Area Under the Curve (AUC) of 0.81 during internal validation. When applied to Cohort 2, only 41 of the 150 rules were available due to missing SNPs, achieving an external validation AUC of 0.73.

Conclusion: Our ML-based model demonstrated strong predictive performance for PBC risk, with AUC values of 0.81 and 0.73 in internal and external validation, respectively. These findings highlight the potential of ML for genetic risk prediction in rare diseases, offering a customizable and interpretable framework for future refinements.

C.O.I. Disclosure:

E. Merelli: None. **C. Caime:** None. **D. Verda:** A. Employment (full or part-time); Significant; Rulex Innovation Labs, Rulex Inc. **A. Piazza:** A. Employment (full or part-time); Significant; Rulex Innovation Labs, Rulex Inc. **M. Muselli:** A. Employment (full or part-time); Significant; Rulex Innovation Labs, Rulex Inc. **P. Invernizzi:** None. **R. Asselta:** None. **A. Gerussi:** None.

EP15.063 Unravelling the Genetic Complexity of Neurodevelopmental and Neuromuscular Symptoms: A Case Report of Rare Homozygous Variant in *SOD1*

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Background: Superoxide dismutase 1 (*SOD1*) is typically associated with amyotrophic lateral sclerosis (ALS), a progressive neurodegenerative disease. Recently, a syndrome with severe childhood-onset spastic tetraplegia and axial hypotonia (STAHP) caused by a rare homozygous variant in *SOD1*, was described. Pathogenic/Likely Pathogenic variants in *SOD1* impair the enzyme's ability to neutralize superoxide radicals, leading to

oxidative stress, causing degeneration of motor neurons, resulting in spastic tetraplegia and axial hypotonia. Less than five cases have been reported. We report the first Pakistani case of STAHP, caused by a rare homozygous variant in *SOD1*.

Methods: Retrospective review of records - Case report.

Results: A 2.5-year-old male, born to consanguineous parents, presented with developmental delays (motor and speech), and neuromuscular symptoms (muscle stiffness and weakness). The patient exhibited macrocephaly at birth, followed by developmental regression at 10 months, characterized by spasticity, hyperreflexia, and progressive muscular weakness. Whole Exome Sequencing (WES) identified a homozygous missesnse variant in *SOD1:* c. 152T > G (p.Phe51Cys) classified as Likely Pathogenic according to ACMG criteria. The phenotype presentation matched with the genetic diagnosis of autosomal recessive STAHP, providing clinical evidence for the variant to be disease causing.

Conclusion: This is the first instance of STAHP associated with a rare *SOD1* variant in a Pakistani, expanding the clinical and genetic understanding of rare variant in a diverse population. This case highlights the importance *SOD1* variants in causing childhood onset progressive neuromuscular disease, and comprehensive testing such as WES in the clinical workup of such cases. Grants: It is not a funded project.

C.O.I. Disclosure:

M. Nasir: None. **F. Akbar:** None. **Z. Hasan:** None. **S. Kirmani:** None.

EP15.064 Genetic heterogeneity of genes responsible for the development of primary ciliary dyskinesia in the Russian population

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Background: Primary ciliary dyskinesia (PCD) is a rare inherited disorder from the group of ciliopathies with autosomal recessive and dominant, less frequently X-linked inheritance. More than 60 genes responsible for PCD have been studied.

Objective: to evaluate genetic heterogeneity of genes responsible for the development of PCD in the Russian population.

Material and methods: the diagnosis of PCD was established using the criteria of the European PCD Society. Genetic studies included exon sequencing and were performed in 188 patients, 75 of them had 2 variants in heterozygous genes.

Results: In 75 patients 97 genetic variants were found, in 23 genes. 93 (95.9%) with autosomal recessive type of inheritance, among them 23 (24.7%) had pathogenic variants in homozygous state, 70 (75.2%) in heterozygous state. Variants in *OFD1* gene (X type of inheritance) were diagnosed in 3 (4%) patients. 1 (1.3%) patient with autosomal dominant type of inheritance in *FOXJ1* gene was registered. Autosomal recessive type of inheritance with compound heterozygous variants predominates, while homozygous variants predominate in other populations. The frequency of variants in the *DNAH5* gene in the results matched the multicenter study (https://doi.org/10.1183/13993003.01769-2023) and ranks first (40% and 22%, respectively). Further gene frequencies were not matched: *CFAP300* (9.3%), *DNAAF11* (6.7%), *DNAH11* (6.7%), *CCDC39* (5.3%), *OFD1* (4%). According to global data, *DNAH11* (11%), *CCDC40* (9%), *NAI1* (6%), *CCDC39* (5%), *SPAG1* (*DNAAF13*) (4%) genes were ranked second and subsequent.

Conclusion: 23 PCD genes were identified in the Russian population, and differences in gene frequencies were found in comparison with Euro-Asian data.

C.O.I. Disclosure:

T. Kyian: None. **M. Avakyan:** None. **O. Ryzhkova:** None. **A. Bukhonin:** None. **O. Shchagina:** None. **E. Kondratyeva:** None. **S. Kutsev:** None.

EP15.065 Trio-genome sequencing reveals a novel de novo inversion in *CYP19A1* and *USP8* in a patient with aromatase excess syndrome

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Background: Aromatase excess syndrome (AEXS, #139300) is a rare autosomal dominant disorder caused by gain-of-function variants in *CYP19A1*, with ~30 reported cases. It manifests as peripubertal gynecomastia, accelerated bone maturation, initially tall stature, and short adult height due to premature epiphyseal closure in males. *CYP19A1* encodes aromatase, the key enzyme in estrogen synthesis, which converts androstenedione to estrone and testosterone to estradiol.

Material and methods: We performed trio-genome sequencing (GS) on a sample from a 12-year-old patient with gynecomastia since age 5, severe hyperestrogenemia, tall stature, macrocephaly, advanced skeletal age of 17 years, previously tested negative for pathogenetic variants in *CYP19A1*. GS was performed using the Twist Library Preparation EF Kit on a NovaSeq 5000 System, with reads aligned to GRCh37/hg19, and using the VarFish platform for variant analysis.

Results: Trio-GS revealed a novel heterozygous de novo inversion (NC_0000015.9:g.50725978_51617877inv) with breakpoints in intron 1 of *USP8* and intron 1 of *CYP19A1*, being the first reported inversion in *USP8*. The inversion results in a chimeric gene consisting of the non-coding exon 1 of *USP8* containing the promoter region, upstream of the coding exons 2 to 10 of *CYP19A1*. Aromatase expression is regulated by tissue-specific promoters in the first non-coding exon. Since *USP8*, which encodes ubiquitin-specific protease 8, is expressed in many tissues, this rearrangement leads to ectopic *CYP19A1* expression and increased estradiol levels.

Conclusion: Trio-GS improves the detection of balanced structural genomic variants with intronic breakpoints and reveals a novel variant in a patient with AEXS, enabling treatment with aromatase inhibitors.

C.O.I. Disclosure:

A. Petukhova-Greenstein: None. **M. Wannack:** None. **H. Sczakiel:** None. **N. Ehmke:** None. **S. Mundlos:** None. **D. Horn:** None. **P. Kühnen:** None.

EP15.066 Breaking the standards: Mosaic trisomy 9 detected through short-read WGS

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Background: Complete trisomy 9 (T9) is usually associated with spontaneous abortion in early pregnancy or affected fetus die *in utero* or shortly after birth. Mosaic form of T9 has a longer survival and wide spectrum of severity in clinical outcome.

Material and methods: We present a 1-year-old female with developmental delay, muscle hypotonia, facial dysmorphism, respiratory difficulties, impaired feeding and kidney function in the immediate postnatal period. Results from the prenatal biochemical test were with increased risk for trisomy 18 and NIPT results suggested trisomy 9, which remained unconfirmed with invasive prenatal and postnatal cytogenetic analysis. We perform short-read WGS, followed by an individual bioinformatic analysis.

Results: Our first-tier bioinformatic analysis algorithms didn't identify causative variant/s and we proceed with multidisciplinary approach and reevaluation of the medical documentation and clinical features. An individual pipeline for bioinformatic and coverage analysis on the WGS data was developed and trisomy 9, was identified. It was confirmed with cytogenetic analysis and targeted FISH, which showed ~17% mosaicism in lymphocytes culture.

Conclusion: Prenatal diagnosis of mosaic trisomy 9 is challenging due to the significant variability in the proportion of trisomic cells in different tissues and probably due to differences in the mitogenic potential of cells with an abnormal karyotype. Diagnosis of such diseases requires a combination of different methods and the examination of non-dividing cells in a sufficient volume to ensure high sensitivity and informativeness of the analysis. Examination of non-dividing cells by targeted FISH analysis is critical, especially in low-grade mosaicism.

C.O.I. Disclosure:

M. Atanasoska: None. **L. Balabanski:** None. **S. Yaneva Staykova:** None. **A. Yosifova:** None. **G. Balatzenko:** None. **S. Angelova:** None. **I. Bradinova:** None. **A. Andreev:** None. **D. Avdjieva-Tzavella:** None. **M. Sredkova:** None. **S. Hadjidekova:** None. **D. Toncheva:** None. **R. Vazharova:** None.

EP15.067 Identification of the heterozygous variant p.Val142Ile in *TTR* gene in a patient with unspecified polyneuropathy

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Background: Polyneuropathy is a major feature of amyloidosis. A hereditary amyloid transthyretin amyloidosis (ATTR) with polyneuropathy is a rare multisystemic progressive disease induced by protein misfolding and aggregation into insoluble amyloid fibrils that deposit in major organs leading transthyretin protein misfolds to form amyloid, which is deposited in the endoneurium.

Material and methods: Herein, we reported a 38 years-old afro descendent man with unspecified polyneuropathy, born to non-consanguineous healthy parents. His clinical evaluation showed distal tetraparesis associated with significant muscle atrophy, hammertoe, areflexia, apalesthesia down to the knees, severe hypopalesthesia in the wrists, foot drop, lumbar scoliosis with convexity to the right, intense, severe and chronic axonal and demyelinating polyneuropathy involving sensory and motor fibers, and hypothyroidism. NGS Panel for Hereditary Peripheral Neuropathy was performed using the Illumina platform.

Results: NGS panel revealed a heterozygous missense variant in *TTR* gene, chr18(GRCh38):g.31598655G > A, ENST00000237014 (p.(Val122Ile)). The variant ATTR p.Val142Ile is the most common cause of cardiomyopathy and usually affects individuals aged > 60 years. Extracardiac diagnosis is occasionally associated with neuropathy, and rates of neuropathy have been reported in up to 10–38% of patients. Our proband presented with polyneuropathy and did not have cardiomyopathy, however he was referred for annual follow-up by a cardiologist.

Conclusion: ATTR amyloidosis with polyneuropathy is the most serious hereditary polyneuropathy of adult onset. The variant p.Val142Ile has an autosomal dominant pattern and the diagnosis is essential for family screening. Carriers at risk should be offered appropriate surveillance, early disease detection and management, and family counselling.

C.O.I. Disclosure:

I.P. Pinto: None. **J.F. da Silva:** None. **T.A. Campos:** None. **M.B. Porto:** None. **F.S. Santos:** None. **E.M. Uchoa:** None. **Z. Raffael:** None. **A.H. Bernardes:** None. **L.B. Minasi:** None. **A.D. da Cruz:** None.

EP15.068 Jervell and Lange-Nielsen syndrome with a pathogenic homozygous *KCNQ1* gene mutation in an Iranian Family

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Background: Jervell and Lange-Nielsen syndrome (JLNS), is the autosomal recessive manifestation of Long QT Syndrome (LQTS) characterized by bilateral sensorineural hearing loss and cardiac phenotype, QT prolongation, ventricular arrhythmias, and sudden cardiac death (SCD) (1,2). This condition arises from homozygous or compound heterozygous mutations on *KCNQ1* or *KCNE1* (3). The proteins produced by these two genes together constitute a potassium channel responsible for facilitating the outward flow of positively charged potassium ions from the cells. (4). Close to 90% of mutations are located in *KCNQ1*, amplifying the risk of developing arrhythmias (5,6). In this study, our objective was to

examine the genetic etiology of JLNS and LQT in a one-year-old child with hearing impairment.

Material and methods: A clinical recruitment was conducted for an Iranian pedigree diagnosed with JLNS, anemia, and suspected LQTS. After obtaining informed consent from the proband and his family members, DNA extraction were performed from blood samples. Whole exome sequencing (WES) was conducted and subsequently validated through Sanger sequencing within the family.

Results: In this research, we identified a homozygous pathogenic variant, c.G1097A:p.R366Q, in KCNQ1 gene (NM_000218) as the possible pathogenic cause of JLNS. This finding implies an elevated risk of cardiac events in a deaf child.

Conclusion: Identifying the mutation in the KCNQ1 gene as the causative factor of the disease facilitates a more rapid diagnosis and treatment of the disease in deaf children with JLNS.

C.O.I. Disclosure:

N. Naderi: None. **M. Maleki:** None. **S. Kalayinia:** None.

EP15.069 Investigation of *GATA4* gene sequence in non-syndromic familial congenital heart disease in 10 Iranian families

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Background: Congenital heart diseases (CHD) are generally caused by defective morphogenesis during fetal development. These abnormalities may be limited to the cardiovascular system (non-syndromic CHD) or occur as part of defined syndromes involving other systems (CHD syndrome) (1). Zinc finger transcription factors of the GATA4 family play a prominent role in regulating of myocardial growth. *GATA4* knockdown in cardiomyocytes reduces the expression of essential cardiac growth-related genes (2).

Material and methods: In this study, among the patients referred to the Rajaie Cardiovascular Medical and Research Institute between Februery 2024 and May 2024, patients with CHD were identified through clinical diagnosis and echocardiography. Blood samples were collected from 10 families with CHD and their family members (patients/healthies) after obtaining informed consent. DNA was extracted, and PCR was performed for *GATA4* coding exons. Sanger sequencing was done to analyze the *GATA4* coding exons.

Results: Among the 17 patients with CHD studied, two pathogen missense mutations c.1223C>A and c.1312G>A, were found in the *GATA4* gene in two unrelated patients from two different families using bioinformatic analysis. Additionally, 14 polymorphisms were detected: 8 variants in intronic regions, 2 in coding regions, and 4 in the 3′UTR.

Conclusion: The identification of family variants in this study shows the significant influence of carriers or affected parents on the risk of CHD in their offspring. These findings highlight the importance of genetic screening for prenatal diagnoses and future pregnancy in families with a history of CHD.

C.O.I. Disclosure:

S. Taherinia: None. **I. Salahshourifar:** None. **S. Kalayinia (*Corresponding Author):** None.

EP15.070 Investigation of *TMEM43* gene sequence in 10 patients with Arrhythmogenic Right Ventricular Cardiomyopathy referred to Shahid Rajaei Cardiovascular Medical and Research Institute

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Background: Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) is an inherited disorder characterized by ventricular arrhythmias and progressive ventricular dysfunction. Mutations in the *TMEM43* gene are associated with an aggressive form of the disease, known as ARVC-5, which carries an extremely high risk of sudden death. *TMEM43* encodes transmembrane protein 43, and mutations in this gene result in dysfunction of cardiac cells.

Material and methods: This study involved 10 families with a history of sudden cardiac death. After obtaining informed consent, genomic DNA was extracted from whole blood. Specific primers targeting all coding regions of the *TMEM43* gene were designed. Sanger sequencing was performed on the coding regions and exon-intron boundaries of the *TMEM43* gene. Segregation analysis within family members was subsequently carried out to confirm the results.

Results: Sequence analysis identified two heterozygous pathogenic mutations: c.1073C>T and c.947G>C, located in exons 12 and 11, respectively. These mutations result in the amino acid substitutions S358L and W316S. In the first family, segregation analysis of the c.947G>C mutation was conducted, but none of the available family members carried the variant. In the second family, unfortunately, all first-degree relatives had passed away, preventing further examination of the c.1073C>T variant.

Conclusion: Genetic and molecular investigations of the exon sequences of the *TMEM43* gene offer valuable insights into the pathways involved in the pathogenesis of ARVC. These studies aid in the identification of familial carriers, facilitate the development of novel therapeutic approaches, and help prevent fatal complications associated with the disease.

C.O.I. Disclosure:

H. Motallebzadeh: None. **S. Kalayinia:** None. **N. Mahdieh:** None. **A. Fazelifar:** None.

EP15.071 Clinical case of Zellweger syndrom caused by PEX2 gene variation

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Background: Peroxisome proliferator-activated receptor-Zellweger spectrum disorder is a rare childhood-onset genetic disorder caused by mutations affecting genes involved in the metabolic disorder. The overall incidence of peroxisome disorders is approximately 1 in 50–100,000 live births.

Material and methods: Medical history, exome sequencing, enzyme activity in DBS.

Results: The patient was a male infant, the first child of non-blood parents, and the pregnancy was uncomplicated. The child's condition deteriorated sharply within 2hours after birth, with convulsions, depression of consciousness, and apnoea. On the 42nd day of life, the child developed skin and mucous membrane lesions, as well as severe postnatal physical developmental delay.

Instrumental examinations revealed a congenital heart defect (perimembranous interventricular septal defect with signs of left heart overload, left atrial dilatation, open foramen ovale, moderate pulmonary hypertension), hepatomegaly, pseudocysts of the choroid plexus and a picture of epileptic encephalopathy.Genetic tests: - a dry spot blood test to determine the enzymatic activity of acid sphingomyelinase - negative result; - exome sequencing, which revealed a pathogenic homozygous variant of the PEX2 gene (autosomal recessive disorder of peroxisome proliferator-activated receptor type 5A). The baby's vital functions and other health indicators were stable, so they had surgery at 7months to reduce the pulmonary artery. However, the condition was severe due to the underlying disease. Her life is supported by constant medical monitoring

Conclusion: This case is unique due to the severity of the condition, successful surgery and relatively long life expectancy (our patient is already 8 months old) and doctors continue to fight for his life.

C.O.I. Disclosure:

V. Hlohus: None. **H. Akopyan:** None. **N. Lukyanenko:** None. **M. Iskiv:** None.

EP15.072 NGS in critically ill infants/neonates

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Newborn genetic diagnosis using genome sequencing has enabled the rapid screening of a wide range of monogenic disorders. The purpose of our study was to evaluate the outcomes of whole exome sequencing (WES) and whole genome sequencing (WGS) in symptomatic neonates and infants. This cohort study enrolled 25 symptomatic neonates and infants, exhibiting a spectrum of mainly neurological symptoms. WES/WGS identified relevant diagnostic variants in 32% of the cases (n = 8 patients) (including single nucleotide variants- SNVs, copy number variants-CNVs and one case of aneuploidy), suggesting that using next generation sequencing (NGS) in critically ill infants/neonates enhances traditional diagnostic capabilities.

C.O.I. Disclosure:

Y.L. Loukas: None. **K. Anagnostopoulou:** None. **G. Thodi:** None. **M. Spanou:** None. **C. Gavalas:** None. **Y. Dotsikas:** None. **M. Alvanou:** None. **R. Pons:** None. **K. Tziouvas:** None. **G. Vartzelis:** None. **E. Skouteli:** None. **E. Loukatou:** None. **A. Charitou:** None. **K. Douros:** None. **S. Siahanidou:** None. **M. Giorgi:** None. **A. Stephanede:** None. **M. Angeli:** None. **M. Nikolaidou:** None. **E. Kokkinou:** None. **I. Kouri:** None. **V. Koute:** None. **A. Dinopoulos:** None.

EP15.073 Investigating the sequence of the KCNQ1 gene in ten patients with Long QT Syndrome referred to Shahid Rajaei Cardiovascular Center

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Background: Long QT Syndrome (LQTS) is a form of ventricular arrhythmia and a hereditary condition that affects cardiac ion channels.(1)It is characterized by a prolonged QT interval on an electrocardiogram (ECG).(2)Common symptoms include palpitations and syncope.(3)LQTS is classified into 17 subtypes, with type 1 accounting for approximately 50% of congenital cases.(4)The most common cause is a mutation in the KCNQ1 gene, which typically follows an autosomal dominant inheritance pattern, although autosomal recessive cases have also been reported.(5)

Material and methods: 10 families with Long QT Syndrome were selected for KCNQ1 gene exon sequencing. Genomic DNA was extracted from blood samples, and specific primers were designed. Sanger sequencing was performed on amplified DNA samples. Results were analyzed using BioEdit software, and segregation analysis was conducted within families to confirm findings.

Results: A pathogenic variant, c.727C>T, was identified in exon 6 of the KCNQ1 gene in one patient, resulting in a heterozygous state. This variant was also heterozygous in the patient's father, while the mother tested negative. Another pathogenic variant, c.1097G>A, was found in exon 9 of the KCNQ1 gene in a different patient, resulting in a homozygous state. Both parents and the patient's sister were heterozygous for this variant, whereas the patient's brother was unaffected and had a normal genotype.

Conclusion: Genetic and molecular analysis of KCNQ1 gene exon sequences provides valuable insights into the pathogenesis of LQTS. These findings can aid in understanding the underlying mechanisms, improving early diagnosis, and developing preventative and therapeutic strategies for affected individuals and their families.

C.O.I. Disclosure:

Z. Abak: None. **S. Kalayinia:** None. **M. Rafie Khorgami:** None. **N. Mahdieh:** None.

EP15.074 Опыт скрининга новорождённых методом полноэкзомного секвенирования когорты российских пациентов

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Background: Newborn whole exome sequencing (WES) can successfully complement the diagnosis of existing newborn screening for severe childhood disorders in newborns by identifying and treating at/before symptom onset. We present the results of the 441 newborn WES screening of cohort of Russian patients from Sakhalin Island.

Material and methods: Cord blood sample was obtained from the newborn and venous blood sample was obtained from their parents. WES was performed on NovaSeq 6000 Sequencing system. Validation of the found variants was verified by Sanger sequencing trio.

Results: There were assessed pathogenic, likely pathogenic and VUS variants for 68 newborns who has signs of the disease (selective screening). There were assessed pathogenic and likely pathogenic for 373 healthy newborns without signs of the disease. As a result, chromosomal pathology was detected for 5 patients, X-targeted recessive type of inheritance was detected for 1 patient, autosomal recessive type of inheritance was detected for 12 patients, AD type of inheritance was detected for 19 patients, of which 14 were with late onset. The variants detected were related to Syndromic conditions (13,8%), Cancer (19,4%), Musculoskeletal (11,1%), Metabolic (8,3%), Cardiovascular (16,6%), Neurologic (5,5%), Ophthalmological (5,5%), Otorhinolaryngological (11,1%), Mitochondrial (5,5%) and Hematological (2,8%) genetics diseases.

Conclusion: Our newborn WES identified an inherited pathology in 8,1% of newborns. There were detected the diseases at a preclinical stage for 69,4% of newborns. Thus, newborn whole exome sequencing obtained make it possible to quickly and accurately diagnose the disease at the preclinical stage, begin its prevention, and, if necessary, treatment.

C.O.I. Disclosure:

V. Demeneva: None. **Z. Ogneva:** None. **S. Lebedev:** None. **E. Markelova:** None. **V. Shumatov:** None. **D. Trofimov:** None. **V. Rosemeiko:** None.

EP15.075 Detection of a novel Alu-insertion in *ATP7B* in a patient with Wilson's disease using long-read sequencing

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Background/Objectives: Wilson's disease (WD) is a rare, inherited, autosomal recessive metabolic disorder caused by pathogenic variants in the *ATP7B* gene. WD is characterized by the accumulation of copper that can cause acute liver failure and brain disease. WD can be treated and severe complications can be prevented with chelating agents. It is paramount to discover both causal variants in *ATP7B* to enable the identification of affected siblings and start their treatment. Here, we present a case describing 9-year-old boy who underwent a liver transplantation because of acute liver failure who met the Leipzig criteria for a clinical diagnosis of WD. He has two siblings and whole-exome sequencing (WES) identified only one pathogenic mutation in *ATP7B* (c.3694A>C, (Thr1232Pro)). We aimed to discover the second pathogenic variant on the other allele.

Methods: Long-read sequencing (LRS) using Oxford Nanopore's adaptive sampling was performed and *ATP7B* was analyzed using the Molgenis Variant Interpretation Pipeline. This approach enables the detection of structural variants and variants in complex genomic regions often missed by WES.

Results: The analysis revealed a novel AluYb9-insertion in intron 5 of *ATP7B* c.1870-10_1870-9insAlu p.?. This insertion is predicted to affect splicing (SpliceAI Δ-score acceptor loss 0.67).

Conclusion: Alu-insertions in *ATP7B* have not been previously described. The Alu-insertion and the previously identified pathogenic mutation are predicted to produce a dysfunctional protein causing WD. CRISPR-activated RNA-sequencing is now being performed to determine the transcriptional consequence of the Alu-insertion. This case report highlights the added value of LRS in identifying complex genetic mutations.

C.O.I. Disclosure:

K.K. van Dijk: None. **A.P. Lampri:** None. **D. Schoonhoven:** None. **E.N. de Boer:** None. **W.S. Lexmond:** None. **C.C. van Diemen:** None. **H. Westers:** None. **B. Raddatz:** None. **D.J. Dijkstra:** None. **R. van Jaarsveld:** None. **F. Imhann:** None.

EP15.076 in silico pathogenicity analysis of CPT-1, CPT-2 and OCTN2 Variants in Carnitine Cycle and Transport Disorders reveals structural and functional impacts

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Background: Carnitine Cycle and Transport Disorders (CCD) are rare metabolic diseases cause by deficiencies in the carnitine cycle leading to impaired fatty acid metabolism, hypoketotic hypoglycemia, hepatic steatosis, and myopathy. CCD is linked to mutations in carnitine palmitoyltransferase 1 and 2 (CPT-1 and CPT-2) and organic cation/carnitine transporter 2 (OCTN2) genes. This study analyzed missense and indel variants of CPT-1, CPT-2 and OCTN2 using Oxford Nanopore Sequencing (ONT).

Material and methods: Whole blood samples from 20 CCD patients (9 male and 11 female patients (3 neonates, 12 children under 10 years and 5 adults) diagnosed via tandem mass spectrometry were subjected to long range PCR amplification and ONT sequencing. Variants were annotated using ANNOVAR and VarAFT tools,prioritized with ClinVar, Franklin by Genoox, and VarSome, and assessed for pathogenicity using MutPred and Missense3D. Structural modeling was performed using Protter, AlphaFold, and SwissModel.

Results: in silico pathogenicity predictions identified 8 pathogenic, 8 (likely pathogenic) LP variants 8 variants of uncertain significance (VUS). Five missense variants were predicted to disrupt protein structure, and the OCTN2:NM_003060.4:c.454G>C:p.Gly152Arg variant showed significant instability, confirmed by both MutPred and Missense3D. Indel variants also resulted in major structural changes.

Conclusion: This study highlights potentially pathogenic CCD-related variants, emphasizing the role of in silico analysis in identifying structural disruptions that may impact protein function. These findings support genetic screening for CCD diagnosis and further functional studies.

C.O.I. Disclosure:

G. Akan: None. **M.C. Balcı:** None. **G. Tuncel:** None. **M. karaca:** None. **A. Ozketen:** None. **H.H. kazan:** None. **Ö. Özgen:** None. **G.F. Gokcay:** None. **F. Atalar:** None.

EP15.077 p.Arg1914His variant reduced the abundance of full-length NBAS protein in patient derived fibroblasts with SOPH syndrome

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Background: SOPH syndrome- rare, genetic, developmental defect during embryogenesis malformation syndrome characterized by severe postnatal growth retardation, craniofacial dysmorphism, Pelger-Huët anomaly of leukocytes and bilateral optic atrophy with loss of color vision and visual acuity. In the Yakut population of the Republic of Sakha (Yakutia) it occurs with a frequency of 18:100,000. It is caused by G5741 → A (R1914H) variant in NBAS gene.

Material and methods: Patients' dermal fibroblasts were cultured in DMEM culture media sodium pyruvate and Glutamax (Capricorn) supplemented with 10% fetal bovine serum (FBS) (Capricorn), 100 IU/ml penicillin and 100 µg/ml streptomycin (Invitrogen) at 37 °C in a humidified atmosphere of 5% CO2. Cell lysates were clarified by centrifugation at 21,000 g Samples were subsequently separated by SDS-PAGE, transferred to PVDF membranes (GE Healthcare) and subjected to incubation with anti-human NBAS (Sigma-Aldrich), and actin (Sigma-Aldrich) antibody followed by secondary antibody. Immunoblotted NBAS bands were quantified by densitometry using Image software, normalized to actin bands. A paired student *t*-test was used to calculate *P*-values.

Results: We measured the level of NBAS protein in healthy and patient derived fibroblasts. The missense mutation reduced the abundance of full-length NBAS protein in patient derived skin fibroblasts with SOPH and causes the production of its truncated form.

Conclusion: The study of the NBAS protein will be very relevant and will contribute to the disclosure of the molecular mechanisms of pathogenesis and fundamental research to identify therapeutic targets for this disease.

C.O.I. Disclosure:

M.T. Savvina: None. **A. Guriev:** None. **N.R. Maksimova:** None. **A.L. Sukhomyasova:** None.

EP15.078 Diagnostic yield of Exome Sequencing in the setting of a General Pediatric Clinic for hospitalized children with suspected genetic disorders

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Background/Objectives: Genetic conditions contribute a significant portion of disease etiologies in children admitted to a General Pediatric Clinic (GPC). Exome Sequencing (ES) is a NGS method, identifying pathogenic or likely pathogenic single nucleotide variants, small insertions/deletions and copy number variants (CNVs). We aim to investigate the diagnostic impact of ES in children with suspected monogenic disorders, while receiving inpatient care.

Methods: We retrospectively reviewed all ES tests performed on pediatric patients, admitted to the GPC at a tertiary medical hospital between November 2022 and December 2024, with various disorders and unrevealing workup before ES. The patients' clinical features and molecular diagnoses based on ES results are reported in this study. ES was performed on DNA extracted from peripheral blood: library preparation was performed with Clinical Exome (Sophia Genetics) or Whole Exome (Twist Bioscience) and sequenced on NextSeq-550 (Illumina). Bioinformatics analyses were conducted by SOPHiA DDM® bioinformatics pipelines. All ES were performed in a specific laboratory.

Results: A total of 117 probands were referred for ES. Neurological phenotype was the most common (26/117), followed by metabolic (22/117), cardiovascular and immune disorders (18/117 each). We identified definitive positive or probable positive results in 50/117 patients (42.7%). 46/50 patients had pathogenic/likely pathogenic variants for monogenic disorders, 1/50 a pathogenic risk factor for multifactorial disease and 3/50 CNVs indicative of genetic syndromes. In 30/117 the results were inconclusive, considering further action.

Conclusion: ES consists a powerful tool, revealing a significant fraction of genetic etiologies among children admitted to a GPC, ending their diagnostic odyssey.

C.O.I. Disclosure:

E. Papadopoulou: None. **G. Christopoulou:** None. **A. Fountoulaki:** None. **I. Konstantinidou:** None. **K. Kavalierou:** None. **M. Kassotaki:** None. **M. Michailou:** None. **R. Poulaki:** None. **P. Konstantoulakis:** None.

EP15.079 HMGA2 - a new candidate gene for Moreno-Nishimura-Schmidt syndrome

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Background: Moreno-Nishimura-Schmidt (MNS) syndrome is a very rare disorder characterized by severe overgrowth as well as various skeletal and other features (OMIM #608811). Since 1974, a total of 6 patients have been reported, with no known genetic etiology to date.

Material and methods: We describe a 4-year-old male patient presented with an extreme tall stature, loss of subcutaneous adipose tissue, facial dysmorphism, neurodevelopmental delay, scoliosis, congenital contractures of the elbow, knee, and hip joints, spondylar dysplasia, dolichocolon and dolichosigma. Proband was clinically diagnosed with MNS syndrome. Trio whole-genome sequencing was performed followed by cytogenetic and molecular cytogenetic analysis.

Results: Signs of a complex chromosomal rearrangement with multiple breakpoints involving chromosomes 12 and 18 were revealed by structural variants calling with Manta algorithm followed by visual inspection in the IGV browser. These changes were not detected in the parents' genomes, indicating the de novo nature of the chromosomal rearrangement. FISH analysis confirmed the presence of a paracentric inversion inv(12)(q13.1q14) with an insertion ins(12;18)(q14;q11.2q?12.3). Seven protein-coding genes (*HMGA2*, *RXYLT1*, *LRIG3*, *NR4A1*, *ZNF521*, *LOXHD1*, *ST8SIA*) were affected by rearrangement, of whicn gene *HMGA2* is known to be implicated in height abnormalities. For the first time germinal fusion gene *HMGA2-ZNF521* formed in 5′-3′ (head-to-tail) orientation with breakpoints in *HMGA2* intron 3 and *ZNF521* intron 5 was identified.

Conclusion: Shared phenotype features regarding extreme tall stature of our patient and the only previously described case of germline rearrangement of *HMGA2* inv(12)(p11.22q14.3) allows *HMGA2* to be proposed as a candidate gene for Moreno-Nishimura-Schmidt syndrome.

C.O.I. Disclosure:

E.V. Vasilyev: None. **I.S. Dantsev:** None. **A.D. Kolotii:** None. **A.A. Kudakaeva:** None. **M.A. Parfenenko:** None. **V.M. Kenis:** None. **I.Y. Iourov:** None. **V.Y. Voinova:** None.

EP15.080 Atypical phenotypes in Prader-Willi syndrome: suggestions from four novel patients

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Background: Prader Willi syndrome (PWS) is a well known condition characterized by low birth weight, severe neonatal hypotonia with feeding difficulties, obesity since the first years of life, and a final phenotype characterized by mild intellectual disability, severe obesity, hypogonadism, short stature and small hands and feet. All these signs allow for a gestaltic diagnosis of PWS in the majority of cases. Literature deals with several atypical clinical presentations of PWS, but the molecular bases of this diversity are largely unknown.

Material and methods: we present on four PWS subjects with atypical clinical presentation, with respect to both the pattern of growth and the degree of the neurodevelopmental impairment. We searched for the molecular basis of this clinical heterogeneity by 1) chromosomal microarray analysis; 2) exome sequencing; 3) type of the genomic defect and its occurrence in a mosaic status.

Results: explaining clinical diversity of PWS was the comorbidity with a second genomic abnormalities, including del(16p11.2) deletion, a pathogenic variant in *NOTCH3* and a mosaic status for UPD(15)mat. The last patient with homogenous 15q11q13 deletion on blood cells presented with very mild intellectual disability and no obesity received an early metabolic and educational intervention. A search for mosaicism for the deletion on different tissues is ongoing.

Conclusion: clinical validity of the present data are discussed.

C.O.I. Disclosure:

M. Palucci: None. **P. D'Ambrosio:** None. **A. Gazzellone:** None. **B. Rosa:** None. **A. Crino':** None. **M. Zollino:** None.

EP15.081 Sequence analysis of hotspot exons (exons 11, 12, 23, and 36) of the *EIF2AK4* gene in 8 families with a patient with Primary Pulmonary Hypertension referred to Rajaie cardiovascular medical and research institute

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Background: PPH is a rare and progressive disease that often leads to patient mortality. Understanding its genetic and molecular mechanisms may lead to novel diagnostic and therapeutic approaches. Accordingly, this study aims to perform the sequence analysis of hotspot exons (exons 11, 12, 23, and 36) of the *EIF2AK4* gene in eight families with members affected by PPH, referred to Rajaie Cardiovascular Medical and Research Institute.

Material and methods: From the patients referred to the Rajaie Cardiovascular Medical and Research Institute in 2024, eight individuals diagnosed with PPH by a cardiologist were selected for this study. Family pedigrees were constructed, and blood samples were collected. Genomic DNA was extracted and DNA concentrations were measured. Primers were designed. Patients DNA were amplified using PCR, and successful amplification was confirmed by gel electrophoresis. Amplified DNA samples were sent for Sanger sequencing. If a pathogenic variant was identified in a patient, additional blood samples were collected from their family members for segregation analysis. Identified variants were analyzed using bioinformatics tools to predict their pathogenicity and potential role in disease causation.

Results: Among the eight patients, a pathogenic variant, c.C4669T, was identified in exon 36 in one patient in a homozygous state. Sequencing of exon 36 in the patient's family members showed that five out of seven carried the c.C4669T variant, while the remaining two did not carry the variant.

Conclusion: Sequence analysis of the EIF2AK4 gene in PPH patients can significantly enhance our understanding of PPH pathology and preventive and therapeutic strategies.

C.O.I. Disclosure:

A. Zare Neyestanak: None. **Z. Noormohammadi:** None. **S. Kalayinia:** None.

EP15.082 A pathogenetic variant in *DYSF* gene in iraninan family with Laing early onset distal myopathy

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Background: Laing early onset distal myopathy (MPD1) is an autosomal dominant myopathy caused by mutations within the slow skeletal muscle fibre myosin heavy chain gene(1). muscle disorders characterized by weakness of the distal muscles of the arms and legs, Weakness of the neck flexors is seen in most affected individuals and mild facial weakness is often present(2). In this study, an Iranian patient with MPD1 was reported due to mutation in *DYSF* gene.

Material and methods: Blood sample of the patient with MPD1 and other available members of her pedigree (healthy/patient) were collected. Clinical evaluations performed. DNA was extracted and NGS analysis of the proband was conducted. Identified variant was confirmed using specific designed pair primers.

Results: Clinical evaluations were consistent with MPD1 in proband and other patient of the pedigree. Our NGS analysis revealed the ptoband is carrier of one pathogenic variant naming *DYSF*, c.C3070T: p.R1024X.

Conclusion: NGS tests could be helpful for finding the causal variants in MPD1. This study highlights the role of genetics in the better and more precise diagnosis of MPD1.

C.O.I. Disclosure:

T. Masoumi: None. **M. Maleki:** None. **S. Kalayinia:** None.

EP15.083 Relevance of Next Generation Sequencing in the diagnosis of thalassemia and hemoglobinopathies the experience of four italian diagnostic hubs

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Background: This study highlighted the efficacy and rapid identification of all types of mutations in the α- and β-globin genes, including silent variants, using the Devyser Thalassemia NGS kit. Furthermore, we report the frequency of mutations identified in a total population of 2649 individuals recruited from four Italian Medical Genetics Laboratories.

Material and methods: All samples were first hematologically characterized, and sequence analysis was conducted by using the Devyser Thalassemia NGS kit. All variants were also validated in an independent sample by a conventional molecular test.

Results: A total of 1789 subjects were identified with genetic variants in the globin genes, of which 966 (53.9%) had variations in the β-gene, 480 (26.8%) had variations in the α-gene; and 307 (17.1%) had variations in both α- and β-genes. Variant analysis evidenced a heterogeneous mutation spectrum enriched with variants not usually observed in the Italian population.

Conclusion: This study showed the high effectiveness and the rapid identification of all mutation types in both α- and β-globin genes, including silent variants. It should be emphasized that the NGS approach greatly shortens turnaround reporting times, overcoming the classic diagnostic flowchart which envisages multistep, subsequent, diagnostic approaches, often requiring long resolution times.

C.O.I. Disclosure:

R. Selvatici: None. **V. Guida:** None. **M. Maffei:** None. **M.A. Irrera:** None. **A. Margutti:** None. **P. Bisceglia:** None. **M. Mogni:** None. **E. Melchionda:** None. **G. Stoico:** None. **N. Grifone:** None. **L. Bocciardo:** None. **S. Salerio:** None. **V. Nagliati:** None. **A. Alberico:** None. **G. Tringali:** None. **C. Melles:** None. **A. De Luca:** None. **A. Ferlini:** None. **D. Coviello:** None. **C. Curcio:** None.

EP15.084 Whole Exome Sequencing Reveals Compound Heterozygous Mutations in *TTN* in a Child with Dilated Cardiomyopathy and Myopathy

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Background: Dilated cardiomyopathy (DCM) and myopathy are classified as a group of genetic disorders characterized by the impairment of muscle fibers, leading to muscle weakness and dysfunction. Various genetic mutations across several genes have been reported to affect the structure and function of muscle tissue, ultimately compromising muscular performance. The *TTN* gene encodes a large protein known as titin, which plays a critical role in skeletal and cardiac muscles. Several nucleotide alterations have been identified that contribute to DCM and early-onset myopathy.

Material and methods: A two-year-old girl diagnosed with DCM and myopathy, born to consanguineous parents, was selected for genetic analysis using Whole Exome Sequencing.

Results: Two compound heterozygous mutations, (c.65488C>T) and (c.51884G>A), in the *TTN* gene were detected in exons 339 and 326, respectively. The mutation c.65488C>T has been previously reported as a pathogenic variant; however, c.51884G>A is identified as a novel variant and is predicted to be of uncertain significance according to ACMG classification.

Conclusion: These findings highlight the potential genetic underpinnings of DCM and myopathy and may assist in prenatal diagnosis for families and carrier detection of other affected family members. Furthermore, prenatal diagnosis can serve as a significant step in confirming clinical diagnoses.

C.O.I. Disclosure:

M. Soveizi: None. **M. Maleki:** None. **S. Kalaeinia:** None.

EP15.085 Analysis of ALPK3 Gene (Exons 5,8) in 10 Families with Hypertrophic Cardiomyopathy

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Background: Hypertrophic cardiomyopathy (HCM) is a relatively common genetic cardiac disease, with mutations in genes encoding sarcomere proteins. These mutations lead to thickening of the left ventricular wall, resulting in heart failure and impaired cardiac function. Recently, mutations in the alpha kinase 3 (ALPK3) gene have been implicated in the development of HCM. It is considered a regulator of cardiac transcriptional activity through the phosphorylation of transcription factors. This study aimed to examine two hotspot exons (exons 5 and 8) of the ALPK3 gene in 10 families with a history of HCM in one or more affected members.

Material and methods: Ten patients diagnosed with hypertrophic cardiomyopathy (HCM) were selected for this study. Informed consent was obtained and whole blood samples were collected and genomic DNA was extracted. Specific primers were designed for exons 5 and 8 of the ALPK3 gene. Selected sequences were amplified through the PCR, and the resulting products were sequenced using Sanger sequencing.

Results: Among the ten patients, two homozygous pathogenic variants were identified: (c.676C>T) in exon 5 and (c.4019A>G) in exon 8, found in separate candidates. Segregation analysis revealed that three out of four relatives carried the c.676C>T variant.Additionally, in the second family, both relatives carried the c.4019A>G variant.

Conclusion: This study aimed to identify mutations in the ALPK3 gene causing HCM, resulting in the identification of homozygous mutations in exons 5 and 8 that lead to the production of a truncated protein, contributing to HCM symptoms. Our findings underscore the importance of genetic analysis in diagnosing HCM by cardiologists.

C.O.I. Disclosure:

S. Kalayinia: None. **F. Farajollahi:** None. **I. Salahshourifar:** None.

EP15.086 Exome analysis applied to routine diagnostics: report on 2 years's experience

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Background: Exome sequencing (WES) is a crucial test for rare genetic diseases diagnosis. We present considerations relating 2023/2024 experience at the Gaslini Institute. The data analysis

allowed us to delve deeper into some points: characteristics of the clinical question, response times, identification rate, limits, frequency and management of secondary findings. This analysis aims to improve the usefulness and concretely respond to clinical needs.

Material and methods: Selection of coding and intronic regions +/−10 bp, IDT xGen Ex Res Panel v2 kit and Novaseq6000 Illumina Sequencing. Raw sequence alignment hg38 BWA, GATK call, variant reading ≥10X. Annotation and prioritization: Ensembl-VEP, Exomiser; population databases: dbSNP, GnomAD, ACMG class: B, LB, VUS, LP, P (Varsome, Franklin). Limits: limited or no sensitivity for indels in homopolymeric sequences and somatic mutations, del/dup of single or multi-exons, structural rearrangements.

Results: Families: 600 (singleton 8.6%, proband + 1 family member 6.6%, trios 80%, ≥ 4 members 4.6%). Clinical question: Syndromic 42%, Neurological 39%, Muscular 6%, Oncological 5%, Other 8%.

Results: positive 28.5%, causative hot VUS 11.6%, real positives 40.1%. Timing: 3–6 months. Re-Analysis of negatives (performed worldwide): analysis of raw data, including candidate genes and unusual variants in disease genes: 3%. Secondary Findings (ACMG v3.2 list): 1.1%

Conclusion: WES detection rate in literature: 20–40%, depending on the clinical question. Problems: incorrect or incomplete family history, loss of deep intronic variants. CNV loss: WES does not appear to be the test of choice for CNV, identified instead with WGS (Detection GS = detection ES + detection CNV).

C.O.I. Disclosure:

F. Madia: None. **P. De Marco:** None. **M. Traverso:** None. **M. Di Duca:** None. **M. Bono:** None. **M. Scala:** None. **V. Capra:** None. **F. Zara:** None. **D. Vozzi:** None.

EP15.087 Whole exome sequencing in neonatal and pediatric critical care units in a tertiary hospital of the Spanish national health system

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Background: Rapid genomic sequencing in neonatal and pediatric intensive care units has proven to be an effective tool for the diagnosis of genetic diseases, allowing rapid and accurate clinical decision-making and access to precision treatments and therapies. Most genetic disorders present a high clinical and genetic heterogeneity that complicates disease recognition and diagnosis with conventional approaches.

Materials and methods: A prospective study was conducted in a Spanish public tertiary hospital during a 30-month period. Critically ill neonates and infants with suspected genetic disease were offered exome sequencing (ES) in trio as a first-line test. Forty-three children with clinical manifestations indicative of a genetic disorder were included. Sequencing and data analysis were performed at the hospital center. Pathogenic and likely pathogenic variants were reported to referring clinical geneticists. Clinical utility was assessed by questionnaires.

Results: A diagnostic yield of 42% was achieved, identifying the genetic cause in 18 of the 43 cases. Genomic testing led to changes in medical management in 32% of the patients. The mean turnaround time for results was 31.3 days.

Conclusion: Genomic medicine in children admitted to the NICU and PICU has the potential to provide faster and more accurate diagnoses, improving clinical management, access to precision treatments or therapies, and favoring the incorporation of personalized medicine in these care settings, which will result in better prognosis and lower morbidity and mortality.

C.O.I. Disclosure:

M. Pacio Míguez: None. **C. Rodríguez Antolín:** None. **C. Rodríguez Jiménez:** None. **M. Gomez Cano:** None. **E. Rikeros Orozco:** None. **S. Siccha Arancibia:** None. **M. Sanchez Holgado:** None. **J. Menéndez Suso:** None. **F. Climent Alcalá:** None. **F. Santos Simarro:** None. **M. Palomares Bralo:** None.

EP15.088 Rapid and Accurate Phenylketonuria Diagnosis: Integrating ONT-Based Molecular Screening into Newborn Programs

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Background: Phenylketonuria (PKU) is an autosomal recessive disorder caused by PAH gene variants, leading to phenylalanine hydroxylase (PAH) deficiency and toxic phenylalanine accumulation. If untreated, PKU causes severe neurological impairment, making early detection via newborn screening crucial. Traditional screening has evolved from the Guthrie test to high-performance liquid chromatography (HPLC), tandem mass spectrometry (MS/MS), and next-generation sequencing (NGS). Oxford Nanopore Technologies (ONT), offers a rapid and cost-effective molecular diagnostic approach, enhancing newborn screening programs.

Material and methods: This study developed an ONT-based molecular screening method to improve early PKU diagnosis, particularly in high-prevalence regions. An in-house gene panel was designed and validated against Illumina sequencing in 40 PKU patients. Gene-specific primers were used to amplify exonic, untranslated, and exon-intron junction regions of the PAH gene, followed by MinION Mk1c sequencing. Variant analysis was performed using Guppy software and bioinformatics pipelines.

Results: Results demonstrated 100% concordance with Illumina sequencing, confirming the ONT-based accuracy and reliability of the ONT-based approach. The most prevalent pathogenic variants identified were NC_000012.12(NM_000277.3):c.1066-11G > A (37.5%) and NC_000012.12(NM_000277.3):c.782G>A (15%).

Conclusion: In conclusion, the ONT-based single-gene testing method provides a fast, accurate and cost-effective solution for early PKU detection, supporting its integration into newborn screening programs. Implementing ONT-based diagnostics can enhance early intervention strategies, improve clinical outcomes, and facilitate large-scale newborn screening in high-prevalence countries like Türkiye.

C.O.I. Disclosure:

G. Tuncel: None. **M.C. Balcı:** None. **G. Akan:** None. **H.H. Kazan:** None. **Ö. Özgen:** None. **A.C. Ozketen:** None. **M. Karaca:** None. **A. Gedikbasi:** None. **F. Atalar:** None. **G.F. Gökçay:** None.

EP15.089 Advanced omics tools in the diagnosis of hereditary neurogenetic disorders

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Background: More than 60% of patients with suspected genetically based neurogenetic diseases remain unresolved after evaluating the exome sequencing data. Moreover in number of patients only one clearly pathogenic heterozygous variant is found in the described gene correlating with the phenotype (monoallelic patients) but the second is not detected. The usage of advanced genomic tools is necessary for diagnostic in those patients, but the newest methods have its own limitations.

Methods: A combination of whole-genome sequencing WGS (Illumina TrueSeq Nano) and Nanopore Minion long-read sequencing (LRS) was performed in five patients with clearly genetically based neurological disease (SPG, peripheral neuropathy) who had repeatedly evaluated exome data with negative results.

Results: Rare variants and CNV analysis (90 in-house controls) were evaluated from WGS data. In monoallelic patients moreover UTRs, promoter, intronic regions of targeted gene were evaluated, but we did not clarify any of sequenced patients. Interpretation of non-coding variants is complicated and needs to be verified by further methods. LRS data were not sufficient from single flowcell (6–13GB; average genome coverage of 3 reads). Therefore, samples were sequenced 3x (3 flowcells) to achieve minimum 6–8 reads average coverage, but even the already known monoallelic heterozygous pathogenic variants were not detected.

Conclusion: WGS is a robust tool with good quality coverage, allows CNV analysis, however, only coding regions can be reliably interpreted. Coverage of long-read sequencing using Nanopore Minion is not sufficient and it seems not to be a good tool in diagnostic of rare neurogenetic disorders.

C.O.I. Disclosure:

A.U. Meszarosova: None. **A. Musilova:** None. **J. Krejcikova:** None. **D.S. Brozkova:** None.

EP15.090 MyomiR signatures in DMD-associated cardiomyopathy

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Background: Duchenne muscular dystrophy (DMD) is a progressive genetic disorder affecting skeletal and cardiac muscles. While myomiRs are linked to skeletal pathology, their role in cardiac deterioration is less studied. This study examines miRNA-133a, miRNA-133b, miRNA-206, miRNA-208a, and miRNA-208b expression in pediatric DMD patients as potential early cardiac biomarkers.

Material and methods: This retrospective study included 30 children with DMD and 30 age-matched controls. MicroRNA extraction was carried out using the MagMAX™ mirVana™ Total RNA Isolation Kit, followed by RT and qPCR in accordance with Thermo Scientific protocols.

Results: Results showed significant differences in miRNA 133a, miRNA 206, and miRNA 208a expression between DMD patients and controls. miRNA 133a ($p = 0.011$) and miRNA 206 ($p = 0.015$) were elevated in DMD, while miRNA 208a was lower ($p < 0.001$). No significant differences were observed for miRNA 133b ($p = 0.213$) and miRNA 208b ($p = 0.606$). miRNA 208a levels varied with disease stage ($p = 0.027$). miRNA 133b was associated with biventricular involvement ($p = 0.030$). ROC analysis identified miRNA 133b as a predictor of biventricular involvement (AUC = 0.742, $p = 0.030$), with a cutoff of 608.62 (54.5% sensitivity, 89.5% specificity). No associations were found with ejection fraction, global longitudinal strain of left ventricle ($p > 0.05$).

Conclusion: miRNA 133a and miRNA 206 were elevated, while miRNA 208a was reduced in DMD, suggesting their role in pathophysiology. miRNA 133b shows potential as a predictor of biventricular dysfunction. Further research is needed to confirm these findings in larger cohorts.

C.O.I. Disclosure:

I. Rodoman: None. **A. Dorif:** None. **I. Palii:** None. **V. Sacara:** None.

EP15.091 Linking Molecular Findings to Patient Priorities: Uncovering Overlooked Phenotypes in Pitt-Hopkins Syndrome

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Background: Pitt-Hopkins syndrome (PHS), due to *TCF4* variants, present with intellectual disability, seizures, respiratory abnormalities, and language impairment. Despite the high prevalence, dysautonomic symptoms as well as behavioural and cognitive phenotyping remain poorly described.

Material and methods: We conducted an ambispective observational multicenter study of confirmed PHS patients with the following objectives: 1) refine genotype-phenotype correlations, 2) identify overlooked symptoms, 3) validate behavioural and cognitive assessment tools, 4) evaluate patients' and caregivers' quality of life (QoL). Molecular data, clinical exams, neurological findings, and adaptive skills were studied. Applying international diagnostic criteria, we evaluated individuals' clinical profiles and correlated with variant localization, reported as a predictor of phenotype.

Results: Thirty patients (11 months to 24 years, fifteen females) presented 20 different single nucleotide variants and ten total or partial gene deletions. Psychomotor and language delays were constant. Among those older than 3 years, 70% achieved independent gait. Epilepsy affected 20%. Altered breathing patterns occurred in over half of patients over six years old, with variable response to drugs. Neuroimaging findings were frecuent but non-specific. Gastointestinal symptoms were very common. 1/3 of individuals presented dysautonomic symptoms, which significantly limit QoL. Adaptive profile, daily living and communication skills were most impaired. Behaviour and sleep were mostly not a problem.

Conclusion: PHS is a well-recognized condition, but many QoL-impacting symptoms remain underreported. Our study broadens the clinical perspective and offer a genothype-phenotype correlation. We provide validated clinical scales that identified key disabilities, which could improve symptom monitoring and treatment evaluation.

C.O.I. Disclosure:

A.G. Rodríguez: None. **F. Epifani:** None. **M. Bolasell:** None. **V. Montiel:** None. **G.A. Nolasco:** None. **C. Carulla:** None. **X. Alonso:**

None. **N.A. Julia:** None. **M. O'Callaghan:** None. **L. Pias:** None. **L. Martorell:** None. **M. Serrano:** None.

EP15.092 Molecular diagnosis of neurofibromatosis type 1 (nf1) in a mosaic state

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Background: Neurofibromatosis type 1 (NF1) (OMIM #162200, ORPHA 636) is an autosomal dominant genetic disorder, caused by heterozygous pathogenic variants in NF1 gene with an incidence of 1:2,500–3,000 and characterized by extreme clinical variability, even within the same family. It primarily affects the neurocutaneous system and is associated with multisystemic complications.More than 5,000 distinct pathogenic (PVs) or likely pathogenic variants (LPVs) have been reported so far. Additionally, deletions and duplications of single or multiple exons, chromosomal abnormalities, and microdeletions involving the entire gene are documented.

Material and methods: 11 patients with negative genetic analysis or NF1 segmental presentation were selected and analysed using a ultradeep NGS customised panel able to detect very low-level mosaic variants

Results: By using DNA extracted from peripheral blood, oral brushing and fresh skin biopsies, we were able to detect variations with a sensibility of around 1% and find the causative PV/LPV variation in 7/11 cases with allele frequencies (VAF) ranging from 1–6%. In detail, we found PVs/LPVs in 4/4 skin biopsy all sampled from paediatric patients, in 1/3 blood samples from adult patients and finally in 2/4 oral brushing samples one paediatric and one adult.

Conclusion: Due to the enormous phenotypic heterogeneity, which is also influenced by the age of the patients, the diagnosis of NF1 is complex. The method developed has proved effective in early identification and diagnostic assessment, which is important for accurate counselling and reproductive risk evaluation.

C.O.I. Disclosure:

D.M. Graziani: None. **F. Tumiatti:** None. **I. Bestetti:** None. **R. Alfano:** None. **F. Grilli:** None. **M. Brena:** None. **S. Tabano:** None. **C. Cesaretti:** None. **F. Natacci:** None. **P. Finelli:** None.

EP15.093 Diagnostic utility of exome sequencing across diverse disease categories

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Background: Whole-exome sequencing (WES) technologies have been routinely used for the diagnosis of genetic diseases for many years. Understanding the diagnostic yield of this method across different disease groups has become crucial for patient management in clinical practice, and various centers have reported differing experiences regarding its contribution to diagnosis. In this study, we aim to share our own experiences.

Material and methods: WES results of patients referred to our center with suspected genetic diseases were compiled. Index patients with clinical presentations consistent with various genetic disorders were included. Variants were evaluated according to current classification guidelines, with pathogenic/likely pathogenic variants categorized under definitive diagnosis and variants of uncertain significance under suspected diagnosis. Variant confirmation and family segregations were performed using next-generation sequencing methods.

Results: The results of 100 index patients were evaluated. Among them, 37 received a definitive diagnosis, while 21 were classified under suspected diagnosis. In 42% of the patients, no variants consistent with the clinical presentation were identified. In three patients with a definitive diagnosis (8.1%), the coexistence of multiple diseases was observed. Definitive diagnostic variants were identified in 46.7% (7/15) of the limited number of prenatal cases with multiple ultrasound anomalies.

Conclusion: Although WES technologies are among the most commonly used genetic methods, their diagnostic utility has not yet reached very high rates. While the number of cases in the preliminary results of our study is quite limited, efforts are ongoing to expand the cohort and determine more informative rates.

C.O.I. Disclosure:

H. Bas: A. Employment (full or part-time); Significant; Intergen Genetics and Rare Diseases Diagnosis Center. **S. Ceylaner:** None.

EP15.094 Genomic Analyses in Rare Paediatric Diseases - 15 years of experience

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Background: Rare diseases are a heterogeneous group of disorders. To diagnose, understand and find appropriate therapeutic strategies for these disorders, genomic analyses are being effectively used as a diagnostic and gene discovery tool.

Material and methods: Over the past 15 years, we studied more than 1000 children and their families with rare diseases of paediatric onset and unknown aetiology using exome sequencing (WES). In 100 cases with negative WES results, we used whole genome sequencing (WGS) and/or RNA sequencing. We started to use long-read sequencing methods to search for molecular diagnosis in unresolved cases.

Results: In 33% of cases we were able to establish a definitive genetic diagnosis using WES, and in a further 10% of cases we identified a potentially causal genetic variant. Selected variants are subsequently characterised in an international collaboration. To date, 14 novel gene-disease associations have been discovered and published in candidate genes based on WES analyses. Re-

analyses of WES data have led to definitive diagnoses in an additional 2% of cases over time. Short-read WGS and RNA analyses helped to clarify a further 2% of cases.

Conclusion: Genomic analysis is not a one-off process; data should be repeatedly reviewed and re-analysed. Interestingly, we observe a reduction in the diagnostic performance of WES in patients who have undergone panel sequencing and/or clinical WES, which includes genes with newly described association with the phenotype. On the other hand, the number of candidate variants is increasing, suggesting that there is still much to discover.

C.O.I. Disclosure:

L. Noskova: None. **V. Stranecky:** None. **M. Tesarova:** None. **H. Hartmannova:** None. **K. Hodanova:** None. **L. Piherova:** None. **H. Treslova:** None. **L. Steiner Mrazova:** None. **M. Zikanova:** None. **T. Honzik:** None. **M. Magner:** None. **J. Zeman:** None. **E. Hruba:** None. **H. Jahnova:** None. **A. Gregorova:** None. **A. Hladikova:** None. **D. Grecmalova:** None. **M. Mohler:** None. **J. Saligova:** None. **L. Potocnakova:** None. **M. Giertlova:** None. **P. Drencakova:** None. **S. Kmoch:** None.

EP15.095 Complex genomic alterations in a patient with rare neurodevelopmental disorder

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Background: We present an ultra-rare case of a patient diagnosed with both Teebi Hypertelorism Syndrome 2 (TBHS2) and 22q11.2 deletion syndrome (22q11.2DS). TBHS2 is a rare craniofacial disorder characterized by a heterozygous mutation in CDH11, and 22q11.2DS is an autosomal dominant deletion characterized by haploinsufficiency in TBX1 responsible for most of the physical malformations.

Material and methods: A 47-year-old man from Central Brazil, with a complex phenotype, born to non-consanguineous healthy parents. Clinical and physical evaluation revealed hypertelorism, posterior embryotoxon, ptosis, blepharophimosis, short palpebral fissure, thick eyebrows, short and bulbous nose, wide nasal bridge, convex nasal root, low-set ears, micrognathia, high palate, thin upper lip and everted lower lip, brachydactyly, flat feet, sialorrhea, anxiety, speech delay, hearing impairment, learning disorder, attention-deficit hyperactivity disorder, lumbar lordosis, and thoracolumbar scoliosis. He also had seizures, low visual acuity, mandibular weakness, chewing difficulties, recurrent infections, dyschezia, hematochezia, splenomegaly, and hepatomegaly. Global Screening Array v3.0, by Illumina, was used for genomic investigation.

Results: SNP array revealed two pathogenic variants, a 3.1 Mb deletion at 16q21 involving the CDH11 morbid gene and a 1.4 Mb deletion at 22q11.21 with nine morbid genes, including TBX1. These CNVs are associated with TBHS2 and 22q11.2DS, respectively. The proband's phenotypes were consistent with the major presentations of both syndromes.

Conclusion: This is the first patient identified with TBHS2 and 22q11.2DS. This complex and nonspecific phenotype was responsible for the 46-year diagnostic journey of our patient. Therefore, molecular diagnosis was essential for genetic counseling, appropriate medical care, and multidisciplinary patient management.

C.O.I. Disclosure:

L.B. Minasi: None. **M. Macedo:** None. **V.C. Santos:** None. **A.H. Bernardes:** None. **R. Zatarin:** None. **I.P. Pinto:** None. **A.D. da Cruz:** None.

EP15.096 Identification of a novel CNV variant in SP*110* gene - insights from exome analysis of patients with inborn errors of immunity

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Background: Inborn errors of immunity (IEI) represent a heterogeneous group of genetic diseases associated with causal DNA variants identified in over 480 genes. The genomic analysis of IEI is significant for improving the health and quality of patient´s life. The timely diagnosis and effective treatment significantly increase their chances of long-term survival or complete healing. The most common causal genetic findings in IEI patients are reported as missense point variants (74%), followed by small indels (11%) and other types e.g. nonsense, intronic, synonymous, splice site (9%). However, CNV variants are also occasionally reported (6%).

Material and methods: In our study, we have altogether analyzed 96 patients (58 males, 38 females), with average age 10,8 (range 1–67) with suspected IEI using WES approach and virtual panel of 573 genes based on the ESID recommendations.

Results: Using CNV analysis we have identified a rare, previously undescribed homozygous large deletion in three unrelated patients. Deletion was localized in *SP110* gene and affects exon 14 and adjacent intronic regions. Further, using PCR primer walking and Sanger sequencing we have identified the breakpoints of deletion. Segregation analysis revealed that the CNV variant was presented in parents of all probands in heterozygousity.

Conclusion: The presented study represents a pilot report of novel large deletion in *SP110* gene in the Slovak population. Our study was primarily focused on the comprehensive genomic analysis of patients with IEI, which may be significant for the diagnosis and further management of patients with IEI.

C.O.I. Disclosure:

G. Krasnanska: None. **G. Blandova:** None. **V. Elias:** None. **M. Baldovic:** None. **J. Krsiakova:** None. **P. Drencakova:** None. **F. Cisarik:** None. **M. Konecny:** None.

EP15.097 Inflammatory biomarker profiling in the follicular fluid of female *FMR1* premutation carriers

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Background: Female *FMR1* premutation carriers are at risk for developing fragile X-associated primary ovarian insufficiency, a condition characterized by amenorrhea before age 40 years. Abnormal immunological patterns have been previously observed in *FMR1* premutation carriers, albeit little is known about cytokine production in females at the cellular level. We investigated cytokine levels in follicular liquid of females *FMR1* premutation carriers under age of 35 years.

Material and methods: Follicular fluid was isolated and bioassayed for IL-6, IL-8, IL-10, IFNγ, MCP-1 and VEGF-α activity from 11 female *FMR1* premutation carriers (<35 years of age) and 4 sex and age matched controls. Cytokines were measured using Luminex liquid chip suspension technology. Milliplex MAP kit (HCYTOMAG-60K; Merck Millipore, Billerica, MA, USA) according to the manufacturer's instructions. Data were acquired using a Luminex MAGPIX System instrument (Luminex Corporation, Austin, Texas) and analyzed using MILLIPLEX Analyst Software (version 5.1; Merck Millipore).

Results: We found a decrease in the concentration of cytokine production in female *FMR1* premutation carriers when compared with controls. Decrease IL-8, IFNγ and VEGF-α levels were statistically significant when compared to control samples ($p = 0.02$, 0.012 and <0.01, respectively). No significant correlation was found between any cytokine level and the *FMR1* CGG repeat length ($p > 0.05$).

Conclusion: Decreased immune responses in adult human female premutation carriers have been previously reported in blood samples. Our results are in agreement with this observation and suggest that impaired immune responses may play a role in the follicular development of *FMR1* premutation carriers.

C.O.I. Disclosure:

L. Rodriguez-Revenga: None. **P. Trilla:** None. **I. Agusti:** None. **A. Borras:** None. **M. Morales-Ruiz:** None. **M. Guimera:** None. **L. Ribera:** None. **Y. Barral:** None. **D. Manau:** None. **M. Alvarez-Mora:** None.

EP15.098 Detection of Prader-Willi Syndrome in a IVF Pregnancy: A Case Report

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Background: Prader-Willi syndrome (PWS) is a genetic disorder that results from either a paternal deletion or maternal uniparental disomy (UPD) affecting the 15q11-q13 region of chromosome 15, or from defects in imprinting mechanisms. We present a case involving a 10 day old female neonate, born prematurely from a pregnancy achieved through in vitro fertilization (IVF). The neonate was referred for a genetic evaluation due to hypotonia and dysmorphic features. At the time of presentation, cranial ultrasonography, echocardiography, and brain MRI were performed to determine any potential anomalies. Both parents, aged 43, are non-consanguineous and report no health issues.

Material and methods: A comprehensive clinical examination was performed, including a review of the patient's prenatal and perinatal history, along with genetic testing. The genetic testing included the following: karyotyping, Affymetrix CytoScan Optima 315 K SNP array analysis (Thermo Fisher Scientific, Waltham, MA, USA), fluorescence in situ hybridization (FISH), and MRC Holland SALSA MLPA Probemix ME028 Prader-Willi/Angelman probe analysis.

Results: The physical examination revealed characteristics consistent with Prader-Willi syndrome, including hypotonia, micrognathia, and reduced neonatal reflexes. SNP array analysis identified a loss of heterozygosity on 15q11.2q22.2-15q23q26.3. Consequently, data from the mother, father, and child were compared. Maternal uniparental disomy was detected. FISH and MLPA analyses confirmed the diagnosis of Prader-Willi syndrome.

Conclusion: This case highlights the importance of screening for imprinting-related syndromes, such as Prader-Willi syndrome, along with chromosomal anomalies in pregnancies achieved through in vitro fertilization. Early detection allows for prompt diagnosis and intervention, thereby preventing potential complications linked to these disorders.

C.O.I. Disclosure:

M. Polat: None. **S. Purtuloglu:** None. **Y.K. Terzi:** None. **Z. Celik:** None.

EP15.099 Forward and reverse phenotyping of neurogenetic disorders in the era of radiogenomics

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Background: Neurogenetic disorders often present with overlapping clinical and radiological findings, making diagnosis challenging. This study aimed to evaluate the consistency of radiological and genetic diagnoses in pediatric patients with suspected neurogenetic diseases using a forward and reverse radiogenomics approach.

Material and methods: Ten pediatric patients who underwent brain MRI due to non-specific clinical findings and were suspected of having neurogenetic diseases were included. Pediatric radiologists attempted to predict diagnoses based on MRI findings before genetic testing. Whole-exome sequencing was then performed, and the final diagnoses were established by integrating radiological and genetic results. The concordance between initial radiological predictions and final genetic diagnoses was assessed.

Results: The final genetic diagnoses included mitochondrial short-chain enoyl-CoA hydratase 1 deficiency (Leigh-like disease), ACTA2-related smooth muscle dysfunction, glutaric aciduria type 1, Fahr disease, a dual diagnosis of Pseudo-TORCH syndrome and 3-methylcrotonyl-CoA carboxylase (3-MCC) deficiency, a dual diagnosis of sepiapterin deficiency and hereditary spastic paraplegia, and Aicardi-Goutières syndrome types 4 and Aicardi-Goutières syndrome types 6. Consistency between radiological and genetic diagnoses was observed in neurogenetic disorders affecting specific brain regions, such as neurometabolic diseases. However, discrepancies arose in conditions with overlapping MRI features (e.g., Aicardi-Goutières syndrome and Fahr disease) and in cases with dual diagnoses, where radiological predictions alone were insufficient.

Conclusion: This pilot study highlights the utility of radiogenomics in diagnosing neurogenetic disorders. As the database expands with more cases, the integration of forward and reverse radiogenomics may facilitate faster and more precise diagnoses, improving patient management.

C.O.I. Disclosure:

A.A. Shukur: None. **E. Eker:** None. **S.K. Şahap:** None. **S. Fitöz:** None. **S. Teber:** None. **Ö. Bektaş:** None. **M. Yıldırım:** None. **E. Köse:** None. **F.T. Eminoğlu:** None. **E.N. Cakinberk:** None. **H. Mutlu:** None.

EP15.100 Overview of the results from the Spanish IMPaCT-Genomics program for the diagnosis of rare diseases in the Valencian Community and the Balearic Islands

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Background: The IMPaCT-GENóMICA Programme, part of the IMPACT initiative led by the Carlos III Health Institute (ISCIII) and coordinated by CIBERER, aims to establish an infrastructure for the coordinated and equitable management of complex genomic diagnoses across Spain, with a particular focus on undiagnosed rare disease patients.

Material and methods: Whole Genome Sequencing (WGS) was performed on over 2,000 undiagnosed rare disease patients from 110 hospitals nationwide. To date, 50% of the samples have been analysed for single nucleotide variants (SNVs) and indels using the CNAG Genome-Phenome Analysis Platform. Occasionally, analysis have also included structural and copy number variants (SVs and CNVs), mitochondrial variants and short tandem repeats (STRs). This study presents the current state of the first 118 cases from Valencian Community and Balearic Islands.

Results: Of the 118 cases, 19 have been diagnosed (16,1%), mainly through SNVs and indels poorly covered in previous exome studies or through reclassification of previously detected variants. Some other patients carried pathogenic variants in new OMIM genes, and one case presented a pathogenic *FMR1* STR. Currently, 26 more cases (22%) harbour candidate variants undergoing expert review, familial segregation or functional studies (e.g. exonic/intronic VUS or variants in candidate genes). The remaining 73 cases are still under study, focusing on SVs, CNVs, mitochondrial variants and STRs.

Conclusion: The IMPaCT-GENóMICA Program is already improving genetic diagnosis for undiagnosed rare disease patients in Balearic Islands and Valencian Community. In the near future, participants will hopefully benefit from enhanced analytical tools, additional functional studies and expert consultations.

C.O.I. Disclosure:

C. Navarro-Moreno: None. **P. Barberán-Martínez:** None. **G. García-García:** None. **J. Rosell-Andreo:** None. **J.M. Millán:** None.

EP15.101 Functional Investigation of Pathogenic Variants in *CCN6* Causing Progressive Pseudorheumatoid Dysplasia

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Background: Progressive Pseudorheumatoid Dysplasia (PPRD) is a rare skeletal disorder inherited in an autosomal recessive manner. The gene for PPRD, Cellular Communication Network Factor 6 (*CCN6*), encodes the WNT1-inducible signaling pathway protein 3 (WISP3). The pathophysiology of PPRD is hypothesized to involve dysregulation of cartilage homeostasis due to variations in *CCN6*, although the exact molecular mechanism underlying the disease remains unclear. The functions of PPRD gene variants were investigated in human chondrocytes through the overexpression of these variants.

Material and methods: Gene expression plasmids including the most common variations in the Turkish population (p.Cys52*, p.Gly83Glu, p.Tyr109*, and p.Cys114Trp) were constructed using WISP3 clones and overexpressed in human chondrocyte cells (HC 402-05A). Different MMP protein levels, as well as *SOX9* mRNA levels, were evaluated. WISP3 localization and mROS levels were investigated through immunocytochemistry studies.

Results: Western blot analysis revealed no changes in MMP-2 and MMP-3 levels, and neither production nor secretion of MMP-9 was detected. However, alterations in MMP-13 levels were observed. *SOX9* levels in cells with missense variations were comparable to those with overexpressed wild-type *CCN6*, whereas nonsense variations resulted in a downregulation of *SOX9* levels. Additionally, increased mROS levels and a redistribution of WISP3 within the cell were observed, with no evidence of mitochondrial localization.

Conclusion: Elevated mROS levels and reduced MMP-13 expression indicate a pivotal role in regulation, contributing to ER stress. Alterations in *SOX9* levels suggest WISP3 variants proteome interactions which require further omic research.

C.O.I. Disclosure:

G. Guven Tasbicen: None. **Z. Tonbul:** None. **A. Tufan:** None. **B. Savsar:** None. **E. Güzel:** None. **G. Bayram Akcapinar:** None. **O. Akgun Dogan:** None. **E. Tahir Turanli:** None. **Y. Alanay:** None.

EP15.102 Whole genome sequencing in patients with inherited retinal dystrophies

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Background: Inherited Retinal Dystrophies (IRDs) are a group of rare diseases characterized by visual loss, which is progressive in

most cases and can lead to legal blindness. Currently, approximately 300 genes are implicated in IRDs. However, the study of these genes using different NGS approaches typically focuses on coding regions and allows for a genetic diagnosis in only 50–70% of patients. The aim of this study is to perform whole-genome sequencing (WGS) to improve the rate of genetic diagnosis in IRD patients.

Material and methods: We performed WGS on a cohort of patients with IRD who had previously undergone other NGS strategies. For the analysis, filtering was applied using a virtual panel that included genes currently associated with ocular pathologies. The analysis also incorporated the study of deep intronic variants through the SpliceAI predictor, CNVs using ExomeDepth and structural variants using MANTA.

Results: The analysis conducted in this study has enabled the genetic diagnosis of 10 families through the identification of variants missed in previous studies due to technical limitations. Six families were solved with variants located in low-coverage regions from previous NGS analyses: four cases harbored variants in *RPGR*-ORF15, one case presented compound heterozygous variants in *PDE6A*, and another presented a CNV in *CRX*. One family showed a structural variant in *PRPF31*. Finally, three families presented novel deep intronic variants in the genes *CEP78*, *CHM* and *BBS4*.

Conclusion: This study demonstrates that WGS is a useful tool for patients with genetic diseases that remain unsolved through other NGS strategies.

C.O.I. Disclosure:

P. Barberán Martínez: None. **C. Navarro-Moreno:** None. **B. García-Bohórquez:** None. **M. Balanzá:** None. **M. Quinodoz:** None. **P. Marin-Reina:** None. **E. Calpena:** None. **C. Rivolta:** None. **T. Jaijo:** None. **E. Aller:** None. **G. García-García:** None. **J. Millán:** None.

EP15.103 Characterization of 21-hydroxylase deficiency Argentinian cohort by long read sequencing

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Background: The gene CYP21A2 is responsible for the 21-hydroxylase deficiency (21OHD). It is mapped to 6p21.3 in the RCCX module that is duplicated in tandem with a centromeric and a telomeric copy. In this work, we used Oxford Nanopore Technology (ONT) Long Reads sequencing (LRS) to analyze 21OHD samples from Argentina previously studied and also new ones.

Material and methods: A total of 34 DNA samples were sequenced in 2 amplicons of 8.5Kb covering the centromeric and telomeric RCCX modules following ONT recommendations.

Results: With the developed workflow, we obtained both known and novel GVs. From the 21 alleles with converted or chimeric genes, we uncovered 5 of them lacking GVs commonly expected to be involved in 21OHD rearrangements. Importantly, we revised the classification of one patient with a deletion that had been overlooked with non-LRS diagnostic technologies. These new findings changed the genetic counseling for a patient and his family related to the presence of a carrier allele for Congenital Adrenal Hyperplasia and Ehlers-Danlos Syndrome (CAH-X). Furthermore, by addressing the study of the centromeric and the telomeric RCCX modules, we found 19 GVs for CYP21A1P and 29 GVs for TNXA not previously described in Latin American populations nor reported in any of the consulted databases.

Conclusion: This study may represent the first one applying ONT LRS in clinical studies in Latin America, highlighting the importance of LRS as a high resolution method of diagnosis and to better understand the diversity of the RCCX modules.

C.O.I. Disclosure:

A. Claps: None. **J.E. Kolomenski:** None. **F. Fernández:** None. **N. Macchiarolli:** None. **M.L. Ingravidi:** None. **M. Delea:** None. **C.S. Fernández:** None. **T. Castro:** None. **J. Laiseca:** None. **L. Kamenetzky:** None. **M. Taboas:** None. **L. Dain:** None.

EP15.104 Genetic profiling of SMN2 and NAIP variations for personalized prognosis in spinal muscular atrophy patients

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Background: Spinal muscular atrophy (SMA) is a neuromuscular disorder caused by deletions or mutations in the SMN1 gene, with disease severity influenced by CNVs in SMN2 and deletions in NAIP. While SMN2 CNVs is a key disease modifier, additional genetic variations may provide a more precise classification of SMA phenotypes, enabling improved patient stratification and therapeutic decision-making. This study aimed to evaluate the correlation between SMA genotypes and phenotypes, emphasizing the role of SMN2 and NAIP in diagnostic approaches.

Material and methods: A cohort of 35 SMA patients was analyzed through molecular genetic testing using the MLPA technique to determine CNVs in SMN2 and detect NAIP deletions. The genetic variations were classified into 12 genotypes (G1-G12) and 4 SMA types. Statistical associations were assessed using Chi-squared and Fisher test.

Results: The analysis revealed a significant association between the obtained genotypes and SMA types, as demonstrated by $\chi^2 = 92.57$, $p < 0.00001$. Specific genotypic patterns were identified, such as G10 being exclusively linked to SMA Type 2 and G12 to SMA Type 4, while G5 and G9 displayed a more heterogeneous distribution across multiple SMA types. Additionally, Fisher's exact test confirmed a strong association between G10 and SMA Type 2 ($p = 0.00096$).

Conclusion: Comprehensive genetic profiling improves SMA diagnosis by enhancing phenotype prediction through SMN2 and NAIP analysis. Newly created genotypes may act as predictive markers, advancing classification beyond SMN2 copy number. This approach enables personalized prognosis and targeted therapies, supporting precision medicine. Further research may explore additional genetic modifiers influencing treatment response.

C.O.I. Disclosure:

I. Coliban: None. **V. Sacară:** None.

EP15.105 Rare case of Bardet-Biedl type 4 syndrome in an infant diagnosed by Whole Genome Sequencing

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Background: Bardet-Biedl syndrome (BBS) belongs to the family of ciliopathies, with multisystemic involvement and a prevalence between 1/125,000 and 1/175,000. At least 22 genes (*BBS1* to *BBS22*) have been identified as responsible for the syndrome's phenotype. It is characterized by obesity, pigmentary retinopathy, polydactyly, kidney abnormalities, learning difficulties, and hypogonadotropic hypogonadism in males. It is inherited in an autosomal recessive manner.

Material and methods: A 45-day-old infant is described with bilateral reversed cortico-medullary differentiation on ultrasound examination, normal renal function, and polydactyly. Born at full term via cesarean section with a birth weight of 3,430g. During pregnancy, oligohydramnios and hyperechoic kidneys were observed. The infant had hexadactyly on the right 5th toe, nystagmus, and increased somatometric measurements. Genetic analysis was performed using whole genome sequencing (WGS).

Results: A 6.2kb deletion (exons 3 and 4) and an intronic variant (c.332+5G > A) were detected in the *BBS4* gene in compound heterozygosity. In silico analysis suggested that c.332+5G > A likely disrupts proper splicing, potentially leading to a loss of function. c.DNA sequencing of the infant's peripheral blood showed that the c.332+5G > A variant causes skipping of exon 5.

Conclusion: WGS led to a rapid diagnosis of Bardet-Biedl syndrome type 4, identifying two genetic alterations, one of which, due to its size, could not have been detected by conventional techniques. This enabled the family to receive targeted genetic counseling and make informed decisions regarding family planning. This case represents a rare form of Bardet-Biedl syndrome and supports the significant diagnostic contribution of WGS in rare genetic diseases.

C.O.I. Disclosure:

K. Anagnostopoulou: None. **A. Poulopoulou:** None. **M. Alvanou:** None. **C. Gavalas:** None. **A. Mitsioni:** None. **H. Kapogiannis:** None. **S. Psoni:** None. **E. Tsoutsou:** None. **S. Amenta:** None. **H. Fryssira:** None. **Y.L. Loukas:** None.

EP15.106 Clinical usefulness of WES secondary findings in a cohort of 100 adult patients with genetic syndromes

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Background: The improvement of molecular techniques, including Whole Exome Sequencing (WES), allowed to improve the yield of genetic diagnosis in patients affected by rare genetic syndromes. However, genetic analysis can lead to the identification of unexpected results unrelated to the clinical suspect, defined as "secondary findings". Although American College of Medical Genetics (ACMG) proposed 81 "actionable" genes, it is actually debated whether WES secondary findings should be reported and heterogeneous laboratory policies exist. We describe a cohort of WES patients to support the clinical usefulness of reporting secondary findings.

Material and methods: 100 adult patients affected by diverse genetic syndromes underwent WES analysis with Illumina platform. Class IV/V variants in ACMG and other clinically relevant genes were considered.

Results: We identified eight patients carrying variants in ACMG genes, mainly associated with cardiac conditions (*SCN5A, TNNT2*), dyslipidemia (*LDLR, APOB*) and predisposition to malignant hyperthermia (*RYR1*). In addition, six variants in clinically relevant genes associated with coagulation defects (*VWF* and *TUBB1*), autoinflammatory (*MEFV*) and neurodevelopmental (*CACNA1E*) disorders were reported.

Conclusion: The strong interaction between geneticists and other physicians like cardiologists and cardiosurgeons improved counselling and management for syndromic patients, for example in preventing possible complications of adult open-heart surgery (i.e. hemorrhages among *VWF* patients or adverse anesthetic reactions among *RYR1* patients) and delivering proper pharmacological therapy (*CACNA1E* patients). Our data suggest that reporting WES secondary findings can be clinically useful and improve patient management. In addition, we support case-by-case evaluation of potential pathogenic variants in genes non included in the ACMG list.

C.O.I. Disclosure:

E. Micaglio: None. **S. Benedetti:** None. **M. Villa:** None. **O. Rondinone:** None. **G. Moresco:** None. **S. Morgan:** None. **A. Giamberti:** None. **M. Chessa:** None. **M. Miozzo:** None. **C. Pappone:** None.

EP15.107 IdentificationofNovelDisease-specific EpigeneticMarkersfor Noninvasive Detection of Fetal Trisomy 18

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Background: Trisomy 18 (T18), or Edwards syndrome, is a congenital disorder caused by an extra copy of chromosome 18. Fetal placenta-specific epigenetic markers for noninvasive prenatal testing (NIPT) have been identified based on tissue-specific epigenetic differences between the placenta and maternal blood. Still, these markers have not been validated in T18 placentas. This study aimed to identify novel T18 placenta-specific epigenetic markers by analyzing differential DNA methylation in T18 placentas compared with maternal blood and normal placentas.

Material and methods: DNA was extracted from chorionic villi of five normal and five T18 fetuses and whole blood of five normal pregnant women. Methyl-capture sequencing (MC-Seq) was performed to assess genome-wide CpG site methylation. Differentially methylated CpG sites (DMCs) and regions (DMRs) were identified, and functional annotations of DMR-associated genes were predicted using bioinformatics tools.

Results: Significant DNA methylation changes were identified in T18 fetal chorionic villi cells, with 1,937 DMCs (900 hypermethylated, 1,037 hypomethylated) and 158 DMRs (67 hypermethylated, 91 hypomethylated). Functional analysis of 148 DMR-associated genes revealed associations with middle ear morphogenesis and cervical spine abnormalities ($P < 0.001$). Methylation-specific qPCR validated the epigenetic characteristics of four DMRs.

Conclusion: This is the first study to examine T18-specific DNA methylation in chorionic villi using MC-Seq. These novel T18

placenta-specific differential methylation patterns may serve as potential biomarkers for noninvasive detection of fetal T18.

C.O.I. Disclosure:

J. Lim: None. **J. Kim:** None. **S. Yang:** None. **Y. Han:** None. **H. Ryu:** None.

EP15.108 Deciphering the transcript-level effect of the c.85G>T variant in *RSPH1* causing Primary Ciliary Dyskinesia in Spanish population

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Background: Primary ciliary dyskinesia (PCD) is a rare genetic respiratory disorder. Although being described with low frequency worldwide, *RSPH1* is one of the most common recessive genes in the Spanish PCD population. The frequency of ENST00000291536.8:c.85G>T variant (p.(Glu29*)) in exon 2 is increased in south-European population. Presumed to cause a premature termination codon (PTC) in a position predicted to escape nonsense-mediated decay (<100 first nucleotides), we have explored its effect.

Material and methods: RNA-sequencing was performed on epithelial nasal cells from five PCD patients carrying the c.85G>C variant (1 homozygous, 1 compound heterozygous and 3 simple heterozygous), and 10 controls. Alterations in gene expression and splicing were analysed using DROP package, and isoform quantification using RSEM software.

Results: Three isoforms of *RPSH1* were detected in the control group: ENST00000291536.8, ENST00000398352.3 -lacking exon 2- and ENST00000493019.1 whose abundances were 74.1%, 24.8% and 1.2%, respectively. Total *RSPH1* expression levels remained unaltered in patients, but the abundance of the two major isoforms resulted imbalanced compared to controls. Their abundances shifted to 37.5%-61.4% in the homozygous patient, 47.2%-51.5% in the compound heterozygous patient and 55.9%-42.5% in simple heterozygous patients. These data suggest that the c.85G>T variant promotes nonsense-associated altered splicing to avoid the predicted PTC in exon 2, rather than triggering nonsense-mediated decay.

Conclusion: RNA-sequencing is a valuable tool for investigating the transcriptomic effects of genetic variants. This study reveals a novel pathogenic effect for *RSPH1*, with the c.85G>T variant inducing in-frame exon 2 skipping and isoform imbalance, offering new insights for PCD therapy.

C.O.I. Disclosure:

L. Carretero-Vilarroig: None. **E. Calpena:** None. **A. Esteve-Codina:** None. **R. Blanco:** None. **I. Ibañez:** None. **N. Muñoz-Fernández:** None. **A. Reula:** None. **J. Millan:** None. **M. Armengot:** None. **T. Jaijo:** None.

EP15.109 "Whole-Exome Sequencing in Consanguineous Populations: Insights from UAE Patients with Rare Genetic Disorders

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Background: Rare genetic disorders pose diagnostic challenges, particularly in consanguineous populations. This study examines genetic, clinical, and inheritance patterns in 693 individuals referred for genetic testing to identify key diagnostic trends and improve rare disease detection.

Material and methods: Whole-exome sequencing (WES) was performed at Dubai Genetics Center, Dubai Health using Agilent V7 and Illumina NextSeq500. Ordering physicians provided clinical data. Diagnostic yield was assessed based on pathogenic/likely pathogenic variants matching clinical indications. Pedigree analysis and statistical tests, including chi-square and Transmission Disequilibrium Test (TDT) with Bonferroni correction, were conducted.

Results: Developmental (27.3%) and metabolic abnormalities (11.4%) were the most common referral reasons. WES identified 922 mutations, diagnosing 36.75% of patients, with higher yields in consanguineous cases ($p < 0.05$). Skin (56.1%) and eye disorders (55.6%) had the highest yields, while immune (11.8%) and respiratory conditions (18.2%) were lower. HBB, ABCA4, and G6PD were the most prevalent genes. ABCA4, GJB2, and TRIO were unique to Emiratis, while ABCA3, DEAF1, and CLPB were distinct to non-Emiratis. MERTK, CRB1, and LOXHD1 were exclusive to consanguineous patients. High yields were noted for MERTK (85.7%), G6PD (83.3%), and HBB (72.7%), while MEFV (40%) and APC (25%) were lower. Developmental and haematological disorders showed significant transmission disequilibrium ($p < 0.05$), suggesting polygenic influences.

Conclusion: This study highlights WES as a powerful tool for diagnosing rare genetic disorders in consanguineous populations, emphasizing high-yield genes and the need for tailored genomic research.

C.O.I. Disclosure:

S. Masalmeh: None. **M. Bahadi:** None. **N. Mankena:** None. **N. Maden:** None. **F. Al Bastaki:** None. **H.S. Elabd:** None. **A. Raza:** None. **H. Yasaei:** None.

EP15.110 Comprehensive genomic analysis reveals dual diagnoses and large copy number variations in pediatric patients with undiagnosed rare diseases

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Background: Identifying the genetic basis of undiagnosed rare diseases in pediatric patients is a challenging yet critical task. This study aims to reach a diagnosis using WES in patients who are suspected of having a rare genetic disease but cannot be diagnosed by conventional clinical, biochemical, radiological, and single gene sequence analysis or custom-designed NGS panel.

Material and methods: We conducted WES analysis on 132 pediatric patients with undiagnosed diseases at a single Rare Disease Research and Application Center. Genomic DNA from the patients was analyzed for SNVs and Copy CNVs, allowing for a comprehensive assessment of genetic variations.

Results: Both previously reported pathogenic variations and novel pathogenic or missense variants of uncertain significance (VUS) were identified. The diagnosis rate was 56%. Our analysis revealed a high rate of dual diagnoses (7 out of 74; 9.5%) in patients who were previously diagnosed by a disease-specific NGS panel but exhibiting a more severe phenotype than expected, due to the presence of an additional SNV affecting the phenotype. CNV analysis identified pathogenic alterations in 8 out of 74 patients (10.8%), including 4 exonic deletions and 4 contiguous gene deletions, with the largest CNV spanning 16 Mb.

Conclusion: Our study highlights the crucial role of CNV analysis in revealing additional diagnostic information beyond standard exonic SNVs. The identification of dual diagnoses and novel variations highlights the complexity and heterogeneity of rare diseases and underscores the importance of comprehensive genetic testing for accurate diagnosis and personalized treatment strategies.

C.O.I. Disclosure:

H. Mutlu: None. **E. Köse:** None. **M. Koç Yekedüz:** None. **D. Akçayöz:** None. **E. Eker:** None. **F. Eminoğlu:** None.

EP15.111 Exploring Genetic Variants in Hypolipidemic Disorders, A Pediatric Hospital Experience

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Background: Familial hypobetalipoproteinemia, abetalipoproteinemia, and chylomicron retention disease are rare lipid disorders. This study explored genetic variants involved in these disorders and their potential clinical implications.

Material and methods: We queried the Le Bonheur Children's Hospital/UTHSC Biorepository and Integrative Genomics data from 9,001 patients. After DNA sequencing, samples were aligned to the GRCh38 human reference genome. DeepVariant with a custom exome model was used for variant calling. The variants were annotated using Variant Effect Predictor(VEP) and gnomADv3, with protein impact assessed via PolyPhen2 and SIFT. High and moderate-impact variants were included. Variants classified as "likely pathogenic" were analyzed.

Results: We identified 936 variants across 10 genes, with 61 variants(6.5%) being "likely pathogenic". Study included 158 patients, all heterozygous, with a mean age of 7 ± 6 years. The cohort was 55% female, 55% White, 26% African American, and 14.5% Hispanic. *APOB* variants were the most frequent(Table 1). Eight patients(5%) possessed 2 abnormal variants. Lipid profiles were only checked in 10%; most were normal. One patient with a novel *APOB* variant(c.4685dup) exhibited persistent hypotriglyceridemia, hypocholesterolemia, and low APOB100 levels, alongside obesity, type-2 diabetes, and hepatic steatosis. Another patient with an *APOB* variant(c.10580G>A) had persistent hyperlipidemia later diagnosed with heterozygous familial hypercholesterolemia. One of 7 individuals with the same variant was tested, showing a normal lipid profile.

Conclusion: This study highlights APOB variants' roles in both hypolipidemia and hyperlipidemia, unlocking the potential for genetic screening to enhance early diagnosis and treatment of lipid disorders.

C.O.I. Disclosure:

F. Ali: None. **A. Mohammed:** None. **L. Chinthala:** None. **D. Black:** None.

EP15.112 The spectrum of rare variants of Noonan syndrome in a cohort of Russian patients

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Background: RASopathies, including Noonan syndrome (NS), are caused by mutations in the RAS-MAPK signaling pathway, with a prevalence of 1 in 1,000 to 2,500 live births. NS is characterized by distinctive facial features, congenital heart defects, and developmental delays. Over 20 genes are associated with RASopathies, with *PTPN11* mutations accounting for approximately 50% of NS cases. Other significant genes include *SOS1*, *RAF1*, *KRAS*, and *RIT1*, each linked to specific clinical features. This study investigates a cohort of Russian patients with NS and related disorders, expanding the analysis beyond PTPN11 mutations to explore broader genetic variability.

Material and methods: DNA from 456 unrelated probands with RASopathies was analyzed using a custom panel for next-generation sequencing, covering 23 genes.

Results: Pathogenic variants were identified in 191 patients, with 106 cases (55.5%) linked to PTPN11. In the remaining cohort, 85 cases (44.5%) had mutations in other NS-associated genes. Clinical data from 78 probands revealed that 82.1% exhibited facial dysmorphism, 55.1% had a short neck, and 48.7% experienced global developmental delay. Additional findings included growth below the 3rd percentile in 25.6% and various other anomalies.

Conclusion: This study highlights the genotypic and phenotypic variability of NS in a Russian cohort, enhancing understanding of its molecular basis. The findings support improved diagnosis, genetic counseling, and personalized management strategies for affected individuals in Russia.

C.O.I. Disclosure:

A. Orlova: None. **O. Shatokhina:** None. **D. Guseva:** None. **N. Demina:** None. **A. Polyakov:** None. **O. Ryzhkova:** None.

EP15.113 Genetic landscape of Kabuki syndrome in a Russian cohort: insights from *KMT2D* and *KDM6A* variants

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Background: Kabuki syndrome (KS), a rare genetic disorder characterized by developmental delays, hypotonia, and a distinctive facial phenotype, is caused by variants in *KMT2D* and *KDM6A*, genes critical for histone modification and embryonic

development. Despite global studies, the genetic landscape of KS in Russian patients remains unexplored.

Material and methods: This pioneering study analyzed 37 Russian patients with suspected KS using next-generation sequencing (NGS), including whole-exome sequencing (WES) and whole-genome sequencing (WGS), to identify pathogenic variants, copy number variations (CNVs), and structural rearrangements.

Results: Pathogenic or likely pathogenic variants were identified in 20 patients, with two findings requiring further validation. Six patients had alternative molecular diagnoses, highlighting KS's phenotypic overlap. In *KMT2D*, we detected 10 frameshift, 4 nonsense, 2 non-frameshift deletions, and 4 missense mutations, consistent with global databases. For *KDM6A*, WES revealed an exon 3–4 deletion, while WGS identified a novel translocation between chromosome X and chromosome 1, with a breakpoint in *KDM6A* intron 28. This is the first study to report such findings in a Russian cohort.

Conclusion: Our findings confirm *KMT2D*'s mutational spectrum and emphasize *KDM6A*'s predisposition to CNVs and structural variants, underscoring WGS's diagnostic value. This study, the first of its kind in Russia, expands the genetic understanding of KS and highlights the importance of advanced genomic methods for accurate diagnosis. These insights improve diagnostic precision and pave the way for future therapeutic advancements in KS management.

C.O.I. Disclosure:

K. Zabudskaya: None. **V. Zabnenkova:** None. **T. Cherevatova:** None. **M. Bulakh:** None. **A. Orlova:** None. **M. Orlova:** None. **O. Mironovich:** None. **T. Vizerov:** None. **F. Bostanova:** None. **I. Anisimova:** None. **D. Gorodilova:** None. **E. Shestopalova:** None. **N. Semenova:** None. **A. Kuchina:** None. **O. Ryzhkova:** None.

EP15.114 Dissecting the molecular mechanisms underlying MDPL genetic disorder using a novel yeast-based model

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Background: Rare diseases are low-prevalence disorders affecting millions worldwide, with 80% having genetic origin. MDPL syndrome (Mandibular hypoplasia, Deafness, Progeroid features, and Lipodystrophy, MIM #615381) is a rare progeroid disorder, caused by de novo *POLD1* variants. *POLD1* encodes the catalytic subunit of Pol δ, critical for DNA synthesis and repair. Due to limited human samples, model organisms are crucial for research, and to investigate MDPL's molecular mechanisms, we developed a novel yeast-based model using plasmid shuffling.

Material and methods: We leveraged the high conservation between human *POLD1* and yeast *POL3* (49.5% sequence identity), with Arg512 and Ser611 in *POL3* corresponding to MDPL-associated mutants Arg507 and Ser605 in *POLD1 (*c.1812_1814delCTC, p.Ser605del and c.1572C>T, p.Arg507Cys). As *POL3* is essential, a knockout strain was created, and WT *POL3* gene was introduced for cell survival. Mutants were then expressed, and WT gene was selectively removed for functional analysis. The study was performed under basal conditions and following DNA damage inductions (ɣ-rays and UV irradiation).

Results: We have successfully developed a novel yeast-based model for MDPL, where the different results compared to WT suggests that mutants affect *POL3* function. The same significant difference was observed after DNA damage induction, highlighting their crucial role in the molecular mechanisms of MDPL for both mutants.

Conclusion: This model provides valuable insights into the functional impact of pathogenic variants on POL3 function under basal conditions. Moreover, our results highlight the significant role of DNA stress in yeast-based model of MDPL, contributing to the investigation of the syndrome's molecular mechanism.

C.O.I. Disclosure:

S. Maccaroni: None. **F. Morales-Polanco:** None. **M.A. Wangeline:** None. **M. Murdocca:** None. **P. Spitalieri:** None. **G. Novelli:** None. **F. Sangiuolo:** None. **J. Frydman:** None.

EP15.115 Investigation of genetic variants in ABCC6 gene from whole exome sequencing results in cohort of liver patients

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Background: A male patient in his twenties presented with clinical aspects of primary sclerosing cholangitis (PSC), chronic cholestatic liver disease, and hypothyroidism. We identified a heterozygote candidate variant in the ABCC6 gene (c.2279G>A, p.(Arg760Gln)). Dysfunctional ABCC6 was described to cause ABCB11 and ABCG5/G8 genes overexpression, leading to imbalanced bile composition resulting in gallstone formation and cholestasis. ABCC6 variants were previously described in cases with low phospholipid-associated cholelithiasis (LPAC) and intrahepatic cholestasis of pregnancy (ICP) (PMID: 36046230, PMID: 37392836). Subsequently, we investigated rare variants in the ABCC6 gene in 138 whole exome sequencing (WES) results of patients with suspected cholestatic liver disease.

Material and methods: Variant evaluation and annotation analysis was carried out on WES results, focusing on loss of function and missense variants with an overall allele frequency of ≤1% in gnomAD. Phenotype related variants were prioritized and classified using ACMG guidelines.

Results: Five different rare heterozygote variants were identified in eight samples of 138 WES results. Among these, the missense variants c.179G>A, p.(Arg60Gln) and c.3139C>T, p.(Arg1047Cys) were found in two LPAC patients. Additionally, the c.1171A>G, p.(Arg391Gly) variant was observed in two related cases: a mother with ICP and her child with elevated gamma-glutamyl transferase (GGT) levels and jaundice. Moreover, this variant was also detected in another patient with elevated transaminases. c.3871G>A, p.(Ala1291Thr) and c.955A>G, p.(Ile319Val) were seen in patients with ICP and cholestasis, respectively. All variants classified as uncertain significance.

Conclusion: In depth analysis of WES results revealed potentially relevant variants in ABCC6 that may contribute to liver phenotypes.

C.O.I. Disclosure:

S. Alinaghi Arjas: None.

EP15.116 Identification of rare missense variant in the *PIGV* gene in two Slovak patients with Mabry Syndrome

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Background: Mabry Syndrome is a rare AR disorder caused by mutations in genes involved in glycosylphosphatidylinositol (GPI) anchor biosynthesis, including *PIGV*. The syndrome is characterized by developmental delay, seizures, hyperphosphatasia and dysmorphic facial features, with prevalence <1:1,000,000 (Orphanet). Here, we report two Slovak patients diagnosed with Mabry Syndrome with the same pathogenic missense variant in the *PIGV* gene.

Material and methods: Whole, respectively Clinical exome sequencing (WES, CES) was performed on both samples to identify genetic variants associated with clinical phenotype of epilepsy. Sanger sequencing was used for confirmation and segregation analysis. For the data analysis Sophia DDM software and Franklin, respectively Varsome tools were used.

Results: The c.1022C>A (p.Ala341Glu) variant in *PIGV* gene was identified in homozygous state in both patients, which was consistent with the clinical presentation of Mabry Syndrome. This variant is localized in the Mannosyl trans-2 functional domain, has low frequency of 0.0119% in GnomAD NFE population, is predicted by Franklin as deleterious (0.85) and co-segregates in the families. Genetic analysis of the probands' parents revealed the heterozygous status of the variant, while no consanguinity reported. However, it is notable that both families reside in the Nitra region of Slovakia.

Conclusion: The identification of the same pathogenic variant in two individuals suggests that the prevalence of the *PIGV* c.1022C>A pathogenic variant may be higher in the Slovak population than previously estimated. This finding highlights the importance of studying regional genetic variations to better understand the distribution of rare variants and make the diagnostic process more effective.

C.O.I. Disclosure:

K. Durisova: None. **G. Krasnanska:** None. **M. Baldovic:** None. **G. Blandova:** None. **V. Elias:** None. **S. Vogelova:** None. **M. Konecny:** None.

EP15.117 GeNewBS: a multiregional, genetic newborn screening study for multiple rare diseases to pilot a nationwide approach

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Background: In Italy the metabolic newborn screening has become mandatory from 1992 and currently 50 diseases are screened. Genetic newborn screening (gNBS) approaches would enable to increase considerably the number of diseases to be screened.

Material and methods: The PNRR project involves 3 Italian regions (Emilia-Romagna, Lombardia and Puglia) and is articulated in 3 studies: i) feasibility: 3000 newborns will be tested using a new gene panel (ENSplus) that contains genes associated with treatable rare diseases; ii) validation: 1200 newborns resulted positive at metabolic screening will be assessed by the ENSplus panel; iii) innovation: WES will be carried out in about 200 infants with discordant results between metabolic and genetic screening, or with uncertain results. Some selected VUS will be tested by in vitro cell studies. Tele-counselling will be adopted for results communication.

Results: Ethical approval has already been obtained in the 3 regions. GeNewBS has just started and we are now in the recruitment phase and ENSplus panel setting. We hope that by our gNBS approach, the first in Italy, we may provide a validated pipeline for the national screening program. Implementing gNBS should improve the detection rate of infants with rare diseases, thus shortening the time to diagnosis.

Conclusion: This project aims to be a cross-validation metabolic and genetic tools to demonstrate the feasibility and the sustainability of gNBS in the National Health System.

C.O.I. Disclosure:

A. Margutti: None. **R. Selvatici:** None. **F. Fortunato:** None. **V. Nagliati:** None. **C. Cereda:** None. **S. Carelli:** None. **A. Mauri:** None. **C. Berardo:** None. **S. Simonetti:** None. **S. Russo:** None. **M. Gentile:** None. **P. Orsini:** None. **F. Gualandi:** None. **A. Ferlini:** None.

EP15.118 *PATL1*: a novel candidate gene for neurodevelopmental disorders with motor impairment

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Background: Neurodevelopmental diseases are heterogeneous disorders leading to disruptions in the brain and nervous system, causing neurological and cognitive problems such as intellectual disability, movement disorders, and speech delays. *PATL1* (PAT1 homolog 1, processing body mRNA decay factor) plays a crucial role in mRNA decay and translation regulation. We identified two patients with biallelic *PATL1* predicted loss-of-function variants presenting with developmental delay, walking difficulty, and speech disorders. This study investigated the *PATL1*'s role in neurodevelopment using *C. elegans* and patient-derived cells.

Material and methods: Loss-of-function mutations in *patr-1*, the *C. elegans* homolog of *PATL1*, were analyzed for development, locomotion, and nervous system defects. Locomotion was assessed with tracking and thrashing assays using ImageJ, and axons were visualized with confocal microscopy. RNA sequencing of *C. elegans* and patient-derived cells is being conducted to identify differentially expressed genes.

Results: Mirroring patient phenotypes, *patr-1* mutants exhibited developmental delay from early larval stages to adulthood and reduced locomotion in both tracking and thrashing assays. Consistent with neuronal roles, the number of axons in glutamatergic neurons was significantly decreased in *patr-1* mutants compared to wild-type, supporting *patr-1* critical role in neural development.

Conclusion: These data suggest that *patr-1* mutations impact development, motor function, and neuronal structure. RNA-seq analysis will reveal affected molecular pathways by altered *PATL1* function. Future studies will investigate whether the movement and developmental abnormalities are due to muscle, neuronal, or neuromuscular defects. These findings provide insights into the

PATL1's role in neurodevelopment and may contribute to future therapeutic strategies for neurodevelopmental disorders.

C.O.I. Disclosure:

F. Yenisert: None. **A. Sezer:** None. **F.E. Perçin:** None. **G. Kayhan:** None. **M. Alders:** None. **O.&. Kaplan:** None. **S. Maas:** None.

EP15.119 Impact of medically actionable secondary findings in genomic data: prevalence, predictive factors, and outcomes in NGS and chromosomal microarray: a systematic review and meta-analysis

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Background: Chromosomal microarray (CMA), exome sequencing (ES), and genome sequencing (GS) are powerful genetic testing tools that can reveal secondary findings (SFs) with significant clinical implications. These findings can lead to interventions such as surveillance, prevention, or treatment. Given the increasing volume of genomic data, a structured approach to returning medically actionable SFs is essential. However, the factors influencing their detection remain underexplored. This review and meta-analysis aim to evaluate the prevalence, predictive factors, clinical outcomes of medically actionable SFs, and the diagnostic and clinical utility of ES, GS, and CMA in patient care.

Material and methods: Following PRISMA guidelines, studies from PubMed and Scopus were included if they evaluated the proportion of patients with medically actionable SFs identified through CMA, ES, or GS. The primary outcome was the rate of SFs (P/LP variants in actionable genes), while secondary outcomes included diagnostic and clinical utility rates. Studies were assessed for quality using the Newcastle-Ottawa Scale. Statistical analyses included meta-regression, pooled proportions, and odds ratios to explore the relationship between exposures and outcomes. Sensitivity analyses were performed to examine heterogeneity.

Results: A total of 1,190 studies from Scopus and 4,190 from PubMed were screened, with 475 duplicates removed. Of the remaining studies, 530 were assessed for eligibility through full-text review, with irrelevant or insufficient data studies excluded. For cohorts with multiple publications, only the most recent and comprehensive study was selected.

Conclusion: This review will provide valuable insights into integrating genetic sequencing into clinical practice and managing SFs more effectively.

C.O.I. Disclosure:

A. Elfatih: None. **Y. A. AlSarraj:** None. **M. Khir A. Kohailan:** None. **D. Velayutham:** None. **R. M. Badji:** None. **H. Mbarek:** None.

EP15.120 A collaborative working group of experts to define the list of diseases to be screened in the French genomic newborn screening project PERIGENOMED

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Background: Advances in genomics and treatment strategies have driven global initiatives in genomic newborn screening (gNBS). The French University Hospital Federation TRANSLAD leads PERIGENOMED, a two-phase project evaluating gNBS feasibility and utility in France. Phase 1 (PGC1) involves 2,500 newborns across five university hospitals, while Phase 2 (PGC2) extends to all maternity units in one French region to assess nationwide scalability. Ancillary studies examine psychosocial, economic, ethics and legal implications.

Methods: To define the gene list for PGC1, a comprehensive dataset was shared with 20 French Rare Disease Networks (FRDN). This dataset combined dyad selections from ten international gNBS pilot studies (e.g., BabySeq, BeginNGS) and metadata on each dyad's characteristics (e.g., age at onset, correlation level). The FRDN evaluated inclusion into two categories: List 1 for treatable pediatric conditions and List 2 for actionable diseases (optional for parents).

Results: Of 1,794 dyads identified (1,525 unique genes), 436 were selected for the PGC1 Treatable List: 34% appeared in more than six pilots, 50% in 2–5 pilots, 8% in one pilot, and 8% were added by the FRDN and absent from all pilots. These dyads primarily involve hematologic, immunologic, metabolic, and hearing-related conditions. The optional Actionable List also includes 436 dyads, mainly in hematologic, immunologic, pulmonary, skeletal, and renal domains: 5% in more than six pilots, 55% in 2–5 pilots, 22% in one pilot, and 18% added by the FRDN. This heterogeneity is being discussed within the international consortium ICoNS, of which PERIGENOMED is a member, to refine global gNBS strategies.

C.O.I. Disclosure:

C. Level: None. **Y. Duffourd:** None. **C. Lenelle:** None. **D. Salvi:** None. **E. Davoine:** None. **F. Huet:** None. **M. Chevarin:** None. **F. Tran Mau Them:** None. **C. Binquet:** None. **A. Ziegler:** None. **J.**

Arnoux: None. **A. Belot:** None. **C. Sevin:** None. **M. Krahn:** None. **D. Cheillan:** None. **V. Tardy:** None. **S. Bezieau:** None. **L. Pasquier:** None. **P. Rollier:** None. **S. Odent:** None. **C. Thauvin:** None. **L. Faivre:** None.

EP15.121 The genetic and phenotypic landscape of orofacial clefts in Ghanaian multiplex families

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Background: Orofacial clefts (OFCs) are among the most common congenital anomalies, affecting approximately 1 in 700 births globally. They arise from incomplete fusion of facial prominences during embryogenesis, leading to cleft lip, cleft palate, or both. OFCs pose a significant public health concern due to their multifactorial genetic and environmental causes. This study aimed to investigate the genetic basis of OFCs in multiplex families while exploring associated facial phenotypes.

Material and methods: The study involved 25 multiplex families and 275 unrelated controls. Saliva or cheek swab samples, clinical data, and 3D facial images were collected from participants. Twenty-seven facial landmarks were annotated to measure 22 features, which were analyzed in R using Shapiro-Wilk tests, Wilcoxon Rank Sum tests, and multiple regression models adjusted for sex, age, ethnicity, and BMI, with Bonferroni correction ($p < 0.00227$). Whole exome sequencing (WES) was performed at 100X coverage using the Illumina HiSeq platform. Variants with a minor allele frequency (MAF) < 0.01 were annotated with Variant Effect Predictor (VEP) and evaluated for pathogenicity using 12 tools from dbNSFP. Prioritization employed ACMG guidelines, inheritance patterns, tissue-specific expression, and OFC-related pathways

Results: Likely causative variants were identified in *GRHL3* (p.Arg520Gln), *CTNND1* (p.Thr113Pro), *IRF6* (p.Arg400Trp), *STAB2* (p.Pro257Gln), *and FAM98C* (p.Trp211Arg). Distinctive facial traits in affected individuals included reduced outer-canthal distance, palpebral fissure length, nasal height, labial fissure length, and increased nasolabial angle.

Conclusion: These findings emphasize integrating genetic and phenotypic data to identify potential markers for OFC risk, particularly in high-burden populations.

C.O.I. Disclosure:

B. Tsri: None. **G.O. Mensah:** None. **C. Asamoah:** None. **E. Tackie:** None. **S.O. Yeboah:** None. **A. Butali:** None. **P. Donkor:** None. **L.J. Gowans:** None.

EP15.122 Revealing local peculiarities of diagnostic yield WGS trio over mono

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Background: The advantages of the trio WGS format for performing WGS are no one doubts. Trio WGS provide identifying causative variants in probands easier and more reliably. However, trio WGS is not always possible in practice, primarily due to the difficulty of involving relatives in the clinical study. The aim of the study is to evaluate the diagnostic output WGS trio vs WGS proband mono & Sanger trio for children with orphan hereditary diseases.

Material and methods: Mono and trio WGS: ~30x, PE150, enzymatic fragmentation-based and PCR-free protocol, DNBseq sequencing platform (T7, G400) (MGI, China); Sanger sequencing: ABI3500, Applied Biosystems.

Results: For 6000 (100%) probands : 85% WGS mono and 15% WGS duo, trio, quadro. From 85% WGS mono: 26,5% no variants, 73,5% identified causative variants: 50% was validated, only 1% was no validated (allele dropout, GC-rich region, pseudogens, wrongly reported variant for validation). Diagnostic output WGS trio (100% (308)): 70% [AD(de novo) 17%; AD (inherited) 16,5%; AR (homo) 4,2%; AR(compound) 16,9%; X-linked (de novo) 1,6%; X-linked (recessive) 3,9%; mt 0,3%, structural 8,7%], 30% no variants identified. Diagnostic output WGS proband mono & Sanger trio (100%(559)): 55,6% [AD(de novo) 26,5%; AR (homo) 3,9%; AR(compound) 14,5%; X-linked (de novo) 1,9%; X-linked (recessive) 6,1%; mt 0,7%, structural 1,9%], 44,4% [AR(cis) 2,3%, AD (inherited) 42%].

Conclusion: For our research the diagnostic yield WGS trio in children with hereditary diseases was shown 15–20% higher than when performing WGS proband mono & Sanger trio.

C.O.I. Disclosure:

O. Groznova: None. **A. Antonenko:** None. **R. Belov:** None. **P. Ulanova:** None. **M. Gubona:** None. **A. Krinitsina:** None. **M. Belenikin:** None.

EP15.123 Two cases of Postmortem and Postnatal Rare Thrombocytopenia Radius Aplasia Syndrome: Genetic Counseling and PGD

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Background: TAR Syndrome is a recessive genetic disorder characterized by absent radii, the presence of two thumbs, and often transient thrombocytopenia, which contributes to a high infant mortality rate due to early hemorrhages. Early prenatal diagnosis is essential for managing complications and guiding decisions regarding pregnancy continuation. Postnatal genetic counseling, however, remains complex due to unclear genotype-phenotype correlations.

Materials and methods:Case 1: A 30-year-old woman, G1P0A0, at 21 weeks' gestation, presented with a well-monitored, low-risk pregnancy. Ultrasound revealed bilateral radial absence and claw-like hands. The family chose termination, and genetic testing (karyotyping, QF-PCR, microarray, and skeletal dysplasia panel) was planned for fetal tissue.

Case 2: A 5-year-old girl with TAR syndrome, born to a 30-year-old G1P1A0 mother without prenatal ultrasound abnormalities. She had a 25-day ICU stay for thrombocytopenia and underwent surgery for bilateral radial clubhands. The family sought genetic counseling and preimplantation genetic diagnosis (PGD) for future reproductive planning.

Results: In Case 1, a 577 KBP deletion in 1q21 and an *RBM8A* c.-21G > A variant were identified. In Case 2, a 619 KBP deletion in 1q21.1, along with the *RBM8A* c.-21G > A variant, was found. Both families underwent segregation analysis and are pursuing PGD-IVF.

Conclusion: TAR syndrome is rare (1/100,000–200,000) and presents challenges in genotype-phenotype prediction. These cases emphasize the importance of early genetic counseling to guide pregnancy decisions and management.

C.O.I. Disclosure:

Z.N. Parali: None. **Z. Sakalli:** None. **E. Pariltay:** None. **B. Durmaz:** None. **E. Isik:** None. **T. Atik:** None. **F. Hazan:** None. **M. Guvenc:** None. **S. Sagol:** None. **H. Akin:** None.

EP16 Diagnostic Improvements and Quality Control

EP16.001 Development of a new droplet digital PCR for the molecular detection of *Rickettsia* spp

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Background/Objectives: Rickettsioses are a group of emerging diseases caused by gram-negative bacteria belonging to the genus *Rickettsia*. These diseases can be categorized into three distinct groups based on their clinical manifestations: 1) the typhus group (R. prowazeki and R. typhi), 2) the spotted fever group (R. conorii, R. africae, …), and 3) the pustular typhus (R. akari). Considering the epidemiological significance of infections in domestic dogs and their potential epizootic role, this study aims to develop a droplet digital PCR (ddPCR) method for the rapid and effective detection of *Rickettsia* spp.

Methods: Using the Primer3 program, the primer design was carried out for the specific detection of *Rickettsia* spp. using the 16S rRNA as a target sequence. Primer efficiency was evaluated using conventional PCR (cPCR), and the PCR amplicon of a clinical sample was cloned with the pGEM-T® Vector Systems to achieve a positive control. A curve of concentrations was used to identify this technique's detection limit, which was set up in a QX200 ddPCR (BioRAD) using ddPCR EvaGreen® Supermix. The ddPCR was assessed in 63 blood samples from domestic canids with prior clinical suspicion of rickettsiosis collected in Tenerife (Spain).

Results: The ddPCR developed demonstrated high sensitivity, with a detection limit of 0,00002 ng DNA/reaction. The optimised concentration for the positive control was 0,0064 ng DNA/reaction.

Conclusion: The new method allows the specific detection of *Rickettsia* spp at a very low load suggesting it is a fast and reliable option for diagnosing and monitoring Rickettsiosis in clinical samples.

C.O.I. Disclosure:

A. García Ramos: None. **O. García Pérez:** None. **M. Gajate Arenas:** None. **A. Domínguez de Barros:** None. **C. Sirvent Blanco:** None. **J. Piñero:** None. **J. Lorenzo Morales:** None. **E. Córdoba Lanús:** None.

EP16.002 Project Baby Panther - assessment of the implementation of the rapid Whole Genome Sequencing as routine diagnostic toll for critically ill neonates and children in Austria

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Background: In critically ill neonates and children, accurate and timely diagnosis is needed to enable targeted treatments, decisions on palliative care and to avoid unnecessary invasive procedures. Exome analysis has become the indispensable diagnostic gold standard. We aim to assess feasibility and utility of rapid trio Whole Genome Sequencing (rtWGS) as a routine diagnostic method in the Austrian healthcare system.

Material and methods: Within two years, we will include 130 patients and their parents who consent to receive rtWGS. Parents and medical personnel are surveyed on psychological well-being and satisfaction with genetic testing. Clinical utility of the genetic testing results is assessed using a questionnaire including the C-Guide (Genetics in Medicine (2022) 24, 430–438). Primary and secondary endpoints include diagnostic yield, time to diagnosis, hospital stay duration, and therapeutic changes. Implementation aspects involve personnel requirements, workload analysis, and time from blood sampling to report generation. Families who fulfil the inclusion criteria and decide not to receive rtWGS (standard diagnostic analysis or no genetic analysis) can still be enrolled to answer the survey. Referring clinicians will receive a questionnaire to assess their attitude towards genome analysis.

Results:

Conclusion: We expect that the study will enable a better assessment of the diagnostic effort, personal and clinical benefits of rtWGS in the health care of seriously ill newborns and children in Austria.

C.O.I. Disclosure:

M. Zaminer: None. **E. Schreiner:** None. **S.T. Bierbaumer:** None. **J. Blatterer:** None. **S. Hammer:** None. **L. Kaufmann:** None. **S. Kühberger:** None. **H. Tichy:** None. **J. Wagner-Skacel:** None. **M. Holter:** None. **E. Eber:** None. **K. Wagner:** None. **S. Verheyen:** None.

EP16.003 Adaptive sampling Nanopore sequencing for molecular diagnostics of Alzheimer's disease

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Background: Alzheimer's disease (AD) is the leading cause of dementia, representing 60–80% of cases and affecting more than 55 million people worldwide, with high economic, social, and human costs. Furthermore, prevalence is projected to continue to increase due to population aging, reaching 132 million in 2050. These data place AD as one of the major public health problems to be addressed in the coming years, as age is the main risk factor for AD. Molecular diagnosis of AD is currently challenging in terms of the speed and accuracy required. Classical genotyping methods are time-consuming and tedious. On the other hand, second-generation short-read sequencing methods are imprecise in detecting large structural variants and haplotypes. Furthermore, in light of recent findings, it is also necessary to determine the methylation changes that occur in AD.

Material and methods: Third-generation methods, using long-reads, allow the determination of SNVs and structural variants, besides direct detection of DNA modifications. On the other hand, Nanopore's adaptive sampling technique allows the selection of genomic regions to be sequenced without library preparation, using genomic DNA.

Results: In this work, we present a new molecular diagnostic technique for AD using a Nanopore adaptive sampling sequencing panel, to simultaneously sequence all AD-related genes with long reads, and to determine their 5-mC methylation.

Conclusion: The results provide a rapid and accurate molecular diagnosis of AD with minor DNA manipulation and library preparation, and allow the simultaneous detection of SNVs, structural variants, and methylation changes.

C.O.I. Disclosure:

F. Cardona: None. **F.B. Valverde:** None. **I. Viñals:** None. **L. Moreno:** None. **J. Perez-Tur:** None.

EP16.004 Covaris truCOVER Library Prep Kit: A Streamlined PCR-Free WGS Workflow for Enhanced NGS Library Performance and Data Quality

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Background: Whole-genome sequencing (WGS) has emerged as a widely applied tool in germline analyses, such as hereditary disease diagnostics and pharmacogenomics (1). As WGS increasingly transitions from research to routine clinical use, optimization of workflows for precision, scalability, and efficiency is essential. Addressing key challenges such as inconsistency in DNA fragmentation, suboptimal library conversion rates, cumbersome optimization for sample input variation, and its resulting sequencing biases, is essential for ensuring high-quality data and variant calling reporting.

Material and methods: DNA from blood, saliva, and FFPE were sheared using Covaris R230 Focused-ultrasonicator to achieve precise fragment sizes optimized for sequencing. Library performance was evaluated based on conversion rates, median insert sizes, and germline variant detection using GATK and DRAGEN pipelines.

Results: Covaris launched the truCOVER WGS PCR-free Library Prep Kit, a streamlined solution for WGS ibrary preparation. This single-vessel workflow integrates Covaris' Adaptive Focused Acoustics® (AFA®)-based DNA shearing, followed by end-repair and adapter ligation, and adds a post-library size selection that results in reproducible and controlled insert sizes (2). By minimizing transfer steps and eliminating the need for post-library QC steps like qPCR, this approach reduces workflow complexity, shortens turnaround time, and enhances overall efficiency.

Conclusion: The truCOVER workflow eliminates fragmentation bias and simplifies library preparation through a single-tube process. It consistently delivers high conversion rates and improved variant detection accuracy across diverse sample types, compared to competitor workflows. **References:** 1. Rosenquist et al., (2022). Seminars in Cancer Biology, 84, 32–39. 2. Process et al., (2024). Covaris Application Note (2024). https://www.covaris.com/wp/wp-content/uploads/M020179.pdf

C.O.I. Disclosure:

E. Soderback: None. **V. Process:** None. **M. Werner:** None. **M. Ambavaram:** None. **T. Dao:** None. **H. Giese:** None. **S. Khanal:** None. **L. Becker:** None. **S. Vasantgadkar:** None. **M.A. Berkeley:** None. **Z.T. Herbert:** None. **G. Endress:** None. **E. Daviso:** None. **U. Thomann:** None.

EP16.005 Newly designed saliva strip may be a useful sample collection method for detecting SARS-CoV-2

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Background: For detecting SARS-CoV-2 virus, the most commonly used sample collection method is nasopharyngeal swab, however by using this method, especially sample collection from pediatric patients is difficult. Therefore there is a processing need for designing a much more useful sample collection technique. The aim of this study was to compare the effectiveness of our newly designed sample collection strip with the commonly used nasopharyngeal swab collection method.

Materials and methods: The samples were collected from 200 pediatric patients. SARS-CoV-2 were analyzed using two different techniques. In the first technique, the collected nasopharyngeal swab samples and newly desined saliva collection strips were purified by nucleic acid isolation and then this purified RNA was analyzed by real-time polymerase chain reaction (RT-PCR). In the second technique, the collected samples were analyzed directly by RT-PCR without any isolation process.

Results: At the end of the study, Ct (Cycle Threshold) values were compared between groups and very similar results were obtained for all of the methods. This reveals that both nasopharyngeal swab samples and saliva collection strips, as well as both extraction and direct RT-PCR methods, gave similar accuracy results in terms of detecting SARS-CoV-2.

Conclusion: The use of our designed strips simplifies the sample collection process, reducing both the difficulty and the risk of contamination. The collection process does not require professional assistance, therefore it helps to reduce the workload on healthcare workers. Therefore it could be a useful sample collection method for detecting SARS-CoV-2.

C.O.I. Disclosure:

A. Orbay: None. **T. Kuruoglu:** None. **D. Bayirli Turan:** None. **E. Erdeniz:** None. **D. Gur Vural:** None. **D. Kirac:** None.

EP16.006 Whole genome sequencing for NF1 molecular diagnosis

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Advances in next-generation sequencing (NGS) have transformed genetic testing by increasing diagnostic yield and decreasing the time to reach a diagnosis. Compared to other molecular methods, Whole Genome Sequencing (WGS) aims to detect simultaneously structural and complex rearrangement in addition to coding and noncoding variants, proposing to become the first-line laboratory approach in the evaluation of patients with a rare disorder. Neurofibromatosis type 1 is an autosomal dominant disorder characterized by multiple café au lait macules, freckling, cutaneous neurofibromas, and learning disability and is caused by neurofibromin 1 loss of function variants. Because different genomic alteration like exonic, intronic, deep intronic variant, deletion and intragenic structural rearrangements have been identified, molecular diagnosis is established using a multistep protocol based specific gene-targeted analysis to identify the different rearrangements. This approach provides a detection rate of about 97% but can be an extremely time-consuming process. In order to validate the WGS as a rapid and complete diagnostic tool for molecular diagnosis of Neurofibromatosis we sequenced 10 positive patient (exonic and intronic variant, deletion, intragenic structural rearrangement) and 7 negative patients. WGS analysis data detect and confirmed most of the characterized cases and through the negative case identified 5 deep intronic variant, leading to the molecular diagnosis in these patients. For a complex rearrangement the result was only detected visualizing the raw data but was not called by the filtering software. Our data confirmed that WGS could increase molecular diagnoses for rare disease with a more complete and faster analysis.

C.O.I. Disclosure:

F. Lepri: None. **S. Genovese:** None. **R. Capolino:** None. **E. Lorefice:** None. **D. Pompili:** None. **V. Alesi:** None. **M. Digilio:** None. **A. Novelli:** None.

EP16.007 Does NGS really improve the diagnostics of rare neurogenetic disorders?

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Background: Rare neurogenetic disorders, mainly hereditary spastic paraplegia (SPG) and peripheral neuropathy (CMT) are heterogeneous disorders with almost 150 genes implicated in each. The clinical symptoms vary from usually mild presenting with spastic gait or weakness and atrophies on lower limbs to severely affecting resulting in wheel chair bound or paitents with cognitive deficite and limited self-care.

Material and methods: In 120 patients the NGS exome sequencing with evaluation of gene panel was performed. The 144 genes in SPG and 287 genes in CMT panels were evaluated. Moreover, the pathogenic variants reported in ClinVar that could be associated with the neurological conditions were inspected.

Results: The diagnostic yield in patients with virtual gene panel for SPG was 22 % and for CMT was 23%. Extended evaluation of ClinVar variant revealed pathogenic conditions in 3 more patients. All three variants were one nucleotide deletions, repeatedly reported as pathogenic in several publications; NRXN1 (NM_001330078.2): c.2792del, p.(Phe931Serfs*8), PTEN (NM_000314.8) c.491del, p.(Lys164Argfs*3) and PRRT2 (NM_145239.3): c.649del, p.(Arg217Glufs*12). These variants were not confirmed with Sanger sequencing.

Conclusion: The NGS method has limited discrimination power for the region of the same nucleotides. This limited discrimination results in variant calling of the false positive variants. These variants are frameshift and are classified as pathogenic. But in reality, they are not present in the DNA and could not be verified with other method. All should be aware of this limitation and first confirm the presence of variant and only after that report it.

C.O.I. Disclosure:

D. Safka Brozkova: None. **J. Krejcikova:** None. **D. Stanclova:** None. **L. Sedlackova:** None. **A. Meszarosova:** None.

EP16.008 A simple and cost-effective real-time PCR method using diluted and heat-treated whole blood lysate

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Background: Although DNA extraction is a crucial step before polymerase chain reaction (PCR), attempts to perform PCR without DNA isolation have been ongoing since the 1990s. While partial success has been achieved with direct conventional PCR, the DNA isolation step has remained indispensable for real-time PCR. Here, we developed a method that does not require complete DNA isolation by lysing EDTA-treated whole blood samples through the application of osmotic pressure and heat.

Table 1. Ct values of the DNA samples

60 °C, DNA	ACT Mean CT	SMN Mean CT	F9E4 Mean CT	F9E7 Mean CT	F9E8B Mean CT	F9E8C Mean CT	F9E8D Mean CT	p53E5 Mean CT	PIK3CAE9 Mean CT
Sample 1	26.16	23.98	22.86	23.07	22.82	22.09	22.77	23.22	23.27
Sample 2	26.35	23.97	22.70	23.01	22.80	22.10	22.78	23.19	23.26
Sample 3	26.46	24.52	23.62	24.00	23.80	23.11	23.70	22.91	23.18
Sample 4	26.21	23.90	23.76	23.99	23.89	23.08	23.81	22.99	23.11
Sample 5	26.11	24.51	24.00	24.63	23.85	22.94	23.83	22.88	23.02
Sample 6	26.42	24.21	24.07	24.38	24.09	23.24	23.95	23.08	23.34
Sample 7	26.45	24.15	22.82	23.15	22.90	22.11	22.99	23.12	23.20
Mean	26.31	24.17	23.40	23.74	23.45	22.66	23.40	23.05	23.19

Table 2. Ct values generated from GG-RT-PCR experiments with 1:5 and 1:10 dilutions of blood lysates

60 °C 1:10 Lysate	ACT Mean CT	SMN Mean CT	F9E4 Mean CT	F9E7 Mean CT	F9E8B Mean CT	F9E8C Mean CT	F9E8D Mean CT	p53E5 Mean CT	PIK3CA E9 Mean CT	61 °C 1:10 Lysate	ACT Mean CT	SMN Mean CT	F9E4 Mean CT	F9E7 Mean CT	F9E8B Mean CT	F9E8C Mean CT	F9E8D Mean CT	p53E5 Mean CT	PIK3CA E9 Mean CT
Sample 1	26.93	26.12	24.50	25.34	25.07	24.17	25.46	26.53	24.26	Sample 1	26.45	29.63	25.74	26.23	25.56	24.62	26.14	26.81	24.25
Sample 2	26.86	25.46	23.72	24.40	24.16	23.45	24.51	25.78	23.45	Sample 2	26.21	29.28	24.62	25.05	24.58	23.65	24.93	26.30	23.68
Sample 3	26.77	26.14	24.50	25.47	25.50	24.56	25.59	25.84	23.61	Sample 3	26.04	29.05	25.52	26.18	25.62	24.77	25.90	26.17	23.72
Sample 4	27.30	26.27	24.60	25.43	25.44	24.43	25.60	26.27	23.61	Sample 4	26.51	29.02	25.49	25.94	25.64	24.58	25.82	26.46	23.58
Sample 5	26.75	24.98	23.94	24.80	24.41	23.54	24.79	25.82	22.75	Sample 5	25.52	27.78	24.58	25.13	24.77	23.77	24.95	26.25	22.76
Sample 6	26.35	24.73	24.17	24.92	24.61	23.75	24.69	25.23	22.85	Sample 6	25.62	27.77	24.78	25.15	24.89	24.03	25.18	25.81	22.98
Sample 7	26.21	25.60	23.96	24.49	24.30	23.49	24.52	25.31	23.74	Sample 7	25.33	28.79	24.37	24.94	24.43	23.67	24.96	25.61	23.72
mean	26.73	25.61	24.19	24.98	24.78	23.91	25.02	25.82	23.47	mean	25.95	28.76	25.01	25.51	25.07	24.15	25.41	26.20	23.52
CT difference 1/10 vs 1/5	1,81	-2,03	-0,20	0,13	0,48	0,62	-0,10	0,58	0,78	CT difference 60 °C vs 61 °C	-0.78	3.15	0.82	0.54	0.29	0.24	0.39	0.38	0.06
Ct difference 10 ng DNA	0,43	1,44	0,79	1,23	1,34	1,25	1,62	2,77	0,27	CT difference 1/10 vs 1/5	0,96	-3,32	1,01	0,92	0,93	0,94	0,99	0,99	1,00
										Ct difference 10 ng DNA	-0,35	4,59	1,61	1,77	1,62	1,49	2,01	3,15	0,33
60°C 1:5 Lysate	**ACT Mean CT**	**SMN Mean CT**	**F9E4 Mean CT**	**F9E7 Mean CT**	**F9E8B Mean CT**	**F9E8C Mean CT**	**F9E8D Mean CT**	**p53E5 Mean CT**	**PIK3CAE9 Mean CT**	**61°C 1:5 Lysate**	**ACT Mean CT**	**SMN Mean CT**	**F9E4 Mean CT**	**F9E7 Mean CT**	**F9E8B Mean CT**	**F9E8C Mean CT**	**F9E8D Mean CT**	**p53E5 Mean CT**	**PIK3CAE9 Mean CT**
Sample 1	25.23	29.19	25.08	25.58	24.90	23.79	26.16	25.79	23.52	Sample 1	25.49	33.11	24.54	25.19	24.65	23.67	25.05	25.88	23.25
Sample 2	25.12	27.86	24.07	24.22	23.88	22.90	24.49	25.43	22.81	Sample 2	25.19	32.05	23.65	24.08	23.69	22.76	23.93	25.29	22.67
Sample 3	24.93	27.68	24.68	25.12	24.72	23.91	25.25	25.06	22.77	Sample 3	24.92	31.73	24.45	24.97	24.68	23.76	24.86	25.05	22.67
Sample 4	25.32	27.26	24.79	25.59	24.70	23.67	25.59	25.83	22.68	Sample 4	25.45	34.07	24.60	25.16	24.66	23.76	24.92	25.56	22.58
Sample 5	24.79	26.20	24.03	24.51	23.90	22.81	24.77	25.15	21.93	Sample 5	24.89	30.64	23.62	24.27	23.81	22.84	24.08	25.24	21.76
Sample 6	24.57	26.27	24.17	24.66	24.07	23.04	24.87	24.79	22.13	Sample 6	24.56	31.22	23.88	24.40	23.87	22.95	24.22	24.82	22.06
Sample 7	24.53	29.00	23.93	24.22	23.93	22.90	24.69	24.68	22.95	Sample 7	24.43	31.72	23.26	24.14	23.62	22.78	23.90	24.61	22.70
mean	24.93	27.64	24.39	24.84	24.30	23.29	25.12	25.25	22.68	mean	24.99	32.08	24.00	24.60	24.14	23.21	24.42	25.21	22.53
Ct difference 10 ng DNA	-1,38	3,46	0,99	1,10	0,85	0,62	1,72	2,20	-0,51	CT difference 60°C vs 61°C	0.06	4.44	-0.40	-0.25	-0.16	-0.07	-0.70	-0.04	-0.16
										Ct difference 10 ng DNA	-1,32	7,90	0,59	0,85	0,69	0,55	1,02	2,15	-0,67

Material and methods: 2 mL of peripheral blood was collected from 8 healthy donors. Blood samples were mixed with distilled water, incubated at 95 °C for 20 min before centrifugation at 14,000 rpm for 5 min. The resulting lysates were used as templates for real-time PCR. Real-time PCRs were performed under identical conditions with lysates and DNA samples using 9 different primer sets.

Results: Target genes were successfully amplified using 1:10 and 1:5 diluted blood lysates both at 60 °C and 61 °C. The PCR efficiency for ACTB and PIK3CA differed by 20% and 14%, respectively, between the DNA samples and blood lysates (Table 1 and Table 2).

Conclusion: We successfully amplified all selected genes using "GG-RT PCR", greater temperature, greater speed method, without the need for additional enzymes or buffers. GG-RT PCR presents a cost effective option for applications such as SNP analysis and deletion detection if appropriate primer designs are made.

C.O.I. Disclosure:

G. Güllü Amuran: None. **B. Polat:** None. **A. Kahraman:** None. **M. Akkiprik:** None.

EP16.009 Integrating a prototype of Quality control management system in Next generation sequencing diagnostic laboratory

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Background: Huge amounts of Next generation sequencing (NGS) data are challenging to explore without proper bioinformatic tools. Rich quality control (QC) assessment report can improve the long and tough data analysis process. Most commercially available QC solutions need IT and/or command-line interface background. This exploration aims to integrate an in-house Quality Control Management System (QCMS) solution supporting non-IT specialists in genetic testing.

Material and methods: Python's Flask was used to upgrade the prototype, creating back-end logic for collecting QC parameters. Front-end was designed with Bootstrap 5. DRAGEN Bio-IT platform (Illumina) emits numerous metrics (depth/mean/uniformity of coverage, mapping quality, and autosomal over sex chromosomes coverage ratio). Based on international NGS QC guidelines a detailed report was generated.

Results: User-friendly interface shows important graphics and fields after a few button clicks. Selected features make it easy to check distribution of samples in the run, evenness of coverage, and compare expected-predicted sex karyotype. Besides the shortened time for QC measurement, the QCMS revealed some interesting aneuploidy cases, previously undetected.

Patient's karyotype		Patient's phenotype
Expected	**Predicted**	
46,XY	47,XXY	Hidden-Kleinfelter syndrome
46,XY	48,XXXY	Neurological clinic
46,XX	46,XY	Nephrotic syndrome,*WT1* mutation
46,XX	46,XY	Pancreatic cancer

Conclusion: QCMS complements the analysis, final results generation, and genetic counseling. It helps the user complete the evaluation with minimal effort, remove unnecessary work, increase efficiency, and save costs. The system's functionality can easily be maintained and supports high-quality NGS QC analysis.

C.O.I. Disclosure:

M. Georgiev: None. **K. Kamenarova:** None. **D. Kachakova-Yordanova:** None. **K. Mihova:** None. **N. Ivanova:** None. **O. Beltcheva:** None. **I. Dimova:** None. **R. Kaneva:** None.

EP16.011 Diagnosis of Angelman syndrome, with 63 years of delay, using DNA methylation profiling

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Background: DNA methylation (DNAm) analysis is increasingly used where a genetic diagnosis cannot be made despite comprehensive DNA sequencing. Diagnostic DNAm analyses are especially useful in reclassification of variants of unknown significance or hypothesis-free screening against known disease specific DNAm signatures. In this study, we report an individual with intellectual disability, microcephaly, speech impairment, and seizures, who remained undiagnosed until age 63 years.

Material and methods: Using exome sequencing two missense variants of unknown significance were identified in *ZNF142*. DNAm profiling was carried out using EPIC DNAm array, and the patients DNAm profile was assessed against the DNAm signature established for biallelic pathogenic *ZNF142* variants. A non-targeted screening for differentially methylated regions was conducted on the generated DNAm data. The DNAm profiling re-sults were verified using methylation-specific multiplex ligation-dependent probe amplifi-cation (MS-MLPA).

Results: As the patients DNAm profiling did not fit in to the DNAm signature of *ZNF142*, a hypothesis free DNAm screening was carried out. This analysis revealed several hypo-methylated regions at chromosome 15q11.2-q13 suggesting Angelman syndrome and the diagnosis was confirmed using MS-MLPA enabling a genetic diagnosis of the patient at age 63 years.

Conclusion: This study highlights the utility of DNAm analysis in undiagnosed patients with neurodevelopmental disorders.

C.O.I. Disclosure:

M. Hildonen: None. **M. Ferilli:** None. **I. Krey:** None. **O. Kohnen:** None. **C. Cappelletti:** None. **R. Jamra:** None. **K. Platzer:** None. **A. Ciolfi:** None. **M. Tartaglia:** None. **Z. Tumer:** None.

EP16.012 Clinical Exome Caveats: Two Case Reports from a Tertiary Center for Rare Diseases in Romania

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Background: Next generation sequencing has become a fundamental diagnostic tool in human genetics, giving its increasing

affordability in the recent years. This has led to an exponential rise in the number of tests performed across laboratories worldwide. Tertiary analysis and clinical reporting have advanced, albeit at a slower pace, and face several limitations, despite the promising contribution of artificial intelligence to this field.

Methods and Results: Herein, we present two cases where clinical exome (TruSight One, TSO) was employed as a first-tier diagnostic test for Neurofibromatosis type 1 (NF1) and Autoimmune polyendocrinopathy syndrome type 1. In both instances, variant calling was not sensitive enough to provide a definitive diagnosis. Subsequent whole exome sequencing (WES) detected two frameshift variants — *NF1*(NM_000267.3: c.6789_6792del) and *AIRE*(NM_000383.4: c.21_43dup), respectively — that were overlooked by the initial test. Additionally, in the NF1 case, both TSO and WES identified a truncating *CHD8* variant in the last exon of the gene (NM_001170629.2:c.7267C>T), but its clinical interpretation was conflicting across laboratories. Retrospective visual inspection of the original sequencing data confirmed the missing variants, but revealed, however, a suboptimal sequencing depth in the target genomic regions.

Conclusion: These findings highlight the importance of visual inspection of sequencing data, particularly when the clinical suspicion is high. However, this process can be very challenging at times, especially for long and complex genes as *NF1*. Furthermore, our cases provide additional evidence for the suboptimal performance of TSO in detecting short indel variants, a limitation already reported in literature for this test.

C.O.I. Disclosure:

C. Munteanu: None. **N. Andreescu:** None. **C. Pantea:** None. **A. Chirita-Emandi:** None.

EP16.013 Clinex pro long: Long reads as an even more powerful tool in precision medicine

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Background: Clinical Exome Sequencing (CES) is widely used in Precision Medicine, but it has limitations. Short reads struggle to discern between highly homologous regions, such as pseudogenes, leading to misalignment issues. Long-read sequencing offers a robust solution by addressing these shortcomings. ClinEX pro Long, a capture-based kit from 4Bases, integrates the benefits of CES with long-read technology.

Materials and methods: Commercial samples from the Coriell Institute (NA12878, NA05170) were analyzed. Genomic DNAs were fragmented using g-Tube Covaris, followed by end-repair and A-tailing for ligation with specific adapters for indexing. The hybridization time was reduced to one hour. CES targets were captured with streptavidin beads and amplified. The library was sequenced on Nanopore PromethION flow cells V10.4.1 for 72 hours, and data analysis was conducted on 4eVar, 4Bases' proprietary analysis platform.

Results: Long reads (4 Kb) significantly improved alignment quality in homologous regions (i.e., HBA1/HBA2, PMS2) and enabled comprehensive analysis of exons and flanking introns, enhancing our understanding of gene structure and regulation. Furthermore, these technologies permit accurate identification and characterization of CGG repeat expansions in the gene associated with Fragile X syndrome (i.e., FMR1). The analysis showed the recognition of exact breakpoints in DMD gene samples (exons 4–43 deletion in NA05170). Analysis of NA12878 replicates demonstrated the kit's robustness and reproducibility across runs, with a Precision of 95% and Recall of 88%.

Conclusion: The advantages offered by CES analysis in the ClinEX pro (4Bases, Italy) are enhanced by the long-read sequencing approach, augmenting the opportunity to solve complex clinical cases.

C.O.I. Disclosure:

A. Degiorgi: A. Employment (full or part-time); Significant; 4Bases SRL. **V. Favalli:** A. Employment (full or part-time); Significant; 4Bases SRL.

EP16.014 Validation of a 2-day lab protocol for genomic array - improving turnaround-time for urgent analysis of neoplasms and prenatal samples

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Background: Genomic array is the standard of care method of choice for copy number analysis in many clinics both for cancer patients and prenatal samples. As these analyses are urgent it is essential to obtain a fast result, but the lab procedure and subsequent analysis takes a minimum of 4 days using for example the high-resolution assay CytoScan HD (Thermo Fisher Scientific). In the fall of 2023, a new accelerated protocol was released, CytoScan HD Accel, allowing analysis results in only 2 days. At the Section for clinical molecular diagnostics in Lund, Sweden, we validated this new assay as soon as it was available and implemented it in clinical routine in April-2024.

Material and methods: 35 samples were included in the validation (5 bone marrows, 8 solid tumors, 3 blood, 1 cultured fibroblasts, 10 chorion villus samples(CVS), 5 Amniocentesis, 3 product of conception (POC)), all of which had been previously analyzed with Cytoscan HD. The DNA input ranged from 22ng-250ng (nanodrop measurement), and all these samples except one had acceptable QC metrics on the CytoScan HD platform.

Results: All bone marrow, tumor, blood, fibroblast and POC samples had acceptable QC and consistent results with both platforms, but out of the 15 CVS and amniocentesis samples, 7 (47%) failed the QC check. In total, 28/29 (96%) samples with a DNA-input of ≥100ng passed, while only 1/6 (17%) with <100ng input passed.

Conclusion: The accelerated protocol provides a shorter turnaround-time but requires higher DNA input compared to the established CytoScan HD platform.

C.O.I. Disclosure:

M. Bååth: None. **L. Arvidsson:** None. **A. Isinger Ekstrand:** None.

EP16.015 Molecular genetic diagnosis of mosaic forms using duplex-specific nuclease in patients with PIK3CA-related overgrowth spectrum

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Background: The detection of genetic variants of interest in mosaic disease can be significantly complicated by the low representation of such variants in the biological material taken for analysis. There is a wide range of methodological approaches for the detection of rare genetic variants in clinical and laboratory diagnostics, but not all of them may be available to many laboratories due to various limitations. A method using duplex-specific nuclease followed by Sanger sequencing aims to detect low-represented single-nucleotide genetic variants virtually anywhere in the genome.

Material and methods: DNA from peripheral blood leukocytes and tissue biopsies was used as a material for the study. To determine the analytical properties of the method, the level of alternative allele representation was modeled by adding DNA samples homozygous for polymorphic and reference variants to the reaction in different ratios.

Results: The minimum reliably detectable level of alternative allele representation was 1.5%. Using the DSN-based method, the *PIK3CA* genetic variants previously detected on the NGS panel with high coverage in 9 patients with clinical manifestations of PROS were confirmed.

Conclusion: In this study, a new approach based on the enzymatic properties of DSN is proposed for the accurate diagnosis of low-represented variants of *PIK3CA*, which can be used in clinical practice. This method can be used both as a validating method and as the first line of diagnosis.

C.O.I. Disclosure:

A. Pustovalova: None. **A. Nikolaeva:** None. **K. Petrova:** None. **E. Bychkova:** None. **V. Sigin:** None. **V. Strelnikov:** None.

EP16.018 Truncating variants in APOB are not VUS when screening for familial hypercholesterolemia

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Background: *APOB* is associated with both Familial Hypercholesterolemia and Hypobetalipoproteinemia, two seemingly opposite conditions, rendering variant interpretation challenging. Our aim was to investigate the clinical impact of *APOB* truncating variants in large unselected populations to streamline variant interpretation when screening for Familial Hypercholesterolemia.

Material and methods: This retrospective study utilizes Helix Research Network™ data from 144,852 participants. Phenotypes evaluated: (i) first diagnosis of elevated LDL-C, and (ii) first prescription of statin.

Results: 93 participants (0.07%) had a truncating variant in *APOB*, and 127 had one of the two *APOB* pathogenic variants (p.Arg3527Gln, p.Arg3527Trp). Participants with an *APOB* truncating variant had fewer and later onset documented elevations in LDL-C compared to those without a variant (Hazard Ratio = 0.09 (0.01–0.6)), or to those with *APOB* or *LDLR* pathogenic variants. The same result was observed for first statin prescription (HR = 0.14 (0.05–0.4). Moreover, truncating variants were associated with lower LDL-C levels, up through the last exon of *APOB* according to the Power Window method (an optimized sliding window approach to identify key associated regions of a gene).

Conclusion: For clinical variant interpretation, *APOB* truncating variants should be returned as benign and not as VUS in the context of Familial Hypercholesterolemia. A similar approach can be used for all genes where haploinsufficiency is not the mechanism of disease. Clear evidence that pLOF variants are not pathogenic in these scenarios will reduce the burden of variant interpretation without decreasing the accuracy of the screening.

C.O.I. Disclosure:

E.T. Cirulli: A. Employment (full or part-time); Significant; Helix. **K.M. Barrett:** A. Employment (full or part-time); Significant; Helix. **S.F. Powell:** None. **D. Stoller:** None. **C.N. Chapman:** None. **C.A. Chahal:** None. **D.P. Judge:** None. **D.A. Olson:** None. **J.J. Grzymski:** None. **A. Bolze:** A. Employment (full or part-time); Significant; Helix.

EP16.019 Robust quality parameters for WGS variants: towards consensus variant validation policies

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Background: Despite a relatively long history of using massive parallel sequencing for clinical tasks, there is still no widely accepted consensus on which variants require orthogonal validation, with different papers citing different quality cutoff values. The knowledge is particularly sparse for low-coverage applications such as WGS and LP-WGS.

Material and methods: A 1756 variant set of clinically relevant variants derived from clinical WGS sequencing were subjected to Sanger validation. Based on this data, quality parameters for variant classification were suggested to formulate validation policies.

Results: Out of 1756 variants, 5 were not confirmed by Sanger sequencing. Using DP ≥ 15, AF ≥ 0.25 caller-agnostic quality thresholds achieved 84 variants in the "low-quality" bin, with all of the unconfirmed ones within this number. By using a single QUAL ≥ 100 threshold for HaplotypeCaller v4.2 the "low-quality" bin shrinked to the size of 21 variants (1.2 % of the initial set) with the same 100% sensitivity for the unconfirmed variants. Using this data, a method for a more robust and precise determination of quality cutoffs was suggested based on downsampling of the well-defined WGS datasets and the outlook on the further work in this direction is provided.

Conclusion: For WGS being widely accepted as a diagnostic tool a preliminary policy for the variant validation is given based on the orthogonal validation data for a large dataset. A path for the determination of the universal policies is presented.

C.O.I. Disclosure:

V. Bogdanov: None. **D. Molodtsova-Zolotukhina:** None. **A. Kopernik:** None. **A. Esibov:** None. **A. Borkovskaia:** None. **A. Rozhkova:** None.

EP16.020 The impact of hemolysis on the results of non-invasive prenatal testing

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Background: The quality of biomaterial at the pre-analytical stage significantly affects genetic testing accuracy. The hemolysis percentage indicates proper blood collection, storage and transport. The hemolysis index (HI, mg/dl) is measured automatically or visually. Hemolysis is detectable visually at HI > 30, and testing may be rejected at HI > 80.

Material and methods: Over 21,000 blood samples from pregnant women undergoing NIPT were analyzed. Hemolysis was visually assessed. NIPT was performed using BGI technology (China) per manufacturer protocols.

Results: Hemolyzed samples with HI > 60 accounted for 0.0046% and were rejected. Samples with visible hemolysis were 0.06%, of which 72.8% had minor hemolysis, 25.2% - severe and

2% - HI 30–50. At HI 30–60, the probability of "no call" NIPT-results increased 3-fold. Hemolyzed samples showed a higher frequency of high-risk findings for chromosomal anomalies. The probability of detecting high risk on chromosome 21 increased 1.5-fold at HI < 30 and 3-fold at HI 30–60; for chromosome 18 at HI 30–60, it increased 3-fold; for sex chromosome aneuploidies (XXY or XO), 4-fold.

Conclusion: Performing NIPT for hemolyzed samples requires careful attention to evaluate the results. The preanalytical stage of sample processing at Evogen LLC meets optimal standards: the proportion of samples with high HI does not exceed 1.2% (according to WG-LEPS quality criteria).

C.O.I. Disclosure:

P. Ulanova: None. **E. Domoratskaya:** None. **A. Bartsiz:** None. **A. Antonenko:** None. **O. Andreeva:** None. **A. Panferova:** None. **M. Kaplanova:** None. **A. Potapov:** None. **A. Krinitsina:** None. **M. Belenikin:** None.

EP17 Bioinformatics, Machine Learning and Statistical Methods

EP17.001 UCSC Genome Browser -- Important Features

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Background: The UCSC Genome Browser is a unique resource for modern genomics. Used by hundreds of thousands of regular users in both the research and clinical space, it is an essential tool for scientists worldwide. The proposed presentation is designed to introduce both experienced and new users to features of the Genome Browser not typically found by the casual user. Because the Browser is very complex, with very many features, not all of them are easily discovered. There are also new features being released all the time, which often fly "under the radar," i.e. not discovered by people using the Browser in their daily work.

C.O.I. Disclosure:

R.M. Kuhn: None.

EP17.002 Exploring the Mutation Landscape of TNNT2: Insights from ClinVar

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Background: TNNT2 encodes cardiac troponin T and is linked to various cardiomyopathies. The mechanisms by which TNNT2 mutations drive disease remain unclear.

Objectives: This study evaluates TNNT2 mutation clustering and genotype-phenotype correlations using ClinVar, assessing its comprehensiveness. The hypothesis by Gomes et al. regarding arginine substitution mutations is revisited.

Methods: ClinVar data were filtered for pathogenic, likely pathogenic, uncertain significance, and missense mutations. Mutation densities across exons and protein domains were analysed using the chi-squared test for clustering. A systematic review of TNNT2 mutations in PubMed (31st Oct 2024) provided additional data for comparison.

Findings: ClinVar identified 351 TNNT2 missense mutations, with significant clustering in exons 9 and 11, while exons 1 and 13 lacked mutations. Of 137 PubMed papers, 109 were included, revealing 39 mutations absent from ClinVar, giving the database a 64% coverage. Forty-four novel arginine substitution mutations were identified. No specific domain mutations were linked to distinct cardiomyopathies.

Conclusion: ClinVar documents more TNNT2 mutations than individual studies but lacks complete coverage of the literature. Clustering in exons 9 and 11 highlights the T1 domain's importance in cardiomyopathies. The Gomes et al. hypothesis regarding arginine substitutions was validated.

C.O.I. Disclosure:

B. Singh: None.

EP17.003 A platform to integrate and manipulate data from physical and digital objects

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Background: Mendelian gene discovery and patient diagnosis require analysts to understand the relationship between biological and digital assets, ensuring cases with sufficient biological material can be revisited using orthogonal genomic or multiomic technologies. As a GREGoR consortium member, we developed a solution to capture these associations. The GREGoR data model records biological and digital assets with high fidelity, yet no platform existed to integrate them—so we built one.During the first 18 months, we found collaborators and sample suppliers had variable awareness of their biorepositories and associated data. Integrating these sources—from LIMS, genomic databases, and labs—is complex. Our goal was to streamline this integration between the central hub, AnVIL, and our site.

Material and methods: Our software team built a relational database (SQLite) interfaced via a Django RESTful API, enabling AnVIL-ready submissions per the GREGoR data model. This system fosters collaboration across the rare disease research lifecycle—from sample acquisition to sequencing, analysis, and reanalysis. The database is accessible through a custom UI and programmatically via API, hosted on AWS EC2 and secured within a HIPAA-compliant UCI Health environment.

Conclusion: This architecture co-evolves with the GREGoR model, improving efficiency with AnVIL and collaborators. It is generalizable to other sites and data models, supporting AI/ML integration. The API features an auto-generated Swagger UI for interactive access, while the Redoc page offers a responsive, mobile-friendly interface.

C.O.I. Disclosure:

C.H. King: None. **L. Auriga:** None. **J. LoTempio:** None. **E. Vilain:** None.

EP17.004 Investigating the potential of grapefruit seed extract as an inhibitor of MRSA's PBP2a receptor through computational methods

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Background: Methicillin-resistant *Staphylococcus aureus* is emerging as a prevalent and highly resistant bacterium, highlighting the growing concern that antimicrobial resistance poses a global health challenge. Our previous in vitro studies have demonstrated that Grapefruit Seed Extract (GSE) shows antimicrobial activity, particularly against MRSA. Methicillin resistance in MRSA is due to the presence of the *mecA* gene, which encodes penicillin-binding protein 2a (PBP2a), a promising target for molecular docking

studies. This study aimed to assess the binding affinity of selected compounds from GSE with the SauPBP2a protein to identify which compounds may exhibit the strongest inhibitory potential.

Material and methods: The Protein Data Bank (PDB) was used to obtain the protein 3D crystal structure, while PubChem provided the Structure Data Files (SDF). Protein and ligand preparation was carried out using AutoDockTools, while molecular interactions were analyzed using PyMOL 2.4 and LigPlot+ v.2.2.8. We used ceftobiprole as a positive control, while methicillin was used as a negative control for binding affinity.

Results: Compounds narirutin and naringin exhibited the highest binding affinities. All GSE components established significant hydrogen bonds with the active site of SauPBP2a, suggesting their potential to inhibit MRSA.

Conclusion: Our findings suggest that the overall anti-MRSA activity exhibited by GSE may arise from its multifaceted inhibitory effects, one of which could be related to the inhibition of specific enzyme activities, contributing to its effectiveness. Further in vivo studies and clinical safety assessments are needed, along with evaluations of both the individual and synergistic effects of these compounds.

C.O.I. Disclosure:

B. Šarić Medić: None. **A. Jerković-Mujkić:** None. **N. Tomić:** None. **J. Ramić:** None. **N. Lojo-Kadrić:** None. **L. Pojskić:** None.

EP17.005 Comparative study for developing an automated similarity assessment model for structural variants

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Structural variants (SVs) are known to be associated with disease onset, and large-scale cohort studies worldwide are accumulating extensive SV datasets. Interpreting detected SV data requires determining whether each variant corresponds to a previously known SV. Additionally, given the vast number of SVs detected across multiple samples, clustering common SVs is essential. However, due to the intrinsic complexity and diversity of SVs, comparing them and assessing their concordance remain challenging.

In this study, we aim to develop a similarity assessment model for SVs that takes polymorphism types into account. As an initial step, we have investigated differences among commonly used SV detection tools to establish criteria for similarity evaluation. Meanwhile, with the advent of updated reference genomes such as CHM13 and Pangenome graphs, new detection tools, and updates to existing ones, it has become necessary to reconsider the investigation.

To address these challenges, we re-evaluated SV detection tools and conducted comparative analyses across multiple reference genomes at the whole-genome level, assessing the variability of different SV types. Based on our evaluation results, we aim to develop a scoring system for SV similarity assessment, enabling the automated classification of SV similarity.

C.O.I. Disclosure:

M. Kamada: None. **Y. Kawai:** None. **T. Katayama:** None.

EP17.006 Exploring advanced frontiers in single-cell transcriptomic bioinformatics: moving beyond differential expression analysis

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Background: Differential expression (DE) analysis, a cornerstone of gene function studies, often yields inconsistent results and detects genes with limited biological relevance. This challenge is particularly acute in single-cell transcriptomics, a rapidly growing field.

Material and methods: To address the underlying limitations of DE and introduce data-driven methods to extract meaningful biological insights from single-cell data, we developed scTenifoldNet, scTenifoldKnk, and scTenifoldXct.

Results: These methods go beyond DE and include: (1) Gene regulatory network construction and comparison—this approach reveals how genes interact and identifies key regulators in different cellular contexts; (2) Virtual gene knockout analysis—this technique simulates gene inactivation to predict functional consequences and identify essential genes; and (3) Manifold learning analysis for cell-cell communication—this method uncovers communication patterns between cells, providing insights into cellular interactions.

Conclusion: By moving beyond DE, these approaches offer a deeper understanding of gene function and cellular processes in single-cell transcriptomics data.

C.O.I. Disclosure:

J.J. Cai: None.

EP17.007 Precision diagnosis in early-onset prostate cancer: a machine learning and transcriptomic approach identifies *PCSEAT* lncRNA as a race-specific biomarker

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Background: Early-onset prostate cancer (EOPC) shows an increasing incidence and late detection, causing poor prognoses. Despite racial differences, race-specific early-diagnosis biomarkers remain undiscovered. Here, we integrated bioinformatics and machine learning (ML) to identify biomarkers in White EOPC patients.

Material and methods: TCGA-PRAD was used to download RNA-seq and clinical data of 10 tumor and 10 normal samples from White individuals under 55 years old. After quality control and variance-stabilizing normalization, WGCNA identified tumor-associated modules. Functional genes were found by intersecting WGCNA driver genes with differentially expressed genes (DEGs) from DESeq2. Enriched pathways were analyzed via clusterProfiler. Given the limited samples available, a nested LOOCV framework with SVM, RF, and ANN selected biomarkers using LASSO. Model performance was assessed using standard classification metrics, including ROC-AUC. Permutation importance and SHAP confirmed feature robustness.

Results: WGCNA revealed 3 tumor-associated modules. The driver genes of these modules intersected with 485 DEGs ($padj < 0.05$, $|log2FC| > 1$), which were enriched in cytoskeleton organization and contractile fiber pathways, suggesting an influence on tumor aggressiveness, metastasis, and stromal interaction. ML models achieved high diagnostic accuracy (AUCs: SVM = 0.86, RF = 0.89, ANN = 0.91), with *PCSEAT* lncRNA as the only common biomarker across all algorithms. *PCSEAT* was overexpressed in tumor samples. Previous studies have also identified *PCSEAT* (*PRCAT38*) as an androgen-regulated lncRNA interacting with *EZH2* to promote tumor progression. *PCSEAT* overexpression was validated in GSE115414.

Conclusion: Our study identifies *PCSEAT* lncRNA as a novel race-specific biomarker for EOPC, highlighting its clinical potential for precision diagnosis and targeted therapy in White patients.

C.O.I. Disclosure:

R. Zohreh: None. **N. Asghari Moghaddam:** None. **M. Ramezani:** None.

EP17.008 Comparative evaluation of long and short sequencing technologies for human trio genotyping

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Background: Recent advances in sequencing technologies have paved the way for personalized medicine and clinical genomics, providing unprecedented insights into the genetic basis of human health and disease. High-throughput sequencing is now recognized as an effective diagnostic tool for patients affected by rare diseases but the use of either Whole Exome Sequencing (WES) or Whole Genome Sequencing (WGS) technologies has shown to be uninformative in most cases. On the contrary, the recent introduction of long read technologies can resolve genomic regions inaccessible to short-read sequencing, but a reliable benchmark assessment is missing.

Material and methods: This study presents a comparative evaluation of long-read and short-read sequencing data, aiming to elucidate their strengths and limitations through the investigation of family pedigrees, which represents a powerful approach for the identification of transmitted alleles and/or de novo mutations. We investigated a family trio with a pediatric proband affected by a rare disease for which WGS analysis based on short reads resulted ineffective for diagnosis.

Results: The trio analysis presented here has provided a high-confidence assessment of short and long read performances in variant calling accuracy, coverage uniformity, and allele specificity.

Conclusion: Our results confirmed the powerful impact of long-read technologies on genotyping accuracy and the identification of complex genomic rearrangements, showing unmatched effectiveness on repetitive regions and structural variations.

C.O.I. Disclosure:

A. Varvara: None. **M. Chiara:** None. **I. Urso:** None. **S.N. Cox:** None. **S. Buonanno:** None. **G. Visci:** None. **A. Ciolfi:** None. **C. Mancini:** None. **A. Bruselles:** None. **C. Gissi:** None. **M. Tartaglia:** None. **G. Pesole:** None.

EP17.009 Copy number variation analysis in genotyping arrays using PCR enrichment workflows

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Genotyping using microarrays is an established technology for determination of single nucleotide polymorphisms (SNP), short insertion / deletion events, and copy number variations (CNV). But non-transcriptional active pseudo gene sequences are blocking certain variants from being analyzed and are a major challenge e.g. in pharmacogenetics. One approach to address these issues was introduced lately in the Illumina enhanced pharmacogenomics product line (GSA ePGX, GDA ePGX). In that workflow the input material is split in normal whole genome amplification workflow known from normal Infinium genotyping and a dedicated polymerase chain reaction (PCR) workflow that enriches the real coding sequences over pseudogene regions. Both DNA aliquots are merged afterwards and submitted subsequently to the following steps.

Downstream analysis is performed using a command line program ("dragena") or analysis in BaseSpace. This workflow is very different from the GenomeStudio analysis, as it is generating vcf output files for genotypes and copy number results. Copy number events are evaluated for a given set of ten regions only. Due to the extra PCR amplified DNA, the arrays cannot be analyzed for whole genome CNVs in GenomeStudio, since the normalization addresses the enriched regions.

We will present our whole genome CNV analysis pipeline for these PCR treated samples. Enabling scientists to use these array products for the detection of larger duplication and deletion events.

C.O.I. Disclosure:

S. Herms: A. Employment (full or part-time); Significant; Life&Brain GmbH, Genomics. **N. Fricker:** None. **C. Mathey:** None. **J. Steinberger:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; YAI PoC AG, St. Gallen, CH. **M. Nöthen:** A. Employment (full or part-time); Significant; Life&Brain GmbH, Genomics. E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Life&Brain GmbH, Bonn. **P. Hoffmann:** A. Employment (full or part-time); Significant; Life&Brain GmbH, Genomics.

EP17.010 GenomeKlinic: Precision Omics by AI & Supercomputing

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Background: Univariate and multivariate methods for association of the genomic variations with the end-or-endo phenotype have been widely used for genome wide association studies. In addition to encoding the SNPs, we advocate usage of clustering as a novel method to encode the structural variations, SVs, that can be used as an independent feature variable value for downstream computation by artificial intelligence methods to predict the endophenotype or end phenotype.

Material and methods: GTEx V7 pilot dataset was used. We introduce a clustering based encoding scheme for structural variations and omics-based analysis. We conducted a complete all genomic variants association with the phenotype using deep learning and other machine learning techniques, though other methods such as genetic algorithms can also be applied. The genomic variants acting as feature sets were optimized for selection.

Results: Applying this encoding of SVs and one-hot encoding of SNPs on GTEx V7 pilot DNA variation dataset, we were able to get high accuracy using various methods of DMWAS and particularly found logistic regression to work the best for death due to heart-attack (MHHRTATT) phenotype. Variant Id P1_M_061510_3_402_P at chromosome 3 position 192063195 was found to be most highly associated to MHHRTATT. We present here the top ten

optimized genomic variant feature set for the MHHRTATT phenotypic cause of death.

Conclusion: Combining methods of structural variation extraction by tools such as GMSECT or third party and deploy machine learning algorithms or tools such as DMWAS can yield remarkable results as we showcase it at www.GenomeKlinic.com.

C.O.I. Disclosure:

A.N. Singh: None.

EP17.011 A multi-omics analysis of a mitophagy-related signature in pan-cancer

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Background: Mitophagy is an essential process within cellular autophagy, with a critical role in regulating key cellular functions such as reproduction, metabolism and apoptosis. Emerging evidence indicates that mitochondrial dysfunction contributes to tumorigenesis, cancer cell survival and resistance to therapy. The investigation of a mitophagy-related gene (MRG) signature should provide valuable insights into the molecular pathways of mitophagy in cancer.

Material and methods: We conducted a comprehensive bioinformatics analysis of a MRG signature in pan-cancer. We used geneset cancer analysis to perform gene set genomic (expression, SNV, CNV and methylation) and immunogenomic analyses, using data from the TCGA, TCPA and ImmuCellAI. TIDE was used to find associations between the MRG signature and immune checkpoint inhibition therapy outcomes.

Results: Pathway analysis revealed a complex interplay between the expression of the signature and potential effects on the activity of various cancer-related pathways in pan-cancer. Immune infiltration analysis linked the MRG signature with certain immune cell types, particularly *OPTN* with immune infiltration in melanoma. Methylation patterns correlated with gene expression and immune infiltration. Mutation analysis also showed frequent alterations in *PRKN*, *OPTN*, *PINK1* and *SRC*, with implications for the TME. We also found various correlations between the MRG expression and sensitivity in different drugs, suggesting that its targeting could improve therapy efficacy.

Conclusion: The comprehensive analysis of gene expression, pathway activity, methylation and immune infiltration provides a robust framework for exploring these mechanisms and their implications in personalized cancer therapy. Overall, the relevance of mitophagy varies across cancer subtypes and their molecular characteristics.

C.O.I. Disclosure:

N. Agir: None. **I. Georgakopoulos-Soares:** None. **A. Zaravinos:** None.

EP17.012 A neural network architecture approach for variant prioritization and annotation using CanVas, a population-specific cancer patient database, as a training set

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Background: The vast number of variants with unknown significance in extensive datasets like ClinVar presents significant challenges for automated annotation tools. Rare variants add an additional layer of complexity in accurately assessing clinical significance. National genetic variation registries, such as CanVaS (Cancer Variation reSource), provide valuable insights into population-specific genetic diversity, enhancing automated annotation approaches.

Material and methods: We propose a framework leveraging machine learning and deep learning to improve genetic variant annotation using ClinVar and CanVaS as complementary datasets. CanVaS integrates germline genetic data from ~11,000 Greek cancer patients, analyzed for up to 100 cancer susceptibility genes, with ~7,000 variants manually and thoroughly annotated. Detailed data include allele frequencies, clinical significance, segregation, and phenotypic traits. First, we will train tree-based algorithms and Support Vector Machines on *BRCA1/BRCA2* variants from ClinVar, chosen due to their extensive study and critical role in cancer. These models will be validated on CanVaS to assess population-specific applicability. Next, we will broaden the approach to annotate missense variants from other cancer genes, using convolutional neural networks to analyze missense variants.To address rare variants, self-supervised learning techniques like contrastive learning will be applied to ClinVar's unlabeled variants, fine-tuned on *BRCA1/BRCA2*, and validated on CanVaS.

Results: We anticipate high accuracy in pathogenicity predictions, with effective generalization to the Greek population and scalability to broader missense variants.

Conclusion: By integrating advanced algorithms with population-specific data, this framework offers robust tools for variant annotation, enhancing personalized risk assessment and clinical decision-making, providing impactful insights on rare and uncertain variants.

C.O.I. Disclosure:

D. Kalfakakou: None. **C. Bampos:** None. **A. Papathanasiou:** None. **F. Fostira:** None. **P. Apostolou:** None. **I. Konstantopoulou:** None. **G.A. Pavlopoulos:** None. **V. Megalooikonomou:** None. **D. Yannoukakos:** None.

EP17.013 Optimising the semantic coding of genetic polymorphisms for predictive analysis in type 2 diabetes

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Background: Genomics has transformed large-scale genetic data analysis, offering key insights into disease biology and phenotypic traits. However, genomic data's high dimensionality and sparsity challenge conventional machine learning models. This study optimizes semantic encoding of genetic polymorphisms to improve predictive models using a case/control Next Generation Sequencing dataset on type 2 diabetes.

Material and methods: A comparison was made of encoding methods, including one-hot encoding (OHE), and inheritance-

based strategies, both separately and in combination. Variants with multiple alleles were converted to a biallelic format, only common variants were retained, and missing values were left unprocessed. Mutual information between variants and the target guided inheritance model selection. Feature selection was performed using SEQENS ensemble feature selection. Survival analysis methods were employed to avoid misclassification of at-risk young individuals. Each model's Concordance Index was measured in a 5-Fold cross-validation, evaluated with: (i) all variables, (ii) selected variables with backward elimination, (iii) SEQENS selected variables.

Results: SEQENS-based feature selection improved predictive performance across encoding strategies. OHE-raw with selected variables achieved the best results (0.574 ± 0.025), followed by OHE-MI-guided inheritance (0.558 ± 0.056) and MI-guided inheritance (0.557 ± 0.016). Recessive and additive models performed similarly (0.535 ± 0.047 and 0.560 ± 0.020, respectively), while dominant encoding was less effective (max 0.515 ± 0.028). These results underscore the importance of optimized encoding strategies, with OHE and MI-guided inheritance proving particularly effective.

Conclusion: Feature selection is key to optimizing genetic data encoding. OHE and inheritance-based methods enhance predictive performance, while backward elimination may discard useful information.

C.O.I. Disclosure:

C. Moya-Valera: None. **I. Sales-Ibáñez:** None. **P. Pons-Suñer:** None. **F. Lara-Hernandez:** None. **M. Gallardo-Espinoza:** None. **M. Quiroz-Rodriguez:** None. **J. Arlandis:** None. **J. Navarro-Cerdán:** None. **F. Chaves Martínez:** None. **A. Garcia-Garcia:** None.

EP17.014 In Silico Analysis Reveals Association of the SLC44A2 (rs22889045) gene with ncRNAs

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Background/Objectives: The SLC44A2 gene encodes a membrane protein expressed in neutrophils and monocytes. Its allelic variants are associated with HNAs, implicated in transfusion complications and pregnancy-related alloimmunization. Studies suggest that polymorphisms in this gene are linked to venous thrombosis and susceptibility to leg ulcers in sickle cell disease patients across five populations of different geographical origins. Given that ncRNAs play a central role in gene regulation and inflammatory processes, our aim was to investigate, in silico, potential miRNAs and lncRNAs associated with SLC44A2.

Methods: In silico analyses of the interactions between SLC44A2 and ncRNAs in human tissues were performed using the STRING, miRDB, and LncTarD2.0 platforms. Molecules with at least two pathway connections were selected to ensure greater robustness in the interaction assessment. Gene functions and associated pathways were obtained from the miRPathDB database.

Results: The STRING analysis revealed functional associations of the SLC44A2 protein with various genes. Based on these connections, we identified common ncRNAs between SLC44A2 and the associated proteins, highlighting 11 miRNAs with over 70% association, including hsa-miR-22-3p and hsa-miR-124-3p, along with the lncRNA NEAT1. Functional enrichment analysis indicated that these miRNAs are involved in processes such as wound healing, inflammation regulation, cell adhesion, and angiogenesis.

Conclusion: The results suggest that SLC44A2 may be involved in regulatory mechanisms mediated by ncRNAs, particularly miRNAs and lncRNA NEAT1. The association of these ncRNAs with the gene and proteins indicates potential regulatory pathways relevant to pathophysiological processes. This may contribute to understanding the epigenetic influence on inflammation and healing.

C.O.I. Disclosure:

C.R. Bonini-Domingos: None. **J.D. Rios:** None. **S. Azouzi:** None. **F.C. Lisoni:** None. **S.M. Oliani:** None.

EP17.015 Investigation of The Prognostic Value and Clinical Significance of canopy FGF signaling regulator 4 in Low-Grade Glioma

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Background: Glioma is a kind of neuroepithelial tumor with the highest incidence and the worst prognosis among brain tumors. Due to the high heterogeneity of gliomas, precise targeted therapy has become a novel therapeutic option for patients with different gliomas on the basis of conventional treatment. Canopy FGF Signaling Regulator 4(CNPY4), as a signaling pathway regulator, is involved in positive regulation of protein localization to the plasma membrane.

Material and methods: Data on CNPY4's expression, prognosis, and clinical phenotypes were sourced from The Cancer Genome Atlas(TCGA) and Chinese GliomaGenome Atlas(CGGA) and the Genotype-Tissue Expression(GTEx) normal tissue database. Analyzed with online and R software. Checked CNPY4's RNA - level differential expression in glioma, verified protein - level by HPA. Studied its impact on glioma survival, its correlation with TIICs via TIMER and TISIDB, and predicted ICB responsiveness using TIDE. Analyzed possible pathways by GSEA and its impact on prognosis and drug sensitivity by COX and IC50.

Results: CNPY4 is highly expressed in gliomas, linked to higher WHO classification. It's an independent prognostic factor for LGG; high expression means poor LGG prognosis. It may affect LGG via TIICs; low expression leads to higher ICB responsiveness. High expression may imply LGG sensitivity to TMZ and cisplatin.

Conclusion: CNPY4 is an independent prognostic factor for LGG. High expression indicates poor prognosis. It could be a potential immunotherapy target and predict drug sensitivity.

C.O.I. Disclosure:

T. Duan: None. **Z. Wang:** None.

EP17.016 The exploratory analysis of singular LD matrices of genome-wide SNPs

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Background: Partitioning single nucleotide polymorphisms (SNPs) into groups of correlated SNPs enhances the power and efficiency of genetic association studies. The correlation between SNPs is usually addressed with pairwise r2 measure of linkage disequilibrium (LD). Genotyping technologies produce genome-wide data. To get merit of them, LD measures accounting for multiple loci are required. We introduced the determinant of LD matrix as integrative measure of SNPs' correlation. This allowed partitioning SNPs into groups represented by singular LD matrices.

Material and methods: Real genome-wide data from dbGaP (phs000615.v1.p1) were partitioned by growing a set of consecutive SNPs until the determinant of LD matrix dropped below ε,

which varied from 10-6 to 10-1. The optimal ε was taken as the maximum of the product of the number of groups to the variance of their sizes, both values being converted into a z-score. The r2 values were estimated with Plink 1.9. The squared matrix (23583 × 23583) with random values distributed uniformly within [0, 1] was partitioned similarly.

Results: Singular LD matrices generally occurred every 8–10 SNPs in the genome. The distribution of their sizes followed the Poisson process with optimal ε being 0.001 and 0.01 for real autosomes and random matrix, respectively. The number of groups showed the power low dependence on ε with the exponent of 1/6.

Conclusion: The determinant of LD matrix is a promising integrative measure of LD that can be efficient in handling multilocus data.

C.O.I. Disclosure:

G. Khvorykh: None. **A. Khrunin:** None.

EP17.017 Performance of polygenic risk score methodologies in the absence of external genome-wide association study summary statistics

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Background: Polygenic risk scores (PRS) have garnered attention for predicting risk based solely on genotype information, often matching the predictive power of traditional clinical risk factors. However, their reliance on genome-wide association study (GWAS) data corresponding to the target trait limits their applicability when such data is unavailable.

Materials and methods: Here, we propose EX-TERR (EXternal Technique with Earth Regional Regression), which leverages Multi-Adaptive Regression Splines (MARS) and single-trait PRS as training data. In scenarios simulating the absence of target trait GWAS, EX-TERR was compared against two established PRS methodologies: baseline PRS (selecting the most predictive single-trait PRS) and PRS_{multi} (an elastic net regression approach using multiple single-trait PRS).

Results: Using UK Biobank participants of British ancestry ($n = 408{,}160$), we generated target outcome PRS for 61 traits with external GWAS summary statistics and 69 UKB outcomes. External GWAS for target traits were masked, and output PRS were assessed for discrimination and calibration the 69 outcomes. Of the 207 PRS validated, 93.7% were significantly associated with outcomes ($p < 0.05$). Predictive performance noticeably decreased without GWAS data. On average, PRS methods were able to recover 13.6% of information without corresponding external GWAS for continuous traits and 80.9% for dichotomous traits.

Conclusions: In the absence of target trait GWAS data, PRS predictive performance diminishes significantly. While no single method performed best universally, approaches integrating multiple GWAS demonstrated superior accuracy for most outcomes. Overall, current methodologies partially mitigate the challenges posed by missing GWAS data.

C.O.I. Disclosure:

A. Le: None. **S. Mao:** None. **A. Canty:** None. **Y. Li:** None. **A. Man:** None. **K.K. Pang:** None. **G. Pare:** None.

EP17.018 In silico analysis of deleterious missense variants in the human SMN1 gene

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Background: The *SMN1* gene encodes the survival of motor neuron 1 (SMN) protein that is taking part in the regulation of gene expression. Mutations in *SMN1* are associated with spinal muscular atrophy (SMA). Unfortunately, the effects of single nucleotide variants (SNVs) in the *SMN1* gene are poorly understood. The aim of this research is to uncover deleterious missense SNVs that may have damaging structural and functional impact on the SMN protein.

Material and methods: 253 missense SNVs, gene and protein sequences were retrieved from the dbSNP and Uniprot databases. Bioinformatics tools fathmm, PANTHER, PhD-SNP, PolyPhen-2, SIFT, and SNPs&GO were used to assess pathogenic effects of SNVs on the protein structure and functions. Protein stability was analyzed by means of predictors i-Mutant2.0, MUpro, and iStable. Residue flexibility of the protein were obtained from MEDUSA server. Evolutionary conserved residues in the protein were identified by ConSurf tool.

Results: All six bioinformatics tools indicated 39 SNVs in the *SMN1* gene as damaging, and all three predictors marked 118 SNVs as decreasing stability. 26 of them were found both damaging and decreasing stability, thus they could destabilize the SMN protein and disturb its functions. SNVs V94G, G106D, G106S, M269V, Y272C, Y272D, G275C, G279S were not listed in bioinformatics databases as pathogenic. Most of those residues were found to be rigid as indicated by MEDUSA server. They were truly highly conserved due to the ConSurf data.

Conclusion: There were revealed nine novel deleterious SNVs in the *SMN1* gene, which would help diagnosis of SMA.

C.O.I. Disclosure:

I. Sadovnychenko: None.

EP17.019 Advancing AI-powered cytogenetic diagnostics: a focus on heterochromatic variations

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Background: Artificial intelligence (AI) may revolutionize cytogenetics by automating critical tasks like detecting and classifying chromosomal abnormalities. Advanced machine/deep learning algorithms and image analysis technologies could greatly improve analytical speed and accuracy, reduce laboratory workloads and enhance clinical reports. This study explores AI's applications and challenges in cytogenetic diagnostics, highlighting the need for better algorithms and datasets to ensure reliable detection of chromosomal variations.

Material and methods: A literature review (2020–2024) was conducted to explore the state-of-the-art of AI in cytogenetics, using the keywords "artificial intelligence", "machine learning", "cytogenetics" and "karyotype" from PubMed, Google Scholar, and Scopus.

Results: The analysis identified key challenges for AI in cytogenetic diagnosis, particularly in detecting chromosomal heteromorphisms - structural heterochromatic variations with no apparent phenotypic impact. While current AI models show promising accuracy in detecting common abnormalities (97.5% on

average), they struggle with subtle variations, especially in underrepresented genomic regions or atypical banding patterns (33.3%). This increases the risk of misinterpreting these variants as pathogenic, leading to false positives, missed diagnoses, and lacking the contextual expertise of cytogeneticists.

Conclusion: To overcome these challenges, AI algorithms must be critically improved by training models on a broader range of heterochromatic variations, alongside a greater investment in the study and scientific understanding of these regions. Continuous collaboration between AI developers and cytogeneticists is essential to integrate expert knowledge and contextual information. This may ensure accuracy, enabling AI to become a transformative tool in cytogenetic diagnosis, improving analytical precision and proper management of benign/pathogenic chromosomal variants.

C.O.I. Disclosure:

S. Pires: None. **A. Mendonça:** None. **P. Jorge:** None. **T. Liehr:** None. **N. Oliva-Teles:** None.

EP17.020 Divergences of in silico analysis of BP4 or PP3 criteria for *MSH2* and *MLH1* missense variants based on gene-specific guidelines from ClinGen InSight and CanVIG-UK

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Background: In order to improve pathogenicity classification of MSH2 and MLH1 variants, ClinGen InSIGHT (CGI) and CanVIG-UK (CV-UK) developed gene-specific guidelines based on the original ACMG criteria and a scaled point system. Regarding the BP4 and PP3 criteria, the recommended in silico tools differ: While CGI recommends HCI prior probabilities of pathogenicity, CV-UK recommends REVEL as a meta-predictor tool. Additionally, the strength of PP3 varies: CGI allows it to be scored as supporting (1 point) or moderate (2 points), while CV-UK recommends it as supporting only. This study aimed to evaluate the in silico results obtained from the application of the BP4/PP3 criteria according to CGI and CV-UK and assess the consequences of the different strengths attributed to PP3.

Material and methods: 216 missense variants (*MSH2* and *MLH1*), reported in ClinVar and reviewed by expert panels, were selected. The scores for BP4/PP3 were obtained using VEP and HCI Prior database.

Results: In silico evaluation for BP4/PP3 was concordant in 75% of the variants. However, the application of PP3 strength diverged among 63% of the variants. Full agreement for criteria and strength (as supporting) were only applicable for 6.9 (PP3) and 3.2% (BP4) of the variants.

Conclusion: Our in silico evaluation highlighted the differences among BP4/PP3 criteria and PP3 strength of both guidelines that can impact the final pathogenicity classification. It is mandatory to standardize gene-specific guidelines for variant classification in order to avoid discrepancies that could lead to different clinical decisions and patient management/treatment based on predictions.

C.O.I. Disclosure:

P.G. Rodrigues: None. **J.M. Gonçalves:** None.

EP17.021 Transcriptomic profiling of iPSC-derived astrocytes from patients with 22q11.2 deletion syndrome

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Background: Neurodevelopmental disorders represent considerable public health challenges.One of the syndromes associated with heightened risk of NDDs is 22q11.2 Deletion Syndrome (22q11.2DS), caused by microdeletion in chromosomal region 22q11.2. However, molecular mechanisms underlying NDDs are largely unidentified. Here we analyzed transcriptomic profiling of 22q11.2DS astrocytes.

Material and methods: Total RNA was isolated from iPSCs-derived astrocytes of two cases with familial 22q11.2DS with 1.5Mb microdeletion and one healthy control. Paired-end RNA-seq was carried out on Illumina NovaSeq 6000 sequencer. Bioinformatic processing of raw data was conducted via NVIDIA platform, while differential gene expression analysis was performed in RStudio using DESeq2 R package. The obtained list of DEGs was used for pathway enrichment analysis by employing EnrichR and WikiPathways.

Results: 125 DEGs with lower expression and 287 DEGs with higher expression in 22q11.2DS astrocytes compared to the control were obtained. For genes with lower expression in 22q11.2DS astrocytes, 22q11.2 Copy Number Variation Syndrome and Axon Guidance were the top enriched pathways, while for genes with higher expression in 22q11.2DS astrocytes we did not identify biological pathways that are enriched in DEG lists more than would be expected by chance. We found 178 DEGs with lower expression and 205 DEGs with higher expression in astrocytes of symptomatic child with 22q11.2DS compared to oligosymptomatic mother with 22q11.2DS. Employing EnrichR and WikiPathways we did not identify biological pathways that are enriched in DEG lists more than would be expected by chance.

Conclusion: We provide preliminary evidence for an altered transcriptomic landscape of 22q11.2DS astrocytes.

C.O.I. Disclosure:

I. Simeunovic: None. **N. Kovacevic-Grujicic:** None. **M. Peric:** None. **O. Petter:** None. **F. Ehrhart:** None. **J. Kostic:** None. **S. Lazic:** None. **G. Cuturilo:** None. **L. de Nijs:** None. **D.E. Linden:** None. **A.J. Harwood:** None. **M. Stevanovic:** None. **D. Drakulic:** None.

EP17.022 Deep-learning based classification of Alzheimer's Disease using common SNPs

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Background: The utility of polygenic prediction has been so far of secondary importance to non-genetic predictors. Deep learning using artificial neural networks (ANN) is a sub-field of machine learning with increasing use in disease prediction. We aimed at

assessing the potential of genome-wide ANNs in predicting Alzheimer's disease (AD) in UKBiobank and how these compare to GWAS and prediction by linear polygenic scores (PGS).

Methods: Study sample included 3,956 individuals with AD and three different control sets of same size. Imputed genome-wide genotypes were QCed and pruned to include independent SNPs by PLINK. SNPs were further pre-selected by their association to AD ($P < 0.05$), resulting in ~2K SNPs. ANN architecture was determined by SNP annotations using ANNOVAR, GeneSCF and KEGG, data were split 60:20:20, trained and tested using GenNet.

Results: Prediction accuracy maximised in models in which full list covariates were included in the model(AUC = 0.65) and higher when features were pre-selected (AUC = 0.86). For the "SNP-only" models (no covariates, fully matched controls) the prediction accuracy was higher when pre-selected SNP features were used (AUC = 0.74 vs. 0.65) and were higher than the linear PGS-based prediction (AUC = 0.50) in the same dataset. All ANN models pointed 19q13.32 locus (*NECTIN, TOMM40, APOE*) SNPs among most important predictors.

Conclusion: This is one of the first studies attempting to compare the traditional GWAS-based linear PGS to ANNs, showing that internally determined feature selection increases the AUC for ANNs. More systematic comparisons with such approaches on real data are needed to enable best practises for machine learning in GWAS.

C.O.I. Disclosure:

C. Gkertsou: None. **A. Parameshwarappa:** None. **A. Shiyanbola:** None. **T. Sakey Ravi:** None. **S. Kouchaki:** None. **Z. Balkhiyarova:** None. **I. Prokopenko:** None. **V. Lagou:** None. **A. Demirkan:** None.

EP17.023 Molecular dynamics simulations and in silico analysis of F9 variations

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Background: Hemophilia B (HB) is a congenital bleeding disorder associated with blood coagulation factor IX (FIX) deficiency. FIX is encoded by the F9 gene located on the X chromosome (Xq27). Understanding the functional consequences of these variations is crucial for establishing genotype-phenotype correlations. This study aims to investigate the effects of eight F9 variants (Cys117Gly, Gly432Arg, Glu159Val, Arg191Gln, Gly58Glu, Glu54Asp, Thr84Arg, Asn138His) identified in HB patients in Turkey.

Material and methods: Molecular dynamics (MD) simulations of the wild-type FIX protein were conducted for 100 ns. Each mutations was then introduced,100 ns simulations were performed. Structural analysis of the mutant protein variants was conducted using parameters like root mean square deviation, root mean square fluctuation, radius of gyration, solvent accessible surface area. These parameters were used to assess the structural differences between the wild-type and mutant proteins. Functional effects of variants were evaluated by using PolyPhen-2.

Results: The MD simulations revealed the structural impacts of the mutations on FIX stability. The Glu159Val and Arg191Gln mutations show more stabe structure, while Cys117Gly caused a substantial destabilization. The Gly432Arg mutation was found to enhance protein stability, whereas Glu54Asp, Asn138His, and Gly58Glu mutations negatively affected stability. PolyPhen-2 analysis suggested that except Glu159Val, all the variants are probabaly damaging.

Conclusion: MD simulations and PolyPhen-2 analysis of the variants revealed 87,5% concordant results. In vitro expression studies would reveal the impact of the variants on the function and expression of F9.

C.O.I. Disclosure:

K. Cansiz: None. **M. Ozbil:** None. **G. Gullu Amuran:** None. **E. Berber:** None. **B. Koc:** None. **B. Zulfikar:** None. **E. Berber:** None.

EP17.024 In silico design of a novel multi-epitope vaccine using neoantigens against triple-negative breast cancer (TNBC) via immunoinformatics approach

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Background: Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer. TNBC poses a significant clinical challenge due to its poor prognosis, aggressive behavior, and lack of targeted therapies. This study presents a comprehensive approach to designing a multi-epitope, neoantigen-based vaccine.

Methods: LncRNA-related peptides were retrieved from the Spencer database, and the most antigenic peptides were selected using the VaxiJen server. The IEDB server was utilized to identify CTL epitopes from the selected antigenic peptides. The vaccine construct was assembled using appropriate linkers and an adjuvant. Antigenicity, allergenicity, toxicity, and physicochemical characteristics were assessed using respective tools. Subsequently, the secondary and tertiary structures of the vaccine were visualized using the PDBsum and trRosetta servers, respectively. Docking of the vaccine-TLR4 complex was performed using ClusPro2.0, followed by molecular dynamics (MD) simulation. In silico cloning was performed for further assessment.

Results: Seven 9-mer epitopes were selected for the vaccine construct, which consisted of 186 residues. IEDB estimated the vaccine's population coverage to be over 89% worldwide. The Ramachandran plot revealed that over 99% of residues were located in favorable and permitted regions. Protein-protein docking and MD simulation demonstrated a strong and stable interaction between the vaccine and TLR4. Immune simulation using the C-ImmSim server confirmed the secretion of IFN-γ, IL-12, and IgG, and showed that the vaccine successfully activates T cytotoxic cells.

Conclusion: The developed vaccine is optimal, effective, and safe for usage; however, our vaccine candidate needs to be validated through in vitro and in vivo studies.

C.O.I. Disclosure:

Z. Zolfkhani: None.

EP17.025 Automating submission of NHS variant data to ClinVar

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Background: East Genomic Laboratory Hub (East GLH) is part of the NHS Genomic Medicine Service. Variants interpretations performed at East GLH are recorded in local systems; these

interpretations determine patient diagnosis and treatment. To enhance the value of this information, we have developed tools and processes to enable locally generated variant interpretation data to be shared with the wider genomics community by routinely submitting to the ClinVar database.

Material and methods: A series of dockerised Python scripts extract variant interpretations from local laboratory records, add these interpretations to a cloud-based, restricted-access variant database, and then submit all new variant interpretations in that database to ClinVar.

Results: East GLH is a network of genomics laboratories; this method is now used to submit all new variant interpretations from one of the labs to ClinVar, with a bulk submission each month. This process will be scaled up to include all East GLH labs. This approach is adaptable; the variant database enables variants from various sources to be captured and submitted to ClinVar using this method. The variant database will also be used in the future to annotate variants in patient cases with their previous interpretation at East GLH.

Conclusion: Increased NHS variant data sharing is a goal supported by the ACGS, BSGM and ESHG, and is now required for NHS GLHs; this project enables this to be automated. Sharing data means that clinical scientists can access additional evidence to conclusively classify variants and provide patients with a diagnosis.

C.O.I. Disclosure:

K.J. Winfield: None. **Y. Kim:** None. **E. Johnston:** None. **K. Bradshaw:** None. **M. Garner:** None. **J. Ahn:** None.

EP17.026 Scaling privacy-preserving federated GWAS across global biobanks with GPU acceleration and AI-driven multi-modal integration

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Background: Genome-wide association studies (GWAS) have linked genetic variants to complex traits and diseases, but key challenges remain: (1) computational bottlenecks limit scalability, (2) federated analysis is hindered by privacy concerns, and (3) AI-driven causal inference is constrained by the difficulty of integrating gene expression and phenotype data. Current frameworks require centralized data aggregation, which is often infeasible due to regulatory restrictions. We are developing an integrated framework combining federated learning, AI-driven causal inference, and GPU-optimized computing to enable secure, scalable, and biologically interpretable GWAS analysis across global biobanks.

Material and methods: We enhanced SAIGE by implementing a GPU-accelerated pipeline for step 1, optimizing genetic relationship matrix computations. We are developing a federated learning framework using Secure Multi-Party Computation and Preconditioned Conjugate Gradient algorithms to enable distributed GWAS without data sharing. Simulated genotype data for 6 million samples across multiple population groups using Hapgen2 is being used to test federated learning. We are also working on AI-driven multi-modal modeling to integrate genotype, phenotype, and gene expression data.

Results: Our GPU-enhanced SAIGE implementation achieved a 20-fold acceleration, enabling large-scale GWAS across 2,068 phenotypes from 635,969 participants. Initial federated learning tests confirm feasibility, with ongoing optimizations to improve efficiency, ensure reproducibility, and scale to real-world datasets. We are also refining AI-driven multi-modal approaches to enhance fine-mapping and integrate genomic and transcriptomic data for improved causal inference.

Conclusion: While initial results are promising, further optimization is needed to enhance AI-driven inference and federated learning scalability, unlocking new discoveries in human genetics.

C.O.I. Disclosure:

A.A. Rodriguez: None. **R. Madduri:** None. **T.N. Nandi:** None. **Z. Li:** None. **Y. Kim:** None.

EP17.027 Detection of fusion gene-induced overexpression in whole transcriptome sequencing data to improve diagnostic algorithms in paediatric cancers

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Background: Understanding the molecular mechanisms underlying paediatric cancers is essential for accurate diagnosis, prognosis, and the selection of effective treatment strategies. Structural variations contribute to tumorigenesis by forming fusion genes and through enhancer hijacking, where distal regulatory elements are positioned near oncogenes, leading to aberrant expression. However, these events are hard to detect by current gene fusion analysis.

Material and methods: We combined the standard computational fusion detection pipeline with outlier detection algorithms (DESeq2, outsingle and z-score calculation for TPM-normalised counts) to identify overexpressed genes in pediatric tumour whole transcriptome sequencing data. Pediatric patients with tumours of the same origin were used as the reference dataset.

The developed workflow was validated using samples where overexpression was known to be the underlying molecular mechanism, and its utility was further shown for fusions with previously uncharacterized effects. Among five validation samples, three (IGH::MYC, TCF3::HLF, and ETV6::RUNX1) were previously reported to drive fusion gene overexpression, while two (Linc::MECOM and IGH::IRF4) had an unknown effect.

Results: We successfully identified overexpressed genes in all samples with previously documented fusion-induced overexpression. For the novel Linc::*MECOM* fusion, significant *MECOM* overexpression (z-score 5,16; p-value 2,44e-7) was detected. In case of the *IGH::IRF4* fusion, despite the breakpoint being located in the noncoding region of *IRF4*, the observed increase in *IRF4* expression (z-score 11,35; p-value 7,16e-30) supported its classification as oncogenic.

Conclusion: Detecting fusion-induced overexpression is a valuable approach improving the diagnostic yield and the classification of fusion oncogenicity utilising the same transcriptome data, without significantly increasing sample processing costs.

C.O.I. Disclosure:

T. Kiselova: None. **D. Rots:** None. **E. Berga-Švītiņa:** None. **D. Enkure:** None. **I. Nartiša:** None. **L. Bārdiņa:** None.

EP17.028 Variant calling framework for highly homological regions in short-read whole genome sequencing that functions independently of prior knowledge of common variants and haplotypes

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Background: In short-read whole genome sequencing (WGS) highly homologous regions (HHR) are regarded as inaccessible for standard variant callers due to read misalignment. More than 6000 genes are fully or partially inaccessible in WGS, of those around hundred are implicated in various diseases (CYP21A2, SMN1) or pharmacodynamics effects (CYP2D6). Common alternatives are gene-specific test systems and gene-specific callers, which detect limited sets of variants and/or rely on existing knowledge of haplotypes and their population frequencies.

Material and methods: We created HHRTyper, a specialized framework for any pairs of HHR in WGS, able to detect variants without prior knowledge of the gene-specific variant set, population frequencies and haplotypes. For in silico validation we designed a pipeline for synthetic HHR generation in WGS. For real-data validation we sequenced (WGS and targeted sequencing of CYP21A2) 135 individuals either affected with congenital adrenal hyperplasia (CAH), CAH-carriers, or CAH-free.

Results: HHRTyper demonstrated high accuracy in synthetic HHRs variant calling, achieved 0.97 accuracy and 0.95 macro f1-score identifying pathogenic variants in CYP21A2. Moreover, HHRTyper also successfully detected all CYP21A2 deletions, rare pathogenic variant rs72552758, and potentially causative variant rs1463196531. Additionally, HHRTyper assessed cis-trans variant configuration, correctly predicting CAH-risk in 91.2% cases. Next we applied HHRTyper to 1000 Genomes data, detecting CAH-carriers and assessing pathogenic variant frequency.

Conclusion: We present a novel approach for calling variants in HHR using short-read WGS without prior gene-specific information. HHRTyper allows processing of large WGS collections to discover new variants, and their population frequencies.

C.O.I. Disclosure:

Z. Antysheva: None. **V. Bogdanov:** None. **J. Krupinova:** None. **I. Kiseleva:** None. **V. Shchekina:** None. **E. Avsievich:** None. **E. Petriaikina:** None. **A. Ivashechkin:** None. **Y. Kacaran:** None. **V. Yudin:** None. **A. Keskinov:** None. **S. Yudin:** None. **D. Svetlichnyy:** None. **P. Volchkov:** None. **M. Woroncow:** None. **V. Skvortsova:** None.

EP17.029 Runs of homozygosity analysis: a powerful bioinformatics approach for gene discovery, diagnostics and profiling population consanguinity

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Background: Runs of homozygosity (ROH) are continuous homozygous regions across the genome that often indicate consanguinity. Their size and frequency reflect the degree of shared parental ancestry. Homozygosity mapping using whole-exome sequencing (WES) or whole-genome sequencing (WGS) can simultaneously identify ROH and disease-causing variants, facilitating recessive diseases' genetic etiology discovery.

Methods: Personalized multigene panels (PMP) creation, based on WES data and ROH, was automated using a two-algorithm bioinformatics solution (ROHMMCLI and HomozygosityMapper), incorporating Human Phenotype Ontology terms, and implemented in a Django web application. Additionally, using Portuguese population 2021 Census data, we reduced our dataset from 12,167 to 3,941 WES samples. Multidimensional Scaling (MDS) was applied to predict consanguinity from ROH features.

Results: Following PMP creation strategy implementation, undiagnosed patients with rare diseases who underwent WES-based testing were reanalyzed. In two siblings with epilepsy, myoclonus, and dystonia, this approach identified *CSTB* as the disease-causing gene. Genetic analysis of another pair of siblings (with intellectual disability, dystonia, and parkinsonism) presently under analysis, focusing on WGS-based ROH calling, will be presented. Additionally, we assessed genome-wide autozygosity from ROH (F_ROH) for 3,941 WES samples, enabling the first extensive ROH profiling of the Portuguese population and identifying districts with the highest F_ROH scores. ROH count, sum, and additional features powered consanguinity classification, achieving a F1 score of 0.96.

Conclusion: This study advances ROH analysis, through the development of new bioinformatics tools and provides unprecedented Portugal population-level insights. By leveraging WGS-based ROH analysis, we enhanced diagnostic capabilities and gene discovery for rare recessive diseases.

C.O.I. Disclosure:

S. Valente: None. **M. Ribeiro:** None. **J. Schnur:** None. **F. Alves:** None. **N. Moniz:** None. **D. Seelow:** None. **M. Magalhães:** None. **J.P. Freixo:** None. **P. Silva:** None. **J. Oliveira:** None.

EP17.030 Refining MGPT accuracy: a new scoring algorithm validated on *BRCA1* primers

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Background: Targeted multi-gene panel testing (MGPT) for clinically significant loci typically requires higher accuracy in primer design, despite complications from primer dimerisation, hairpin structures, repeat sequences, and off-target amplification. To address these, we developed ALPlex—an ALgorithm for multiPLEXed primer analysis—and tested it on the *BRCA1* gene

as a proof of concept, chosen for its established clinical relevance in breast cancer and the abundance of pathogenic variants.

Material and methods: Using the MPPrimer tool, we designed 56 primer pairs targeting 7,953 bases of canonical *BRCA1* exons plus 20 bp of flanking regions. ALPlex then systematically evaluated primer-primer interactions in a sliding window approach that iterates base-by-base. Therefore, it checks self-annealing and partial or complete cross-complementarity between primers. Each complementary base was awarded one point, with an additional point for G-C pairs, resulting in a "dimerisation score." This scoring enabled the preemptive removal or replacement of problematic primers before wet lab testing. We additionally validated our pipeline across distinct PCR setups. Our design achieved 99.31% coverage, missing only 55 bases.

Results: In vitro applications revealed six different multiplex PCRs producing proper amplicons with minimal off-target products. Although low-level primer dimers were observed in negative control lanes, they did not compromise the main amplification signals. These findings underscore ALPlex's capacity to enhance primer specificity through in-silico scoring.

Conclusion: This work demonstrates that coupling MPPrimer with ALPlex creates a robust pipeline for primer design before high-throughput DNA sequencing applications, providing a scalable framework for high-coverage MGPT in more extensive and complex genomic targets.

C.O.I. Disclosure:

A. Akkus: None. **R. Uyanik:** None. **N. Agaoglu:** None. **O. Ozdemir:** None. **O. Hatirnaz Ng:** None.

EP17.031 Standardizing and harmonizing NGS analysis workflows within GHGA to create a unified omics data resource for Germany

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Background: The rapid adoption of high-throughput DNA and RNA sequencing in human research and clinical settings has created a dire need for adequate infrastructure to facilitate the management, storage, analysis, and secure access control for this data. With such an infrastructure, a unified omics data resource available for broader secondary use, is not only possible but in demand. To meet this demand, the German Human Genome-Phenome Archive (GHGA) is building a secure national human omics data infrastructure with an embedded ethico-legal framework for data protection compliance. However, GHGA should be more than an archive.

Material and methods: Therefore, we are developing, maintaining, deploying, and benchmarking scalable and reproducible next-generation sequencing (NGS) workflows. By leveraging our connection to the nf-core community, we ensure these pipelines meet GA4GH standards and uphold the FAIR principles (findability, accessibility, interoperability, and reusability). This ensures they are reproducible and scalable within cloud computing infrastructures for massive datasets of diverse modalities. In addition, by creating and testing runtime configurations for each data modality and workflow, we can continuously evaluate workflow performance.

Results: Here we present our progress in developing and deploying a range of workflows, such as nf-core/spatialxe and spatialqc dedicated for spatial transcriptomics, nf-core/variant-benchmarking for evaluating variant calling pipeline accuracy, and nf-core/drop for RNA-seq-based expression and splicing outlier detection.

Conclusion: By processing available and incoming data in GHGA, researchers will have controlled access to raw and harmonized processed sequence data, driving discovery and innovation and ultimately helping bridge the gap between research and healthcare.

C.O.I. Disclosure:

D. Behrens: None. **K. Narci:** None. **F. Heyl:** None. **P. Menges:** None. **L. Gadelha:** None. **S. Kher:** None. **S. Chakraborty:** None. **F. Hölzlwimmer:** None. **Y. Wang:** None. **C. Mertes:** None. **J. Gagneur:** None.

EP17.032 Integrating genetic datasets to improve Genotype-Phenotype prediction

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Background: Genotype-phenotype prediction relies on the integration of diverse data sources, including genotype data, functional annotations (FA), linkage disequilibrium, polygenic risk scores (PRS), covariates, and genome-wide association study (GWAS). Robust predictors can be made by combining multiple GWAS, PRS scores, and FA. We propose a framework to systematically evaluate and combine individual datasets to create a strong predictor for a specific phenotype.

Material and methods: We used genotype data from the UK Biobank, GWAS files from the GWAS catalog, and functional annotations from publicly available databases. The analysis proceeded in three main steps: (1) The suitability of GWAS files from the catalog for PRS calculation was assessed using the GWASPoker tool; (2) We benchmarked 46 PRS tools and selected the top 4 for generating PRS; (3) Various combinations of all datasets were systematically evaluated for optimal prediction for migraine.

Results: A total of 176 datasets were generated by combining GWAS files, PRS tools, covariates, PCA, and genotype data, both with and without functional annotations. Machine learning algorithms were applied to select the top-performing datasets based on validation performance. The composite dataset (covariates and top 500 SNPs with functional annotations) achieved a training AUC of 0.965 (±0.0372), a validation AUC of 0.7588 (±0.1468), and a test AUC of 0.7082 (±0.0886), while the best individual dataset test performance was 0.62 (±0.144).

Conclusion: This study proposes a framework that integrates diverse datasets, optimizing data combinations and selection strategies to improve the accuracy of genotype-phenotype prediction.

C.O.I. Disclosure:

M. Muneeb: None. **D. Ascher:** None.

EP17.033 Reconstructing cell-type-specific enhancer-promoter interaction regulatory networks with massively parallel reporter assays

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Background: Enhancers are cis-regulatory elements, pivotal to cell-type-specific gene regulation. While a consensus that enhancers are hubs for disease-associated variants has been reached, little is known about the mechanisms through which these elements mediate their effects on gene expression. Additionally, the map of these enhancers' locations and their target genes remains partial. Computational models, which extrapolate epigenetic markers to regulatory activity, have proven immensely successful in predicting enhancer-promoter-interactions (E-P-Is). Still, further improvements are needed in these models' abilities to capture cell-type-specificity and generate more accurate E-P-I predictions.

Material and methods: Massively parallel reporter assays (MPRAs) are a powerful technique for assessing the functionality of regulatory elements and their perturbations under varied conditions. However, computational methods for analyzing MPRA data in a cell-type-specific manner remain limited. Using a convolutional neural network trained on MPRAs from K562, HepG2, and WTC11 cell-lines in combination with epigenetic-datasets, we defined and scored enhancer regions and linked them to target genes. The K562 E-P-I network was benchmarked against existing models, using a CRISPRi dataset as a ground truth.

Results: We performed a series of analyses on these three cell-type-specific networks, including mapping eQTLs and GWAS variants, dissecting regulatory substructures, and integrating TF interactions into the network. We observed improvements in our framework's ability to predict cell-type-specific E-P-Is compared to existing approaches.

Conclusion: The dissemination of our model could promote the broader adoption and generation of MPRA, a breakthrough technology, across the scientific community. Notably, this approach is generalizable and can be applied to diverse cellular contexts.

C.O.I. Disclosure:
A. Kreimer: None. **W. Degroat:** None.

EP17.034 Machine learning based personalized model for predicting sodium valproate-induced weight gain in Persons with Epilepsy from South India

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Background: Sodium valproate (VPA), a widely prescribed anticonvulsant for epilepsy management, is associated with adverse effects, with weight gain being the most prevalent. The genetic predisposition to VPA-induced weight gain remains unexplored in the genetically distinct South Indian population. Our objective is to develop and cross-validate a predictive model for sodium valproate-induced weight gain by integrating genetic factors and clinical parameters in Persons with Epilepsy (PWE) from South India.

Material and methods: This Nested Case control study involved 216 PWE of South Indian origin, aged 18–65 years of both genders, within 1 month of VPA monotherapy. Predictive models for VPA-induced weight gain were developed using algorithms, such as Random Forest(RF), K-Nearest Neighbour(KNN), support vector machine(SVM) and classic logistic regression. The dataset was split into training (80%) and testing (20%) sets for model development and cross-validation, with performance evaluated using ROC, sensitivity and specificity.

Results: Out of 194 participants, 97 individuals were classified as cases (weight gain ≥ 5% from baseline), while 97 were controls. Predictive models were developed using genetic, biochemical and demographic data. The SVM model demonstrated highest median Receiver-operating Curve (ROC) value at 0.79 (0.50–0.83), indicating strong discriminative ability. In comparison, the GLM model had an area under the curve ROC of 0.78 (0.62–0.88), and the highest median specificity of 0.6714(0.46–0,82), suggesting good performance. RF had a median ROC of 0.5901 (0.48–0.66) and KNN displayed least median ROC of 0.48(0.38–0.79).

Conclusion: Developed and cross-validated predictive models incorporating clinical and genetic data that can be used to predict valproate-induced weight gain in PWE.

C.O.I. Disclosure:
A. Sekhar: None. **K. Ramasamy:** None.

EP17.035 A novel ensemble tool for hereditary cancer genes variant detection HCSPred

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Background: Existing in silico tools perform less effectively in classifying cancer-related variants compared to those for rare diseases, leaving over half of hereditary cancer gene variants as variants of unknown significance. Thus, optimizing current algorithms and developing new approaches to predict pathogenicity more accurately is essential.

Material and methods: As of December 2024, ClinVar contained 3,110,973 variants from 52,815 genes. Based on the OncoKB database (January 30, 2025), 1,174 genes were identified. Cancer-related variants were selected using semantic similarity algorithms and expert panels, resulting in a training dataset of 262,468 variants. Classification categories were refined using ABC stepwise variant classification, and ACMG criteria were harmonized through pairwise comparison. Fifty machine learning and deep learning algorithms were implemented, and prediction values were validated on both internal and external datasets.

Results: Ensemble learning was used to retrain 100 pathogenicity prediction tools on the dataset. The newly trained model was applied to predict variants in hereditary cancer panels for 4,102 patients evaluated at Etlik City Hospital between 2022 and 2024, with each variant reviewed by at least three medical genetics specialists. The novel algorithm achieved 99% accuracy and an equivalent F1-metric on known variants.

Conclusion: HCSPred, demonstrating high performance on both internally and externally validated datasets, emerges as an accessible and robust tool to support routine clinical practice.

C.O.I. Disclosure:
M. Alay: None. **H. Erdem:** None. **T. Bahsi:** None. **A. Onat:** None. **H. Saat:** None.

EP18 Large scale genomics (GWAS) and other -omics association studies

EP18.001 Multi-stage genome-wide association studies showed new insights to subarachnoid hemorrhage in the multi-ethnic populations

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Backgrounds: Subarachnoid hemorrhage (SAH) due to intracranial aneurysm rupture is a life-threatening condition with high mortality (up to 50%) and morbidity in the survivors (up to 30%). Previous genome-wide association studies (GWASs) have still limitations to explain a genetic heritability of SAH. Here, we performed a two-stage GWAS and meta-analysis to identify genetic susceptibilities to SAH in the large-scale populations.

Material and methods: Two-stage Korean GWAS involved 151/175 SAH and 99/175 non-ruptured patients at discovery (2015–2019 years)/validation (2020–2023 years) stage, respectively, sharing 6,429,498 markers after a high-captured genotype-imputation process after pruning imputation score < 0.5. Independent GWASs collected 31/1539 SAH and 11/447 non-ruptured patients in Asian-/European-ancestry populations, respectively, from UK Biobank genotype-imputation data, sharing 93,095,623 markers. Genome-wide meta-analyses were performed by weighting each GWAS by the inverse variance approach.

Results: Nine variants within a serine/threonine-protein kinase 3 (*STK3*:8q22.2) gene showed a genome-wide significant association with SAH formation and complete linkage disequilibrium (LD) at the combined stage ($OR = 0.46$, $p = 4.51\times10^{-8}$), dramatically replicated at discovery and validation stages ($p = 0.0456$ and 2.76×10^{-7}, respectively). Twenty-seven of gene regions showed a suggestive association with SAH formation ($p < 1\times10^{-5}$), including two large LD structures such as *SLC2A13-LRRK2* (12q12) and *CCL15-CCL14* (17q12). None of any loci improved an association in either Asian- or European-ancestry GWAS meta-analyses including Korean GWAS results.

Conclusions: Our study shows novel insight modifiers in *STK3* susceptibility to SAH in the large-scale samples. Our findings will be warranted to validate in an independent population, thereby understanding genomic aspect to the pathology and etiology of SAH.

C.O.I. Disclosure:

E. Hong: None. **H. Jo:** None. **J. Rhim:** None. **J. Park:** None. **J. Jeon:** None.

EP18.003 Genetic modifiers in wild-type TTR amyloidosis: a genome-wide association study

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Background: Wild-type TTR amyloidosis (ATTRwt) is an acquired condition caused by the misfolding of transthyretin (TTR) protein, leading to amyloid deposits, primarily in the heart, tendons, and ligaments. Although once thought to be a rare condition, it is increasingly recognized as a frequent cause of heart failure in the elderly male population. Mechanisms underlying wild-type TTR deposition are poorly understood. Therefore, a role of genetic factors in shaping disease risk has been considered. This work aimed at identifying genetic modifiers of ATTRwt amyloidosis by a genome-wide association study (GWAS).

Material and methods: A case-control GWA analysis has been performed on a cohort of ATTRwt patients paired with age-, sex- and ancestry-matched healthy controls (HCs). DNA samples have been genotyped by Infinium™ Global Clinical Research Array-24 v1.0 (*Illumina*, standard backbone of ~1,204,769 SNPs) customized with ~3,000 additional SNPs.

Results: Overall, 1290 ATTRwt patients have been enrolled. They were predominantly males (1192/1290, 92%) of Caucasian ethnicity (907/1290, 70%) with mean age of symptom onset of 76,5 ± 7,4 years. Clinical status was available for 765 patients, and most were symptomatic (698, 91%). 1142 DNA samples from ATTRwt and 393 from HCs of Western European ancestry have been genotyped and 1370 samples passed quality control. The GWAS analysis is underway.

Conclusion: We present the baseline features of the largest cohort of patients affected by ATTRwt amyloidosis. The ongoing GWA analyses will aim at identifying loci associated with increased risk of developing this progressive condition and hopefully shed light on possible druggable pathways.

C.O.I. Disclosure:

E. Vegezzi: None. **S. Facchini:** None. **I. Quartesan:** None. **R. Mussinelli:** None. **Y. Razvi:** None. **K. Knoll:** None. **A. Argirò:** None. **H. Pernice:** None. **P. Iruzubieta Agudo:** None. **T. Yoshinaga:** None. **M. Tagliapietra:** None. **C. Christodoulou:** None. **P. Milani:** None. **K. Kleopa:** None. **G. Fabrizi:** None. **S. Kapetanovic Garcia:** None. **Y. Sekijima:** None. **S. Menao:** None. **M. Posadas Martinez:** None. **M. Nuvolone:** None. **G. Palladini:** None. **P. Mandich:** None. **A. Lopez De Munain Arregui:** None. **K. Hahn:** None. **F. Cappelli:** None. **T. Trenkwalder:** None. **D. Rowczenio:** None. **J. Gillmore:** None. **U. Hegenbart:** None. **L. Obici:** None. **A. Cortese:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant;

FRRB (project ID 1751723), Fondazione Regionale per la Ricerca Biomedica.

EP18.004 miRNA and Gene Expression profile to disentangle Diabetic Cardiomyopathy

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Background: Diabetic cardiomyopathy (DCM) is a heart condition, which develops in some individuals with type 2 diabetes mellitus (T2DM) in the absence of ischemic heart disease or high blood pressure. The underlying mechanisms driving DCM are not yet fully understood. As part of the CARDIATEAM Consortium, we aimed to conduct multi-OMICs analyses to delineate the distinctive OMICs profiles associated with clinically defined phenotypes pertinent to DCM.

Material and methods: We perfomed small RNA sequencing and RNA sequencing in a set of deeply phenotyped participants without overt heart failure divided in two groups defined by the severity of cardiac dysfunction at echocardiography, with a high prevalence of T2DM among more severe individuals.

Results: From small RNA sequencing profiles, plasma levels of 12 miRNAs were significantly differentially expressed between the two omics groups confirmed by a cross-validated generalized linear model (GLM) with a p-value < 0.05. From RNA sequencing, levels of 34 genes were significantly differentially expressed between the two omics groups, confirmed by a cross-validated GLM with a |log2 Fold change (log2FC)| > 0,5 and a p-value < 0.05. These miRNA and genes have been previously reported to be associated to DCM progression and/or pathogenesis.

Conclusion: The integration of RNA-sequencing and miRNA sequencing can be helpful to identify individuals at high risk to develop severe T2DM-related cardiac dysfunction. The Consortium is currently working on other omics level analyses and future studies will aim to validate these interactions and their clinical utility.

C.O.I. Disclosure:

R. Filomena: None. **C. Di Primio:** None. **K. Hafeez:** None. **A. Allione:** None. **M. Rosselli:** None. **A. Russo:** None. **É. Audureau:** None. **A. Pizard:** None. **S. Luce:** None. **F. Sam:** None. **Y. Devaux:** None. **L. Zhang:** None. **J. Lascano Maillard:** None. **M. Ibberson:** None. **E. Casalone:** None. **G. Derumeaux:** None. **G. Matullo:** None.

EP18.005 Identification of SNPs in IL1 related genes and periopathogenic bacteria and its association with periodontitis

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Background: Periodontitis is a multifactorial inflammatory disease of the oral cavity affecting 20–50% of the adult population worldwide. It represents a chronic disease, which requires the presence of a specific bacterial infection and simultaneously uncontrolled immune response of the host organism. The identification of relationships among pathogenic bacteria associated with periodontitis, as well as predisposing DNA polymorphisms, play an important role in the diagnosis and treatment of periodontitis.

Material and methods: The analysis was performed on DNA samples obtained from the gingival sulcus using Real-Time PCR and TaqMan sequence specific probes. The potential association was assessed by OR with 95% CI.

Results: In our study, we have determined the periodontal pathogenic bacteria including A. actinomycetemcomitans, F. alocis, P. gingivalis, T. denticola, T. forsythia, P. intermedia, P. micra, F. nucleatum, C. rectus, E. nodatum, E. corrodens, and C. gingivalis and additionally SNPs in genes IL1A (rs1800587), IL1B (rs1143634), IL1RN (rs419598) in the group of 107 patients with periodontitis and 87 healthy subjects. Periodontal indexes for all samples were measured during dental examinations. The results indicate that the main risk factor is suggested to be the presence of periopathogenic bacteria, especially red complex bacteria and A. actinomycetencomitans. Also, the combination of genetic risk factors and high pathogenic bacteria revealed the high OR values, indicating potentially the most relevant risk markers.

Conclusion: The identification of periopathogenic bacteria and risk DNA polymorphisms associated with periodontitis using DNA techniques enable the identification higher risk patients and supports a comprehensive approach in diagnostics.

C.O.I. Disclosure:

M. Konecny: None. **V. Elias:** None. **O. Roshko:** None. **G. Krasnanska:** None. **G. Blandova:** None. **M. Baldovic:** None. **E. Kovalova:** None.

EP18.006 Molecular genetic markers of rheumatoid arthritis

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Background: Rheumatoid arthritis is a chronic systemic inflammatory disease that primarily affects the small joints of the hands and feet and leads to an average reduction in life expectancy by 3–10 years. RA is considered a multigenic disease with a significant genetic component. RA characterized by symmetrical peripheral polyarthritis due to an autoimmune inflammatory reaction concentrated in the synovial membrane, which leads to joint deformation.

Material and methods: Molecular genetic testing using next-generation sequencing (NGS) was performed in 26 patients. The panel was created specifically for the study and includes 120 candidate genes associated with autoimmune diseases. Bioinformatics analysis was performed with the distribution of significant variants by pathogenicity classes according to the ACMG recommendations.

Results: In the LY96 gene, the detected variant chr8:74922341 CT/C occurs in 18 patients, while in the control group only 3 times. The variant is classified as a variant of unknown clinical significance (VUS). Variants of the IRAK1 gene: chrX:153278833 GCCCG/GCC occurs in 4 patients and 1 participant in the control group, and the variant chrX:153278833 GCC/GCCG was found in 3 patients of the study group, and was not found in the control group. Both variants are classified as variants of unknown clinical significance. The variant chr12:10137558 A/C was detected in 3 patients, not registered in the control group. It is classified as VUS.

Conclusion: Changes in many genes have been studied as risk factors for rheumatoid arthritis. Most of these genes are known or suspected to be involved in immune system function.

C.O.I. Disclosure:

Z. Zhabakova: None. **L. Zaripova:** None. **A. Baigenzhin:** None. **L. Kozina:** None. **A. Boltanova:** None.

EP18.008 Genome-wide association study among Lithuanian elite athletes

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Background: The multifactorial trait of elite athletic performance is the result of multiple genetic and environmental factors. This pilot study represents the genome-wide association study (GWAS) exploring genetic markers among Lithuanian elite athletes, aiming to uncover genetic factors contributing to athletic performance.

Material and methods: The study included 96 Lithuanian elite athletes (55 males, 41 females participating in endurance and sprint/power sports) and 424 non-athlete controls (healthy unrelated 211 males, 213 females). Whole-genome genotyping was performed using Illumina DNA microarray Infinium Global Screening Array24 v3.0 Kit. PLINK 1.9 and Chi-squared test was used for GWAS to investigate the association of single-nucleotide polymorphisms (SNPs) with athlete status. After quality control, 77607 SNPs were included in the analysis, with genome-wide significance threshold set at 5×10^{-8} and suggestive significance threshold set at 5×10^{-5}.

Results: The results of GWAS showed no statistically significant associations between SNPs and elite athlete status. However, we identified eight SNPs that were suggestively significantly associated with elite athlete status: three intergenic variants (rs10957507, rs9453403, rs4359550), three intronic variants (rs12485289, rs16873417, rs1347756), one non-coding transcript exon variant (rs2001078), one missense variant (rs3213445, *CPT1B*:c.196A>T(p.Ile66Phe)). The *CPT1B* gene encodes carnitine palmitoyltransferase 1B, an enzyme essential for beta-oxidation of long-chain fatty acids in muscle mitochondria—a crucial aerobic metabolic pathway for energy production during endurance activities.

Conclusion: The findings emphasize the potential role of genetic factors in elite athletic performance. However, further investigations with larger cohorts and functional studies are required to validate these associations and clarify the underlying mechanisms.

C.O.I. Disclosure:

G. Anikevičiūtė: None. **V. Ginevičienė:** None. **A. Urnikytė:** None.

EP18.009 Pilot study on whole-genome associations with sarcopenia and frailty in Lithuanian older adults

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Background: Population aging is an ongoing issue around the world. Sarcopenia and frailty (S&F) are common geriatric syndromes, defined by loss of muscle mass, strength and function. The aim of the study was to identify genetic variants associated with S&F in older adults.

Material and methods: We examined 192 seniors (aged 82.2 ± 7.6 years) that were classified as non-S&F controls (135 community-dwelling adults, aged 80.2 ± 7.5 years), and 57 S&F individuals (aged 85.3 ± 6.7 years) according to the diagnostic criteria of the European Working Group on Sarcopenia in Older People (EWGSOP2), and Fried's criteria. Phenotypes (questionnaires, scales anthropometric and physiological characteristics) of S&F were used for genome-wide association study (GWAS). Whole-genome genotyping was performed using DNA microarray Infinium Global Screening Array24 v3.0 Kit (Illumina). SNPs were included in the analysis, with a genome-wide significance threshold set at $<5 \times 10\text{-}8$ and suggestive significance threshold set at $1.0 \times 10 - 5$.

Results: Using a genome-wide approach, no statistically significant association of SNPs with S&F status was found. However, we identified two SNPs rs75652203 (regulatory region variant) and rs17102732 (intergenic variant) significantly associated with grip strength, and two suggestively significant intergenic variants rs60001950 and rs10903128 associated with walking pace.

Conclusion: The present study provides preliminary evidence for the genetic basis of S&F, highlighting specific SNPs that may play a role in the development of this conditions. Further genetic studies are needed to better understand the genetic risk underlying sarcopenia and frailty.

C.O.I. Disclosure:

K. Gutauskaite: None. **V. Gineviciene:** None. **A. Urnikyte:** None. **E. Pranckeviciene:** None. **R. Dadeliene:** None. **J. Kilaite:** None. **I.E. Jamontaite:** None. **A. Mastaviciute:** None. **I.I. Ahmetov:** None. **V. Alekna:** None.

EP18.010 Remodeling Inflammatory Bowel Diseases through transcript mics, microbiome and polygenic risk scores

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Abstract Background: Inflammatory Bowel Diseases (IBDs) and Functional Gastrointestinal Disorders (FGIDs) are chronic conditions affecting pediatric patients. IBDs are immune-mediated diseases with alternating inflammation and remission phases, while FGIDs result from gut-brain axis dysregulation. Both conditions involve complex interactions between genetics, the microbiome, and the immune system.

Materials and methods: This study employs a multi-omics approach, integrating genomics, transcriptomics, and microbiome analysis across three patient cohorts: IBD patients, FGIDs patients, and healthy controls. Samples were collected at different disease phases. RNA sequencing of intestinal biopsies and stool microbiome analysis were performed alongside genotyping and metagenomic profiling.

Results: RNA-seq identified distinct transcriptional profiles between IBD and FGIDs, with limited differentially expressed

genes in some cases due to localized inflammation. Microbiome analysis revealed significant differences in bacterial diversity, identifying 2,238 genera. Key microbial and inflammatory markers correlated with disease activity, providing potential diagnostic targets.

Conclusion: This multi-omics approach enhances understanding of pediatric IBDs and FGIDs. Identified biomarkers may aid in early diagnosis and personalized therapies. Further validation and deeper sequencing will refine these predictive tools for improved clinical management.

C.O.I. Disclosure:

A. Grimaldi: None.

EP18.011 Genetic variants of the human host influencing the COVID-19 phenotypes: an overview of systematic reviews

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Background: Since the onset of the pandemic, several studies investigated the human genomic variants associated with COVID-19-related phenotypes. This overview aims to identify the host variants that were consistently replicated across multiple studies.

Methods: We designed a synthesis of systematic reviews published from 2020, following the Cochrane recommendations for overviews of systematic reviews.

Results: We searched PubMed, Scopus and Cochrane databases and identified 26 systematic reviews; of these, 18 reported findings from meta-analyses; 8 did not provide quantitative synthesis. Based on the Odds Ratio (OR) values reported, we identified 12 genes (by descending OR: *ACE2*, *HLA*, *ACE1*, *VDR*, *IFITM3*, *LZTFL1*, *SLC6A20*, *AGTR1*, *TNF-α*, *APOE*, *TMPRSS2*, *DPP9*) significantly associated with the severity of COVID-19 and 6 genes (*IFITM3*, *APOE*, *ACE1*, *KIR*, *CCR5*, *SLC6A20*) significantly associated with susceptibility to SARS-CoV-2 infection. The highest OR for severity was for the rs2285666 variant of the *ACE2* gene (OR = 10.01, 95% CI 5.39–18.56), while for susceptibility was for the rs12252 variant of the *IFITM3* gene (5.87, 1.05–32.76). Additionally, we found that the blood groups A (1.33, 1.14–1.56), B and AB, as well as the Rh-positive group are associated with susceptibility to SARS-CoV-2 infection; blood group A is also linked to increased severity of COVID-19 (1.25, 1.02–1.52).

Conclusion: The present synthesis confirms that variations in receptor expression, immune response and inflammatory pathways contribute to the complex interplay between genetic factors and COVID-19 outcomes. Future studies may use this evidence-based panel of genetic variants to further investigate the role for genetic determinants in the variable response to SARS-CoV-2.

C.O.I. Disclosure:

E. Muscolino: None. **C. Gualco:** None. **D. Giacobbe:** None. **G. Icardi:** None. **E. Di Maria:** None.

EP18.012 Updates to the GWAS Catalog Diagram and REST API access

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Background: The GWAS Catalog is a core resource of human genome-wide association studies, which continues to grow exponentially - with >7,000 publications, >125,000 studies (1,000% increase since 2020), 67% with hosted full genome-wide summary statistics, >15,000 mapped traits, and >700,000 lead associations.

Material and methods: This year, we made a number of software updates to enhance accessibility of our data, including improved API endpoints and scalable diagram visualisation.

Results: Curated Catalog data is accessible programmatically via our REST API, facilitating integration into subsequent workflows or tools developed and utilised by the community. In response to user feedback, we have upgraded our RESTful infrastructure for more robust data handling, and incorporated requested features to allow more sophisticated queries and retrieval of metadata previously only available through the user interface or downloads. Endpoints now offer access to pre-published submission details and enhancements to existing query responses, including external variant information from Ensembl.The GWAS Catalog diagram has provided an illustrative overview of all reported associations across the genome for more than a decade. Following the huge growth in data seen, we have refreshed our methods of generation to improve scalability, now achieving faster visualisation speeds, easier navigation and sustainability for the future.

Conclusion: The GWAS Catalog continues to provide the most comprehensive database of human GWAS, adapting to data growth with innovations in functionality. We welcome feedback on the new software and suggestions for future enhancements.

C.O.I. Disclosure:

E. Lewis: None. **A. Abid:** None. **M. Cerezo:** None. **P. Hall:** None. **Y. Ji:** None. **S. John:** None. **A. Mosaku:** None. **K. Ozan Bircan:** None. **S. Ramachandran:** None. **E. Sollis:** None. **M. Inouye:** None. **H. Parkinson:** None. **L. Harris:** None.

EP18.013 Frataxin locus may affect susceptibility to many common human diseases

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Background: Complex diseases are highly comorbid. Studies exploring local genetic correlations between human phenotypes become increasingly popular. Having lower mapping resolution compared to GWAS, they can detect loci with multiple modest effects, as well as multiallelic tandem repeats that have opposite effect directions depending on the repeat length.

Material and methods: Using SAIGE summary statistics for UK Biobank phenotypes, we calculated global genetic correlations for >200 most common diseases with the LDSC software. We selected a group of 45 phenotypes, in which all pairwise genetic correlations were nominally significant. For these, we calculated local genetic correlations using the LAVA tool.

Results: Excluding the MHC, the only region with at least one nominally significant local correlation for each of 45 phenotypes was centromere region of chromosome 9 (LAVA regions 1415–1417). We performed a gene-based analysis for 48 genes

located in regions 1414–1418 and 45 traits. The lowest P-value was observed for the *FXN* gene and alcohol-related disorders (P = 0.0002). For two more phenotypes, *FXN* showed P < 0.01. Other genes showed two or less signals at P < 0.01 (lowest P = 0.0016). *FXN* encodes frataxin, a mitochondrial protein protecting from oxidative stress. Expanding tandem repeat of its first intron causes Friedreich's ataxia in homozygous state, while intermediate length or heterozygous repeats can be beneficial.

Conclusion: Centromeric region of chromosome 9 is a potential pleiotropy hotspot, with *FXN* being its top candidate gene.

C.O.I. Disclosure:

N. Belonogova: None.

EP18.014 Examining genetic pathways for age at menarche: Insights from cross-ancestry analysis

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Background: The average age at menarche (AAM) has declined globally with most genetic studies focusing on early menarche (EM) risk factors. However, twin studies suggest that EM and late menarche (LM) may have distinct genetic architectures. This study investigates this hypothesis using large-scale genetic data across multiple ancestries.

Material and methods: EM and LM were defined as the lowest and highest 10% of AAM within birth-year distributions across 9 cohorts from European, Asian, Black, and Hispanic ancestry groups (UKB, KoGES, WHI). GWAS was conducted separately for EM and LM, followed by a meta-analysis. SNP-based heritability and genetic components were estimated using LDSC. Functional analyses included gene mapping, association testing, and enrichment tests. PRS for EM and LM was calculated using lassosum and validated across ancestries.

Results: Meta-analysis identified shared and distinct variants between EM and LM, including overlapping genes like LIN28B, EEFSEC, and ANF483. While most signals were specific, LM variants showed ovarian tissue enrichment without statistical significance. PRS demonstrated stronger predictive power for LM-associated variants than EM(particularly in Whites), and PRS models with overlapping variants better predicted LM than EM.

Conclusion: The presence of both shared and distinct genetic loci between EM and LM, along with LM-specific loci and tissue-specific gene expression in ovarian tissue,suggests EM and LM involve different biological mechanisms, extending beyond simple directional effects of EM-associated variants. PRS results further indicate LM has unique genetic determinants rather than being solely explained by inverse EM effects. The functional implications of these genetic differences across populations warrant further study.

C.O.I. Disclosure:

Y. Oh: None. **H. Pyun:** None. **W. Jeong:** None. **J. Kim:** None. **S. Lee:** None. **J. Sung:** None.

EP18.015 Genetic underpinnings of clusters of multiple long-term conditions

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Background: Multiple long-term conditions (MLTCs) is synonymous with poorer quality of life, increased mortality, and healthcare burdens. Patterns of co-occurring LTCs vary between individuals, reflecting common biological pathways or antagonism (inverse comorbidity). We investigate whether these relationships might be attributable to genetic factors.

Material and methods: Using 265,384 White British UK Biobank participants aged 65 and over (data obtained under project #90865), we mapped ICD10 diagnoses to 59 disease categories and identified clusters of MLTCs leveraging fuzzy c-means clustering. Genome-wide association analysis of cluster membership was performed, followed by genetic correlation estimation, conditional analysis and functional annotation of prioritised SNPs.

Results: Six clusters were identified: Psychiatric (C1), Cardiovascular (C2), Gastrointestinal (C3), Musculoskeletal & Inflammatory (C4), Metabolic Syndrome (C5), and Cancers & Eye Disorders (C6). Top SNPs included rs429358 (missense variant in *APOE*, modulating lipid metabolism) for C1, rs72900157 (intergenic, nearest gene: *PITX2*, involved in transcriptional regulation), rs55730499 (intron variant in *LPA*, associated with cardiometabolic multimorbidity) and rs10757277 (intergenic, nearest gene: *CDKN2B-AS1*, associated with cardiovascular disease) for C2, rs1065853 (intergenic, nearest genes: *APOE, TOMM40*, involved in mitochondrial translocation) for C4 and C5, and rs71611783 (intergenic, nearest gene: *CEP44*, associated with Retinitis Pigmentosa) for C6. C1/C6 and C2/C6 pairs demonstrated highly significant negative phenotypic and genetic correlations: rg(C1/C6) = -0.87 (p = 1.35e-43) and rg(C2/C6) = -0.61 (p = 3.61e-14), reflecting inverse comorbidity.

Conclusion: This study highlights genetic factors associated with MLTCs cluster membership and reveals potential underpinnings of comorbidity and inverse comorbidity. Research is needed to explore causal pathways and mechanisms modulating the phenomenon of non-random association of diseases.

C.O.I. Disclosure:

S. Bauer: None. **R. Bowyer:** None. **L. Bravo Merodio:** None. **D. Williams:** None. **G. Gkoutos:** None. **D. Vetrano:** None. **T. Jackson:** None. **C. Steves:** None. **M.B. Freidin:** None.

EP18.017 Identifying genetic variants associated with recurrent pregnancy loss in the Uzbek population

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Background: Recurrent pregnancy loss (RPL) is defined as the loss of two or more pregnancies before the 20th week of gestation and affects approximately 15% of pregnancies. Despite advances in infertility diagnostics, 35–60% of RPL cases remain unexplained, suggesting genetic, epigenetic, and environmental contributions. In this study, we analysed the genetic factors associated with RPL among women in Uzbekistan.

Material and methods: This study included 161 patients diagnosed with RPL and 511 healthy individuals. Genetic screening was performed using the Global Screening Array v.3.0. Hardy-Weinberg equilibrium test and logistic regression analyses were conducted using the SNPassoc software package to identify statistically significant associations between genetic variants and disease risk under an additive genetic model.

Results: Several genetic variants showed significant associations with RPL. Protective effects were observed for rs4871372 (*LOC105375725*, $p = 3.7 \times 10^{-5}$, OR = 0.58) and rs2704035 (*SULF1*, $p = 3.9 \times 10^{-4}$, OR = 0.63). Increased RPL risk was linked to rs4916848 (*ADGRV1*, $p = 1.8 \times 10^{-6}$, OR = 1.82) and rs478993 (*TENM4*, $p = 4.5 \times 10^{-4}$, OR = 1.58).

Conclusion: Our study identified novel genetic associations with RPL in Uzbek women. The lncRNA *LOC105375725* (rs4871372) may function as an epigenetic regulator, while rs4916848 likely acts as an enhancer. Additionally, *TENM4* has been implicated in mesoderm induction during early embryogenesis. Therefore, these variants can be used as diagnostic and prognostic markers. Further research is needed to understand their functional role of these variants in RPL pathogenesis.

C.O.I. Disclosure:

A. Punko: None. **A. Abdurakhimov:** None. **Y. Kapralova:** None. **D. Zakirova:** None. **A. Rakhmatullayev:** None. **G. Esonova:** None. **X. Meylikov:** None. **A. Abdullaev:** None. **D. Dalimova:** None. **S. Turdikulova:** None.

EP18.018 A multi-omics strategy identifies novel genes and variants associated with Type 2 Diabetes in Cilento populations

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Background/Objective: Type 2 diabetes (T2D) is a complex disease with heritability estimates of 40–70%. While numerous genetic factors have been identified, they explain only a fraction of the observed heritability. This study aimed to identify novel T2D-associated genes and variants by employing a multi-omics approach combining transcriptomics and expression quantitative trait locus (eQTL) mapping in genetically isolated populations.

Methods: Total RNA was extracted from peripheral blood mononuclear cells (PBMCs) of 166 individuals (62 T2D cases and 104 controls) from three villages in the Cilento region. Transcriptomic profiles were generated using RNA-Seq on the Illumina NovaSeq 6000 platform. Differential gene expression (DGE) analysis was executed using EdgeR and DESeq2 tools. Cis- and trans-eQTL mapping was subsequently conducted for the differentially expressed genes, using the FPKM values as quantitative traits and ~6.7 million genome-wide SNPs. The identified eQTLs were then tested for association with T2D in an expanded sample of 113 cases and 581 controls from the same populations.

Results: DGE analysis identified novel T2D-associated genes and confirmed previously reported lower ABCG1 expression in T2D patients. Several variants were detected as novel cis- and trans-eQTLs for the identified genes. Notably, five of these eQTLs also showed an association with T2D in the larger validation sample.

Conclusion: This multi-omics approach identified novel T2D-associated genes and eQTLs in genetically isolated populations, highlighting the power of integrating transcriptomic and genotypic data for understanding complex diseases. These findings may contribute to the identification of novel T2D biomarkers and therapeutic targets.

C.O.I. Disclosure:

P. Cennamo: None. **T. Nutile:** None. **D. Ruggiero:** None. **F. Marzano:** None. **A. Tullo:** None. **C. Telegrafo:** None. **F. Zambelli:** None. **G. Pesole:** None. **M. Ciullo:** None.

EP18.019 A map of genetic and phenotypic associations across male reproductive phenotypes

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Background: Male reproductive health problems have increased over the last two decades, yet relevant studies remain scarce, and men's reproductive health remains understudied. Understanding the genetic underpinnings of male reproductive health and diseases can reveal risk factors, improve diagnostics, and enable precision treatments. This study maps the genetic background of phenotypes related to male reproductive health and provides an atlas of genetic and phenotypic correlations.

Material and methods: GWAS meta-analyses (~532,376 men) were conducted across 53 phenotypes (ICD-10 codes) and 12 biomarker measurements, using data from three large biobanks (EstBB, FinnGen, and UKBB). Analyses included GWAS annotation, causal gene identification using a four-tier prioritization approach, heritability estimation, genetic correlation analysis via LDScore, and phenotypic overlap assessment using the Jaccard Index.

Results: Significant genome-wide signals were detected for 13 of 65 phenotypes, totalling 151 signals, including 80 novel findings and 50 not previously associated with reproductive health. Seven phenotypes, including "Hydrocele" (6 signals) and "Premature ejaculation" (1 signal), showed genome-wide signals for the first time. Ten variants enriched in Finnish and Estonian populations suggested population-specific effects. Across genomic risk loci, 260 candidate genes were selected. Heritability estimates for common SNPs were generally low (0.2 to 13%). Genetic correlations were predominantly positive, with some exceptions, such as negative correlation between "BMI" and "Chronic prostatitis". Phenotypic overlap was higher among closely related phenotypes.

Conclusion: This study constitutes the largest effort to date in mapping genetic determinants of male reproductive health, uncovering novel findings and population-specific alleles critical for future research.

C.O.I. Disclosure:

J. Figueredo: None. **T. Laisk:** None. **R. Mägi:** None.

EP18.020 An integrative genotype-first study of *APOH* rs1801689 (p.C325G) variant as an independent risk factor for hepatic steatosis via altered triglyceride metabolism

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Background: The *APOH* rs1801689 (p.C325G) missense variant has been linked to liver injury and reduced plasma triglycerides (TG). However, its impact on protein function and risk of liver disease in metabolically low-risk individuals remains unclear.

Methods: Whole exome sequences were analyzed for carriers of *APOH* rs1801689 in the UK Biobank (N = 500,000) and Penn Medicine BioBank (PMBB, N = 40,000) amongst the whole population and low-risk cohorts (BMI ≤ 25, non-diabetic, non-hypertensive). In silico analysis of mutant protein were made in AlphaFold and in vitro studies in HuH-7 cells evaluated Apolipoprotein H (apoH) function in VLDL-TG secretion. In PMBB, a gene burden of 35 predicted deleterious rare *APOH* variants from gnomAD was also assessed. Multivariate regression models were adjusted for demographics and ancestry, with statistical significance at $p < 0.05$.

Results: *APOH* rs1801689 carriers showed reduced plasma TG (−5.3%, $p < 0.001$) and large VLDL particles (−8.4%, $p < 0.001$), clinically elevated liver transaminases (OR:1.12, $p < 0.01$) and higher risk of dyslipidemia diagnosis (OR:1.1, $p < 0.001$). These effects were replicated in PMBB and also in low metabolic risk cohorts. However, rare predicted deleterious variants in the PMBB were associated with lower TG and a 4-fold hepatic fibrosis risk (OR:4.3, $p < 0.05$). Structural predictions revealed the C325G substitution to disrupt the apoH domain V, impairing phospholipid binding. ApoH knockdown in hepatocytes reduced VLDL-TG secretion and increased TG accumulation, suggesting loss-of-function directionality.

Conclusion: The phenotype observed in *APOH* rs1801689 carriers are compatible with loss of protein function which leads to steatosis via TG retention in hepatocytes even in absence of metabolic syndrome.

C.O.I. Disclosure:

H.Y. Huang: None. **C. Vitali:** A. Employment (full or part-time); Significant; CV is currently employed at Arrowhead Pharmaceuticals. At the time of study, CV was employed at the University of Pennsylvania. **M.D. Rendel:** None. **D. Conlon:** None. **D. Zhang:** None. **K.M. Schneider:** None. **D.J. Rader:** None. **C.V. Schneider:** None.

EP18.021 Rare variant associations with cardiac phenotypes in the Cilento isolates

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Background/Objectives: Although several genetic loci have been identified so far through GWAS for complex cardiovascular phenotypes, a large part of the heritability of such traits is still missing, due largely to rare variants not sufficiently tagged by common SNPs. In isolated populations, the use of local reference panels improves the imputation of rarer alleles, helping to discover functional variants associated with complex phenotypes. This study aims to identify rare variants associated with cardiovascular phenotypes in some Cilento genetic isolates.

Methods: Twenty-one cardiovascular phenotypes were collected for ~2,000 individuals living in three Cilento villages. Genome-wide genotypes and WES data were used to build a Local Reference Panel, to perform a genome-wide imputation of 355K exonic variants. A gene-based analysis was performed for each trait, using SKAT-O, for 19,381 genes capturing rare variants (MAF < 0.01) in 1000G and/or gnomAD samples. Then, a single SNP analysis was carried out for the associated genes, to identify the variant(s) with a stronger effect on the phenotype.

Results: Ten genes were associated with cardiac function traits at a genome-wide statistically significant level ($p < 2.5E-6$). Among them, a gene already linked to heart failure was identified for Fractional Shortening, carrying an intron variant extremely rare in gnomAD (MAF = 0.02 in Cilento) showing a strong effect on the phenotype.

Conclusions: Our study identified rare variants in genes possibly implicated in clinically relevant cardiac phenotypes. It also showed that homogeneous populations in Cilento might contribute to filling the gap of missing heritability in the genetics of complex traits.

C.O.I. Disclosure:

D. Ruggiero: None. **T. Nutile:** None. **P. Cennamo:** None. **M. Ciullo:** None.

EP18.022 Consideration of chronic pain facilitates major depressive disorder gene discovery: family-based study findings replicate in the UK Biobank

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Background: Although major depressive disorder (MDD) has a strong genetic component, identified susceptibility loci explain minimal genetic variance. Chronic pain frequently co-occurs with MDD, possibly reflecting a partially overlapping genetic etiology.

Material and methods: We performed genome-wide linkage and association analyses using MDD with chronic pain as a narrow subphenotype in the GenRed family-based study of early-onset recurrent depression. Participants were assessed using DSM IV criteria and DIGS, with affection status incorporating both MDD diagnosis and chronic pain classification. We attempted replication in the UK Biobank (UKB), comparing 314 early-onset (age<30) recurrent depression cases with multiple chronic pain sites against 344 pain-free controls.

Results: Using Bayesian analysis (posterior probability of linkage, PPL) in KELVIN, we identified linkage signals at chromosome 8p23 (PPL = 0.35) and 16q12 (PPL = 0.51). The 8p23 region contains *CSMD1*, previously associated with depression and bipolar disorder, while 16q12 represents a novel finding containing approximately 15 genes. Our strongest association was with *HCN1* on chromosome 5p12 (PPL = 0.36), implicated in ketamine response, depression, and pain. The 8p23 and 16q12 findings replicated in the UKB.

Conclusion: The identification of established depression-related genes and novel regions, with replication in a large independent cohort, suggests that considering chronic pain in depression genetics may enhance gene discovery and provide new therapeutic targets.

C.O.I. Disclosure:

A.V. Grant: None. **D. Nolan:** None. **W. Valentine-Cooper:** None. **S. Seok:** None. **J. Burian:** None. **V. Vieland:** None.

EP18.023 Cross_body site and cross_population evidence for shared genetics in chronic pain

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Background: Chronic pain, defined as pain lasting >3 months, has a heritable component and manifests as site-specific, widespread, or count of affected sites (multisite chronic pain, MCP). With genetic correlations of 0.6–1.0 among pain traits, we aim to identify and characterize their shared genetic architecture.

Material and methods: We analyzed genome-wide associations for MCP and site-specific chronic pain across 9.2 million variants in 457,461 European-ancestry UK Biobank participants. Using MiXeR, we estimated causal effects and variant overlap between phenotypes. We tested polygenic risk scores derived from UK Biobank in the Canadian Longitudinal Study on Aging (CLSA) (N = 25,261) across 9 trait pairs. We consolidated the gene sets from Gene Ontology terms to test unique and common biological pathways underlying chronic pain traits using Multimarker Analysis of GenoMic Annotation software (MAGMA).

Results: MCP, hip, knee, back, and neck/shoulder pain showed similar polygenicity with 95% causal variant overlap. Headache, facial, stomach, abdominal, and widespread pain showed lower polygenicity but >80% shared causal variants. MCP polygenic scores successfully predicted chronic pain risk in Canadians, with significant differences between the highest and lowest quintiles for migraine, back, and knee pain. We tested biological pathways enrichment on the list of MAGMA-based gene sets, identifying 43 common pathways across chronic pain traits with the most significant enrichment in neurological and immunological pathways including neuromuscular process, synapse, pain behavior, and T cell antigen processing and presentation.

Conclusion: Different chronic pain conditions share substantial genetic architecture, with findings transferable between UK and Canadian populations.

C.O.I. Disclosure:

G. Koli Farhood: None. **M. Parisien:** None. **L. Diatchenko:** None. **A. Grant:** None.

EP18.025 Genetic determinants of metabolomic traits in the Amish: Insights into cardiometabolic disease pathways

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Background: Ex/GWAS identify genetic contributors to cardiometabolic diseases (CMDs). Integrating genomics with metabolomics enhances understanding of biological mechanisms. We investigated the genetic determinants of metabolomic traits in the Amish population to identify genes and gene variants influencing CMD-related metabolic pathways.

Material and methods: We profiled the levels of 1288 plasma metabolites from 6270 Amish adults with genetic data. Ex/GWAS was conducted on 1015 metabolites and 5981 samples that passed QC. Fine-mapping and stepwise conditional analyses were performed to identify independent likely causal variants.

Results: We identified 150 independent functional or likely functional (CADD ≥ 20) variants associated with 414 metabolites. Among these, 62 variants (including 16 predicted loss-of-function) are highly enriched in the Amish. We confirmed six previously reported hypertension-associated *ENPEP* stop-gained variant (p.Trp413Ter)-metabolite level associations and discovered four new ones. Additionally, the *B4GALT1* missense variant (p.Asn352Ser), previously shown to be associated with lower LDL-C and cardioprotection, is linked to 12 metabolites involved in amino acid and energy metabolism pathways. We also found a novel association between 9 metabolites and a *PCK2* splice donor variant (c.1234+1G > T)s, which is 58-fold enriched in the Amish compared to non-Finnish Europeans. Furthermore, the Amish-enriched familial hypercholesterolemia *APOB* variant (p.Arg3527Gln) is associated with 138 lipid metabolites, including cholesterol.

Conclusion: We performed Ex/GWAS in the Amish and identified 62 missense and pLoF variants that are highly enriched in this population and are associated with metabolite levels. We aim to explore the functional effects of these variants on disease phenotypes toward a better mechanistic understanding of CMD.

C.O.I. Disclosure:

M. Jallow: A. Employment (full or part-time); Significant; Regeneron Pharmaceuticals, Inc. **G. Tzoneva:** A. Employment (full or part-time); Significant; Regeneron Pharmaceuticals, Inc. **B. Ye:** A. Employment (full or part-time); Significant; Regeneron Pharmaceuticals, Inc. **V. Zavala:** A. Employment (full or part-time); Significant; Regeneron Pharmaceuticals, Inc. **O. Sosina:** A. Employment (full or part-time); Significant; Regeneron Pharmaceuticals, Inc. **M. Kapoor:** A. Employment (full or part-time); Significant; Regeneron Pharmaceuticals, Inc. **J. Backman:** A. Employment (full or part-time); Significant; Regeneron Pharmaceuticals, Inc. **M. Montasser:** None. **B. Mitchell:** None. **A. Shuldiner:** A. Employment (full or part-time); Significant; Regeneron Pharmaceuticals, Inc. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Regeneron Pharmaceuticals, Inc.

EP19 Genetic Epidemiology and Mendelian Randomisation

EP19.002 The Causal Relationships between Lipid Profiles and Kidney Function: A Mendelian Randomization Study

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Background: The global prevalence and mortality of chronic kidney disease (CKD) had been increasing. Epidemiological studies had identified the association between lipid profiles and kidney function as well as CKD. This study aimed to elucidate the causal relationships between lipid profiles and kidney function using Mendelian Randomization (MR) in Taiwan.

Material and methods: The study was a one-sample MR design. A total of 128,632 individuals and 9,814,944 genetic variants were obtained from the Taiwan Biobank. The outcome variables included renal failure, stages of renal failure, and estimated glomerular filtration rate (eGFR). Significant genetic variants identified from the genome-wide association study (GWAS) of lipid profiles from Taiwan Biobank were used as instrumental variables (IVs). Two-stage least squares (2SLS), Inverse-variance weighting (IVW), MR-Egger, MR-pleiotropy residual sum and outlier (MR-PRESSO) were used for balanced, directional, and horizontal pleiotropy, respectively.

Results: Using individual genetic variants or polygenic risk score (PRS), we observed that higher LDL-C could significantly increase eGFR (2SLS: β = 0.401, 95%C.I.:[0.035, 0.768]; MR-PRESSO: β = 0.340, 95%C.I.:[0.043, 0.637]; PRS 2SLS: β = 0.333, 95%

C.I.:[0.062, 0.604]). We also found higher TG could significantly increase the risk of renal failure (IVW: OR = 1.281, 95%C.I.:[1.088, 1.511]; PRS 2SLS: OR = 1.288, 95%C.I.:[1.001, 1.658]) and the risk of stages of renal failure (MR-PRESSO: OR = 1.092, 95%C.I.:[1.028, 1.160]; PRS 2SLS: OR = 1.095, 95%C.I.:[1.014, 1.181]). Moreover, a decreased risk of renal failure per 1 mmol/L increase in HDL was observed (IVW: OR: 0.654, 95%CI:[0.441, 0.971]).

Conclusion: Our study indicated that the higher TG and lower HDL-C could increase the risk of renal function, while higher LDL-C could increase eGFR.

C.O.I. Disclosure:

M. Lin: None. **S. Yang:** None. **Y. Lin:** None. **Y. Lin:** None. **H. Weng:** None. **S. Tsai:** None.

EP19.003 Spectrum of birth defectst among newborns in Lviv region (Ukraine) for 2002–2024

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Background: Birth defects (BDs) and hereditary pathology consistently ranks second in the structure of mortality of children in the first year of life. Every year in Ukraine, about 12,000 children are born with BDs, and in 20% of children they are the cause of disability. Aim of our study: Determine the spectrum of BDs among newborns in Lviv region (Ukraine) for 2002–2024 to improve the efficiency and timeliness of diagnostics various organs and systems.

Material and methods: Prospective and retrospective analysis of medical documentation of maternity hospitals in Lviv region among newborns was conducted using the "case-control" study in 1424 cases with BDs and 1417 healthy children.

Results: The spectrum of BDs among 1424 newborns was as follows: BDs and deformities of the musculoskeletal system −322 (22.6%), cleft lip and palate −233 (16.3%), chromosomal anomalies −213 (14.9%), BDs of the genital organs −195 (13.6%), BDs of the nervous system −153 (10.7%), multiple BDs −108 (7.5%), BDs of the circulatory system −98 (6.8%), BDs of the digestive system −65 (4.5%), BDs of the urinary system −22 (1.5%), BDs of the eye, ear, neck −11 (0.7%), BDs of the respiratory system −4 (0.2%).

Conclusion: The high prevalence of musculoskeletal deformities, cleft lip and palate, and chromosomal anomalies suggests that early detection and specialized care for these conditions could significantly improve outcomes. Prevention strategies should focus on maternal health, prenatal screenings and genetic counseling of individuals from the high-risk group when planning the next pregnancy.

C.O.I. Disclosure:

N. Kitsera: None. **S. Genyk-Berezovska:** None. **N. Lukyanenko:** None. **O. Hnateiko:** None. **V. Hlohus:** None.

EP19.004 The Dual Impact of Telomere Length in Multiple Sclerosis: Insights from Experimental to Computational Studies

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Background: As the etiology of MS remains unknown, identifying risk factors contribute to MS mechanisms.Telomere length (TL), a marker of biological aging, serves as a potential biomarker in MS.

Material and methods: Observational, experimental data, systematic reviews and Mendelian Randomization (MR) studies were gathered. Finally, a comparative analysis was employed to reach a robust finding.

Results: The data implies that MS risk and progression are multifaceted, with both and shorter TL associated. Observational and experimental research indicated that MS patients, particularly those with progressive form, have shorter TL. Shorter TL is associated with an elevated risk of conversion to secondary progressive. However, MR showed a causal relation between longer TL and MS. MR has limitations in this area. As MS primarily affects younger people, age-specific variations in analysis must be considered. Children conceived by older men have typically longer TL. TL also exhibits gender or racial differences, even in newborns. TL is longer in females than males, and longer in people with African ancestry than European ancestry. Different tissues have different TL due to replication rates. Telomere Profiling showed that the end of certain chromosome is either longer or shorter. Genes near chromosomal ends may be regulated by telomere chromatin interaction, impacting telomerase regulation. Longer TL may increase cell proliferation via higher telomerase activity, leading to more mutations. Lastly, the classic MR method detect linear correlations while there might be a U-shaped link between them.

Conclusion: This dual impact needs further research to understand the role of TL in MS.

C.O.I. Disclosure:

F. Aliazami: None. **F. Aliazami:** None.

EP19.005 The association of TNFα and IL6 gene polymorphisms with injury susceptibility in elite athletes of Kazakhstan

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Background: Elite athletes are focused on intensive training and physical overload to achieve peak performance. Therefore, athletes are highly prone to acute injuries and musculoskeletal damage. Injuries can significantly affect performance, and career overall. Cytokine-related genes play an important role in the inflammatory response, a key component of injury recovery. This study explored the influence of polymorphisms in immune and inflammatory response genes on injury risk among athletes, aiming to identify potential biomarkers.

Material and methods: The study included 92 elite male athletes from Kazakhstan representing power sports (wrestling, boxing and weightlifting). All recruited athletes were of Asian descent. We have collected data on acquired injury from medical records. Genotyping of cytokine gene polymorphisms of TNFα (rs1799964), TNFα (rs1799724), TNFα (rs1800629) and IL6 (rs1800795) genes was performed using TaqMan allelic discrimination assay.

Results: In this study cohort IL6 (rs1800795), TNFα (rs1799964) and TNFα (rs1800629) polymorphisms did not show any significant association with injury susceptibility. Nevertheless, TNFα (rs1799724) showed a significant association with injury risk in athletes, suggesting that the T allele increases the risk of injury compared to carriers of the C allele (C/C-C/T vs. T/T, OR = 6.34, 95% CI = 1.18–34.09, $p < 0.05$).

Conclusion: The T/T genotype of TNFα (rs1799724) polymorphism could be used as a potential biomarker for non-contact muscle injury susceptibility in Kazakhstan's elite athletes. These

findings underscore the importance of genetic screening for personalized injury prevention strategies. Despite this, further research on a larger cohort of athletes is required.

C.O.I. Disclosure:

A. Gabdulkayum: None. **S. Amangeldikyzy:** None. **A. Akilzhanova:** None. **D. Yerezhepov:** None.

EP19.006 Estimation of different STR allele frequencies in HTT and FXN genes for the general population of the Republic of Moldova

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Background: Both Huntington disease and Friedreich ataxia have a ruinous impact upon patients' and their relatives' quality of life. Moreover, recent findings show that short tandem repeat (STR) expansion disorders are more common than previously thought. However, there is a limited number of studies aimed at estimating different allele frequency and populational risks in the general population, as most research is restricted to high-risk groups or high-income geographical areas only. Most of these research is done either for known-high risk populations or in generally high income countries. Here we aim to estimate different STR allele frequencies in *HTT* and *FXN* genes for the general population of the Republic of Moldova.

Material and methods: Confidential DNA samples stored in local biobank were used. Samples were analyzed through triplet-primed PCR (TP-PCR) followed with capillary electrophoresis using ABI 3500 Dx Gene Analyzer with POP7 polymer and 50 cm capillary array. Primers for TP-PCR are designed in house.

Results: For the first time, there were estimated frequencies of different STR alleles in *HTT* and *FXN* genes for the general population of the Republic of Moldova, including at-risk alleles.

Conclusion: Frequencies of different STR alleles *HTT* and *FXN* genes for the general population of the Republic of Moldova were estimated and compared with corresponding data for other populations. Primary risk assessment was performed for general population of the Republic of Moldova.

C.O.I. Disclosure:

A. Dorif: None. **V. Sacara:** None.

EP19.007 VDR gene polymorphism and its association with vitamin D status in National Olympic team members

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Background: Vitamin D plays an important role in bone and muscle health, and its deficiency is associated with bone fractures and muscle damage. Its receptor which is encoded by the VDR gene, is found in many tissue types of the body. Any variation in vitamin D pathway genes can affect serum 25-(OH)D levels. Our study explored the influence of VDR gene polymorphisms on the status of vitamin D in elite athletes of Kazakhstan.

Material and methods: 109 elite athletes of Asian origin have been recruited for the study. All athletes are members of the Kazakhstan National Olympic team. We collected the serum samples and identified the levels of 25-(OH)D by electrochemiluminescence immunoassay. Four VDR gene polymorphisms were identified using a qPCR assay and pre-designed TaqMan probes (FokI, rs2228570; BsmI, rs1544410; ApaI, rs7975232; and TaqI, rs731236).

Results: More than 70% of recruited athletes had vitamin D insufficiency and one-third were deficient. FokI, BsmI, and ApaI polymorphisms did not show any association with the vitamin D status of athletes. G/G genotype of VDR TaqI was significantly associated with insufficient serum vitamin D levels in athletes (A/A-A/G vs. G/G, OR = 4.07, 95% CI = 1.03–16.08, $p < 0.05$), but not with deficiency.

Conclusion: Our findings suggest that the G/G genotype of TaqI (rs731236) polymorphism can be used as a potential biomarker for the prediction of insufficient vitamin D levels. However further studies of a larger group of athletes and general populations are required.

C.O.I. Disclosure:

D. Yerezhepov: None. **A. Gabdulkayum:** None. **S. Amangeldikyzy:** None. **A. Akilzhanova:** None.

EP19.008 Integrating polygenic scores and clinical factors for elevated cIMT risk assessment: Evidence from Taiwan Biobank

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Background: Carotid intima-media thickness (cIMT) indicates early atherosclerosis and serves as a cardiovascular surrogate. This study aimed to develop a risk assessment model for elevated cIMT risk in Han Chinese.

Material and methods: Our study utilized the Taiwan Biobank data, randomly split into training (n = 17,829) and validation (n = 4,814) sets at an 80:20 ratio. GWAS was performed to identify SNPs for elevated cIMT, followed by polygenic risk scores (PRS) construction at $p = 0.1$ and $p = 0.0001$. Logistic regression ($p < 0.25$) identified clinical predictors, with AUROC, NRI, and IDI assessing model performance.

Results: A total of 22,643 participants (36.15% male, mean age 55.4 ± 10.2 years) were included in this study. AUROC (95% CI) for the training set was 0.81 (0.80, 0.82) for the clinical factors only model, and 0.83 (0.81, 0.84) for the validation set. The incorporation of PRS into the risk assessment model enhances the predictability of elevated cIMT risk, with AUROCs of 1.00 (1.00, 1.00) and 0.83 (0.82, 0.84) at $p = 0.1$ and $p = 0.0001$, respectively, in the training set. However, the predictability of elevated cIMT risk did not improve in the validation set, with an AUROC of 0.83 (0.81, 0.84). NRIs were 0.36 (training) and 0.02 (validation) at $p = 0.1$; 0.17 and 0.00 at $p = 0.0001$. IDIs were 0.42 and 0.00 at $p = 0.1$; 0.02 and 0.00 at $p = 0.0001$.

Conclusion: Incorporating PRS improves elevated cIMT risk prediction in the training set, which supports genetic studies in Han Chinese. Further optimization enhances model stability and clinical applicability for cardiovascular risk assessment.

C.O.I. Disclosure:

Z. Yu: None. **L. Liao:** None.

EP19.009 *Impact of the implementation of Whole Exome Sequencing for the diagnostic confirmation in Catalonia's Newborn Screening Program using the initial Dried Blood Spots*

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Background: Hospital Clinic of Barcelona (HCB) centralizes the Catalonia's Newborn Screening analyses. For positive detections related to inborn errors of metabolism (IEM), after referring the baby to the Clinical Unit for diagnosis confirmation and treatment, DNA is obtained from peripheral blood and sent to HCB for genomic analysis, primarily whole exome sequencing (WES). This study aimed to validate a WES workflow using DNA extracted from the initial dried blood spots (DBS) to reduce the turnaround time for diagnostic confirmation.

Material and methods: An automated WES workflow was developed and validated using DNA from DBS of 30 patient samples with known genotypes. In three of those patients, WES data from both peripheral blood DNA and DBS DNA were compared.

Results: WES data from DBS and peripheral blood DNA showed comparable coverage. The mean coverage across 30 DBS samples was 89.3× (range:43.2×-117.0×). The corresponding mutations were successfully identified in all newborns studied, involving 17 different genes associated with IEMs. The average time to obtain results was 14 days (range:7–20). Implementing WES from DBS reduced the diagnostic confirmation time by 4–10 days. Among 93 WES analyses for IEM confirmation, using peripheral blood or DBS samples, 67 (72%) identified causative mutations. In 13% of cases, cDNA studies using skin biopsies were needed to detect a second mutation.

Conclusion: WES using DBS provides a reliable, less invasive alternative to peripheral blood, streamlining diagnostic processes and reducing delays. Transitioning to genome sequencing might improve the diagnostic yield of newborns with complex genetic variants.

C.O.I. Disclosure:

J. Garcia Villoria: None. **A. Paredes-Fuentes:** None. **E. Verdura:** None. **J. Villanueva:** None. **X. Sole:** None. **A. Montalban:** None. **A. Argudo-Ramírez:** None. **J. Gonzalez de Aledo-Castillo:** None. **J. Laguna:** None. **R. López-Galera:** None. **L. Gort:** None. **J. Puig-Butille:** None.

EP19.011 Preventive CYP2C19 pharmacogenetic analysis and clopidogrel failure of treatment risk in a healthy cohort

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Background: The CYP2C19 gene encodes an enzyme key in the metabolism of multiple drugs. Its importance in pharmacogenetics has been studied in the metabolism of clopidogrel, proton pump inhibitors and antidepressants. Clopidogrel is a prodrug that requires hepatic activation by CYP2C19 to exert its antiplatelet effect. Variants associated with reduced enzymatic function may lead to an insufficient therapeutic response and an increased risk of thrombotic events. The increased enzymatic activity may increase the risk of bleeding.

Material and methods: 2324 patients were included for a prospective pharmacogenomic study. DNA was extracted using Qiasymphony and Illumina DNA PCR-Free WGS was performed. GRCh37 reference assembly was used. CYP2C19 status and its relationship with clopidogrel was analyzed specifically following CPIC and DPWG guidelines. For that purpose, the following CYP2C19 alleles were included: *1, *2, *3, *4, *5, *6, *8, *17, *35.

Results: Among the 2324 patients studied, 615 individuals (26%) showed the non-functional alelle *2, 23 showed *4 and 131 showed the increased function alelle (*17). After checking clinical consequences, 593 of our patients were found to be intermediate metabolizers (25%) and 63 individuals were found to be poor metabolizers (2.71%). This information was actioned in each patient's Electronic Clinical Reports (ECR).

Conclusion: The results indicate that a considerable amount of patients (27%) show reduced metabolizing features in CYP2C19 related drugs. Considering that, a preventive pharmacogenomic panel should be tested in healthy people before treatment with clopidogrel or antidepressants is established, in order to avoid adverse reactions or treatment failure.

C.O.I. Disclosure:

C. Ferrer: None. **B. Paumard:** None. **S. Pasalodos:** None. **F. Yelamo:** None. **B. Bustillo:** None. **S. Fernández:** None. **L. Delgado:** None. **A. de Vasconcelos:** None. **S. Gutiérrez:** None. **C. Morcillo:** None. **J. Suela:** None.

EP19.012 Gastrointestinal cancer risks in Israeli *BRCA1/BRCA2* mutation carriers

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Background: The risk of developing gastrointestinal (GI) malignancies in BRCA1/BRCA2 mutation carriers and the subsequent proposed surveillance schemes are clinically important and debated. Here, we evaluated the risks of GI cancers in Israeli *BRCA1/BRCA2* mutation carriers to define effective cancer risk management.

Material and methods: 1. Data from Israeli *BRCA1/BRCA2* carriers for risk assessment by Kaplan-Meier analysis and compared to general population risks. 2. Long-term follow-up of an unaffected male cohort from a large population-based male study.

Results: This study included: 1. 6,554: 3,814 (58.2%) *BRCA1* and 2740 (41.8%) *BRCA2* carriers, 5,432(82.9%) women and 1122(17.1%) men. Median age and years of follow-up were 50(39,65) and 7(2,12).Lifetime risk for pancreatic cancer in *BRCA1* carriers was 2.49%[CI95:0.6%-4.3%] and in *BRCA2* carriers 6.42% [CI95:3.2%-9.5%]. The general population risk is 1.3. The risk for colorectal cancer (CRC) in *BRCA1* carriers was 3.2%[CI95:1.1%-5.3%] and in *BRCA2* 1.95%[CI95:0.3%-3.6%], lower than population risks of 3.6. Risks for gastric and esophageal cancer in *BRCA1* carriers were 2.2%[CI95:0%-4.3%] and 2.5%[CI95:0.05%-4.5%], respectively. In *BRCA2* risks were 1.6%[CI95:0.3%-2.9%] and 0.3%[CI95:0%-0.9%]. Population risks are 0.85% for gastric and 0.045% for esophageal cancers.

Conclusion: Two cohorts of *BRCA1/BRCA2* carriers demonstrated a modest increase in pancreatic, gastric, and esophageal cancer risks, whereas no increased CRC risk was observed. These data need to be validated in larger ethnically diverse cohorts. Yet, it seems clear that heightened CRC surveillance is not warranted in *BRCA1/BRCA2* carriers.

C.O.I. Disclosure:

O. Murik: None. **S. Lieberman:** None. **A. Tomer:** None. **E. Levy-Lahad:** None.

EP19.013 Clinical and Genetic Features of Inherited Retinal Dystrophies in the Eastern European Population

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Aim: The study aimed to analyze the genetic heterogeneity and clinical variability of hereditary retinal diseases among Russian patients.

Materials and methods: The study included 3,465 patients from 2,307 families, of whom 1,163 underwent molecular genetic testing. Blood samples were used for DNA analysis. All patients underwent a comprehensive ophthalmological examination, which included various clinical and instrumental methods such as optical coherence tomography and electrophysiological testing. Molecular genetic analysis involved Sanger sequencing and next-generation sequencing (NGS), which was customized for specialized panels or used for clinical and exome analysis.

Results: The study identified key genes responsible for the development of retinitis pigmentosa (56.25% of cases). The most significant of these were RPGR (10.9% of all cases), SNRNP200 (9.4%), EYS (7.81%), ABCA4 (7.8%), PRPF31 (7.8%), USH2A (6.25%), and PRPF8 (6.2%). Additionally, the most common genes associated with cone-rod dystrophy and congenital night blindness were identified—ABCA4 and PITPNM3, each occurring in 17.4% of cases. Furthermore, three major genes associated with Leber's amaurosis were determined: RPE65 (31.2%), GUCY2D (21.9%), and CEP290 (15.6%). In half of the cases related to RPE65 mutations, the changes were localized in exon 4.

Conclusions: The established clinical and genetic spectrum of hereditary retinal diseases enabled accurate diagnosis of specific pathologies, facilitated medical and genetic counseling, guided practical healthcare interventions, identified patients eligible for gene replacement therapy, and helped form study cohorts for further research and the development of promising treatment approaches for various posterior segment disorders of the eye.

C.O.I. Disclosure:

V. Kadyshev: None. **A. Stepanova:** None. **O. Shagina:** None. **S. Kutsev:** None. **R. Zinchenko:** None.

EP19.014 Integrated clinical risk prediction of type 2 diabetes with a multifactorial polygenic risk score

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Background: Combining information from multiple GWASs for a disease and its risk factors has proven a powerful approach for development of polygenic risk scores (PRSs). This may be particularly useful for type 2 diabetes (T2D), a highly polygenic and heterogeneous disease where the additional predictive value of a PRS is unclear.

Material and methods: A meta-scoring approach was used to develop a metaPRS for T2D that incorporated genome-wide associations from both European and non-European genetic ancestries and T2D risk factors. Its performance was evaluated and benchmarked against existing genome-wide PRS in 620,059 participants and 50,572 T2D cases amongst six diverse genetic ancestries from UK Biobank, INTERVAL, the All of Us Research Program, and the Singapore Multi-Ethnic Cohort. We further evaluated the metaPRS for enhancing guideline-recommended screening when used alongside conventional risk factors (QDiabetes risk scores).

Results: The metaPRS was the most powerful PRS for predicting T2D in European population-based cohorts and had comparable performance to the top ancestry-specific PRS, highlighting its transferability. In UK Biobank, the metaPRS had stronger predictive power for 10-year risk than all individual risk factors apart from BMI and biomarkers of dysglycemia. The metaPRS modestly improved T2D risk stratification of QDiabetes risk scores for 10-year risk prediction, particularly when prioritising individuals for blood tests of dysglycemia.

Conclusion: Overall, we present a highly predictive and transferrable PRS for T2D and demonstrate that the potential for PRS to incrementally improve T2D risk prediction when incorporated into UK guideline-recommended screening and risk prediction with a clinical risk score.

C.O.I. Disclosure:

S.C. Ritchie: None. **H.J. Taylor:** None. **Y. Liang:** None. **H.D. Manikpurage:** None. **L. Pennells:** None. **L. Pennells:** None. **C. Foguet:** None. **G. Abraham:** A. Employment (full or part-time); Significant; CSL Ltd. **J.T. Gibson:** None. **X. Jiang:** None. **Y. Liu:** None. **Y. Xu:** None. **L.G. Kim:** None. **A. Mahajan:** A. Employment (full or part-time); Significant; Genentech. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Roche. **M.I. McCarthy:** A. Employment (full or part-time); Significant; Genentech. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Roche. **S. Kaptoge:** None. **S.A. Lambert:** None. **A. Wood:** None. **X. Sim:** None. **F.S. Collins:** None. **J.C. Denny:** None. **J. Danesh:** F. Consultant/Advisory Board; Modest; AstraZeneca, Novartis, UK Biobank. **A.S. Butterworth:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; AstraZeneca, Bayer, Biogen, BioMarin, Bioverativ, Novartis, Regeneron, Sanofi. **E. Di Angelantonio:** None. **M. Inouye:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; AstraZeneca, Nightingale Health, Pfizer. F. Consultant/Advisory Board; Modest; Open Targets. F. Consultant/Advisory Board; Significant; Public Health Genomics Foundation.

EP19.015 Clinical-genealogical and molecular-genetic study of hereditary diseases in Yakutia (Russia)

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North-Eastern Federal University, Yakutsk, Russian Federation.

Background: The study aimed to determine the spectrum of hereditary diseases in Yakutia.

Material and methods: Clinical-genealogical methods based on the Genetic Register of Hereditary Pathology of Yakutia (data from 2000–2024) and molecular-genetic methods were used.

Results: Yakutia is one of the biggest regions in the North-East of Russia. There were 180 nosological forms of hereditary diseases.

In total, out of 2708 patients with diseases, 56.8% had AD, 36.3% had AR, 6.4% had X-linked. In terms of the number of patients, the most common AD diseases in the Republic of Sakha (Yakutia) are spinocerebellar ataxia type 1 (SCA1) - 323 patients, neurofibromatosis types 1 and 2–198, myotonic dystrophy 1 (MD1) - 173, oculopharyngeal muscular dystrophy (OPMD)- 119. Among AR diseases, are Gilbert's syndrome - 199 patients, methemoglobinemia type 1–91, SOPH syndrome - 89, Three M syndrome - 74, among X-linked - ichthyosis and hemophilia A. Yakuts significantly predominate - 80.0%, and Russians are 16,8%. 57.3% of patients live in rural areas, and 42.7% - in the city of Yakutsk. High prevalence in the ethnic group of Yakuts of such diseases as SCA1, OPMD 1, methemoglobinemia type 1, SOPH syndrome, Charcot-Marie-Tooth neural amyotrophy (type VI C), Tree M-syndrome 1, Cataract 18, Ichthyosis congenital autosomal recessive 3, NCL type 6 was shown. Phenylketonuria and cystic fibrosis were detected only among Russians.

Conclusion: A significant contribution to the structure of hereditary diseases is made by both AD diseases and AR diseases.

C.O.I. Disclosure:

N.R. Maksimova: None. **A.L. Sukhomyasova:** None. **P.I. Golikova:** None.

EP19.016 Exploring the causal relationship between body mass index and kidney function using tissue-partitioned mendelian randomization

Artemis Briasouli[1], *Genevieve Leyden*[2], *Aristomo Andries*[1], *Stein Ivar Hallan*[1], *Bjørn Olav Åsvold*[1], *Tom Richardson*[2], *Humaira Rasheed*[1], *Ben Michael Brumpton*[1]

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Background: BMI is a risk factor for chronic kidney disease (CKD), a significant contributor to global mortality. Mendelian randomization (MR) studies confirm BMI's causal role in CKD, but identifying specific BMI pathways remains challenging. Genetic studies suggest BMI-associated loci influence body composition through appetite regulation in the central nervous system (CNS) and fat distribution linked to adipose tissue, indicating distinct mechanisms. We used tissue-specific gene expression to disentangle BMI-related phenotypes' effects on kidney function and CKD.

Material and methods: We applied univariable and multivariable MR using genetic variants associated with BMI that colocalize with gene expression in brain (140 loci) or subcutaneous adipose tissue (86 loci). GWAS estimates were obtained for glomerular filtration rate (eGFR) using serum creatinine (N = 1,046,070), eGFR using cystatin C (N = 460,826), blood urea nitrogen (BUN) (N = 852,678), CKD (eGFRcrea<60 ml/min/1.73m^2; 41,395 cases) and eGFR decline (34,874 cases).

Results: Univariable MR showed BMI pathways were associated with kidney function and CKD. However, multivariable MR found no differential effects on eGFR assessed by eGFRcrea ($beta_{adipose}$ = -0.01, 95% CI: −0.04 to 0.01, $beta_{brain}$ = 0.005, 95% CI: −0.02 to 0.02) or any other kidney function measurement (eGFRcys, BUN). Similarly, no differential effects were observed for CKD ($OR_{adipose}$ = 1.2, 95% CI: 0.87 to 1.65, OR_{brain} = 1.18, 95% CI: 0.9 to 1.54) or eGFR decline.

Conclusion: Our findings suggest no clear distinction between BMI pathways linked to kidney function via brain or adipose tissue. These findings imply that BMI reduction could mitigate CKD risk, without the need for tissue-specific interventions.

C.O.I. Disclosure:

A. Briasouli: None. **G. Leyden:** None. **A. Andries:** None. **S.I. Hallan:** None. **B.O. Åsvold:** None. **T. Richardson:** None. **H. Rasheed:** None. **B.M. Brumpton:** None.

EP19.017 The effect of interactions between genes involved in glucose metabolism and total energy and protein levels on lipid parameters

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Background: The single nucleotide polymorphism (SNP), rs780094, of glucokinase regulator (GCKR) gene is associated with triglycerides (TGs). Glucose-dependent insulinotropic polypeptide receptor (GIPR) SNP rs10423928 is associated with metabolic health. In this study, we aimed to analyze the association between GCKR SNP rs780094 and GIPR SNP rs10423928 with lipid and glucose levels, and to examine their individual interactions with total energy and protein intake in lipids and glucose concentrations.

Material and methods: A total of 268 healthy Saudi adults participated in the study. Dietary intake data was assessed using a 136-item validated semiquantitative food frequency questionnaire. Blood samples were obtained to analyze lipid profiles, including total cholesterol (TC) and TGs and two SNPs were genotyped using real-time polymerase chain reaction. Univariate regression was used to examine the interactions between SNPs and total energy and protein intake on lipids and glucose concentrations.

Results: A significant interaction was observed between protein intake and GCKR SNP rs780094 in determining TC levels (P interaction = 0.004), protein intake shown to decrease risk of TC among CC carriers (-0.4 ± 0.1) than T allele carriers consume high protein intake. Furthermore, marginal interactions were observed between total energy intake and SNP rs780094 in determining TGs (P interaction = 0.008), also, with SNP rs10423928 on TC (P interaction = 0.04) and TGs (P interaction = 0.02).

Conclusion: Protein intake may be associated with decreased TC levels among carriers of CC genotype with GCKR SNP rs780094. Additional studies on the Saudi population with larger sample sizes and longitudinal design are needed in the future.

C.O.I. Disclosure:

I.M. Shatwan: None. **M. Al-odinan:** None. **N.M. Aljefree:** None. **N.M. Almoraie:** None. **M. Bakarman:** None. **H.A. Alhadrami:** None.

EP19.018 Introducing a precision effectiveness model that predicts the 1-year weight loss response to semaglutide

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Background: The weight loss response to the GLP-1 receptor agonist semaglutide varies considerably among individuals, with many possible contributing factors. Leveraging data from a network of clinico-genomic cohorts, we developed a precision effectiveness model that predicts the likelihood that patients will achieve ≥10% weight loss within one year of semaglutide treatment.

Material and methods: This longitudinal study utilized clinical-grade exome sequencing and electronic health record data from seven U.S. cohorts contributing Helix Research Network™ data

(n = 134,806). This analysis included patients initiating semaglutide by February 2024 with a BMI ≥ 27 kg/m^2 and no type 2 diabetes diagnosis. A BMI polygenic score (PGS) was calculated using 26,941 variants (PGS001228). Weight loss likelihood was modeled using logistic regression, incorporating dose information, demographics, BMI PGS, comorbidities, and other medications. Model performance was assessed through 5-fold cross-validation.

Results: Among 1,153 semaglutide users, the mean pretreatment BMI was 37 kg/m^2. Overall, 37% achieved ≥10% weight loss within one year; 49% of those with ≥1.7-mg dosing achieved ≥10%. The prediction model demonstrated good discriminative ability (average AUC = 0.714). After computing sex-and ancestry-normalized ranks, the weight loss success rates among users with ≥1.7-mg dosing increased with increasing prediction likelihood: 36% success in the bottom quintile vs 67% in the top quintile (Table).

Conclusion: These findings highlight the importance of precision medicine in obesity management. Integrating genetic and metabolic risk profiling enables more accurate weight loss expectations, optimized patient selection, personalized dosing strategies, and tailored counseling to improve treatment outcomes.

Predicted treatment success and proportions achieving 10%+ weight loss on 1.7- to 2.4-mg semaglutide

Ranked prediction likelihood quintile	% achieving ≥10% weight loss (95% CI)
Low (quintile 1)	35.6% (25.6% to 45.7%)
Lower than average (quintile 2)	41.7% (31.8% to 51.5%)
Average (quintile 3)	49.5% (40.2% to 58.9%)
Higher than average (quintile 4)	48.5% (38.9% to 58.2%)
High (quintile 5)	67.3% (58.4% to 76.2%)

C.O.I. Disclosure:

M.E. Levy: A. Employment (full or part-time); Significant; Helix. **N. Telis:** A. Employment (full or part-time); Significant; Helix. **L.M. McEwen:** A. Employment (full or part-time); Significant; Helix. **K.M. Barrett:** A. Employment (full or part-time); Significant; Helix. **A. Bolze:** A. Employment (full or part-time); Significant; Helix. **S. White:** A. Employment (full or part-time); Significant; Helix. **N.L. Washington:** A. Employment (full or part-time); Significant; Helix. **W. Lee:** A. Employment (full or part-time); Significant; Helix. **J. Lu:** A. Employment (full or part-time); Significant; Helix. **E.T. Cirulli:** A. Employment (full or part-time); Significant; Helix.

EP19.019 Mammographic density, breast cancer susceptibility gene variants and breast cancer risk

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Background: Mammographic density (MD) and protein-truncating variants (PTVs) in breast cancer susceptibility genes are major determinants of breast cancer risk, but their association and joint effects on breast cancer risk are unclear.

Material and methods: We analysed data from 6,809 cases and 18,189 control participants with targeted sequencing or array genotyping and MD data as part of the BCAC. MD measures, including dense area (DA), non-dense area (NDA), and percentage density (PD), were scored using Cumulus or Stratus. We investigated the association between MD measures and burdens of PTVs in ATM, BARD1, BRCA1, BRCA2, CHEK2, PALB2, RAD51C and RAD51D and their joint effects on breast cancer risk. Mendelian Randomisation (MR) analyses were used to investigate causality between MD measures and breast cancer outcomes.

Results: There was no evidence of an association between the overall burdens of PTVs and any MD measures. Some evidence for a negative interaction between the overall burden of PTVs and PD (OR = 0.79, 95%CI$_{int}$ = 0.62, 1.00) was found, largely driven by a positive interaction with NDA. MR analyses indicated attenuated effects among *BRCA1* but not *BRCA2* carriers (for PD and breast cancer risk, OR = 1.54 (95% CI: 1.31, 1.80) in the general population, 1.02 (95% CI: 0.78, 1.34) in *BRCA1* carriers).

Conclusion: There was no evidence for an association between MD measures and breast cancer susceptibility genes, but some suggestion that the association between MD and breast cancer risk may be weaker, particularly in *BRCA1* PTV carriers.

C.O.I. Disclosure:

X. Zhang: None. **M. Eriksson:** None. **N. Mavaddat:** None. **J. Dennis:** None. **C.M. Vachon:** None. **P. Hall:** None. **D.F. Easton:** None.

EP20 Pharmacogenomics and Drug Repurposing

EP20.001 Analysis of the association between *MMP-9* gene polymorphisms and the occurrence of hemorrhagic transformations in acute ischemic stroke patients treated with thrombolysis

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Background: Acute ischemic stroke (AIS) is a leading cause of disability and mortality worldwide. The administration of recombinant tissue plasminogen activator (rtPA) has shown potential in mitigating the long-term effects of AIS, yet it carries a risk of hemorrhagic transformation (HT). Matrix metalloproteinase-9 (MMP-9) has been implicated in exacerbating cerebral injury following AIS. Genetic variations, specifically the rs3918242 and rs3918249 polymorphisms within the MMP-9 gene, may influence its expression and, consequently, the susceptibility to rtPA-induced HT. This study aimed to investigate the relationship between these polymorphisms, their haplotypes, and the occurrence of rtPA-related HT.

Material and methods: The study involved a cohort of 158 consecutive AIS patients who received rtPA therapy. HT was monitored throughout their hospitalization. Genotyping was conducted using Real-Time PCR methodology, while haplotype analysis was carried out using the Haploview software.

Results: No statistically significant association was observed between the frequencies of the rs3918242 and rs3918249 genotypes and the occurrence of HTin patients with AIS (p = 0.787, p = 0.428, respectively). Similarly, no statistical

significance was identified when genotypes were grouped according to dominant or recessive inheritance models for either polymorphism, nor during haplotype analysis. Haplotype block was found between MMP-9 gene rs3918242 and rs3918249 polymorphisms ($D' = 0.96$, $r2 = 0.35$). Our patients had TT, TC and AC haplotypes (71.5%, 15.6%, 12.6%, respectively).

Conclusion: The results suggest no significant association between rs3918242 and rs3918249 polymorphisms, their haplotypes, and HT risk in AIS patients, highlighting the need for larger studies to confirm these findings.

C.O.I. Disclosure:

M.G. Dusanovic Pjevic: None. **L. Sarenac:** None. **M. Grk:** None. **B. Jekic:** None. **L. Vojvodic:** None. **T. Damnjanovic:** None. **M. Rasic:** None. **D. Perovic:** None. **N. Maksimovic:** None.

EP20.002 Pharmacogenomic-guided opioid therapy for pain: a systematic review and meta-analysis of randomised controlled trials

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Background: Inter-individual variability in opioid response is significantly influenced by genetics, impacting both analgesic efficacy and adverse effect profiles. Pharmacogenomic-guided opioid therapy offers a promising approach to personalise pain management. This meta-analysis of randomised controlled trials investigates whether genotype-informed opioid prescribing improves clinical outcomes compared to standard care.

Materials and methods: A systematic review was conducted following PRISMA guidelines. Embase, Medline, Web of Science, and Cochrane databases were searched. Risk of bias was assessed using the Cochrane ROB 2 tool, and evidence certainty was evaluated using GRADE criteria. A meta-analysis was performed using a random effects model.

Results: From 2,496 screened articles, six randomised controlled trials met inclusion criteria. Meta-analysis revealed that pharmacogenomic-guided prescribing significantly reduced opioid consumption (SMD −0.38; 95% CI −0.67 to −0.08; $p = 0.01$). While pain intensity at follow-up trended lower in the pharmacogenomic group, this difference was not statistically significant (SMD −0.21; 95% CI −0.65 to 0.24; $p = 0.36$).

Conclusion: This meta-analysis provides evidence that pharmacogenomic-guided opioid prescribing leads to a significant reduction in opioid consumption. These findings suggest the potential of genetic information to optimise opioid therapy and improve patient outcomes. Further high-quality trials with larger cohorts are warranted to validate these results and explore the clinical impact on pain intensity and adverse events.

C.O.I. Disclosure:

S. Jethwa: None. **K. Langlands:** None. **M. Ball:** None.

EP20.003 Integrating pharmacogenomics into pediatric care: a scoping review of test counseling, expectations, concerns, and attitudes of patients and their families

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Background: "How can pharmacogenomics testing help my child's health?" is a frequent question by families when informed about pharmacogenomic (PGx) testing. PGx testing personalizes medicines by determining genetic variations that can alter a drug's response, potentially improving drug efficacy and reducing adverse effects. This scoping review explores current literature on pediatric patients' and their parents' expectations, concerns, and perceptions of the PGx testing.

Method: The scoping review followed the recommendations of the *Scoping Studies Towards Methodological Framework*. Inclusion Criteria were peer-reviewed, English-language journals with full-text access that addressed communicating PGx test results to pediatric patients or their parents/caregivers or described experiences, perspectives, attitudes, and concerns regarding PGx testing up to Dec 2024. A total of 1,604 articles were screened, resulting in 75 full-text studies being assessed. Ten studies were selected for data extraction.

Results: All studies indicate a positive attitude of pediatric patients and their parents and caregivers towards PGx testing. Key themes included: understanding and interpreting PGx test results, the expectation of PGx testing utility and perceived benefits, psychological reactions to results, the significance of informed consent and shared decision-making for future treatment, impact on the patient-provider relationship, and ethical, legal, and social implications.

Conclusion: As PGx testing continues to expand in pediatric care, effective and clear communication between healthcare providers, patients, and parents/caregivers is essential. Ensuring mutual understanding of the tests, result interpretation, benefits, and limitations can help families make informed decisions regarding their child's health.

C.O.I. Disclosure:

N. Tabassum: None. **M. Le:** None. **M. Hasan:** None. **M. Aziz:** None. **A. Maruf:** None.

EP20.004 Influence of CYP2C9 and VKORC1 gene polymorphisms on the anticoagulant efficacy of warfarin

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Background: Warfarin is a commonly prescribed oral anticoagulant with narrow therapeutic range. Polymorphisms in CYP2C9 and VKORC1 are known to influence warfarin metabolism and dosage requirements, but data on their impact in the Taiwanese population remain limited.

Material and methods: We retrospectively enrolled 356 adult patients (mean age 58.37 ± 14.41) from a single medical center in Taiwan. All participants received warfarin for ≥3 months, and stable daily dose was defined by consecutive INR assessments. Genotyping for CYP2C9*3 (1075A > C) and VKORC1 c.-1639 G > A was performed using the Affymetrix TWB 2.0 array, followed by standard quality control. Associations between genotypes and stable warfarin dose, as well as risk of over-anticoagulation (INR > 4.0), were analyzed.

Results: The most prevalent genotype was CYP2C9*1/1 (91.57%), while 8.15% had CYP2C9*1/3, and 78.93% carried the VKORC1 AA variant. Carriers of CYP2C9*3 or VKORC1 c.-1639 A required significantly lower warfarin doses ($p < 0.001$). In particular, the mean stable dose for CYP2C9*1/3 was 2.27 ± 1.06 mg/day versus 2.83 ± 1.53 mg/day for CYP2C9*1/*1. Patients with VKORC1 AA genotype also showed higher risk of over-anticoagulation compared to those with the GG genotype.

Conclusion: Our findings demonstrate that CYP2C9 and VKORC1 genotypes strongly influence warfarin dosage requirements and susceptibility to over-anticoagulation in Taiwanese patients. Incorporating genetic testing into clinical practice may help optimize warfarin therapy and reduce adverse events.

C.O.I. Disclosure:
Y. Chang: None.

EP20.005 Distribution of *NAT2* allelic frequencies in the moroccan population and its impact on personalized tuberculosis treatment

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Background: Several pathophysiological and genetic factors influence susceptibility to antituberculosis drug-induced hepatotoxicity (ATDH), particularly with isoniazid (INH). One of the major susceptibility genes for ATDH is N-acetyltransferase 2 (*NAT2*), which determines an individual's acetylator status (fast, intermediate, or slow) for metabolizing drugs and xenobiotics. Polymorphisms in the *NAT2* gene vary significantly across populations of different ethnic backgrounds.

Material and methods: This study aimed to determine the frequencies of different acetylator profiles in an in-house Exomes database of 220 Moroccan individuals, using in silico analysis, to predict the efficacy and potential adverse effects of tuberculosis treatment, particularly hepatotoxicity.

Results: Among the 11 identified *NAT2* variants, three were predominant: *NAT2*5* (c.341T>C; p.Ile114Thr) and *NAT2*6* (c.590G>A; p.Arg197Gln), both associated with a slow acetylator profile, with allele frequencies of 27% and 11.6% respectively, and *NAT2*12* (c.803G>A; p.Arg268Lys), associated with a fast acetylator profile, with an allele frequency of 25.5%.

Conclusion: The Moroccan population appears to be heterogeneous, with both slow and fast acetylators. This genetic variability should be considered in personalized medicine. A dose adjustment based on the *NAT2* genotype may be necessary for Moroccans to determine the optimal INH dose for tuberculosis treatment, thereby reducing adverse effects and improving treatment efficacy.

C.O.I. Disclosure:
I. Ratbi: None. **B. Nada:** None. **L. Jaber:** None. **R. Yasmina:** None. **C. Imane:** None. **S. Abdelaziz:** None.

EP20.006 Complex pharmacogenomic variants in the Slovenian reference genome

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Background: Most of the currently applied genotyping technologies for pharmacogenomic variation profiling are based either on single nucleotide variant microarrays or short-read next-generation sequencing (NGS). Both approaches are limited in characterizing complex regions, as several pharmacogenes are partly located in complex genomic regions or contain tandem repeats and pseudogene hybrid conformations.Our aim is to assess the limitations of the short-read whole genome sequencing (WGS) in providing clinically relevant pharmacogenomic information and to develop and provide a comprehensive validation of a pipeline for extracting and reporting actionable pharmacogenomic data from long-read WGS data.

Material and methods: Anonymized WGS data from The Slovenian Reference Genome Project, launched in January 2025 will be used. The first step is the evaluation of the capacity of short-read WGS data to provide evidence-based drug treatment recommendations in PharmCat (version 2.12.0). The next step is the construction of a pipeline for extracting and reporting on the actionable pharmacogenomic data from long-read WGS data. Finally, a validation comparing results from both NGS techniques will be provided.

Results: Our preliminary analysis of the short-read WGS data showed that PharmCat (version 2.12.0), enables calling of only 9 out of 22 relevant genotypes. We expect that long-read WGS data will resolve complex pharmacogenomic variants (e.g., deletions, duplications, or rearrangements in the *CYP2D6* genes) and enable calling and reporting recommendations for all clinically relevant pharmacogenes.

Conclusion: Establishing a national Slovenian reference genome and bioinformatics pipeline for long-read WGS data will enable better prediction of the efficacy and safety of drug treatment.

C.O.I. Disclosure:
U. Janžič: None. **B. Jenko Bizjan:** None. **J. Kovač:** None. **T. Battelino:** None. **V. Dolžan:** None.

EP20.007 Genetic variability of *HIF1A* and treatment response to cisplatin based chemotherapy in malignant mesothelioma

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Background: Malignant mesothelioma (MM) is a rare and aggressive tumor of the pleura and peritoneum, predominantly associated with asbestos exposure. Despite multimodal treatment strategies, MM prognosis remains poor as most patients are diagnosed at an advanced disease stage and the tumor is not very chemosensitive. Current chemotherapy regimens usually include cisplatin/pemetrexed or cisplatin/gemcitabine doublets. Chemosensitivity of mesothelioma cell spheroids was shown to be related to their ability to adapt to hypoxic conditions. As such conditions increase the levels of hypoxia-inducible factor 1A (HIF-1A), this may lead to increased resistance to cisplatin-based treatment. Our aim was to evaluate the association of *HIF1A* polymorphisms with chemotherapy response in Slovenian MM patients.

Methods: This retrospective study included 234 patients with histologically confirmed MM treated with cisplatin/pemetrexed or cisplatin/gemcitabine doublets at the Institute of Oncology in Ljubljana. *HIF1A* rs11549465, rs11549467 and rs2057482 were genotyped by competitive allele-specific PCR. Logistic regression, non-parametric tests or the Cox proportional hazards model were used to assess the associations of these polymorphisms with response to chemotherapy, progression-free survival, and overall survival in MM.

Results: *HIF1A* rs11549467 and rs2057482 were not associated with any of the cisplatin-based treatment outcomes. However, carriers of the *HIF1A* rs11549465 CT genotype showed a slightly reduced, albeit statistically significant response to chemotherapy

after adjustment for weight loss and C-reactive protein (CRP) ($p = 0.044$).

Conclusion: Among the investigated *HIF1A* polymorphisms, only rs11549465 plays some, but minor role in treatment response to cisplatin based chemotherapy in MM.

C.O.I. Disclosure:

Ž. Doljak: None. **M. Šetina:** None. **E. Šetina:** None. **K. Goričar:** None. **V. Kovač:** None. **V. Dolžan:** None.

EP20.008 A pilot study in the Bulgarian population about the impact of genetic polymorphisms in the IL6R gene and the NPR3, C5orf23 locus on clopidogrel response variability

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Background: Dual antiplatelet therapy with aspirin and clopidogrel is routinely used to prevent thrombotic events after coronary stent implantation. Several studies have associated high residual platelet reactivity after clopidogrel treatment with an increased risk of ischemic events. The present study investigated the potential association between polymorphisms in the *IL6R* gene rs2229238 and the *NPR3,C5orf23* locus rs1173771 and the benefits of clopidogrel therapy.

Material and methods: A total of 104 patients with coronary stent placement and an increased risk of thrombosis were divided into two groups: those with a good response to clopidogrel therapy($N = 54$) and with a poor response($N = 50$). Inhibition of ADP P2Y12 receptors at clopidogrel treatment was determined by measuring ADP-induced platelet aggregation (ADP 6.5 µM) in whole blood. Genomic DNA was isolated from frozen whole blood by phenol-chloroform extraction. The selected polymorphisms were genotyped by TaqMan SNP Genotyping Assay. Non-adjusted χ^2-based analysis was applied for evaluation of genotype and allele association with clopidogrel response by PLINK1.07.

Results: The presence of a polymorphic G allele for rs1173771 in the *NPR3,C5orf23* locus was associated with a twofold increased risk of clopidogrel resistance compared to wild-type (OR2.49(1.41–4.39) $p = 0.016$). The presence of a polymorphic T allele for rs3918226 in the IL6R gene showed a favorable influence on clopidogrel resistance compared to wild-type (OR0.48(0.24–0.96) $p = 0.04$).

Conclusions: The present study sought to ascertain the potential contribution of polymorphic variants in *IL6R* gene rs2229238 and *NPR3,C5orf23* locus rs1173771 to platelet reactivity and variability in response to clopidogrel therapy. These findings must be clarified by further genetic and functional analyses.

C.O.I. Disclosure:

R.S. Tzveova: None. **V. Baycheva:** None. **D. Pendicheva-Duhlenska:** None. **D. Dineva:** None. **V. Mitev:** None. **I. Paskaleva:** None. **R. Kaneva:** None.

EP20.009 Synthetic ACTH analogue impacts on gene expression pattern of SH-SY5Y human neuroblastoma cells under peroxide oxidative stress

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Background: Oxidative stress (OS) is important pathogenic mechanism of many diseases, including stroke. Synthetic ACTH-analogue (Met-Glu-His-Phe-Pro-Gly-Pro, ACTH(4-7)PGP) are being actively studied as neuroprotective drugs for stroke therapy. Additionally, ACTH(4-7)PGP demonstrates compensatory effect on gene expression pattern disrupted by ischemia-reperfusion in rat brain. However, genesis of its impact on individual stages of the ischemic cascade remains unexplored. We aimed to study the effect of ACTH(4-7)PGP on the gene expression of SH-SY5Y human neuroblastoma cells under H_2O_2-induced OS.

Material and methods: SH-SY5Y cells, H_2O_2-induced OS, real-time RT-PCR.

Results: Twenty-four hours after 20min exposure to 40µM H_2O_2, 48% of cells had round shape and smaller volume, which are signs of cell death. When 1mM ACTH(4-7)PGP was added after cells exposure to H_2O_2, a decrease in the proportion of round cells to 28% was observed after 24h. Real-time RT-PCR analysis showed that the expression level of *Bax, IL1b, Tnf, Hspb1*, and *Nr4a1* genes was increased by 1.3 to 3.9 times under the influence of OS, while it was decreased by 10 to 30% under the influence of ACTH(4-7) PGP.

Conclusion: ACTH(4-7)PGP increases the survival of SH-SY5Y cells a day after H_2O_2-induced OS. Furthermore, the peptide could exert its neuroprotective effect, including by controlling the genetic response to OS-induced inflammation and apoptosis. We expect that further genome-wide gene expression studies may provide known more about regulation of peptide activity in cells under OS. The work was carried out within the state assignment of NRC "Kurchatov Institute" (gene expression analysis) and grant (24-14-00027) of RSF (cell culturing).

C.O.I. Disclosure:

O.Y. Sudarkina: None. **E.V. Gurianova:** None. **D.A. Ales:** None. **N.F. Myasoedov:** None. **S.A. Limborska:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Russian Science Foundation (24-14-00027). C. Other Research Support (supplies, equipment, receipt of drugs or other in-kind support); Significant; The state assignment of NRC "Kurchatov Institute". **L.V. Dergunova:** None. **I.B. Filippenkov:** None.

EP20.010 Allele frequencies of clinically relevant *DPYD* polymorphisms in Bulgarian population

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Background: The *DPYD* gene encodes dihydropyrimidine dehydrogenase (DPD), enzyme playing key role in metabolism of pyrimidine nucleotides and certain chemotherapy drugs. Variations in this gene can impair DPD activity, leading to reduced/absent enzyme function. This can affect individual's response to chemotherapy drugs (fluorouracil (5-FU) and capecitabine). Four variants are recommended by CPIC to be tested related to 5-FU treatment (Amstutz et al,2017). Data about their allele frequencies in Bulgarian population is limited and mainly from clinical studies. It is essential to have better estimates of their population

frequency to explore their impact on toxicity risks and optimize pharmacotherapy.

Material and methods: The allele frequencies of *DPYD* polymorphisms were estimated in a cohort of 444 Bulgarian patients, from a previous COVID-19 cohort study. Following informed consent, genomic DNA was isolated, and whole exome sequencing (WES) was conducted using the Illumina DNA prep with enrichment protocol on the Illumina NovaSeq6000 platform. Allele frequencies of the selected variants were calculated and compared with European (non-Finnish) reference population using gnomAD database.

Results:

Variant	Population allele frequencies	
	Bulgarian	European (non-Finnish)
*2A, rs17376848, (c.1896T>C)	3,15%	4,22%
*13, rs2811178, (c.1740+40A > G)	5,54%	5,92%
HapB3, rs56038477, (c.1236G>A)	2,47%	2,11%
HapB3, rs56293913, (c.1129-15T > C)	14,07%	11,77%
rs67376798, (c.2846A>T)	0,37%	0,51%

Conclusion: Our results show that the allele frequencies of the tested polymorphisms in Bulgarian population were similar to reference European population with slightly increased HapB3, rs56293913. The knowledge about the *DPYD* allele frequencies is important for evidence-based pharmacotherapy in Bulgarian cancer patients.

C.O.I. Disclosure:

K. Stefanova: None. **K. Mihova:** None. **K. Kamenarova:** None. **M. Georgiev:** None. **D. Georgiev:** None. **I. Dimova:** None. **R. Kaneva:** None.

EP20.011 Bulgarian patients with Chronic lymphocytic leukemia - IGHV mutational status genotyping

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Background: The most common leukemia in adults is Chronic lymphocytic leukemia (CLL). There are many well-known markers associated with CLL, but the best-established prognostic marker is the mutational status of the immunoglobulin heavy chain variable region (IGHV) genes.

Material and methods: IGHV genotyping technology has been optimized and applied for the first time in routine diagnostics in Bulgaria. ERIC recommendations are strictly followed throughout the testing process. Sanger sequencing was applied to determine IGHV mutation status is determined on gDNA or cDNA extracted from venous blood. All sequencing profiles were analysed by the IMGT/V-QUEST tool, and IG-blast was also used for some of the samples.

Results: A total of 449 patients were analyzed, of which 215 were genotyped as unmutated IGHV (U-CLL), 215 - mutated (M-CLL), and 18 - borderline (B-CLL). In 1/449 patients an unproductive rearrangement was detected. Out of 449 patients, 281 were male and 168 were female. The most frequent HV genes in our samples are: IGHV1-69 (n = 65), IGHV3-23 (n = 39), IGHV1-2 (n = 22), IGHV3-7 (n = 24) and IGHV4-34 (n = 44). Subsets CLL#2 (n = 7) and CLL#8 (n = 2) were determined in 9/449 (2%) patients. During the IGHV genotyping in one patient an interesting case with multiple rearrangements was detected. In that one patient 2-70*01/3-30*01/4-4*02 alleles were detected with discordant status.

Conclusion: The present data from IGHV genotyping greatly contributed for the proper treatment of the Bulgarian CLL patients.

C.O.I. Disclosure:

A. Yosifova: C. Other Research Support (supplies, equipment, receipt of drugs or other in-kind support); Modest; Johnson & Johnson Bulgaria. **A. Todorova:** C. Other Research Support (supplies, equipment, receipt of drugs or other in-kind support); Modest; Johnson & Johnson Bulgaria.

EP20.012 Pharmacogenetic variability in the Iranian population: Insights from 123 variants across nine key genes

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Background: Pharmacogenomics (PGx), as a growing field of personalized medicine, aims to optimize the efficacy and safety of medications by studying the association between germline genetic variations and drug responses. The present cross-sectional study aims to evaluate the allele frequency of the 123 genetic variations of nine pharmacogenes, including CYP2C9, CYP2C19, CYP2D6, DPYD, SLCO1B1, UGT1A1, VKORC1, TPMT, and NUDT15, in the Iranian population to provide insights into personalized treatment decisions in the Iranian population. Notably, DPYD results are based on previously published data.

Material and methods: A representative sample set of 1142 unrelated healthy Iranian individuals over 18 years of age, regardless of disease or medication use, were genotyped using the Infinium Global Screening Array-24 BeadChip.

Results: We identified 27 out of 123 pharmacogenetic variants with minor allele frequency (MAF) ≥ 1%, including *CYP2D6* (*41), *CYP2C9* (*3), *CYP2C19* (*2), *UGT1A1* (*6 and *80), *DPYD* (*4, *5, *6, 9A, HapB3; c.1236-T; c.1129-5923-C, c.1896-G, c.496-C), *NUDT15* (*2 and *3), *SLCO1B1* (*15; c.388A/G; c.521-C, *SLCO1B1*5* and *20), and nine variants of *VKORC1* among the present studied population,

which may explain the substantial variability in drug response phenotypes among different populations.

Conclusion: The results reveal 27 more important variants in nine important pharmacogenes due to their high prevalence in the study population that could significantly influence clinical decision-making. These variants, with their potential to explain the substantial variability in drug response phenotypes among different populations, shed light on a crucial aspect of pharmacogenomics.

C.O.I. Disclosure:

N. Sarhangi: None. **N. Niknam:** None. **F. Sharifi:** None. **S. Nikfar:** None. **B. Larijani:** None. **G. Patrinos:** None. **M. Hasanzad:** None.

EP20.013 Monogenic Disorders Associated with Adverse Drug Reactions in the Russian Population: Implications for Pharmacogenomic Testing

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Background: Adverse drug reactions (ADRs), up to 50% of which are attributed to genetic variability, remain a significant public health issue. Pharmacogenetic testing helps reduce ADR risk by optimizing drug selection and dosage, particularly in monogenic disorders.

Material and methods: Whole-exome sequencing of 6739 samples from the Russian population was performed using the MGIEasy Universal DNA Library Prep Set on the DNBSEQ-G400 platform (MGI). Variants in 47 genes were examined, focusing on channelopathies (Long QT syndrome, Timothy syndrome, Andersen-Tawil syndrome, Brugada syndrome), enzyme deficiencies (Glucose-6-Phosphate Dehydrogenase Deficiency, porphyrias), and malignant hyperthermia. All identified variants had been reported at least once as pathogenic (P) or likely pathogenic in ClinVar, along with those occasionally classified as variants of uncertain significance (VUS). Each variant was manually re-evaluated according to ACMG criteria.

Results: A total of 61 unique variants in 13 genes were observed in 103 individuals (1.53%), including 17 carriers and 13 women with a *G6PD* mutation. Of these, 46 variants were classified as P, 14 as LP, and 1 as VUS. Missense variants accounted for the largest proportion (70.49%). The most affected genes were *KCNQ1* (24/103), which exhibited the highest number of unique variants (18), *RYR1* (20/103), and *G6PD* (20/103). Regarding associated conditions, mutations linked to Long QT syndrome or Brugada syndrome were found in 44 individuals, malignant hyperthermia in 33, Glucose-6-Phosphate Dehydrogenase Deficiency in 20, and porphyrias in 6.

Conclusion: This study reveals that at least 1.28% of the sampled population already has a genetically determined increased risk of ADRs.

C.O.I. Disclosure:

A.A. Buianova: None. **V.V. Cheranev:** None. **A.O. Shmitko:** None. **I.A. Vasiliadis:** None. **G.A. Ilyina:** None. **O.N. Suchalko:** None. **M.I. Kuznetsov:** None. **V.A. Belova:** None. **D.O. Korostin:** None.

EP20.014 miR-21 and miR-34a expression and genetic polymorphisms as biomarkers of radiotherapy response in ductal carcinoma in situ

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Background: Ductal carcinoma in situ (DCIS) is non-invasive breast cancer treated with surgery and adjuvant radiotherapy (RT). RT enables good disease control, but also causes various adverse events. Epigenetic regulation, such as miRNA expression, could contribute to the inter-individual variability of RT treatment outcome. Our aim was to determine if RT affects expression of miR-21 and miR-34a and if miRNA expression or genetic variability is associated with RT toxicity in DCIS.

Methods: Our prospective study included 119 DCIS patients treated with adjuvant RT. Skin and cardiac toxicity were evaluated immediately after RT and after 6 months. Quantitative PCR was used to determine plasma miR-21 and miR-34a expression before and after RT. Allele-specific PCR was used for genotyping of *MIR21* rs1292037 and *MIR34A* rs2666433 polymorphisms. Nonparametric tests and logistic regression were used in statistical analysis.

Results: Relative expression of miR-21 and miR-34a increased after RT ($P = 0.001$ and $P < 0.001$, respectively). Increased miR-34a expression after RT was associated with more skin toxicity after RT (OR = 1.13 (1.02–1.26), $P = 0.026$) and after 6 months (OR = 1.13 (1.01–1.27), $P = 0.035$). Increased miR-21 expression after RT was associated with more cardiac toxicity after RT: higher NYHA score (OR = 3.24 (1.34–7.87), $P = 0.009$) and higher NT-proBNP levels ($P = 0.044$). Higher miR-21 and miR-34a expression after RT was also associated with higher NT-proBNP after 6 months ($P = 0.023$ and $P = 0.028$, respectively). Investigated polymorphisms were not associated with RT toxicity.

Conclusion: Our results suggest RT affects miRNA expression and that miR-21 and miR-34a expression could serve as a biomarker of RT treatment outcome in DCIS.

C.O.I. Disclosure:

S. Redensek Trampuz: None. **A. Cvetkoska:** None. **V. Dolzan:** None. **T. Marinko:** None. **K. Goricar:** None.

EP20.015 Implementation of Pharmacogenetics in routine-diagnostics of patients with rare diseases

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Background: Pharmacogenetic testing has the potential to enable an individualized pharmacotherapy. While theoretical benefits have been demonstrated widespread implementation and concrete guidelines for pharmacogenetic testing strategies are still lacking in many countries e. g. Germany, hindering a transition from theoretical discussions to concrete clinical care.

Material and methods: We present preliminary data from an ongoing study, where we reanalyze NGS-short read genome data from rare disease patients for pharmacologically relevant variants. The lists of relevant variants were taken from two panels, the ubiquitous pharmacogenomics (U-PGX) panel and the association for molecular pathology pharmacogenomics working group (AMP-PGX Tier 1) panel. If available, trio data is used to overcome limitations of short-read sequencing data for the identification of complex star alleles. An interdisciplinary team of human geneticists and clinical pharmacologists carries out the genetic and bioinformatic analysis and interpretation of the results as well as the communication with the patients.

Results: We demonstrate that the majority of known highly relevant pharmacogenomic variants within these panels can be addressed using routine genome-data-sets normally used for the identification of rare genetic diseases. Our preliminary data show a

noticeable percentage (85 percent) of patients presenting clinically relevant variants in pharmacogenes.

Conclusion: Patients who undergo genome sequencing for identification of a rare genetic disease can benefit twice from the genetic analysis. Parallel pharmacogenetic testing might be of great added value for treatment strategies, particularly in the case of hereditary cancer, epilepsy and all other rare diseases where (especially long-term, multi-drug) pharmacotherapy is needed.

C.O.I. Disclosure:

J. Krause: None. **T. Krüger:** None. **K. Just:** None. **M. Elbracht:** None.

EP20.016 Comprehensive analysis of CYP2D6 genetic variations and their impact on recurrence in Syrian breast cancer patients treated with tamoxifen

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Background: In our previous study, we identified the frequency of five CYP2D6 allelic variants (2*, 4*, 10*, 41*, 69*) and their impact on recurrence rates in breast cancer patients treated with tamoxifen. To enhance our understanding of the interaction between CYP2D6 genetic variation and clinical outcomes, we expanded our research to include the detection of copy number variations (CNVs) and a total of 30 alleles.

Material and methods: We re-genotyped 99 eligible breast cancer patients for 19 CYP2D6 single nucleotide variations (SNPs) using reverse hybridization techniques (StripAssay), while CNVs were assessed through real-time quantitative PCR. Corresponding metabolizer phenotypes were allocated based on the updated activity score. Patients were categorized into two groups based on their response to tamoxifen therapy: 57 with recurrence and 42 without recurrence over five years. The relationship between genotypes and disease-free survival (DFS) was evaluated using the Kaplan-Meier method and log-rank test.

Results: Analysis of CYP2D6 phenotypes revealed that 66% of participants were normal metabolizers, with intermediate and ultra-rapid metabolizers accounting for 22% and 9%, respectively, while poor metabolizers constituted only 3%. The DFS analysis indicated that poor metabolizers showed a trend towards reduced survival compared to other metabolic categories (P-value = 0.053). Additionally, whole genome analysis identified three specific SNPs that caused allele drop-out, leading to homozygous outcomes in certain samples.

Conclusion: These findings underscore the need for careful interpretation of CYP2D6 genotyping results and highlight the importance of comprehensive genetic profiling in pharmacogenetic studies involving this complicated gene.

C.O.I. Disclosure:

L. Alkebbeh: None. **A. Berndt:** None. **C. Oberkanins:** None. **M. Kenj:** None. **J. Trupke:** None. **L. Youssef:** None.

EP21 Population Genetics and Evolutionary Genetics

EP21.001 Carrier screening for *MEFV* variants in Dagestan Republic RF

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Background/Objectives: Familial Mediterranean fever (FMF) has a certain ethnic specificity. Some ethnic groups inhabiting the Republic of Dagestan have a common origin with the Mediterranean populations in which FMF is widespread. There are no information about FMF and *MEFV* gene mutation carrier studies in Dagestan Republic.

Methods: The Sanger exons 9 and 10 *MEFV* gene sequence was performed for 390 healthy individuals from the indigenous populations of Dagestan.

Results: 36 people were found to have variants in the *MEFV* gene. The most common variants were p.Ile591Thr (allelic frequency = 0,015), p.Arg761His (0,009), p.Met694Val (0,006), p.Val726Ala (0,005), p.Ala744Ser (0,005). In addition, 4 VUS have been found by us in the investigated *MEFV* regions. We have determined the estimated frequency of the disease for 1:445.

Conclusion: Variants p.Ile591Thr and p.Ala744Ser have been described in literature in patients with mild phenotype. Based on this, it can be concluded that these variants cannot be used to calculate the FMF frequency. FMF frequency calculation based on pathogenic variants is 1:2105, which is significantly lower than in other Mediterranean populations.

C.O.I. Disclosure:

D. Lendoeva: None. **V. Kadnikova:** None. **A. Polyakov:** None. **G. Radzhabova:** None. **M. Radzhabov:** None. **O. Shchagina:** None.

EP21.002 Analysis of genetic ancestry with Y-Chromosome Haplotypes in an Latina American admixed population from Southern Caribbean: A report of the Cartagena Cohort Study -CaReS-

Eduer Pérez-Castilla[1], *Violeta Escudero-Morales*[1], *Maria Stephany Ruiz-Diaz*[1], *Gustavo Mora García*[1], *Sara Mestra-Meriño*[1], *Luisa Jimenez-Rodriguez*[1], *Diana Mena-Yi*[1], *Marilyn Jimenez-Agamez*[1], *Elmer Fernandez*[2], *Rodrigo Villegas*[3], *Vanessa Garcia-Larsen*[4]

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Background: Genetic structure in Latin-America and Caribbean (LAC) has been driven by a sex-biased admixture. Over the Spaniard colonial period, European males shaped the socio-demographic dynamics, which reflects on current distribution of European, African and Native American ancestry groups. Aim: Analyze the patrilineal ancestry of an admixed LAC population settled.

Material and methods: Unrelated males living in Southern Caribbean, enrolled in the Cartagena Cohort Study -CaReS-, were recruited. 17 Y-chromosome short tandem repeats (Y-STR) in non-recombinant regions were amplified, and fragment lengths were determined by capillary electrophoresis. Haplogroups were resolved through a Bayesian approach.

Results: A total of 300 males were included. Haplogroups for European ancestry were predominant (45%), followed by African ancestry haplogroups (26%). Haplogroups J lineage (Middle Eastern) accounted for 11%, while haplogroup Q (Native American) were 8%.

Conclusion: Patrilineal ancestry showed a complex diversity. Four relevant ancestry groups contributed to the composition of

contemporary genetic structure. These findings coincide with social history and demographic records.

C.O.I. Disclosure:

E. Pérez-Castilla: None. **V. Escudero-Morales:** None. **M. Ruiz-Diaz:** None. **G. Mora García:** None. **S. Mestra-Meriño:** None. **L. Jimenez-Rodriguez:** None. **D. Mena-Yi:** None. **M. Jimenez-Agamez:** None. **E. Fernandez:** None. **R. Villegas:** None. **V. Garcia-Larsen:** None.

EP21.003 Widespread differences in sequence context around rare and common nonsense variation in the human genome

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Background: Emergence of large-scale catalogues of human genome variation, such as the Genome Aggregation Database (gnomAD) allows researchers to identify various factors that determine the functional impact of genetic variants. In this study, we tested the impact of sequence context on the frequency of protein-truncating variants (PTVs) in the human genome.

Material and methods: Variants annotated as causing premature stop codons by Ensembl VEP were extracted from gnomAD v.4.1. Additional filtering of variants was performed based on gene-level constraint (LOEUF < 0.35) and expression levels (pext > 0.5). The resulting set of 15,655 variants was stratified according to predicted nonsense-mediated decay (NMD) status (2,373 PTVs were predicted to escape NMD). Variants were then grouped into singleton (AC = 1) and non-singletons (AC > 1). Equal number of singleton and non-singleton variants were drawn from each gene (514 variants in 313 genes for NMD-escaping subset), and several sequence features were compared between groups.

Results: Our analysis showed that singleton PTVs display a lower proportion of variants in the 1st base of a codon (64.5% compared to 73.1%). Among the stop codons, TGA was substantially less prevalent among singletons (24.7% compared to 51.8%). Besides, we detected significant differences in the sequence context in the position immediately preceding the premature stop codon.

Conclusion: Our results show widespread differences in sequence context at rare and common nonsense variant sites, suggesting that nonsense suppression may alleviate the negative effects of PTVs in some cases.

C.O.I. Disclosure:

Y.A. Barbitoff: None. **A. Ivanova:** None. **I.V. Grishchenko:** None. **A.V. Predeus:** None.

EP21.004 Insights into the variation of insulin resistance-related genes in the Lithuanian clean-up workers of the Chornobyl nuclear disaster cohort

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Background: Ionising radiation (IR) is extensively studied due to its carcinogenic consequences; moreover, IR increases the risk of non-cancer diseases and causes adverse metabolic effects. The main metabolic clinical manifestations resulting from exposure to IR include increased body mass index (BMI), insulin resistance and dyslipidemia. Therefore, we aimed to perform the analysis in genes linked to insulin resistance in the cohort of IR-exposed Lithuanian clean-up workers of the Chornobyl nuclear disaster (LCWC).

Material and methods: The study group consisted of 51 male LCWC. The control group included 57 non-exposed to IR males of Lithuanian descent (LTU). Both groups comprised individuals with BMI ≥ 28 and the age range between 50–80 years. A list of variants in genes related to insulin resistance (KEGG PATHWAY database) was extracted from high-throughput beadchip genotyping data. We performed quality control and association analysis using linear regression, adjusted for two genetic principal components using PLINK v2.0.

Results: Clinical data revealed that 10 of the 51 LCWC and 1 of the 57 self-reported LTU individuals are suffering from type 2 diabetes. Three statistically significant variants in the *SLC27A4* gene were identified: rs7048106 ($p = 0.008$, $OR = 0.4$), rs10987965 ($p = 0.017$, $OR = 0.19$), rs6478827 ($p = 0.021$, $OR = 0.2$).

Conclusion: Our established possibly protective variants are less frequent in the LCWC cohort, and these findings confirm clinical data. Despite IR's ability to influence metabolic changes, IR potentially does not affect predisposition to type 2 diabetes in the LCWC cohort.

C.O.I. Disclosure:

K. Samaite: None. **I. Domarkiene:** None. **G. Žukauskaite:** None. **K. Baronas:** None. **L. Ambrozaityte:** None.

EP21.005 Investigating the role of the Tri-allelic variant at DYF387S1 and its significance in Forensic data interpretation

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Background: The Y chromosome markers are used widely in DNA forensic cases like paternity disputes, individual identification, mass disaster and sexual assault. While using Y-STRs, infrequent artefacts may arise from mutation or a contaminated source, in such a scenario, the interpretation becomes crucial. Atypical singularities must be addressed for proficient interpretation.

Material and methods: 88 healthy male individuals from the Gujarati Brahmin population of India, were screened for the Y-STR analysis. DNA was extracted from peripheral blood using a silica-based column and amplified using a YFiler™ Plus PCR amplification kit. The amplicons were separated using capillary electrophoresis and analyzed using GeneMapper ID-X™ software.

Results: Out of all the samples analyzed, two samples exhibited a tri-allelic pattern, and eleven displayed a single peak at the multi-copy marker DYF387S1. The samples were validated using autosomal STRs to ensure a single source, additionally, no such artefacts were observed in the same.

Conclusion: Based on the observation, it can be determined that the tri-allelic pattern is observed due to the non-allelic homozygous recombination (NAHR) occurring due to the presence of a palindromic sequence on the Y chromosome. The single peak on the multi-copy marker indicates either deletion or genetic recombinational loss of heterozygosity (recLOH). Such artefacts are critical while interpreting forensic DNA profiles, as they may result in misinterpretation of the same. In addition to that, such artefacts can help in drawing clinical inferences since most of the markers are present on the coding region of the Y chromosome.

C.O.I. Disclosure:

V. Dave: None. **A.A. Fulkar:** None. **M.A. Shukla:** None.

EP21.006 Possible founder effect of glycine encephalopathy: an evidence of a GLDC c.2714T>C p.Val905Gly common variant in the paisa community based in Cali, Colombia

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Background: Non-ketotic hyperglycinemia (NKH) is an autosomal recessive disorder caused by a deficiency in the mitochondrial glycine cleavage system. This condition is characterized by a complex and variable neurological phenotype with genetic heterogeneity. Our aim was to report and analyze cases of patients from the Paisa community in Colombia with a common variant in the *GLDC* gene.

Material and methods: Case series from retrospective information. Sociodemographic, clinical, and molecular analysis was performed. IRB approval was obtained.

Results: Seven patients with origins or ancestry from the Paisa community in Colombia were clinically, biochemically, and molecularly evaluated. Parental consanguinity was not reported, but intra- and interfamilial isonomy was observed, suggesting a potential link. All participants exhibited symptoms such as hypotonia, lethargy, feeding difficulties, and seizures refractory to conventional treatment. Biochemical studies revealed elevated glycine levels in the amino acid profile, confirmed in cerebrospinal fluid in 3/7. Molecular studies identified the same homozygous variant in *GLDC* (NM_000170.3:c.2714T>G; p.Val905Gly) in all patients, confirming the diagnosis of NKH. Pharmacological and non-pharmacological supportive management was initiated, with clinical follow-up revealing variability in disease progression among the patients.

Conclusion: This study is the first comprehensive Colombian report of NKH cases, providing sociodemographic, clinical, biochemical, and molecular data. We identified a recurrent homozygous *GLDC* variant (p.Val905Gly), indicating a possible founder effect in the Paisa community. Despite the absence of reported parental consanguinity (except one case), intrafamilial and interfamilial isonomy suggests ancestral links. Further studies are needed to confirm these findings and explore the implications of this founder effect.

C.O.I. Disclosure:

S.E. Sinisterra Diaz: None. **E.Y. Gómez Pineda:** None. **H.M. Pachajoa Londoño:** None.

EP21.007 Variants of the PKLR gene are associated with rates of hospitalization in Angolan children with sickle cell anemia

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Background: Sickle cell anemia (SCA), caused by the c.20 A > T (p.Glu6Val) mutation in the HBB gene, is one of the most prevalent hereditary diseases in Sub-Saharan Africa. Patients with the HbSS genotype exhibit variable phenotypic expressions and disease severity, often reflected in hospitalization rates. Reduced activity of pyruvate kinase (PKR, gene: PKLR), a key enzyme in glycolysis, impacts SCA pathophysiology through 2,3-DPG accumulation and ATP deficit. This study analyzed the association of three PKLR variants with hospitalization and clinical parameters in Angolan children with SCA.

Material and methods: Sixty-three SCA children (3–12 years) were monitored in a prospective study (2019–2022). Polymorphisms rs8177970 and rs3020781 (intron 3) and rs1052177 (3' UTR) in the PKLR gene, were analyzed via PCR-RFLP. Allele frequencies and Hardy-Weinberg equilibrium (HWE) were assessed using Arlequin 3.5, and SPSS 27 was used for statistical analyses. Association studies were performed using the Mann-Whitney U test, combining heterozygous and homozygous individuals for the minor allele into a single group.

Results: Minor allele frequencies for rs8177970, rs3020781, and rs1052177 were 0.214, 0.221, and 0.377, respectively, with all genotype distributions consistent with the HWE. Polymorphism rs1052177 was significantly associated with hospitalization rates ($p = 0.025$), while rs8177970 showed a near-significant association ($p = 0.068$). No associations were found with hematological parameters.

Conclusion: This study conducted in SCA children from Angola found potential links between two PKLR SNPs and hospitalization rates. The PKLR gene may act as a genetic modifier of the clinical progression of SCA, as these SNPs can affect gene expression levels.

C.O.I. Disclosure:

L. Manco: None. **B. Santos:** None. **P. Faustino:** None. **C. Ginete:** None. **M. Brito:** None. **A.P. Arez:** None.

EP21.008 "Sardinian MICA and MICB allelic diversity: deciphering immune regulation for possible personalised therapies"

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Background: Characterising MHC class I chain-related genes *MICA* and *MICB* deepens our understanding of the mechanisms underpinning immune system activation and regulation and offers prospects for diagnosing and treating complex conditions such as tumours, autoimmune diseases, and post-transplant complications. This holds significant importance in Sardinia, a region where a high incidence of immune-mediated disorders and tumours has been documented.

Material and methods: This study aimed to assess the distribution of MICA and MICB gene alleles in the Sardinian population and to compare these distributions with those

observed in other regions of Italy. We sequenced the MICA in 1607 and MICB and 1589 healthy Sardinians and 612 (MICA) and 590 (MICB) Italians from Northern, Central, and Southern Italy, using an NGS 17-loci typing kit.

Results: The most notable finding when comparing the Sardinian population with other Italian populations was the higher frequency of *MICA*001:01* [16.33% (525/3214) vs.2.2% (27/1224)], and *MICA*002* [19.1% (614/3214) vs.14.95% (183/1224)] alleles. In contrast, the other Italian populations showed higher frequencies of *MICA*008* [13.8% (444/3214) vs.23.28% (285/1224)] and *MICA*009* [9.11% (293/3214) vs.13.2% (162/1224)] alleles. Regarding *MICB* alleles, the most frequent allele in the Sardinian population was *MICB**008 [12% (379/3178) vs. 6.77% (80/1180)], whereas the *MICB*002* allele was more frequent in other Italian populations [20.5% (652/3178) vs. 24.4% (288/1180)].

Conclusion: The findings underscore the importance of characterising these genetic markers not only to enhance our understanding of immune system regulation but also to inform the development of targeted diagnostic and therapeutic strategies, potentially paving the way for more personalised approaches in clinical practice.

C.O.I. Disclosure:

M. Murgia: None. **C. Sanna:** None. **R. Littera:** None. **S. Mocci:** None. **S. Lai:** None. **A. Mascia:** None. **E. Giuressi:** None. **S. Rassu:** None. **E. Zedda:** None. **F. Cannas:** None. **C. Mereu:** None. **M. Lorrai:** None. **C. Cocco:** None. **L. Serventi:** None. **G.M. Tosone:** None. **F. Marongiu:** None. **G. Nutile:** None. **M. Frick:** None. **V. Tedde:** None. **N. Marongiu:** None. **M.G. Mattana:** None. **M. Boi:** None. **S. Giglio:** None.

EP21.009 Improved Clinical Management Following Diagnosis with Familial Hypercholesterolemia in the Helix Research Network Population Genomics Screening Program

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Background: The Helix Research Network TM is a large population genomics program that screens an all-comers population of adult patients for CDC Tier 1 genetic conditions, including familial hypercholesterolemia (FH). We evaluated changes in clinical management and LDL cholesterol (LDL-C) levels among patients identified to have FH.

Materials and methods: Participants across six U.S. health systems provided samples that underwent clinical-grade exome sequencing. Individuals with positive screening results for a CDC Tier 1 conditions were offered no-cost genetic counseling through their health system. Using medication and laboratory testing records, we assessed whether patients with FH received new lipid-lowering therapies and LDL-C changes.

Results: Among 124,200 individuals enrolled between 2017–2024, 580 (~1/215) had a pathogenic or likely pathogenic FH variant in LDLR (75%), APOB (24%), or PCSK9 (1%). Of the 420 with retrospective and prospective EHR data, 83% lacked a prior FH diagnosis. Over a median 1.5 years (IQR:0.8–3.0), 34% received new/modified lipid-lowering therapy within 3 years. The proportion with new/modified therapy was higher in those with a FH diagnosis code newly documented after screening (54% vs 21% for those without documentation, $p < 0.001$). Patients with new/modified therapies had a mean LDL-C reduction of 61 mg/dL, compared to only 20 mg/dL in patients with no therapeutic change (difference = 41 mg/dL, $p < 0.001$).

Conclusions: Identification of FH through the Helix Research Network TM led to improvements in clinical management and LDL-C levels for many patients. EHR documentation of the FH diagnosis code was associated with greater likelihood of therapeutic changes and larger LDL-C reductions.

C.O.I. Disclosure:

C. Hajek: A. Employment (full or part-time); Significant; Helix, LLC. **M. Levy:** A. Employment (full or part-time); Significant; Helix, LLC. **K. Schiabor Barrett:** A. Employment (full or part-time); Significant; Helix, LLC. **M. Betts:** None. **D. Kann:** None. **A. Bolze:** A. Employment (full or part-time); Significant; Helix, LLC. **J. Cauwels:** None. **D. Stoller:** None. **C. Chapman:** None. **A. Chahal:** None. **D. Judge:** None. **D. Olson:** None. **J. Grzymski:** None. **L. Cirulli:** A. Employment (full or part-time); Significant; Helix, LLC.

EP22 Functional Genomics

EP22.001 Identification of inflammation-associated genes linked to ischemic stroke

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Background: Inflammation plays a key role in the pathogenesis of many diseases, including ischemic stroke (IS). We analysed genes linked to IS and inflammation to find shared associations.

Material and methods: The human genes associated with IS (N = 1177) were retrieved from DisGeNet platform. The inflammation and immune response genes were obtained from Gene Onthology, NCBI and Reactome databases. We also considered rat gene orthologues expressed differentially (fold change>1.5 and *Padj* < 0.05) under experimental brain ischemia (tMCAO) implemented by us previously. The data were analysed and visualized with R language.

Results: We selected genes that were found simultaneously in both the inflammatory and IS gene lists. As a result, combined gene list included 1300 inflammatory genes. The *NFKB1*, *RELA*, *IL1B*, *TLR2*, *CD14*, *CD40*, and *HMGB1* genes were in top of the various sets of inflammatory genes.

Conclusion: Using combination of experimental and bioinformatic approaches, a representative list of inflammatory genes important for IS was formed. The genes identified may hold significant relevance for the advancement of anti-inflammatory therapeutic strategies of IS.

The work was carried out within the state assignment of NRC "Kurchatov Institute" (bioinformatics) and grant 24-14-00027 of Russian Science Foundation (gene expression data).

C.O.I. Disclosure:

G.V. Khvorykh: None. **I.B. Filippenkov:** None. **A.V. Khrunin:** None. **L.V. Dergunova:** None. **S.A. Limborska:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Russian Science Foundation (24-14-00027). C. Other Research Support (supplies, equipment, receipt of drugs or other in-kind support); Significant; The state assignment of NRC "Kurchatov Institute".

EP22.002 Cohort-based Integrated Multiomics from Single Sample Blood Draws Provides Comprehensive Holistic Phenotypic Insights

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Background: Omics era has expanded the repertoire of approaches for researchers and clinicians to unravel the complexity underpinning human health: Next Generation Sequencing and Mass Spectrometry can characterize genomes, epigenomes, transcriptomes, proteomes and metabolomes. Advanced DNA barcoding and automated microfluidics can take this to the next level, enabling multiomic single-cell characterization. PBMC offer a non-invasive approach that, when combined with omics tools, can provide a holistic view of immune processes across patient cohorts.

Material and methods: We detail a proof-of-principle workflow utilizing a single blood draw to rapidly produce a diverse set of multiomics results. Blood draws using heparin tubes were collected and processed within 24 hours of the primary blood draw to ensure high viability and yield of PBMC, along with plasma separation and collection. PBMC and plasma samples are then processed such that whole exome, whole genome methylation, single cell RNAseq, proteomic and metabolomic analysis can all be collected from patient blood draw.

Results: Integrated analysis across multiple data modalities allows for holistic views of pathways and processes that are highly impacted. While genomics, transcriptomics, and proteomics provide information about genetic and functional potential, inclusion of metabolomics grants deeper phenotypic insights within and across individuals in the patient cohort. All these datatypes can be produced within days of sample collection using minimal amounts from a single blood draw, to address the biological questions at hand.

Conclusion: With increased accessibility of omics approaches, integrative workflows as described here continue to gain broader adoption and drive greater insights and innovation in human health applications.

C.O.I. Disclosure:

M. Yammine: None. **N. Zoulias:** None. **A. O'hara:** None. **T. Cohen:** None.

EP22.003 Detection of bacterial ribosomal proteins in human extracellular vesicles derived from *Staphylococcus aureus*-spiked blood during antibiotic treatments

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Background: Sepsis is responsible for 20% of all global deaths, with over 70% of those linked to antibiotic-resistant infections. Bacterial membrane vesicles (bMVs), ranging from 20 to 400 nm, contain proteins and virulence factors that influence host immune responses and may play a role in organ damage. However, the proteomic differences between human extracellular vesicles (EVs) and bMVs during infection and antibiotic treatment are not yet fully understood.

Material and methods: EDTA-treated blood from healthy donors was spiked with Staphylococcus aureus (SA), either alone or in combination with antibiotics: Piperacillin-Tazobactam (Pip-Tazo: 25, 50, 100 µg/ml), Vancomycin (6.25, 12.5, 25 µg/ml), and Moxifloxacin (0.18, 0.73, 2.9 µg/ml). EVs were isolated using the EV Isolation Kit (Miltenyi), followed by incubation of unbound plasma with 1 µm magnetic beads coated with anti-OmpA/GroEL antibodies (bacterial markers). EVs and bMV-BacBead complexes were stained with CellMask™ Deep Red (CMD), anti-PanEV, and anti-SA antibodies for flow cytometry analysis. High-resolution liquid chromatography-mass spectrometry (HRLC-MS/MS) was employed to identify proteins from plasma, EV, and bMV fractions.

Results: Flow cytometry revealed EVs carrying bacterial proteins (CMD + CD45+ PanEV+ SA+) with higher levels at the highest Vancomycin and lowest Piperacillin-Tazobactam (Pip-Tazo) concentrations compared to the SA-only group. Two SA ribosomal proteins were found to be shared across all bacterial treatment groups.

Conclusions: EVs carrying bacterial proteins could be detected and were more prevalent during Vancomycin and Pip-Tazo treatments than under SA-only conditions.These findings suggest that these EVs may play a role in either supporting host defense or promoting SA proliferation.

C.O.I. Disclosure:

M. Reithmair: None. **D.M. Chiang:** None. **G. Schelling:** None. **C. Ludwig:** None. **S.I. Wudy:** None. **B. Kirchner:** None. **M.S. Yu:** None. **C. Zenner:** None. **M.W. Pfaffl:** None.

EP22.004 Comparison of ipsilateral striatum and frontal cortex differential expression gene patterns in rat brain following experimental stroke

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Background: Ischemic stroke is a multifactorial disease that leads to brain tissue damage and severe neurological deficit. Transient middle cerebral artery occlusion (tMCAO) model are actively used for molecular genetics study of stroke. Previously, tissues with different degree of ischemic (tMCAO) damage were selected. Using RNA-Seq we revealed thousand differential expressed genes (DEGs) in both penumbra-associated region of frontal cortex (FC) and infarct-associated striatum of rats at 24h after tMCAO. Here, we compared the gene expression pattern in the both tissues.

Material and methods: RNA-Seq; real-time RT-PCR; bioinformatics.

Results: As a result, 2609 DEGs (e.g. *Ccl2, Socs3, Neurod2*) were overlapped in striatum and FC, whereas 1800 (e.g. *Itpr1, Prim1, RT1-Ba*) and 1165 DEGs (e.g. *Acsl1, Ccl11, Grin2a*) were specific for striatum and FC, respectively. Functional analysis revealed associations of DEGs with inflammatory, neurosignaling and metabolic systems in both studied tissues. Concomitantly, unique DEGs identified in striatum were mainly associated with neurotransmission, cellular senescence, antigen processing and presentation, DNA replication and thyroid hormone systems, whereas FC unique DEGs were predominantly related to biosynthesis of cofactors, ferroptosis and IL-17 responses.

Conclusion: Thus, spatial regulation of the ischemic process in ipsilateral hemisphere of rat brain at the transcriptome level was revealed after tMCAO in rats. We believe that targeted adjustment of genome responses identified can be key for induction of regeneration processes in brain cells after stroke and following its treatment. The work was carried out within the state assignment

of NRC "Kurchatov Institute" (bioinformatics) and grant (24-14-00027) of RSF (gene expression analysis).

C.O.I. Disclosure:

I.B. Filippenkov: None. **Y.Y. Shpetko:** None. **S.A. Limborska:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Russian Science Foundation (24-14-00027). C. Other Research Support (supplies, equipment, receipt of drugs or other in-kind support); Significant; The state assignment of NRC "Kurchatov Institute". **L.V. Dergunova:** None.

EP22.005 Gene expression signature of the severity of coronary stenosis

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Background: The purpose of the work is to relate the expression levels of genes related to high-density lipoprotein metabolism and atherogenesis and underlying metabolic pathways to the severity of coronary artery stenosis, represented by the number of stenotic vessels.

Material and methods: The expression of 65 preselected genes in the peripheral blood mononuclear cells of control patients (n = 63) and coronary artery disease (CAD) patients with one or two (low stenosis group, n = 35) or three or four (high stenosis group, n = 41) stenotic vessels, confirmed by coronary angiography, was measured with qRT–PCR. The differentially expressed genes were annotated by functional enrichment analysis.

Results: Differentially expressed genes in CAD patients compared to controls were characterized by metabolic pathways connected to plasma lipoprotein assembly, remodeling, and clearance and signaling and regulation of gene expression linked to cholesterol transport and efflux. However, different expression profiles and metabolic pathways existed for high versus low stenosis comparison. The expression of the *CETP, PLTP, CD36, IL18, ITGB3, S100A8, S100A12*, and *VEGFA* genes increased with the increase in the number of stenotic vessels, which suggests the involvement of these genes in stenosis expansion via various pathways, including lipoprotein metabolism, inflammation, angiogenesis, and innate immunity. The *ITGB3, VEGFA*, and *CETP* hub genes were selected as a new signature of the expansion of coronary artery stenosis, which was further validated with the GSE12288 dataset.

Conclusion: The expression levels of *ITGB3*, *VEGFA*, and *CETP* genes may be used for the diagnosis, prognosis estimation, and treatment of coronary stenosis.

C.O.I. Disclosure:

M.A. Vinogradina: None. **A.D. Dergunov:** None. **E.V. Nosova:** None. **A.V. Rozhkova:** None. **V.B. Baserova:** None. **M.A. Popov:** None. **S.A. Limborska:** None. **L.V. Dergunova:** None.

EP22.006 Secondary hereditary erythrocytosis due to *in frame* loss of 54 amino acids in the catalytic site of EPAS1

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Background: Secondary hereditary erythrocytosis due to elevated erythropoetin levels is caused by pathogenic germline variants in genes encoding proteins of the oxygen-sensing pathway, such as inactivating *EGLN1* (encoding PHD2) and *VHL* (pVHL) variants or activating *EPAS1* (HIF-2α) missense variants. In normoxia HIF-2α is hydroxylated by PHD2 and targeted to pVHL-mediated ubiquitination and proteasomal degradation. In hypoxia HIF-2α hydroxylation and degradation are prevented, enabling its nuclear translocation and dimerization with HIF-1β to upregulate the transcription of >200 genes, incl. *EPO*.

Material and methods: Massively parallel sequencing, cDNA sequencing and minigene experiments were performed to investigate the underlying genetic cause in a patient presenting with erythrocytosis with a suspected defect in the oxygen-sensing pathway.

Results: We discovered the de novo variant *EPAS1*:c.1555-1_1559del, which leads to loss of the splice acceptor site of exon 12. Analysis of patient's *EPAS1* transcripts and minigene experiments revealed two aberrant transcripts: (1) skipping of the *out-of-frame* exon 12, associated with a premature stop codon and LOF and (2) use of a cryptic splice acceptor site 162 nt downstream in exon 12 resulting in an *in-frame* loss of 54 amino acids, p.Thr519_Gln572del.

Conclusion: Most known *EPAS1* missense variants cause hereditary erythrocytosis by preventing hydroxylation of p.Pro531. This amino acid is also lost by the *in-frame* deletion p.Thr519_Gln572del, while the HIF-2α domains required for heterodimerization with HIF-1β and DNA binding remain unchanged and correctly folded according to 3D structure prediction data. In conclusion, our data suggest that this splicing effect renders HIF-2α constitutively active, causing erythrocytosis in our patient.

C.O.I. Disclosure:

C. Lutz-Nicoladoni: None. **A. Amberger:** None. **P. Traunfellner:** None. **J. Kokot:** None. **J. Zschocke:** None. **K. Wimmer:** None.

EP22.007 DNA methylation changes in Wnt signaling pathway genes in overgrowth patients

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Background: Overgrowth syndromes are clinically and genetically heterogeneous disorders characterized by excessive somatic growth. Increasing evidence points to epigenetic dysregulation, especially in developmental pathways such as the Wnt signaling pathway, as a key factor in these conditions. We therefore investigated DNA methylation changes relevant to Wnt pathway genes.

Material and methods: We profiled genome-wide DNA methylation using the Infinium MethylationEPIC v2.0 array in Overgrowth patients (n = 30) and unaffected controls (n = 2). Differentially methylated probes (DMPs) were identified with the SeSAMe R package using a significance threshold (p-value adjusted < 0.001). Key Wnt-associated genes were then prioritized for detailed analysis.

Results: In total, we identified multiple significant DMPs across Wnt regulators. Notably, *TNKS2* (logFC = 0.1797) and *PYGO1*(-logFC = 0.1333) displayed hypermethylation, while *SFRP2* (logFC = -0.0655), *RSPO2* (logFC = -0.0872), *WNT2* (logFC = -0.1180), and WNT7B (logFC = *-0.0252*) were hypomethylated. These findings reveal a complex pattern of Wnt pathway dysregulation. Several DMPs were found in promoter regions, suggesting potential regulatory consequences.

Conclusions: Our results indicate that epigenetic alterations in key Wnt pathway components may contribute to the overgrowth phenotype, underscoring the potential relevance of targeted methylation changes in the pathogenesis of overgrowth syndromes.

C.O.I. Disclosure:

K. Baronas: None. **L. Ambrozaityte:** None. **A. Utkus:** None.

EP22.008 Investigating the effects of prematurity and age on methylated C-reactive protein in early life

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Background: Methylated C-reactive protein (CRPm), a proxy of inflammation, has been shown to be associated with gestational age in infants[1], but its trajectory in early life has not been investigated. This study examined changes in CRPm from birth to 18 months in term and preterm infants.

Material and methods: Participants were enrolled in the Developing Human Connectome Project.[2] Infants were recruited at varying gestational ages at birth (range 23 - 42^{+7} weeks). Saliva samples for DNA methylation were taken at the preterm timepoint (<37 weeks if applicable, n = 93), term-corrected age (37 - 45 weeks corrected gestation, n = 589) and toddlerhood (17.3 - 34.5 months corrected age, n = 283). CRPm score was calculated at each available timepoint. Regression covariates included maternal smoking, maternal BMI, gestational age, birthweight, sex, ancestry, and Index of Multiple Deprivation.

Results: Higher gestational age at birth was associated with lower CRPm at term-corrected age (B = -0.078, p = 0.002), but there was no association between gestational age and CRPm in toddlerhood (B = -0.008, p = 0.721). Being born prematurely versus at term was associated with higher CRPm at term-corrected age (t(120.4) = -2.5, p = 0.014), but there was no between-group difference in CRPm in toddlerhood (t(71.2) = -1.1, p = 0.272). CRPm declined significantly between birth and toddlerhood in preterm- (t(18) = 5.4, p < 0.001) and term-born (t(186) = 15.5, p < 0.001) children.

Conclusion: CRPm declines from birth to toddlerhood, regardless of prematurity. Preterm infants have higher CRPm than term-born peers at term-corrected age, but not at 18 months. This suggests that the heightened inflammatory state seen in preterm infants around birth does not persist into early childhood.

C.O.I. Disclosure:

I. Kleine: None. **K. Dimitrakopoulou:** None. **D. Fenchel:** None. **K. McKinnon:** None. **V. Kyriakopoulou:** None. **C. Curtis:** None. **H. Patel:** None. **D. Edwards:** None.

EP22.009 Exploring the role of non-coding RNAs in regulating CDH13 during adipogenic differentiation of human MSCs

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Background: Recent research has highlighted the significant role of T-cadherin in regulating adipogenic differentiation, with T-cadherin-deficient cells showing increased spontaneous differentiation into adipocytes and greater adipogenic potential in the presence of adipogenic inducers. This study proposes that non-coding RNAs, particularly microRNAs (miRNAs), may mediate T-cadherin's involvement in this differentiation process.

Material and methods: Human mesenchymal stromal/stem cells (MSCs) derived from adipose tissue were studied under standard conditions and during adipogenic induction. Single-cell RNA sequencing data were analyzed using the Seurat R package, while miRNA profiling utilized the mirNet database, and target gene analyses were conducted through the STRING database.

Results: Comparative analysis of miRNA expression revealed hsa-mir-34a with over a 1.5-fold decrease and three miRNAs—hsa-mir-181a-2, hsa-mir-210, and hsa-mir-497—exhibiting over two-fold increases under adipogenic conditions. Hsa-mir-34a was linked to 100 target genes related to endothelium development, whereas hsa-mir-181a-2 had 587 targets, including the CDH13 gene (T-cadherin), associated with various biological processes such as endothelial cell proliferation and blood vessel morphogenesis.

Conclusion: These findings suggest a regulatory interaction between non-coding RNAs, especially miRNAs like hsa-mir-181a-2, and the CDH13 gene, indicating their crucial role in adipogenic differentiation. The involvement of these miRNAs in essential biological processes further underscores the need for in-depth exploration of their molecular targets.

C.O.I. Disclosure:

M. Arbatskiy: None. **V. Sysoeva:** None. **E. Semina:** None. **K. Rubina:** None.

EP22.010 Fetal and maternal genetic contribution to placental DNA methylation levels of CpGs associated with birth weight

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Background: The placenta is situated at the interface between the fetal and maternal systems. As such, its epigenome may be influenced by both the maternal and fetal genomes. The aim of this study was to identify fetal and maternal placental methylation quantitative trait loci (mQTLs) of 1,106 CpGs associated with birth weight, as identified in a previous study conducted by our group.

Methods: Genome-wide genetic data (Illumina GSA array) and placental villi DNA methylation measurements (Illumina EPIC array) were available for 528 mothers and 408 newborns from BiSC, a population-based birth cohort. mQTLs were identified by fitting linear regression models adjusted for fetal sex, gestational age, five genetic principal components, and six placental cell type proportions using the TensorQTL tool.

Results: At a p-value < 5E-08, we identified 17,033 fetal mQTLs, 16,402 acting in cis (±0.5 Mb) and 631 acting in trans, representing 520 and 236 unique CpGs, respectively. In the mothers, we identified 500 trans-maternal mQTLs, representing 99 unique CpGs. Of these, 51 (9 CpGs) had a ratio between the fetal and maternal estimates <0.6, suggesting maternal effects. Notably, one of these CpGs was annotated to the *MIR193B* gene, which is

involved in trophoblast differentiation and deregulated in preeclampsia and gestational diabetes.

Conclusions: These results highlight a potential genetic contribution of the maternal genome on the placental DNA methylation of CpGs associated with birth weight. Further analyses in the subset of mother-child pairs of European origin and validation in additional cohorts are required.

C.O.I. Disclosure:

M. Bustamante: None. **M. Cosín-Tomàs:** None. **S. Aguilar Lacasaña:** None. **O. Sánchez:** None. **Z. García-Ruiz:** None. **E. Llurba:** None. **L. Gómez-Roig:** None. **J. Sunyer:** None. **M. Vrijheid:** None. **P. Dadvand:** None.

EP22.011 Astrocytes in the mouse suprachiasmatic nuclei respond directly to glucocorticoids feedback

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Background: The circadian timing system allows the anticipation of daily recurring changes in the environment improving physiological fitness. Differences in development of the circadian clock or, e.g. antenatal glucocorticoid treatment, affect the risk of behavioral disorders. In mammals, the circadian system is hierarchically organized, with a master pacemaker located in the hypothalamic suprachiasmatic nuclei (SCN). Glucocorticoid (GC) release from the adrenal gland is under SCN control, peaking before the active phase to synchronize systemically "awake" functions. While the mouse SCN is responsive to GCs during the perinatal period, the adult circuit is traditionally viewed to be resistant. Here we challenge this traditional view, presenting evidence that indicates that adult SCN astrocytes indeed respond to GC feedback.

Material and methods: sn-RNAseq of mouse SCN at different developmental timepoints and validation via RNAscope, Proximity ligation assay (PLA), Ca2+ transient imaging, luminescence assay in SCN slices, and in vivo functional assay in a jet lag mouse model.

Results: We show that the GC receptor (GR) is down regulated in neurons along SCN maturation, but astrocytes continue to express it. Indeed, astrocytic GR is activated by circadian GCs changes, demonstrating for the first time that the mature SCN receives direct GC-feedback. We prove in vivo in a mouse model that GC feedback in the SCN has functional consequences for the adaption to jet lag.

Conclusion: Our data provides novel insight into the gain of cellular and transcriptional heterogeneity of the mouse SCN along development and the essential role of SCN astrocytes in timing physiology.

C.O.I. Disclosure:

K. Händler: None. **V.K. Sreenivasan:** None. **V. Pilorz:** None. **C. Martinez-Perez:** None. **I. Elorduy:** None. **M. Lehmann:** None. **J. Olano Bringas:** None. **L. Escobar Castañondo:** None. **N. Bengoa-Vergniory:** None. **F.N. Soria:** None. **H. Oster:** None. **M. Spielmann:** None. **M. Astiz:** None.

EP22.012 Spatial Transcriptomics Explores Potential Epileptogenic Cell Populations in Absence Epilepsy

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Background: Absence epilepsy, a common form of genetic generalized epilepsy, is marked by recurrent spike-and-wave discharges, but its underlying cellular and molecular mechanisms remain poorly understood. Using the GAERS model, this study applies spatial transcriptomic technologies to achieve high-resolution insights at spatial and cellular levels. The aim is to identify potential epileptic foci, characterized by epileptogenic cell populations, and uncover molecular pathways involved in the onset and progression of epilepsy through spatial transcriptomics, high-resolution RNA sequencing, and LCM.

Material and methods: Cortical regions from P15 (pre-epileptic) and P30 (epileptic) GAERS, as well as age-matched control Wistar rats, were analyzed using EEG and 10XGenomics Visium spatial transcriptomics. Cryosections from the somatosensory cortex were mounted onto Visium slides for spatial transcriptomic analysis. A tailored bioinformatics pipeline, incorporating SpaceRanger, LoupeBrowser, and differential expression workflows, identified transcriptional patterns linked to epileptogenesis. LCM was used to isolate cortical regions of interest for follow-up high-resolution RNA sequencing.

Results: EEG confirmed the absence of seizure activity in P15-GAERS, whereas hallmark spike-and-wave discharges were detected in P30-GAERS. Transcriptomic analyses identified differentially expressed genes (*Nlrp1*,*Csnk1d*,*Hdac11*,*Egr1*,*Cox5a*) associated with neuroinflammation, mitochondrial dysfunction, and epigenetic remodeling. Spatial profiling highlighted cortical regions with distinct transcriptional patterns, including increased expression of hyperexcitability-associated markers, suggesting epileptogenic processes. These findings require further validation.

Conclusion: These findings support the feasibility of spatial transcriptomic approaches in identifying epileptic foci and hyperexcitability signatures at the molecular level. Ongoing work will validate cell-specific contributions using LCM and high-resolution RNA sequencing to refine understanding of epileptogenesis and its translational potential.

C.O.I. Disclosure:

E. Aydin: None. **N. Sevinc-Ozdemir:** None. **S. Ozdemir:** None. **Y. Eken:** None. **A. Akkus:** None. **N. Mutlu:** None. **E.T. Erdeve:** None. **T.T. Turan:** None. **O. Sariyildiz:** None. **N. Carcak-Yilmaz:** None. **F. Onat:** None. **O. Ozdemir:** None.

EP23 New Technologies

EP23.001 Genomic medicine in the digital health landscape. Are we crossing the ocean on a raft?

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Background: The global push for genomic medicine enhances diagnostics, treatment, and healthcare efficiency, with countries like the UK, US, and China sequencing millions of genomes. Research and clinical practice are increasingly interconnected, promising proactive care. However, challenges remain—fragmented health records, data privacy concerns, and limited equitable access hinder progress. Genetic conditions require lifelong data integration, but current infrastructure confines data to isolated pools, making comprehensive analysis nearly impossible. While AI-driven advancements may eventually bridge these gaps, the existing healthcare infrastructure remains too outdated to fully support the potential of genomic medicine in the near future.

Material and methods: This will be a discussion of opportunities and barriers

Results: We will discuss topics around integration, structured data and use of recognised ontology

Conclusion: This would be an opportunity to present solutions and next steps for ESHG members to work together towards progressing research and treatments in rare and inherited diseases. Solutions lie in modernizing health data systems, enhancing interoperability, utilising secondary data uses, and implementing policies that promote secure data sharing.

C.O.I. Disclosure:
D. Georgiou: None.

EP23.002 Advancing Drug Discovery: Using mMPS High-Throughput Synthesis Technology to Elevate Variant Library Precision

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The Variant library is a critical tool in modern drug discovery because it provides the necessary infrastructure to analyse genetic variations, understand their implications in disease, and translate this knowledge into actionable insights for drug development. Its role in supporting precision medicine, identifying novel drug targets, and improving drug safety makes it indispensable in the rapidly evolving field of drug discovery. The mMPS (microchip-based Massive in Parallel Synthesis) platform significantly improves the construction of variant libraries for drug discovery by offering higher yields, enhanced product quality, scalability, and precision. Unlike the conventional high-throughput oligonucleotide synthesis methods which depend on chip manufacturing and complex microfluidic systems for precision, the mMPS platform introduces an innovative sorting mechanism in biomolecule synthesis using a novel microchip design. This system centralises synthesis, ensuring consistent product quality and uniformity. Notably, mMPS produces yields at the pmol- to nmol-scale per sequence, enabling direct use in subsequent processes without the need for PCR amplification, thus reducing potential amplification bias. By eliminating the need for amplification and reducing errors, mMPS ensures that the variant library is more accurate, diverse, and representative of the genetic variants of interest. These advantages make mMPS a powerful tool for advancing drug discovery, particularly in the context of personalised medicine and high-throughput screening efforts.

C.O.I. Disclosure:
M. Dong: None.

EP23.003 Orthogonal genetic network fusion empowers the genotype-to-phenotype relationship

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Functional genetic screens in human cell lines have accelerated systematic gene annotation efforts. The ultimate goals of these efforts would hopefully culminate in a highly accurate model of the genetic circuitry of a human cell. We compare and contrast global functional information obtained from large-scale genome-wide pooled CRISPR screening efforts covering single-phenotype fitness screens across diverse cell lines and multi-phenotype high-content transcriptomic and imaging approaches. Each data type and its underlying genetic network effectively resolve most cellular processes, each with distinct strengths and weaknesses. Single-cell expression phenotypes excel in reconstructing cellular machinery related to transcription, translation, and protein turnover, while co-essentiality networks better resolve processes such as chromatin remodeling, cell communication, and adhesion. Additionally, genetic interaction screens with and without extracellular matrix coating demonstrate that simple fitness phenotypes effectively resolve functional modules required for tissue organization biology, where expression phenotypes lack resolution. Importantly, the fusion of orthogonal CRISPR-based functional screening datasets assigns gene function with substantially higher precision.

C.O.I. Disclosure:
A. Shaw: None.

EP23.004 Interpreting visual transformer RETFound in retinal phenotyping: Insights from temporal angle predictions

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Background: Deep learning models like visual transformer RETFound are reshaping retinal image analysis by predicting complex vascular traits. Notably temporal angles, key features of retinal vasculature, exhibit higher heritability when predicted by a fine-tuned RETFound model than when measured using classical image processing. We aim to understand the factors driving this discrepancy and improve the interpretability of RETFound's predictions through attention-based visualization techniques.

Methods: To assess whether refining measurement methods could improve heritability, we developed alternative temporal angle definitions designed to better align with deep learning representations. However, these measurements did not surpass the heritability of RETFound's predictions. We then investigated the model's attention mechanisms, leveraging attention maps to explore which regions of the images contribute the most to temporal angle predictions. Both individual and cohort-averaged attention maps highlight areas around the optic disc and main vessel arcs, suggesting that the model focuses on biologically relevant structures. To further quantify the reliability of these attention patterns, we are developing metrics that systematically assess their spatial precision and relevance.

Conclusion: Our work highlights the need to open the "black box" of deep learning models to refine phenotyping strategies. By understanding how foundation models like RETFound encode vascular traits, we can move beyond conventional measurements, potentially unlocking new approaches for image-derived traits

that are both biologically more meaningful and genetically more informative.

C.O.I. Disclosure:

S. Bors: None.

EP23.005 Parent-of-Origin-Aware Genomic Analysis: Ethical Considerations and Provider Attitudes Towards an Emerging Genetic Test

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Background: Parent-of-Origin-Aware Genomic Analysis (POAga) is a novel genetic testing method that can predict the parental origins of specific variants using only the proband's sample. This approach represents a major shift from standard-of-care testing, in which testing in multiple family members is used to determine inheritance patterns. POAga may provide clinical benefit across many genetic diseases. However, understanding its ethical implications is critical.

Material and methods: This knowledge translation (KT) project characterizes specialist attitudes towards POAga. We recruited experts from among clinical geneticists, laboratory geneticists, oncologists, surgeons, neurogeneticists, cardiac geneticists, and biochemical disease specialists. Recruitment commenced in June 2024 in British Columbia, Canada, using a purposive sampling approach. To preface feedback, participants were invited to view a video regarding POAga's potential benefits and implications. Data was collected using semi-structured virtual focus groups and questionnaires.

Results: This project is ongoing and will present an overview of data collected so far. Broadly, providers perceive that POAga offers clinical benefits, including increased testing efficiency, expedited initiation of secondary prevention of affected individuals, and reduced genetic counsellor burden. While optimistic about the potential benefits of POAga, providers highlight a need to address issues related to duty to warn predicted carriers and the need for adequate pre-test patient counselling.

Conclusion: POAga promises multiple benefits. However, transitioning POAga into clinical care will require knowledge translation efforts, patient and provider education, and ongoing ethical and practical discussions among healthcare providers and patients. The results of this project will provide a foundation for co-designed educational resources targeted to ordering providers.

C.O.I. Disclosure:

A. Roston: None. **S. Pollard:** None. **J. Nuk:** None. **S. Sun:** None. **S.J. Jones:** D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Modest; Oxford Nanopore Technologies, Illumina Inc. **P. Lansdorp:** None. **D. Regier:** None. **A. Virani:** None. **K. Schrader:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; AstraZeneca Canada.

EP23.006 Validation of the MassARRAY technique for detecting common mitochondrial DNA mutations

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Background: Mitochondrial diseases are caused by mutations in mitochondrial DNA (mtDNA), leading to impaired energy production, cellular dysfunction, and tissue damage.

Material and methods: This study assessed the diagnostic performance of MassARRAY for detecting mtDNA mutations compared with the routinely used MLPA technique. Both methods analyzed ten EDTA blood samples from patients with suspected mitochondrial disorders.

Results: The results were fully concordant, with five samples testing negative and five samples testing positive for mutations: m.11778G > A (3 samples) and m.14484T > C (2 samples). MassARRAY demonstrated several advantages, including a short turnaround time (8 hours) and, most importantly, cost-effectiveness, making it a practical choice for clinical diagnostics. Its greater assay flexibility, allowing probe customization to target 14 loci, compared to MLPA's fixed six loci, further enhances its practicality. These findings indicate that MassARRAY is a reliable and efficient method for mitochondrial mutation detection, offering significant benefits for clinical diagnostics.

Conclusion: The limitation of this study was the small sample size. However, the promising results suggest that MassARRAY could be a valuable tool in the clinical setting, particularly for its ability to detect a wide range of mutations cost-effectively and timely. Further investigations in larger, more diverse cohorts must validate its broader clinical utility. In conclusion, MassARRAY exhibits high diagnostic accuracy and efficiency, representing a reliable and flexible detection of mitochondrial DNA mutations.

C.O.I. Disclosure:

T. Chareonsirisuthigul: None.

EP23.007 Achieving cost-effective NGS through automated miniaturization of Illumina Nextera XT library preparation

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Advances in next-generation sequencing (NGS) technology have dramatically improved sequencing speed and throughput. However, library preparation remains a bottleneck in terms of cost, resources, and manual effort, involving numerous repetitive and time-intensive liquid-handling steps. Automated systems capable of handling low volumes offer a promising solution by enabling reaction miniaturization without sacrificing accuracy. This approach reduces reagent costs and alleviates the labor-intensive nature of manual pipetting. In this poster, we demonstrate how SEQ-IT, a Germany-based sequencing service provider, successfully miniaturized the Illumina Nextera XT library preparation protocol using SPT Labtech's mosquito® nanoliter liquid handler. Miniaturization reduced reaction volumes by up to 80%, delivering substantial cost savings while maintaining high sequencing data quality. Key metrics, including sample fragmentation, mapping rates, GC bias, and sample distribution, consistently met or exceeded standards, even with 5X miniaturization. Additionally, the streamlined workflow significantly shortened preparation times, allowing higher throughput for large-scale projects with lower sample input requirements. This miniaturized NGS workflow not only reduces costs and manual pipetting efforts but also minimizes plastic waste, offering a sustainable and scalable solution for broader NGS applications. By addressing critical bottlenecks, it paves the way for more accessible and environmentally responsible sequencing technologies.

C.O.I. Disclosure:

J. Couto: None.

EP23.008 Spatial transcriptomics in HPV-positive and HPV-negative squamous cell carcinoma of the larynx

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Background: Head and neck squamous cell carcinoma (SCC) affects various anatomical sites and is mostly associated with distinct risk factors: exposure to carcinogens or infection of human papillomavirus (HPV). While the incidence of non-viral HNSCC is decreasing, HPV-associated HNSCC incidence has dramatically increased in last decades. Recent studies highlight the importance of spatial transcriptomics for investigating the role of immune cell infiltration in HPV-positive compared to HPV-negative oropharyngeal SCC. However, there are limited data about spatial profiling of HPV-positive non-oropharyngeal SCC.

Material and methods: Our experiment included formalin-fixed paraffin-embedded (FFPE) tissue samples of patients with laryngeal HPV-positive or HPV-negative invasive SCC and macroscopically normal mucosa. We performed whole-genome spatial transcriptomics (18000 genes) by Visium technology (10X Genomics). We performed functional annotation and pathway enrichment analysis of differential expressed genes between normal mucosa, HPV-positive and HPV-negative laryngeal SCC.

Results: We were able to identify six gene clusters with distinct expression profile between HPV-positive and HPV-negative laryngeal SCC. Of these, three gene clusters were specific for HPV-positive and three for HPV-negative laryngeal SCC. We observed similar expression profile of certain genes already identified in HPV-positive oropharyngeal SCC in comparison to HPV-negative one.

Conclusion: In conclusion, we observed specific pattern of gene expression for HPV-positive as well as for HPV-negative SCC in the larynx. We identify candidate genes that might play a significant role in cancerogenesis of the SCC of the larynx, dependent on HPV-status. We showed that the used approach is a promising technology to study cancerogenesis in FFPE biopsy samples.

C.O.I. Disclosure:

E. Boštjancic: None. **N. Zidar:** None.

EP23.009 Copy Number Variations as Biomarkers of Accidental Exposure to Ionizing Radiation from Cesium-137 in Goiânia (Central Brasil)

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Background: In 1987, the radiological accident involving Cesium-137 in Goiânia exposed and contaminated 249 people to varying doses of ionizing radiation (IR) potentially damaging their DNA, which could enhance the formation of copy number variations (CNVs). Researchers have worked to identify and validate biomarkers to assess the populational risk of IR exposure.

Material and methods: this study quantified CNVs as potential biomarkers of exposure in somatic cells of 20 people accidentally exposed to IR and 25 healthy controls. Both groups were analyzed using Chromosomal Microarray Analysis with the GeneChip® HD CytoScanHD™ (Thermo Fisher Scientific, USA) and its software.

Results: the radioexposed population had 70% more microdeletions and 46% more microduplications than the control group. No statistical difference was observed in CNV sizes between the two groups. A linear regression model suggested that for every 1 Gy increase in absorbed dose, there was a corresponding increase of 0.65 in loss and 0.58 in gain of CNVs. This model accounted for 24% of the variance in microdeletions ($p = 0.040$) and 12% in microduplications ($p = 0.008$). The dose and age predictable variables were tested within the explanatory model, revealing that when combined CNVs of loss increased by 30.1%., similarly, CNVs of gain increased by 12.7%. The increased risk was calculated using logistic regression, thus the odds ratio (OR) for the exposed group was found to be 12.6% and 28.9% for having genomic loss and gain, respectively.

Conclusion: Our findings suggested CNVs could serve as biomarkers to estimate retrospective risk of IR exposure in humans.

C.O.I. Disclosure:

A.D. da Cruz: None. **S.S. Pereira:** None. **I.P. Pinto:** None. **J.F. da Silva:** None. **L.G. Oliveira:** None. **C.C. da Silva:** None.

EP23.010 Validation of long read sequencing technology as a comprehensive diagnostic tool in the study of hereditary ataxias

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Background: The spinocerebellar ataxias represent a genetically and clinically heterogeneous group of rare neurodegenerative disorders. Although, whole exome sequencing (WES) has revolutionized the field of medical genetics, a large proportion of patients still lack a definitive underlying genetic diagnosis, partly due to technical limitations for detecting structural variants and repeat expansions. The aim of this study was to assess the implementation of long-read sequencing (LRS) technology as a comprehensive tool to study the genetic basis of hereditary ataxia.

Material and methods: LRS has been performed to 10 patients who remain undiagnosed after discarding the most common expansions disorders as well as disease causing variants detected by WES. Our study included 7 patients without candidate variants (cohort 1) and 3 patients heterozygous carriers of the same pathogenic variant (NM_003119.4: c.1045G>A, p.Gly349Ser) in the *SPG7* gene (cohort 2).

Results: In cohort 1 we found that 5 of the 7 patients (70%) presented a pathogenic expansion in the *FGF14* gene associated with the recently form of autosomal dominant cerebellar ataxia named SCA27B. These expansions have been further validated by PCR methods. In cohort 2, we are exploring the deep intronic and regulatory variants identified in *SPG7* gene by using several in silico approaches (splice AI scores, regulome database…)

Conclusion: LRS successfully identified pathogenic variants in 50% of the cohort, all of them related to SCA27B, which supports that this entity might represents one of the most common causes of late-onset hereditary ataxia among European populations.

C.O.I. Disclosure:

M.I. Alvarez-Mora: None. **P. Trilla:** None. **I. Madrigal:** None. **L. Rodriguez-Revenga:** None. **J.E. Muñoz:** None.

EP23.011 Direct comparison of short-read genome sequencing and long-read genome sequencing as genetic evaluation for undiagnosed neurological diseases

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Background: Long-read GS (lrGS) was proposed as an alternative to short-read GS (srGS) for diagnosing rare diseases, particularly neurological disorders (NDs). The Hong Kong Genome Project (HKGP) has achieved 18% diagnostic yield in patients with NDs using srGS as the first-tier diagnostic test, with lrGS applied to resolve complex cases. This study aims to establish criteria for selecting patients who may benefit from lrGS over srGS by directly comparing their performance.

Material and methods: A cohort of 224 families from HKGP with NDs underwent both srGS (Illumina) and lrGS (Oxford Nanopore). Primary and secondary analyses were performed following manufacturers' protocols and best practice. To ensure unbiased comparison, blinded tertiary analyses were performed independently by two equally experienced teams of curators.

Results: Diagnostic yields were 19.6% for lrGS and 19.2% for srGS. srGS identified four SNVs near homopolymeric regions that were missed by lrGS. Further analysis revealed that 7.6% of genes in the targeted panel contained ClinVar P/LP variants in these regions. lrGS provided superior resolution for a complex SV and a large STR. lrGS uniquely detected three expanded large GGC repeats in the 5'UTR of *NOTCH2NLC* and provided allele-specific DNA methylation data that refuted a diagnosis in one patient by demonstrating hypermethylation and silencing of the expanded allele.

Conclusion: While srGS excels in detecting variants near homopolymeric regions, lrGS offers better resolution for complex SVs, large STRs, and DNA methylation analysis. lrGS cannot fully replace srGS and should be selected based on the suspected disease and prior genetic tests performed.

C.O.I. Disclosure:

D. Chan: None. **H. Tam:** None. **D. Yeung:** None. **W. Ma:** None. **A. Tong:** None. **A. Chu:** None. **H. Chung:** None.

EP23.012 Optical Genome Mapping allows decoding the clinical significance of a balanced inversion on chromosome X that dysregulates *POU3F4*

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Background: Structural variations are a significant source of genetic variation in human diseases, yet their full impact remains poorly understood. Predicting the clinical contribution of apparently balanced rearrangements presents a particular challenge. Recent advances in genomic analysis techniques provide a new approach for studying these variants and their potential effects on clinical outcomes.

Material and methods: We applied Optical Genome Mapping (OGM) to further investigate an apparently balanced pericentric inversion in chromosome X, previously identified in a male patient with an inner ear malformation consistent with incomplete partition of the cochlea of type III, bilateral severe mixed hearing impairment and autism spectrum disorder, as well as in his normohearing mother. Previous studies of array CGH and exome sequencing did not identify pathogenic variants.

Results: OGM allowed us to accurately characterize an hemizygous ~26Mb balanced inversion on chromosome X, revealing that one breakpoint impacts the upstream regulatory region of *POU3F4*, transcription factor associated with X-linked deafness-2 (DFNX2). No other variants were detected that could contribute to the proband's phenotype.

Conclusion: Other structural rearrangements affecting *POU3F4* or its upstream regulatory sequences have been previously associated with DFNX2 and additional phenotypes. OGM finally provided a molecular explanation for the patient's hearing impairment, confirming the clinical suspicion based on the pathognomonic cochlear malformation. This study demonstrates the clinical utility of next-generation cytogenomic tools for characterizing previously difficult-to-study structural variants and identifying the underlying causes of complex genetic conditions, facilitating accurate molecular diagnosis and supporting clinical decision-making and genetic counselling.

C.O.I. Disclosure:

C. Hernando-Davalillo: None. **D. Salinas Chaparro:** None. **S. Cardelús Vidal:** None. **Á. Díaz Anadón:** None. **M. Russi:** None. **M. Montiel Herrera:** None. **P. Fernández Pareja:** None. **G. Márquez Zambudio:** None. **A. Alcalá San Martín:** None.

EP23.013 Middle-read sequencing: bridging the gap between short-read and long-read sequencing with superior performance and cost efficiency

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[1]MGI Tech, Shenzhen, China, [2]Complete Genomics Incorporated, San Jose, CA, USA.

Background: Sequencing technologies are dominated by two main read lengths: short-read (50–500 bp) and long-read (>1000bp) sequencing. Short-read sequencing is cost-effective and highly accurate for detecting small variants but struggles with challenging regions. Conversely, long-read sequencing excels in de novo assembly of large genomes and SV detection, but its broader application is hindered by high costs and reduced accuracy, particularly in homopolymer regions. Middle-read (500–1000 bp) sequencing can provide affordable solution that offers balanced performance.

Material and methods: We conducted a comprehensive comparison of short-read, middle-read, and long-read sequencing using NIST NA12878 and NA24385 WGS data. This assessment focused on sequencing accuracy, coverage, and variant detection (SNP/INDEL/SV/STR) to highlight the strengths and limitations of each read length. Additionally, we developed a best-practice WGS analysis pipeline and an assembly-based SV caller tailored for middle-read sequencing to enhance performance.

Results: Middle-read sequencing achieved 99.99% accuracy (Q40) for over 90% of reads in the 600–1000 base range. It improved coverage and significantly reduced mis-mapping issues in genomic regions challenging for short-read. Data from GIAB samples showed over 99.5% F-score in SNP detection and 99.2% in indel detection without machine learning method, accurate

detection of STR expansions in genes like *FMR1*, and 94% SV precision comparable to long-read performance.

Conclusion: Middle-read sequencing introduces a new paradigm for applications such as advanced WGS, fl-cDNA, 16S RNA, TCR/BCR sequencing, and amplicon sequencing. It offers cost-effective (sub-$1/Gb) sequencing with extended accurate reads, high mapping quality in challenging regions, and exceptional full haplotype-phased variant detection of SNPs, indels, and SVs.

C.O.I. Disclosure:

H. Yi: None. **D. Radoje:** None. **B. Song:** None. **M. Long:** None. **C. Xu:** None. **M. Ni:** None. **Y. Ding:** None. **X. Chen:** None. **F. Liu:** None. **Y. Zhou:** None. **F. Chen:** None. **J. Liu:** None.

EP24 Treatments for Genetic Disorders

EP24.001 Next-Generation Gene Therapies: Synthetic Biology Innovations in Human Genetics

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Synthetic biology is revolutionizing human genetics by enabling precise and efficient therapeutic interventions for genetic disorders. Advances in DNA and RNA synthesis, coupled with viral and non-viral gene delivery systems, have expanded the potential of gene therapy, offering new treatment paradigms for previously incurable conditions.

Viral vector-based gene therapy has shown remarkable success in treating inherited disorders. Adeno-associated virus (AAV) vectors are widely used for their ability to achieve long-term gene expression with minimal immunogenicity, making them ideal for monogenic diseases such as spinal muscular atrophy and hemophilia.

Simultaneously, non-viral delivery methods, such as synthetic RNA and CRISPR-based systems, offer transient, precise, and scalable alternatives for gene modulation. mRNA-based therapies have demonstrated their potential in protein replacement for metabolic disorders, while CRISPR/Cas technologies are advancing in clinical applications for diseases like Duchenne muscular dystrophy and hereditary blindness. These approaches reduce the risks associated with viral integration while enabling programmable genome editing.

To support the rapid advancement of gene therapies, GENEWIZ offers comprehensive services in AAV and lentiviral vector packaging, as well as high-purity RNA synthesis, empowering researchers in the development of next-generation treatments for genetic disorders. These technologies are driving the transition from experimental therapies to clinically viable, personalized genetic medicine, bringing curative solutions closer to reality.

C.O.I. Disclosure:

P. Rao: None. **C.M. Richardson:** None. **M. Karney:** None. **S. Kumar:** None.

EP24.002 The role of *MECP2* testing in advancing novel pharmacotherapies for Rett syndrome

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Background: Rett syndrome (RTT), is a rare neurodevelopmental disorder primarily affecting females, characterized by a period of normal development followed by a loss of motor, social, and communicative skills. RTT is caused by mutations in the *MECP2* gene, leading to dysfunction in neural development and synaptic plasticity. Recent advancements in research have significantly expanded the syndrome therapeutic landscape, offering renewed hope for affected individuals.

Material and methods: A cohort of 42 females and 1 male RTT patients were diagnosed molecularly applying Sanger sequencing, next-generation sequencing, MLPA, or array CGH.

Results: Novel *MECP2* variants were identified, including the presence of two likely pathogenic variants in *cis* orientation: *MECP2* (NM_001110792.2):c.[871_873delinsA;c.941_968del], p.(Ser291_Leu323delinsX[23]). To our knowledge, no RTT patients described to date exhibit the co-occurrence of two causative variants in *MECP2*. We also reported a known hemizygous variant in a single diagnosed male patient - *MECP2* (NM_001110792.2):c.455C>T, p.Ala152Val. Furthermore, a detailed genotype-phenotype analysis was conducted for all individuals, followed by in silico variants pathogenicity evaluation.

Conclusion: Identifying mutations in the *MECP2* gene, the primary cause of RTT, provides diagnostic confirmation and distinguishes it from other neurodevelopmental disorders with overlapping features. Nowadays, this precision is crucial not only for genetic counselling but also for selecting eligible patients for therapies such as **trofinetide** (approved in U.S.), which has demonstrated benefits in treating RTT core symptoms. Additionally, AAV9-delivered gene therapies approach such as TSHA-102 (NCT05606614 and NCT06152237; both recruiting) and NGN-401 (NCT05898620; recruiting) have shown early promising results.

E.B.O. and K.M. contributed equally to this work

C.O.I. Disclosure:

E. Bukowska-Olech: None. **K. Matuszewska:** None. **M. Telenga:** None. **A. Jakubiuk-Tomaszuk:** None. **R. Śmigiel:** None.

EP24.003 Mutation-specific therapy for CYLD Cutaneous Syndrome (CCS) - screening for premature termination codon readthrough agents

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Background: CYLD cutan syndrome (CCS) is a rare monogenic skin disease characterized by the development of benign skin appendage tumors which are often disfiguring and painful. CCS is caused by pathogenic variants of the cylindromatosis (*CYLD*) gene: approximately 90% of tumor-predisposing germline mutations in CCS are stop codon mutations. Since currently the only treatment for CCS is surgical removal of the tumors, exploring the possibility of causative therapeutic treatments is very important. Premature stop codon readthrough induced by various drugs is a promising strategy for the treatment of hereditary diseases caused by nonsense mutations and has already proven effective in several diseases.

Method: The truncating pathogenic variants of CYLD most frequently identified in CCS patients (S371X, Y585X, R758X, R936X) were generated by in vitro mutagenesis and cloned into the pcDNA3.1 plasmid vector for in vitro experiments. NF-κB-luciferase expressing HEK293 cells were transfected with these constructs

and treated with compounds that potentially induce early stop codon readthrough. Western blot analysis was performed to detect the appearance of the full-length CYLD protein.

Results, conclusion: We tested several compounds using the CYLD mutant constructs, one of them caused partial readthrough of the CYLD R758X nonsense mutation in a dose-dependent manner in vitro. To follow the function of the restored full-length CYLD protein, NF-κB luciferase activity was monitored and the expression of NF-κB-induced, CYLD-regulated genes were measured. Together, these two in vitro methods allowed us to demonstrate that by inducing premature stop codon readthrough, CYLD functions can be partially restored with this compound.

C.O.I. Disclosure:

A. Hajdu: None. **J. Danis:** None. **J. Cintrat:** None. **N. Nagy:** None. **N. Rajan:** None. **M. Szell:** None. **E. Adam:** None.

EP24.004 Repurposing of perampanel in White-Sutton syndrome (WHSUS): first results from an Italian cohort of patients

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Background: Pathogenic mutations in *POGZ* gene are associated to White-Sutton syndrome (WHSUS), a rare neurodevelopmental disorder mainly characterized by psychomotor delay, behavioral abnormalities and facial dysmorphic features. Recent studies have suggested the efficacy of perampanel (Fycompa, an antiepileptic medication) in improving many of the behavioral and social issues of the syndrome.

Material and methods: Nineteen subjects with a diagnosis of WHSUS were characterized through clinical, instrumental and molecular data, creating a detailed cohort of patients. Among them, eligible subjects were enrolled in the clinical trial where, according to the study protocol, they have been extensively evaluated before and during treatment with perampanel. Particular attention was given to adaptive and behavioral tests (i.e. VABS-II, CBCL). Study duration is 12–24 months.

Results: To date, 3 WHSUS patients have been following the study. Range of improvements seems variable, however all subjects have shown positive results in their adaptive levels, anxiety issues and personal independence with perampanel therapy - i.e. VABS-II composite scores before treatment (T0), after 6 months (T1) and 12 months (T2) were:

	PT1	PT2	PT3
T_0	60	38	85
T_1	74	49	81
T_2	94	50	to be assessed

Conclusion: First results suggest that all participants benefit from treatment with perampanel. The study will continue to evaluate its role in ameliorating the behavior and the quality of life of WHSUS patients. Five additional subjects are about to be enrolled. Updated data will be presented and discussed. Moreover, by collecting the first and largest Italian cohort of WHSUS patients, our study gave an insight into this rare condition.

C.O.I. Disclosure:

A. Facchini: None. **M. Concas:** None. **S. Zampieri:** None. **M. Carrozzi:** None. **M. Bin:** None. **A. Arbo:** None. **I. Scala:** None. **C. Graziano:** None. **A. Innoceta:** None. **M. Trivisano:** None. **A. De Dominicis:** None. **G. Trimarchi:** None. **L. Garavelli:** None. **M. Baldassarri:** None. **I. De Maggio:** None. **F. Mari:** None. **D. Greco:** None. **P. Gasparini:** None.

EP25 Genetic Counselling/Services/Education

EP25.002 Structuring a Genetics and Rare Diseases Care Network in the largest metropolis in Latin America

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Background: São Paulo (SP) is a city with 11.4 million inhabitants, and an estimated 680,000 People with Rare Diseases (PRD). In Brazil, the SUS is the public health system that guarantees access to health for population. Its management is part of the federative model, where responsibility is divided into three levels: federal, state, and municipal. In 2014, the National Policy for Comprehensive Care for PRD was established. In 2019, the Municipal Health Department of São Paulo (MHD-SP) has implemented the Program Caring for PRD in SP (PCPRDSP). The objective of this article is to report on the implementation of public policies for PRD in SP based on the PCPRDSP.

Material and methods: With the establishment of the PCPRDSP, the creation of a technical area was implemented; publication of the Guidelines Document and Educational Plan. In partnership with the Jo Clemente Institute and Jeffrey Modell Foundation, the expansion of the Newborn Screening Program for Inborn Errors of Metabolism and Immunity and Genomic Diagnostic Tests for PDR were implemented.

Results: Since the implementation of the Program, the following exams have been carried out: see table.

Conclusion: The SUS is one of the largest and most complex public health systems in the world. The management of actions

must be supportive among the three level. The implementation of a Municipal Program for Rare Diseases assists in the planning, organization, control, evaluation and execution of actions in the comprehensive care of PDR.

C.O.I. Disclosure:

C.F. Andrade: None. **C.S. Aranda:** None. **C.G. Bruzaca:** None. **D. Christofolli:** None. **A. Condino-Neto:** None. **F.D. Rabelo:** None. **V.L. Tavares:** None. **G.Y. Hayashi:** None. **D.L. Neves:** None. **K.M. Dib:** None. **A.M. Mauro:** None. **L.M. Borgianni:** None.

EP25.003 Students' view on a new type of evaluation at the completion of workshops in Medical Genetics

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Background: At the end of the first term students of the Carol Davila Medical and Pharmacy University enrolled in the Medical Faculty have to first pass the examination from the material studied during workshops. To pass this examination is mandatory for being accepted to participate in the final multiple choice examination from the subjects of the lectures. The present study investigates medical students' opinion on a task during their examination at the end of the workshop training in human genetics.

Material and method: 106 students handed in an analysis of a pedigree. Trying to prevent plagiarism I asked them to draw and analyze their own pedigree on whether attending medical university is an inherited trait. I evaluated the diagrams and the interpretation, counting for 50% of their mark in the workshop examination. At the end of the term students were asked to answer a *questionnaire*. 98 answered it and 95 accepted their answers to be made public.

Results: showed that only for 2 students drawing the pedigree seemed difficult, while for 39 it seemed easy. Gathering the data was easy for 48.4% from the total number of students. 41% admitted they knew the family history, and 47.4% considered it a modality to discuss about it with their family members. 91 students (95.8%) stated that they would have not preferred an examination only with multiple choice questions at the end of the workshops.

Conclusion: Nowadays students themselves feel the need to not be evaluated only by answering questions in classical way.

C.O.I. Disclosure:

R.M. Dragotoiu: None.

EP25.004 ERN-ITHACA Genetics educational and training materials

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Background: Education and training are one of the goals of the European Reference Networks. ERN-ITHACA has developed, organised and provided a wide range of variety of educational materials for learners at different levels and specialties.

Material and methods: This poster begins with a general overview of the educational materials offered by ERN-ITHACA, and then it continues with a focus on several textbook projects, namely the APOGeE medical genetics textbook, Dysmorphology handbook and the Survival Guide to Genetics. The main chapters of the APOGeE medical genetics textbook are available online. The Dysmorphology handbook is available with photos and descriptions of symptoms, which will be linked to the syndromes.

Results: The Dysmorphology handbook is available with photos and descriptions of symptoms, which will be linked to the syndromes.

Conclusion: To maximise its reach in the European Union and beyond, the Survival Guide to Genetics has been translated into several languages and more translations will be available.

C.O.I. Disclosure:

N. Szeto: None. **M. Gerard:** None. **Y. Gillerot:** None. **J. Berg:** None. **M. Genuardi:** None. **M. Krahn:** None. **U. Moog:** None. **E. Tobias:** None. **P. Turnpenny:** None. **J. Zschocke:** None. **D. Dan:** None. **S. Douzgou Houge:** None. **M. Le dref:** None. **L. Faivre:** None. **A. Hugon:** None. **J. Jouannic:** None. **K. Tjitske:** None. **D. Koolen:** None. **G. Mosiello:** None. **A. Renieri:** None. **M. Tartaglia:** None. **Z. Tumer:** None. **B. Tumiene:** None. **A. van Eeghen:** None. **L. Vissers:** None. **K. Vyshka:** None. **D. Wieczorek:** None. **G. Zampino:** None. **C. Zweier:** None. **A. Verloes:** None.

EP25.005 ERN-ITHACA: European Reference Network on congenital malformations and rare neurodevelopmental disabilities

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Background: The ERN-ITHACA brings together experts in rare multiple congenital anomalies and rare neurodevelopmental disorders, the latter field mainly covering intellectual disability (ID) and autism spectrum disorder (ASD). ITHACA's field of expertise covers the clinical and biological/genetic diagnosis of

these developmental anomalies, the coordination of their multidisciplinary care and their treatment, and their prenatal diagnosis and fetal pathology. The name ITHACA stands for Intellectual disability, TeleHealth, Autism and Congenital Anomalies. It also echoes the diagnostic odyssey experienced by so many patients with developmental anomalies.

Material and methods: Led by Prof. Alain VERLOES at APHP-Robert DEBRE University Hospital, the network today consists in more than 70 Clinical Genetics Departments, coming from 25 Member States, as well 47 European Patient Advocay Groups (EPAGs).

Results: By networking patients' representatives, clinical experts and researchers, we aim to improve early diagnosis, care and cure of patients with rare developmental anomalies. Here are some of the projects we've completed or are currently working on: producing clinical guidelines and consensus statements on rare developmental disorders (including Phelan McDermid, Rubinstein-Taybi, Cornelia de Lange syndromes.); developing a "meta" patient-registry called ILIAD, connecting 37 HCPs, databases, and biobanks in 13 countries for patients with dysmorphic and/or ID; supporting the development of telemedicine and tele-expertise tools to allow discussion between referring doctors and RD experts scattered in the EU, like the CPMS (Clinical Patient Management System); producing advanced training and e-learning tools dedicated to health professionals or lay persons; organising webinars, conferences and workshops at the European level.

Conclusion:

C.O.I. Disclosure:

M. Le dref: None.

EP25.006 The implementation of the Clinical Patient Management System (CPMS) for data sharing of neuromuscular patients: the ERN EURO-NMD project

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Background: European Reference Networks (ERNs) are virtual networks connecting healthcare providers across Europe to facilitate the diagnosis and treatment of rare diseases. The Clinical Patient Management System (CPMS) is a web-based digital platform used by healthcare professionals within ERNs to virtually discuss patient cases and to securely submit patient data to registries and databases.

Material and methods: From October 2023 to October 2027 (under a multi-beneficiary grant, call EU4H-2023-ERN-IBA) ERN EURO-NMD will organize CPMS activities in the context of Work Package 4 (WP4), whose leadership was entrusted to Sant'Anna Hospital in Ferrara.To support the implementation of CPMS for highly specialized virtual discussions, seven tasks were designed. These include the organization of virtual CPMS teaching courses and CPMS "focus group", or thematic days dedicated to the discussion of specific diseases or atypical cases. A pilot reimbursement scheme has also been defined to encourage medical discussion through the CPMS, as well as the development of guest virtual centres to address coverage issues within the EU. Moreover, a new, user-friendly version of the platform, CPMS 2.0, has recently been launched.

Results: The activities of WP4 have been designed to expand the provision of highly specialised virtual multidisciplinary advice to patients with rare neuromuscular diseases. First results are expected in the course of 2025.

Conclusion: The CPMS serves as an excellent tool and valuable opportunity to enhance the service provided to patients with rare neuromuscular diseases. Fully leveraging this remarkable digital tool is one of the core missions of the ERNs.

C.O.I. Disclosure:

F. Fortunato: None. **C. King:** None. **I. Vivaldi:** None. **M. Neri:** None. **F. Gualandi:** None. **A. Atalaia:** None. **T. Evangelista:** None. **A. Ferlini:** None.

EP25.007 Predictive testing for Huntington's disease in a digital age; patient power with potential pitfalls

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Background: Advances in digital healthcare impacts genetic counselling and testing for hereditary disorders, including Huntington's Disease (HD). The Danish Digital Health Strategy was implemented from 2018 but has recently announced an extension to include the automatic release of all results, including genomic laboratory reports.

Material and methods: The European Huntington's Disease Network (EHDN) Working Group for Genetic Counselling and Testing have reviewed the existing Recommendations for Predictive Testing in Huntington's Disease and the literature concerning digital health records.

Results: Patient engagement, potential severity of results, timing of results, impact on wellbeing and potential for discrimination emerged as common themes from the literature.

Conclusion: We present recommendations to maintain the integrity of genetic counselling practise. Clinician controlled release of digital results rather than automatic release is recommended in the case of predictive testing in HD and other inherited neurodegenerative disease.

C.O.I. Disclosure:

N. Lahiri: None. **V. Mocanu:** None. **S.G. Lindquist:** None. **L.E. Hjermind:** None. **J. Heilmann:** None. **R. Macleod:** None.

EP25.008 The European Long Read Innovation Network (ELRIN)

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Background: Genome sequencing in medicine has revolutionized clinical and basic research, disease diagnostics in particularly of rare diseases and cancer, and is fundamental for personalized treatment approaches. While short-read sequencing (srGS) technology has advanced in throughput and accuracy, ongoing challenges remain including the accurate assembly of complex

genomic regions, the identification of rare copy number variants, and investigating the role of the epigenome in diseases.

Material and methods: Novel long-read sequencing (lrGS) technologies overcome these limitations and allow the identification of structural variants and genomic regions that were previously inaccessible. Recent advancements have made lrGS comparable to srGS in both throughput and accuracy, while data analysis and interpretation remains a challenge. While lrGS technology is already integrated as part of genome diagnostics in some centers in Europe, it's full potential in terms of diagnostic sensitivity and specificity still needs to be explored in clinical routine.

Results: Thus, a European long-read initiative (ELRIN) was formed by human genetic institutes across Europe to develop new standards for ONT-based lrGS technology, to clearly define its field of advantage compared to srGS and to generate novel reference data sets. The ELRIN consortium, consisting out of 73 people from over 25 institutes, serves as experts for clinical questions and collaborates closely with networks such as ERDERA or the 1 + MG (Genomes of Europe) initiative.

Conclusion: With these approaches, ELRIN aims to explore the full potential of lrGS in genomic diagnostics to be integrated as a first-line diagnostic tool in clinical patient care in the near future.

C.O.I. Disclosure:

B. Kristmann: A. Employment (full or part-time); Significant; University Hospital Tübingen. **O. Riess:** A. Employment (full or part-time); Significant; University Hospital Tübingen.

EP25.009 Genetic counseling for stepwise genetic testing of patients with gait disturbance

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Hereditary spinocerebellar degeneration (SCD), is a neurodegenerative disease with cerebellar ataxia as its main symptom. Here, we describe a case of spinocerebellar ataxia, autosomal recessive (SCAR), which was identified based on gait disturbance.Patient is a 59-year-old man, who had been experiencing "wobbling" at walking for approximately three years. He had undergone genetic testing for autosomal dominant SCD at previous hospital, but no pathogenic variant was detected. The patient visited our department of clinical genetics in hopes of receiving prompt treatment. Therefore, at first, genetic testing for spinal muscular atrophy (SMA) and Becker muscular dystrophy (BMD) was performed, but no pathogenic variants were detected. When listening carefully to the patient's thoughts about the test results, we realized he started showing signs of resignation due to the lack of a definite diagnosis, yet remained hopeful about identifying the cause. Therefore, whole exome sequencing analysis was performed, indicate a pathogenic variant and a variant of uncertain significance (VUS) were detected in*VSP13D*, a gene associated with a rare type of SCAR4. Since the patient's parents were deceased, genetic testing of the daughter was performed, who was also found to carry the VUS variant.The genetic diagnosis of gait disturbances is challenging, as it can present in multiple different conditions such as hereditary spastic paraplegia or spinocerebellar ataxia. Therefore, in the diagnostic process, it is important not only to present genetic testing, but also to discuss the patient's frustrations and thoughts about the genetic test as we proceed.

C.O.I. Disclosure:

T. Ohye: None. **N. Ishihara:** None.

EP25.010 ECACC Human Genetic Services - supporting global genetic research for 40 years

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Background: Human Genetic Services (HGS) of European Collection of Authenticated Cell Cultures (ECACC), is a not-for-profit resource centre within the UK Health Security Agency. Over 40 years, HGS has provided essential services to the global genetic research community, isolating peripheral blood mononuclear cells (PBMCs) from whole blood, and subsequently transforming these cells to quality controlled lymphoblastoid cell lines (LCLs) using the Epstein-Barr virus (EBV). LCLs are an invaluable resource, providing a potentially limitless source of biomolecules such as DNA, RNA and proteins, offering a cost-effective in vitro model, particularly for rare genetic diseases.

Material and methods: LCLs were established from human PBMCs isolated by density gradient centrifugation. EBV infection was achieved by culturing 2×10^6 PBMCs in RPMI 1640 with 20% (v/v) foetal bovine serum (FBS), 1% (v/v) phytohaemagglutinin and 20% (v/v) EBV (B95-8) supernatant at 37 °C, 5% CO_2 for 3–4 days. 50% media changed every 3–4 days with RPMI-1640, 20% (v/v) FBS until the transformed cell clusters reached 100 µm in diameter and displayed characteristic 'hand-mirror' morphology.

Results:

Key projects supported by HGS

Study	Number of samples processed
GSK	124,577
ARTHRITIS UK	2780
DIABETES UK	5284
GENERATION SCOTLAND	1452
LACUNAR STROKE	3356
MNDA	3107
CANCER RESEARCH UK	522
ProBAND STUDY	2199
TWINS RESEARCH UNIT	5206
WERN	909
MRC ALZHEIMER STUDY	3441
ASTHMA/ECZEMA	4383
AUTISM STUDY	1904

HGS has supported over 100 organisations including pharmaceutical companies, charities and universities and has processed over 80,000 samples to ISO 9001 and HTA standards. At first attempt, the successful transformation rate was 95%.

Conclusion: HGS offers a reliable, high-throughput, not-for-profit service for the genetic research community. HGS is seeking to enhance its service portfolio by offering CD34+ haematopoietic stem cell isolation which can be used to support regenerative medicine research.

C.O.I. Disclosure:

K. Ahmed: None. **J. Cooper:** None. **E. Burnett:** None.

EP25.011 Importance of family history in indicating HBOC genetic testing in a resource-limited setting

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Background: Genetic screening for hereditary breast and ovarian cancer (HBOC) relies on clinical criteria such as early-onset diagnosis and/or a family history of cancer. In centers with limited access to next-generation sequencing (NGS), optimizing patient selection for genetic testing is crucial to maximize the identification of at-risk individuals. This study aims to compare the frequency of pathogenic mutations based on these inclusion criteria.

Material and methods: We analyzed an extended HBOC panel comprising 28 genes in 62 patients who met at least one of the following criteria: diagnosis before the age of 40 and/or a family history of breast or ovarian cancer. Variants were classified according to ACMG recommendations.

Results: Group "Young without family history" (n = 41): Pathogenic/likely pathogenic mutations were identified in 4 patients, affecting BRCA1(2), ATM(1), MUTYH(1), and APC(1). Variants of uncertain significance (VUS) were found in 13 patients, involving ATM(3), BRCA1(2), RAD50(2), MRE11(2), NBN(1), CDKN2A(1), MLH1(1), MUTYH(1), CDH1(1), and APC(1). Group "With family history" (n = 21): Pathogenic/likely pathogenic mutations were identified in 15 patients, affecting BRCA1(7), BRCA2(6), ATM(1), and MUTYH(1). VUS were identified in 4 patients, involving ATM(2), NBN(1), CHEK2(1), APC(1), and NF1(1).

Conclusion: Our findings suggest that family history is a stronger predictor of pathogenic mutations than young age alone in HBOC genetic screening. In a resource-limited setting, prioritizing genetic testing based on family history could enhance the identification of at-risk patients and optimize the use of NGS.

C.O.I. Disclosure:

A. Bouramtane: None. **B. El hejjioui:** None.

EP25.012 Pathogenic variant *MYO7A* (c.5648G>A, p.Arg1883Gln): a case of Usher syndrome type 1B in two sisters from Ukraine

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Background: Usher syndrome is a rare autosomal recessive genetic disorder characterized by a combination of hearing loss and progressive vision loss. The pathogenic variant MYO7A (c.5648G>A, p.Arg1883Gln) is a primary cause of Usher syndrome type 1B. Aim of our study: to assess the genetic basis of congenital deafness and ocular pathologies (myopia, cataract) in siblings.

Material and methods: NGS analysis of 576 genes ("Invitae", USA), genealogical method, clinical audiological, ophthalmological assessments.

Results: Father aged 40, healthy, pathogenic variant heterozygous MYO7A (c.5648G>A, p.Arg1883Gln). Mother aged 27, with sensorineural hearing loss pathogenic variant heterozygous MYO7A (c.5648G>A, p.Arg1883Gln), heterozygous variant of uncertain significance (VUS) *PITX3, SIPA1L3*, and *COL4A2*. Elder daughter aged 8, with congenital deafness and congenital cataract in the right eye, high myopia and complex myopic astigmatism - homozygous pathogenic variant in *MYO7A* (c.5648G>A), (VUS) *PITX3, SIPA1L3*, and *COL4A2*. Younger daughter aged 7, with congenital deafness, high myopia and complex myopic astigmatism- homozygous pathogenic variant in *MYO7A* (c.5648G>A), (VUS) *PITX3, SIPA1L3*, and *COL4A2*.

Family history uncomplicated by vision and hearing pathology. Both sisters had speech delay, language comprehension issues, and learning difficulties were noted in both children from age 3. Both children had received cochlear implants.

Conclusion: genetic testing confirmed the presence of Usher syndrome type 1B as the primary cause of hearing loss in the children. Ophthalmological issues may be partially associated with other variants, which require further investigation to determine their clinical significance.

C.O.I. Disclosure:

M. Drobchak: None. **N. Helner:** None. **H. Akopyan:** None. **N. Kech:** None. **I. Vijtovych:** None. **K. Sosnina:** None. **M. Vlokh:** None.

EP25.013 Genetic counseling for two cases with *BRCA1*-associated hereditary breast and ovarian cancer syndrome who underwent risk-reducing salpingo-oophorectomy in their 30s

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Background: Center for Medical Genetics (CMG) in Shinshu University Hospital offers genetic diagnosis as well as long-term follow-up to deal with problems and concerns related to the hereditary cancer through genetic counselling (GC). In this report, we describe GC for two contrasting cases with *BRCA1*-associated hereditary breast and ovarian cancer syndrome (HBOC) who underwent risk-reducing salpingo-oophorectomy (RRSO) in their 30s to discuss GC-based decision-making process for RRSO.

Case 1: A 38-year-old woman with two children visited CMG for GC. She experienced bilateral breast cancer and her mother experienced breast cancer and ovarian cancer. When the chemotherapy was discontinued by side-effects, she was eager for RRSO and underwent RRSO at age 39 years.

Case 2: A 31-year-old woman with no child visited CMG for GC. She experienced bilateral breast cancer and melanoma, and her mother experienced breast cancer. She had infertility treatment and had surveillances for ovarian cancer. She had her first child at age 34 years. One year later, she began to consider RRSO. However, she couldn't decide to have RRSO for ambivalent feelings between fear for ovarian cancer and hope for another child. Finally, she underwent RRSO at age 36 years.

Conclusion: Although both cases underwent RRSO in the 30s, the attitudes for RRSO were contrasting and different GC approaches were provided: supportive to the decision in Case 1 and organizing the feeling in Case 2. In the management of HBOC, GC plays a crucial role in the decision-making process especially in the setting of preventive but invasive intervention.

C.O.I. Disclosure:

E. Kise: None. **T. Kojima:** None. **A. Sakyu:** None. **T. Miyamoto:** None. **H. Kobara:** None. **T. Kosho:** None.

EP25.014 Genetic counseling of the siblings with developmental epileptic encephalopathy exhibiting a missense variant in G*ABRA2*

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Background: Developmental epileptic encephalopathy (DEE) is a refractory epilepsy that onset in infancy and is associated with developmental delay. Numerous causative genes have been reported, and treatment strategies and prognosis may differ depending on the specific gene involved, making genetic determination useful. In this study, we performed genetic analysis to investigate the underlying cause of DEE in a sibling pair with a refractory epilepsy and profound developmental delay. Genetic counseling played a crucial role in this case, and therefore, we report the process and outcomes.

Material and methods: The subjects are two siblings, a proband 6-year-old boy and his sister 3-year-old girl, both of whom developed epilepsy at 4 months of age. The proband was clinically diagnosed as DEE in the first year of life, but the causative gene could not be determined in routine clinical practice. As a result, he was enrolled in the Initiative on Rare and Undiagnosed Diseases (IRUD) project, a research initiative in Japan, and trio analysis using WES was performed.

Results: A heterozygous missense variant in the *GABRA2* was identified in the proband. This variant was found in a mosaic state in his father. The sister, who was born after enrollment in the IRUD project, a same variant was identified through single-site analysis.

Conclusion: In this case, it raised suspicion of germline mosaicism in one of the parents. Genetic disorders are often subject to shifting responsibility onto the parental generation, and in this regard, genetic counseling plays a crucial role in addressing these concerns.

C.O.I. Disclosure:

N. Ishihara: None. **T. Ohye:** None. **H. Inagaki:** None.

EP25.015 Contribution of a rare *NPHS2* variant in steroid resistant nephrotic syndrome in Tunisia

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Background: Several genes are known to be involved in hereditary steroid-resistant nephrotic syndrome (SRNS) In autosomal recessive forms, *NPHS2*, encoding podocin, is the most implicated gene that is associated with nephrotic syndrome type 2 (OMIM#600995). The disorganization of podocyte cytoskeleton is histologically expressed as Focal Segmental Glomerulosclerosis (FSGS) lesions. Among the 250 identified variations in *NPHS2*, the substitution c.538G>A(p.V180M) is frequently reported in North Africa.

Material and methods: Targeted sanger sequencing of exon 5 of the *NPHS2*(*NM_014625.4)* gene was performed for the c.538G>A variation in patients with SRNS.

Results: We have collected nine SRNS cases belonging to four unrelated families from the Sfax region in south Tunisia. Two families were consanguineous. Onset age was variable from infancy to adulthood. The kidney biopsy, performed for adults, objectified FSGS lesions. Targeted sequencing of *NPHS2* identified the presence of the c.538G>A variation in the homozygous state, thus confirming the diagnosis of nephrotic syndrome type 2 for all patients. This missense variation leads to a modification of the three-dimensional conformation of podocin, which affects the podocin/nephrine interaction, destabilizing the podocyte cytoskeleton.

Conclusion: The recurrence of the c.538G>A variant in *NPHS2* in Tunisia supports its founder effect and makes the etiological diagnosis of SRNS faster in our country, where access to high-throughput sequencing is not yet a routine test in genetic diagnostic laboratories.

C.O.I. Disclosure:

I. Boujelbene: None.

EP25.016 Bridging the Gap: A Review of the First Adult Genetic Service in Singapore

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Background: The demand for adult genetic services has grown, driven by the need for diagnostic testing, counseling, and managing genetic disorders in adulthood[1,2]. The establishment of an adult genetics clinic in Singapore aims to provide accurate molecular diagnostics and personalized care for adults with suspected non-cancerous genetic conditions in Southeast Asia.

Material and methods: This retrospective study evaluates the early outcomes of a multi-disciplinary adult genetics clinic at a tertiary hospital in Singapore and examines challenges impacting service delivery.

Results: Between January 2021 and December 2023, 134 patients visited the clinic. Referrals were primarily for connective tissue disorders (37%), renal disorders (22%), cardiovascular conditions (18%), metabolic disorders (13%), and others (10%). Most referrals (73%) came from specialist services, while 14% involved patients transitioning from pediatric care. Genetic counseling and testing were offered to 68% of patients, with 36% consenting. The diagnostic yield was 24%, with common findings including pathogenic variants in *GLA* and *PKD1*. The service enabled targeted interventions, informed reproductive planning, and reduced patient anxiety. Barriers included limited availability of geneticists and counselors, high test costs, and low patient awareness, restricting broader accessibility.

Conclusion: Adult genetic services address critical diagnostic gaps and improve patient outcomes. Our findings also highligh the need for enhanced public health awareness to improve service integration and accessibility.

C.O.I. Disclosure:

J. Hsieh: None.

EP25.017 Model process for care pathway development for Rare Diseases

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Background: There is an existing major need for multidisciplinary care pathways (CPs) for rare diseases (RDs). In contrast to agreed best practice methodology for the appraisal and development of clinical practice guidelines, there is lack of an agreed definition and approach for RDs. Under the 'JARDIN' EU4Health current joint action, a multi-stakeholder group developed a model process for ERN CPs for adoption into national healthcare systems.

Material and methods: A systematic review of existing CP development methodologies[1] was completed. A 'model process' for the development of RD care pathways was agreed by comparing key activities identified in the systematic review, aligning common characteristics and identifying gaps. The model process was co-created and validated by a multi-stakeholder

group of clinical experts, experts by lived experience, methodologist and health authorities.

Results: The model process comprises 5 phases with 18 steps: Planning, Scoping, Development, Implementation and Evaluation. This study focussed on the first 12 steps: Selection of Topics & Defining Outline Scope; Identification of Experts & Key Stakeholders; Governance & Planning; Defining Detailed Pathway Scope and Parameters; Gathering & Appraisal of Published Evidence; Mapping of Current Clinical Practices; Collecting Patient Needs; Triangulation of Patient Needs, Baseline Pathway & Published Evidence; Designing Ideal Care Pathway; Consensus on Evidence Gaps; Revising Care Pathway & Supporting Material; Approval & Publication.

Conclusion: The model process was co-created with a patient-centred approach by a multi-stakeholder group, to be published with a supporting toolkit, and piloted under 'JARDIN' in 2025 with development of lead ERN care pathways.

C.O.I. Disclosure:

M.R. Bolz-Johnson: None. **A. Ward:** None. **G. Sweeney:** None. **T. Eileen:** None.

EP25.018 Clinical outcome of a patient with mosaic tetrasomy 18 detected by NIPT

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Background: Non-invasive prenatal testing (NIPT), which analyzes cell-free DNA in maternal plasma, is widely employed to assess the likelihood of fetal chromosomal abnormalities and aneuploidies. While NIPT demonstrates high sensitivity and specificity, it cannot predict the phenotypic manifestations of the fetus.

Case Report: A 40-year-old Japanese woman, due to advanced maternal age, underwent NIPT at a non-obstetric clinic. The test revealed an abnormal quantity of chromosome 18 segments. To further investigate, array analysis was performed using amniotic fluid samples. The array results suggested mosaicism involving either partial trisomy or tetrasomy of chromosome 18. If array results reflect a mosaic trisomy, the estimated mosaicism is 15%. Prenatal ultrasound examination showed no abnormalities. Following birth, G-banding chromosomal analysis of the infant's peripheral blood confirmed the presence of 2% 47,XX,+mar cells. Until six months of age, the child exhibited no significant developmental or growth issues.

Assessment: This case underscores that some fetuses with mosaic chromosomal abnormalities may not exhibit any overt phenotypic anomalies. Cell line with partial gain of chromosome 18 may be decrease during pregnancy and/or the percentage of mosaicism can vary significantly between fetus and placenta. Consequently, multiple consultations were arranged to thoroughly discuss the correlation between the array testing results and the postnatal symptoms, necessitating comprehensive genetic counseling.

Conclusion: The presence of relatively mild or normal phenotypes in neonates with such conditions highlights the complexities and challenges in providing prenatal genetic counseling for cases involving fetal mosaic chromosome abnormalities.

C.O.I. Disclosure:

M. Ozawa: None. **O. Migita:** None. **M. Ogiwara:** None. **S. Ohori:** None. **C. Zhu:** None. **N. Shimizu:** None.

EP25.019 Unmasking childhood syndromes in adults: diagnostic utility of genetic testing for rare conditions

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Background: Childhood-onset rare disorders are frequently overlooked in adult patients, particularly when presenting with atypical or attenuated phenotypes. This oversight can lead to missed diagnoses, delayed interventions, and inadequate reproductive counseling. We present a case of a rare yet well-known condition diagnosed at an unusual age when the milder phenotype complicates the diagnostic process and recognition.

Materials and methods: A 36-year-old male with medullary thyroid cancer was referred for *RET* gene testing due to suspected MEN type 2. During pre-test genetic counseling the medical geneticist noticed short stature and dysmorphic features suggestive of Noonan syndrome. Thus, Whole-exome sequencing (WES) was recommended, replacing the initial single-gene testing plan.

Results: WES identified a heterozygous pathogenic *PTPN11* variant (c.922A>G), confirming Noonan syndrome. This diagnosis explained the patient's phenotype and significantly impacted medical management and reproductive planning. RASopathies are often recognized in childhood due to distinctive features that may diminish with age. Consequently, adult presentations can be subtle, involving seemingly unrelated symptoms that obscure the underlying genetic etiology. These characteristics emphasize the critical need for comprehensive clinical evaluation and detailed history taking in adults, even out of the context of planned visits.

Conclusion: This case highlights the importance of thorough genetic counseling and a broadened diagnostic strategy to identify syndromic conditions beyond initial clinical suspicions. Healthcare providers should remain vigilant for rare genetic syndromes in adults with complex or atypical phenotypes. Combining thorough clinical assessment with increasingly accessible genetic testing can unmask underlying genetic etiologies, ultimately enhancing patient care and supporting informed reproductive planning.

C.O.I. Disclosure:

M. Stoyanova: None. **M. Hachmeriyan:** None. **D. Yahya:** None. **A. Yaneva:** None. **M. Levkova:** None.

EP25.020 Clinical implications of pericentric X-inversion - case report

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Background: A 35 year-old healthy female proband and her partner were genetically tested after repeated failures of IVF. The couple has a healthy daughter from the 4th embryo transfer.

Material and methods: Conventional G-banded karyotype and chromosomal microarray analysis (CMA) of peripheral blood lymphocytes.

Results: Cytogenetic examination showed pericentric inversion on X chromosome in the proband. Karyotype 46,XX,inv(X)(p11.2q24). No copy number variants were detected by CMA. The same pericentric inversion was found in the phenotypically

normal proband's sister, mother, maternal aunt and maternal grandmother.

Conclusion: The overall risk for a carrier of pericentric inversion to have a child with an unbalanced chromosome rearrangement is estimated at 5–10%. Based on the size of the inverted segment, it can be concluded that the unbalanced aberration would be most likely lethal in males and variable phenotype would be possible in females. The risk for a male with a proven inversion is still unclear (e.g. due to a gene disruption at the inversion sites). So far, the inversion has only been found in females in the reported family (and also in 2 female embryos in IVF using PGT-A). None of them has been found to have an unbalanced rearrangement. Preimplantation genetic testing can improve chance for a succesfull pregnancy.

C.O.I. Disclosure:

M. Pokorná: None. **M. Koudová:** None. **D. Stejskal:** None. **J. Horák:** None. **E. Račochová:** None. **A. Inovecká:** None.

EP25.021 RARE AND UNUSUAL CASE OF FRAGILE X TRANSMISSION

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Background: Fragile X syndrome is a genetic disorder characterized by intellectual deficits and dysmorphia of varying degrees, which are more pronounced in male patients. The mode of inheritance is X-linked dominant, with transmission generally from mother to son (asymptomatic females or minimal signs). We report the rare and unusual case of a generation-skipping transmission in a family branch.

Methods (case presentation): The patient was 3 years old and presented with language delay. Her maternal grandfather's cousins, her mother's paternal cousins and her maternal cousins had psychomotor retardation and intellectual disability of varying severity in relation to fragile X syndrome. In view of the clinical signs and family history, a search for the number of CGG repeats in the promoter of the FMR1 gene was carried out.

Results: Molecular testing confirmed the presence of over 200 repeats on one allele, establishing the diagnosis of Fragile X Syndrome. The generation skipping of the pathology in this family branch can be explained by the presence of the premutation in the grandfather, as well as by the variability of the mutation transformation in the different women. Premutation of the FMR1 gene corresponds to a number of repeats between 55 and 199. The appearance of the mutation depends not only on the number of repeats, but also on the sex of the transmitting parent. The father transmits the same number of repeats to his daughters.

Conclusion: This case highlights the complexity of genetic counselling in cases of triplet expansion mutations such as Fragile X syndrome.

C.O.I. Disclosure:

K. Zerrouki: None. **F. Aouni:** None. **A. Jihane:** None. **S. Meziane:** None. **K. Ahmidouch:** None. **A. Chyadmi:** None. **A. Elammari:** None. **M. Tajir:** None.

EP25.022 Genetic counseling dilemmas resulting from delayed carrier testing: a case study

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Background: Rare genetic diseases often present with severe phenotypes and challenging management in clinical practice. Cascade testing can be highly beneficial, particularly for reproductive planning. This case study represents the complicated genetic counseling of a Duchenne Muscular Dystrophy (DMD)- affected family.

Material and methods: We consulted a family referred by the OBG for expecting a second child after a DMD-affected first pregnancy. Her son was incidentally found to have increased creatinine kinase levels at two weeks of age, followed by confirmation of a hemizygous likely pathogenic variant - DMD c.1892–1893dup, p.(Lys632fs*). Unfortunately, the family was not accordingly referred for genetic counseling; the maternal genotype and reproductive risks and options remained unclear. The family history included a maternal uncle who died at age 24 with a severe undiagnosed myopathy.

Results: During the initial counseling session in the second pregnancy's tenth gestational week, we presented three options: sequential, simultaneous maternal plus prenatal testing, or fetal sex determination either parallel to or following maternal testing. We addressed their strengths and limitations. Emphasis was placed on relevant ethical principles and financial implications, as most options were patient-funded. She selected simultaneous testing and was confirmed as a heterozygous carrier while awaiting CVS.

Conclusion: This case highlights the difficulty in managing families with rare diseases, including timely and informed prenatal care. Gaps in standard healthcare practices, not including pre- and post-analytical genetic counseling and genetic testing, can interfere with the necessary follow-up.

C.O.I. Disclosure:

D. Yahya: None. **M. Hachmeriyan:** None. **M. Stoyanova:** None. **A. Yaneva:** None. **M. Levkova:** None.

EP25.023 Preconceptional genetic screening in the Republic of Sakha (Yakutia)

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Background: Reproductive screening for autosomal recessive diseases is extremely important for providing medical and genetic care, planning and implementing measures to prevent hereditary diseases using genetic technologies. Genetic screening for 9 common autosomal recessive hereditary diseases was conducted in Yakutia: 3-M syndrome 1, autosomal recessive deafness 1A, methemoglobinemia type 1, tyrosinemia type 1, SOPH syndrome, neuronal ceroid lipofuscinosis type 6A, mucopolysaccharidosis-plus syndrome, congenital ichthyosis autosomal recessive 3 and cataract 18 (autosomal recessive congenital 2).

Material and methods: 158 married couples (a total of 316 women and men) were examined. Informed consent was obtained from all. Genotyping was performed by Real-time PCR.

Results: Heterozygous carriage was detected for all 9 hereditary diseases with varying frequencies. The highest frequency was found for autosomal recessive deafness 1A - 10,1%, with the lowest - congenital ichthyosis autosomal recessive 3 (0,6%) and tyrosinemia type 1 (0,3%). The overall frequency of heterozygous carriage was 24,1%. When high risk is detected in families, there is a choice of early diagnostics, including preimplantation genetic testing.

Conclusion: A high frequency of heterozygous carriage of autosomal recessive diseases is shown, which indicates the importance of genetic screening in Yakutia.

C.O.I. Disclosure:

A.L. Sukhomyasova: None. **E.E. Gurinova:** None. **R.N. Ivanova:** None. **A.N. Sleptsov:** None. **V.M. Sofronova:** None. **A.L. Danilova:** None. **N.R. Maksimova:** None.

EP25.024 Difficult Prenatal Genetic Counseling in Congenital Anomalies - 8 cases at local tertiary prenatal center -

Akane Kondo[1,2], *Mikio Morine*[1], *Tatsuro Sugimoto*[1], *Takaaki Maeda*[1], *Aki Nagao*[1], *Naoto Yonetani*[1], *Nobuhiko Okamoto*[3], *Kazuhisa Maeda*[1]

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Background: Advances in ultrasound diagnosis allow early detection of many congenital malformations. In addition, genetic testing can be performed in a prenatal setting on a variety of biological samples obtained by invasive and noninvasive procedures: chorionic villus sampling, amniocentesis, cordocentesis, or cell free DNA from maternal blood collection. Since we have a time limit for termination, case with diagnosis at late gestation is always difficult. We reviewed difficult cases at our center to see better supportive care.

Material and methods: We reviewed medical chart of 120 fetal anomaly cases which were delivered at out hospital between 2020 to 2024. In those cases, we focused on 8 cases which had difficult genetic counselling to see the factor related difficulties.

Results: Those difficult cases include down syndrome, Noonan syndrome, bone dysplasia, OI, TS, Cornelia de Lange syndrome, Prune belly syndrome and Lissencephaly. Those case's primary diagnosis were done after 20th week. Common reason of difficulty was related to timing of diagnosis, cost of genetic test and primary explanation at local clinics. Religious background is also important to provide personalized genetic counselling.

Conclusion: Since it is not allowed termination after 22nd week in Japan, genetic counselling for the patients who is diagnosed after 20th week can be more difficult. In addition, genetic test is not covered by insurance in Japan. Therefore, uncertainty can be remain in correct diagnosis and this condition can cause very difficult situation for patients and also genetic counselling providers.

C.O.I. Disclosure:

A. Kondo: None. **M. Morine:** None. **T. Sugimoto:** None. **T. Maeda:** None. **A. Nagao:** None. **N. Yonetani:** None. **N. Okamoto:** None. **K. Maeda:** None.

EP25.025 Assessment of reproductive history in women with child with a birth defects in Lviv region (Ukraine) for 2002–2024

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Background: In-depth investigation of potential causes of birth defects (BDs), especially the reproductive history of women, is a key component of prenatal education and primary prevention. Aim of our study: to assess risk factors for BDs among newborns by comparing reproductive history in women who had children with BDs and the control group for 2002–2024 in the Lviv region (Ukraine).

Material and methods: The reproductive history of 1424 mothers who had a child with BDs and 1417 mothers of healthy children in maternity hospitals of the Lviv region was studied using the "case-control" method.

Results: A statistically significant difference was found in the percentage of stillbirths (3.0%) in mothers who gave birth to a child with BDs compared to women in the control group (0.84%), $p < 0.05$,OR = 3,64,(1,91–6,94). Also statistically significant difference was found in the percentage of infertility (5.1%) compared to women in the control group (2%), $p < 0.05$, OR = 2,68, (1,72–4,17). There was no statistically significant difference in the number of spontaneous miscarriages in the 1st trimester in women who gave birth to a child with BDs (7.5%) compared to the control group of (6.7%), $p > 0,05$, OR = 1,13 (0,84–1,50).

Conclusion: A history of stillbirth and infertility is important in the context of a child born with developmental disabilities because it may indicate underlying genetic, environmental, or maternal health factors that increase risk. Understanding this history can guide early intervention and tailored medical care for both the child and family.

C.O.I. Disclosure:

S. Genyk-Berezovska: None. **N. Lukyanenko:** None.

EP25.026 ReproNAGEN: advanced genomic evaluation in couples with fertility problems

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Background: Approximately one in six people in the world experience fertility problems during their reproductive life. These problems have a great emotional and economic consequences for the couple. Therefore, the ReproNAGEN studied the genetic causes behind repeated miscarriage, implantation failure or poor embryo quality.

Material and methods: At the Assisted Reproduction Unit, 137 couples were recruited over two years. All of them received dedicated genetic counselling. In this Project we analysed the genes related to fertility problems, HLA-C/KIR compatibility, personal and reproductive risk, pre-implantation embryonic aneuploidy and pharmacogenetic profiles of each couple using whole genome sequencing (WGS). To further elucidate the genetics behind fertility issues, we offered WGS of those pre-embryos transferred that failed to implant. The objective was to guide couples towards efficient and personalized treatment, increasing the success rate of assisted reproduction treatments.

Results: We recruited 75, 54 and 3 couples with repeated miscarriage, recurrent implantation failure and poor embryo quality, respectively. Of these, 96.35% agreed to participate. All participants wanted to know both the risk of embryo rejection and pharmacogenetics, whilst 90.53% wanted to know their personal risk of developing a genetic disease, 98.86% wanted to know their reproductive risk and 98.48% consented for the WGS of their embryos to be analysed. On average, the results were delivered within two months.

Conclusion: We highlight the great acceptability of the project by the participants. The results obtained have directly influenced clinical decisions for assisted reproduction treatments. This study demonstrates the importance of implementing genomic analysis in Assisted Reproduction.

C.O.I. Disclosure:

M. Fernández: None. **S. Ciria Abad:** None. **A. Sagardia Fernández:** None. **M. Arasanz Armengol:** None. **M. Miranda Pérez:** None. **D. Cobos Hermosa:** None. **N. Sota Echarri:** None. **A. Martínez Carrasco:** None. **Á. Alonso Sánchez:** None. **J. Zabaleta Jurío:** None.

EP25.027 For self-genetic counselors': What roles for health education and perspective for hemoglobinopathies prevention?

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Background: In Tunisia, the primary prevention of hemoglobinopathies relies on behavioral changes related to screening and genetic counseling.

Methods: To assess potential for genetic self-counseling applied to sickle cell disease (SCD), we designed a questionnaire on SCD knowledge (SCDKA), then recruited 356 students. Statistical analysis of the results was carried out using chi-square tests and Fisher's, to compare the demographic and educational characteristics of the participants.

Results: The analysis of responses to the various SCDKA items shows a lack of awareness about the hereditary origin of hemoglobinopathies. 97.8% of respondents did not recognize electrophoresis as a diagnostic technique. In terms of genetic literacy, the autosomal and recessive nature of hemoglobinopathies is not well understood. This explains why 41% and 74% of these students could not answer items on genetic transmission. The perception of controllability of hereditary diseases is higher among science students, as is the intention to inform their partner before procreation (56.5% vs 24.35%, $p < 0.001$). Responses reveal that science section respondents have higher proactive preventive intentions compared to literature section students, as they recognize the usefulness of genetic counseling (75.5% vs 47.43%, $p < 0.001$), premarital diagnosis (57.5% vs 18.59%, $p < 0.001$), and prenatal diagnosis (61.5% vs 13.46%, $p < 0.001$).

Conclusion: Currently, levels of health literacy and functional genetic literacy do not ensure genetic self-counseling for hemoglobinopathies prevention. The survey shows that having a scientific background was an advantage, but a detailed analysis of these students' results reveals average to low SCDKA scores.

C.O.I. Disclosure:

F. Maaoui: None. **I. Moumni:** None. **I. Safra:** None. **S. Menif:** None.

EP25.028 Hereditary cancer syndrome assessment in patient undergoing somatic mutation analysis: a missed opportunity?

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Background: Somatic mutation analysis, increasingly used to guide targeted cancer therapies, also offers an opportunity to identify individuals at risk for hereditary cancer syndromes (HCS). However, systematic HCS assessment is often not integrated into routine cancer care. Consequently, many patients undergo only somatic testing, even with suggestive personal or family histories.

Material and methods: We retrospectively reviewed records of 214 patients with metastatic colorectal carcinoma who underwent free-of-charge somatic mutation analysis after onsite genetic counselling at our institution. Referral criteria for germline testing included age < 50 years, positive personal/family history (NCCN criteria), and/or Microsatellite Instability-High (MSI-H) status.

Results: Of 214 patients, 20 (9.3%) were <50 years old and 54 (25.2%) had a positive personal or family history warning further evaluation. MSI status was assessed in 161 patients where 19 (11.8%) were MSI-H. Although all evaluated at-risk were offered a germline testing during genetic counseling, none proceeded with testing within the one-year study period.

Conclusion: A significant proportion of patients undergoing somatic testing have HCS risk factors but do not pursue germline testing, even post-counseling. While integrating family history assessment into pre-somatic testing protocols is a start, it is insufficient. Addressing this gap requires systemic changes, including mainstreaming germline testing discussions into oncology care, addressing cost barriers, and implementing strategies to support informed decision-making to ensure comprehensive care and identify individuals at risk for HCS.

C.O.I. Disclosure:

M. Hachmeriyan: None. **M. Levkova:** None. **R. Valeva:** None. **D. Yahya:** None. **M. Stoyanova:** None. **E. Dimitrova:** None.

EP25.029 A comparative analysis of dysmorphic and non-dysmorphic pediatric patients with neurodevelopmental disorders

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Background: Neurodevelopmental disorders (NDs) are characterized by impairments in personal, social, academic, or occupational functioning. While NDs can arise independently, many dysmorphic syndromes are often associated with autistic behaviors or neurodevelopmental delay.

Material and methods: This retrospective study analyzed data from 2019–2024 on pediatric patients presenting with at least one clinical feature: developmental delay, intellectual disability, or autistic-like behavior. Genetic analysis, based on the indication, and counseling were performed for all of them. Participants were stratified by age, sex, presence of dysmorphic features or congenital anomalies and referring unit. Chi-square and Mann-Whitney U tests were applied for statistical analysis.

Results:A total of 407 patients (265 males, 142 females), aged 3–18 years (mean age: 6.9 years), were included.The first group (ND only) comprised 110 participants (89 males, 21 females), while the second group (ND with dysmorphic features) included 297 participants (176 males, 121 females). Patients with dysmorphic features had a significantly higher likelihood of having a positive result from genetic analysis compared to ND-only patients ($p = 0.008$). Males were more frequently affected in both groups ($p = 0.001$), with 80.9% male representation in the ND-only group and 59.2% in the dysmorphic group. Furthermore, dysmorphism reduced the discrepancy in the sex ratio ($p = 0.0004$).

Conclusion: Patients with neurodevelopmental disorders and dysmorphic features have a significantly higher diagnostic yield, especially among males. Dysmorphism also reduces sex-based differences in ND incidence. Therefore, a thorough examination by a geneticist is essential to enhance diagnostic accuracy.

C.O.I. Disclosure:

A. Yaneva: None. **M. Stoyanova:** None. **M. Hachmeriyan:** None. **D. Yahya:** None. **M. Levkova:** None.

EP25.030 Telehealth in genetic counselling consultations: the impact of COVID-19 in a Portuguese genetic healthcare service

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Telemedicine has experienced a significant surge with the onset of the COVID-19 pandemic, highlighting the need for a deeper understanding of patient experiences in genetic counseling via telehealth. This shift has required healthcare professionals to adapt to new technologies while ensuring effective communication and patient-centered care. To explore these aspects, we conducted a study using a mixed-methods approach, employing questionnaires to collect patient perspectives on telehealth-based genetic counseling. The study sample primarily consisted of individuals aged 26 to 45 years (65%), with 33.6% having completed the 12th grade of secondary education. As a direct consequence of the pandemic, we observed an 84.5% increase in teleconsultations, emphasizing the rapid adoption of remote healthcare services. Patient satisfaction was notably high, with 67.3% feeling comfortable discussing personal and family health concerns, 53.6% reporting that they felt well-informed, and 59.1% not experiencing difficulty in establishing a doctor-patient relationship. However, 64.5% of participants stated that they would prefer telemedicine primarily for follow-up consultations rather than initial assessments. Despite some limitations, our findings underscore the critical role of telemedicine in improving healthcare accessibility. Continuous professional training is essential to enhance the quality of genetic counseling, ensuring accurate information delivery and emotional support for patients. Strengthening telehealth services can lead to a more efficient, patient-centered healthcare system, benefiting both patients and their families.

C.O.I. Disclosure:

M.A. Dantas: None. **J.D. Da Silva:** None. **N. Tkachenko:** None. **M. Paneque:** None.

EP25.031 The pitfalls of noninvasive prenatal testing: case examples and the essential role of genetic counseling

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Background: Non-Invasive Prenatal Testing(NIPT) is a screening test that is widely recommended by all relevant guidelines. However, its limitations are frequently overlooked. Notably, the source of cell-free DNA(cf-DNA) used in this test is placental rather than fetal, which can complicate the interpretation of results and potentially lead to misdiagnoses.

Material and methods: We present four cases of misdiagnosis by NIPT that were referred to us for evaluation and genetic counseling. The first two cases had false-negative results for trisomy 21 and trisomy 13, respectively. Ultrasound investigation of the first case only revealed nasal bone hypoplasia whereas the second case had cardiac defects. The third and fourth cases involved ongoing pregnancies where the fetal gender was inconsistent between ultrasound findings and NIPT results. None of the patients received genetic counseling beforehand.

Results: For the first two cases, postnatal investigations revealed that both placentas were in fact euploid,confirming fetal-placental discordance. A detailed history of the third patient revealed that she initially had two gestational sacs, one of which had spontaneously aborted. The fourth patient was identified as a renal transplant recipient, complicating the cf-DNA analysis.

Conclusion: These cases clearly demonstrate the importance of genetic counseling before conducting NIPT. While the technical limitations of NIPT are widely acknowledged, insufficient counseling and incomplete history-taking can still lead to misdiagnosis and severe consequences. Last but not least, it is essential to emphasize that this test is intended solely for screening purposes and that its results should not take precedence over ultrasound findings when making clinical decisions regarding pregnancy.

C.O.I. Disclosure:

E. Baltaci: None. **Y. Terzi:** None. **Z. Celik:** None.

EP25.032 Exploring genetic counselors' opinions on wide application of non-invasive prenatal testing

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Background: Non-invasive prenatal testing (NIPT) is a widely used screening tool, but its expanded application raises ethical and clinical concerns, particularly due to potential false-positive results.

Material and methods: Between October and December 2024, we surveyed 50 randomly selected genetic counselors (GCs) worldwide to assess their opinions on NIPT's clinical utility, accuracy, and applications for single-gene disorders (SGDs), sex chromosome aneuploidies (SCAs), and microdeletion/microduplication syndromes (MMSs). Participants rated their agreement on a Likert scale (1 = lowest, 5 = highest). Regression analysis was performed to evaluate the data.

Results: The majority of respondents were female (73%),and most were aged between 31 and 50 years (75%). Nearly half (51%) had 1–10 years of experience as genetic counselors, and 35% worked in university hospitals. The reported accuracy of NIPT according to the GCs for SGDs was 33%, for SCAs - 51%, and for MMSs - 35%. For SGDs, 45% of counselors recommended testing only in specific cases, whereas 88% supported routine NIPT use for SCAs and 58% for MMSs. A statistically significant difference emerged based on the counselors' workplace (e.g., university hospitals vs. private clinics), with those involved in research more likely to recommend testing for MMSs.

Conclusion: While there is strong support among genetic counselors for the routine use of NIPT for certain indications, such as SCAs, hesitations persist regarding its application for conditions like SGDs and MMSs. Furthermore, our findings highlight that the setting in which genetic counselors practice and their involvement in research significantly influence their recommendations for testing.

C.O.I. Disclosure:

M. Levkova: None. **A. Yaneva:** None. **M. Stoyanova:** None. **D. Yahya:** None. **M. Hachmeriyan:** None.

EP25.033 Empowering clinical teams in genomic variant classification

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Background: Accurate variant classification is key to genetic diagnosis and management, and a necessary skill for clinical geneticists. Clinical teams struggle with evaluating reports, managing variants of uncertain significance and integrating clinical information especially when results conflict or evidence is limited. The ACMG/AMP guidelines, introduced in 2015, are continuously updated to refine classification criteria and integrate new evidence sources. Structured training to better navigate these challenges is needed.

Material and methods: We developed a three-day training workshop to enhance proficiency in variant classification. The program covered ACMG/AMP guidelines, hands-on classification exercises, and training in the use of genomic databases such as UCSC browser, ClinVar, gnomAD, etc. Eleven courses were held between 2022–2025, with over 200 participants. Feedback was analyzed at the end of each workshop and a questionnaire was sent to all participants to collect retrospective feedback in January 2025.

Results: Sixty-two participants replied to the retrospective feedback questionnaire including medical geneticists, counselors, bioinformaticians, and lab staff. Half of the responders had up to five years of experience, while the remainder had more extensive tenure. Although 85% had prior experience with variant classification, 63% reported improved usage following the course. Notably, 79% stated that training significantly strengthened their ability to critically evaluate variant classifications. Overall, 94% expressed interest in further training, highlighting the need for continued education.

Conclusion: The workshops enhanced participants' confidence in their personal ability to classify variants and judgmentally assess genomic test results. To sustain learning, we initiated follow-up meetings for case discussions and peer learning.

C.O.I. Disclosure:

L. Bazak: None. **G. Lidzbarsky:** None. **M. Lifshitc Kalis:** None. **N. Ruhrman-Shahar:** None. **N. Asia Batzir:** None. **S. Farage Barhom:** None. **L. Basel Salmon:** None. **I. Maya:** None.

EP25.034 Access to counselling supervision as part of genetic counselling education in Europe: Are we promoting reflective practice?

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Background: Genetic Counselling Supervision (GCS) plays a critical role in professional development, fostering reflective practice, and mitigating burnout among genetic counselling professionals. Despite its significance, GCS integration in educational settings remains inconsistent. This study aimed to evaluate how GCS is integrated as part of genetic counselling educational programmes accredited by the European Board of Medical Genetics (EBMG), identify barriers and facilitators, and explore strategies for its implementation.

Material and methods: Directors of EBMG-accredited MSc genetic counselling programmes in Cardiff, Manchester, Glasgow, Marseille, Barcelona, Porto, Siena, Innsbruck, and Linköping were invited to participate. Of the nine eligible programmes, eight provided responses. Data were collected via a structured questionnaire with open- and closed-ended questions, Likert scales, and multiple-choice items. Quantitative data were analysed descriptively, and qualitative data underwent thematic analysis.

Results: All responding MSc programmes included GCS in their curricula; however, integration at MSc curricula varied widely. During clinical placements, GCS was accessible in 62.5% of programmes, supported by institutional resources and EBMG guidelines. Barriers included a shortage of senior genetic counsellors and limited professional recognition in certain countries. Time dedicated to GCS and its continuity post-training were found to be insufficient, limiting long-term benefits.

Conclusion: This study highlights the need to strengthen GCS integration in European MSc programmes. To ensure graduates are equipped for reflective and high-quality care, educational pathways must prioritize GCS. Collaborative efforts among educators, policymakers, and practitioners are essential to addressing existing barriers. These findings provide a foundation for future actions aimed at enhancing genetic counselling education and professional practices across Europe.

C.O.I. Disclosure:

L. Guimarães: None. **I. Costa:** None. **M. Paneque:** None.

EP25.035 Genetic Counselling for Psychiatric Conditions: exploring current perceptions of family physicians and psychiatrists in Portugal

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Background: Genetic counselling emerged as a specialized healthcare discipline in the 1960s, and since then, various subspecialties have developed globally, namely and more recently, psychiatric genetic counselling. This study provides a pioneering exploration of family physicians and psychiatrists' perceptions regarding genetic counselling provision and its potentialities in the context of psychiatric illnesses in Portugal.

Material and methods: A qualitative methodology with semi-structured interviews was used. Among the participants, five were family physicians, and six were psychiatrists.

Results: Thematic analysis revealed three themes: (1) the role of genetics in healthcare, (2) barriers to psychiatric genetic counselling implementation, and (3) perceived benefits associated with its implementation. Results show that while the importance of genetics in psychiatric disorders is acknowledged, there is low literacy about genetic counselling practice from the professional groups interviewed. Also, the availability and mainstreaming of genetic testing seem to influence how genetic counselling is perceived and utilized. There is a perceived need for training and guidelines that foster the dissemination of genetics into healthcare, specifically mental healthcare. A holistic and patient-centred approach is considered essential in managing psychiatric disorders and, by extension, in psychiatric genetic counselling, as it addresses both medical and psychosocial factors.

Conclusion: Although psychiatrists and family physicians are keen to integrate psychiatric genetic counselling into their

patients' care, it seems that certain fundamental challenges still persist in genetic healthcare provision. Future research should contribute for a more comprehensive evaluation of the readiness for psychiatric genetic counselling implementation in the country.

C.O.I. Disclosure:

B. Ribeiro: None. **I. Homem de Melo:** None. **A. Sequeira:** None. **R. Moldovan:** None. **M. Paneque:** None.

EP25.036 Development and use of psychological support systems in cancer genomic medicine

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Background: The implementation of cancer genomic medicine (CGM) in Japan, including National Health Insurance coverage of cancer gene panel testing from June 2019, has created new therapeutic opportunities for cancer patients. However, it has been revealed that less than 10% of patients receiving cancer gene panel testing can be matched to targeted treatments based on their results. Additionally, secondary findings suggest that approximately 10% of patients may have hereditary tumors. These circumstances pose new psychological stressors for both patients undergoing testing and healthcare professionals providing and following up on such testing.

Material and methods: A qualitative interview study was conducted with healthcare professionals engaged in CGM, including physicians, nurses, pharmacists, and genetic counselors. Based on the interview results, a preliminary support system was established within the hospital, and its effectiveness was evaluated through practical cases.

Results: The analysis identified essential psychological support needs for patients and healthcare professionals. Patients require structured guidance to better understand CGM, while healthcare professionals benefit from an interdisciplinary support system to manage their psychological burden. Preliminary implementation of the support system demonstrated its utility in providing comprehensive care for patients and relieving stress on healthcare providers.

Conclusion: A comprehensive mental health support system is crucial to addressing the psychological challenges associated with CGM. The proposed framework includes team-based interventions, structured follow-up strategies for hereditary tumors, and enhanced knowledge-sharing among regional medical facilities. The establishment of mutual support structures between genetic counseling and psycho-oncology departments has facilitated better access to genetic counseling for patients in need.

C.O.I. Disclosure:

H. Fukushima: None. **M. Ishida:** None. **H. Onishi:** None. **T. Hamaguchi:** None.

EP25.037 Is tele-genetic counselling for disclosing APOE genotype for risk of Alzheimer's disease to healthy and with mild cognitive impairments as effective as the onsite interventions?

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Background: This study examines behavioral and psychological responses following the disclosure of genetic results of AD risk to first-degree relatives of people with AD (PwAD) in Greece. The primary aim was to evaluate the impact of GC on participants' empowerment, psychological well-being, behavioral adaptations, and risk recall over time.

Material and methods: Participants (n = 93) were randomly assigned to one of two GC interventions. Additional grouping variables included the cognitive status (healthy or had mild cognitive impairment-MCI), and the genetic test result (ε4 positive or not). Throughout the three time points (baseline/before GC, T1/3 months post-disclosure, and T2/6 months post-disclosure), participants completed the 21-item Beck Depression Inventory, the Short Anxiety Screening Test, the Impact of Event Scale, the Genetic Counseling Outcome Scale, risk recall and behavioral questions. Repeated-measures ANOVA, Mann-Whitney U tests, logistic regression, Friedman test, and chi-square tests were used to examine changes in different scores.

Results: 93 adults (mean age 64.77, range 37 to 83 years, 61% female, mean years of education 13.72) were randomly assigned to online (46 participants) and onsite (47 participants) groups. There were no statistically significant differences between the groups in GCOS-24, SAST, BDI and IES scores regardless of the time and between healthy and MCI participants. However, MCI has substantially lower odds of recalling risk after six months than healthy participants. People with ε4 were much more likely to endorse behavior change and indicated higher scores in IES-R

Conclusion: Tele-genetic counseling can serve as an effective intervention for APOE disclosure for healthy and MCI relatives of PwAD.

C.O.I. Disclosure:

M. Makri: None. **I. Aggelidou:** None. **L. Fidani:** None. **T. Tegos:** None. **M. Tsolaki:** None.

EP25.038 Genomic Pathway Manager: A novel role in the genomic medicine care pathway in France - Overview and perspectives

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The French Genomic Medicine Initiative 2025 (*PFMG2025*) aims to make high-throughput next-generation sequencing accessible to all, ensuring equitable access across the entire French territory. To achieve this goal, a robust organisation was established, including the creation of the first two laboratories and the prioritization of 70 pre-indications validated by the French Health Authority covering rare diseases, oncogenetics, and oncology. Deployment of specific care pathways for each pre-indication has disrupted existing structures, requiring clinicians to learn new tools, and providing them with additional support. As a result, after two waves of funding 2020 and 2021, a total of 51 Genomic Pathway Manager (GPM) have been created for rare diseases, oncogenetics

and cancers. This role was conceived to assist clinicians in prescribing analyses and managing dedicated multidisciplinary meetings. We focus on the GPM role, aiming to better understand it, assess its contributions, and identify its needs. Using a dedicated questionnaire, a quantitative study was conducted among the 40 GPMs recruited by May 10th 2023. The survey explored their professional profiles, activities, the organisational setup for genomic medicine prescription within their institutions and territories, and their job satisfaction. Out of the 40 GPMs, 36 (90%) responded, revealing the heterogeneous nature of recruited profiles and practice, as well as the diversity of their activities. The attractions and challenges of the job are also highlighted. Describing this new role and conducting this initial assessment is crucial for adapting practices, optimizing the GPM function, and evaluate the relevance of its sustainability within the *PFMG2025*.

C.O.I. Disclosure:

L. Gaudillat: None. **L. Patay:** None. **J. Santenard:** None. **A. Baurand:** None. **A. Beaudoin:** None. **D. Salvi:** None. **C. Level:** None. **L. Faivre:** None. **F. Nowak:** None. **C. Peyron:** None. **C. Thauvin-Robinet:** None.

EP25.039 Building blocks of change: Developing modular digital tools to scale genetic counseling services

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Background: Current genetic counseling service delivery faces significant challenges: demand exceeds capacity, with wait times reaching 2 years and 70–85% of eligible candidates missing appropriate counseling. While digital solutions exist, their rigid structures and clinic-specific designs limit widespread adoption, highlighting the need for more adaptable tools.

Material and methods: We developed a customizable digital genetic counseling tool using design thinking methodology. The process involved: (1) systematic analysis of traditional counseling workflows across different clinical settings, (2) stakeholder consultations to identify key customization requirements, and (3) development of a modular digital platform enabling pathway modification. This approach specifically addresses the challenge of implementing digital solutions in diverse clinical environments.

Results: The developed tool provides comprehensive customization through: (1) configurable landing pages with adaptable risk assessment, (2) flexible educational content management supporting multiple formats, (3) customizable knowledge verification systems, and (4) modifiable consent workflows. The platform allows the clinics to modify all components through an intuitive interface without technical expertise, enabling adaptation to various clinical scenarios and patient populations.

Conclusion: This approach to digital genetic counseling tools offers a practical solution for clinics seeking to implement alternative service delivery models. By emphasizing adaptability and ease of implementation, it provides a potential pathway for scaling genetic counseling services while maintaining clinic autonomy and quality of care.

C.O.I. Disclosure:

A. Narravula: E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; GeneLinx GmbH.

EP25.040 Mainstreaming Genomics in Neurology - Nurse-Led Neurogenetics Clinic Service Evaluation

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Background: An innovative nurse-led neurogenetics clinic was set up in St George's University Hospitals NHS Foundation Trust. The aim of the service is to improve the diagnosis and health outcomes of patients with rare neurogenetic diseases. The objective of the service is to mainstream genomics in neurology by providing equitable access to genetic testing, education, and counselling, signposting to hospital and community services, and to promote clinical research. The service initially focused on neuromuscular disorders and complex epilepsies. This has now expanded to include movement disorders, mitochondrial disorders, neurodegenerative disorders, white matter disorders, and neuro-metabolic disorders.

Materials and methods: Retrospective service evaluation of the nurse-led neurogenetics clinic between March 2022 to March 2024 assessing the clinic activity from referral to reporting of genetic result.

Results: There were 654 referrals to the nurse-led neurogenetics clinic in total between March 2022 and March 2024. 556 patients were seen by the neurogenetics CNS within the same period. 516 patients were seen within 3 months of referral. 476 individuals consented to genetic/genomic testing, with 437 probands and 39 sibs. 309 patients participated to National Genomic Research Library. There were 125 cases with an identified causative genetic variant. 11 patients are receiving disease-specific treatment.

Conclusion: The nurse-led neurogenetics clinic demonstrates a service model that effectively mainstreams genomics and genetic testing in neurology improving equitable access to genetic testing and informing management.

C.O.I. Disclosure:

M. McQueen-Mencias: None. **F. Elmslie:** None. **E. Matthews:** None. **E. Caruana-Galizia:** None.

EP25.041 The family letter for hereditary diseases: perceptions of patients and professionals

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Background: In autosomal dominant clinically actionable diseases, such as cardiovascular diseases or cancer syndromes, cascade genetic counselling and testing are important since preventive measures and/or treatments can reduce morbidity and mortality. At-risk relatives (ARR) are mostly informed through the proband via a family-mediated approach. Unfortunately, the resulting uptake of genetic counselling and testing among ARR is ~50%. Family letters are known to help in informing relatives but are also perceived as difficult to understand.

Material and methods: We explored the perceptions on the family letter in patients/ARR and professionals using focus groups. Our semi-structured topic list addressed personal experiences, understandability, content, format, and needs with respect to the family letter and the process of informing relatives. Findings were analysed using thematic analysis.

Results: Patients/ARR preferred family letters to be tailored to ARR, but also mentioned that this was not possible because of their wish for uniform letters and counsellors not having knowledge of relatives' needs. A follow-up contact or letter was mentioned as a method to improve the process of informing relatives. Counsellors and patients/ARR stressed the importance of a short letter written in easy to understand language using positive framing. They also mentioned that additional media like

animations, telephone calls, and websites could improve understandability, uptake rates of counselling, and potential needs for detailed information.

Conclusion: Short uniform family letters are preferred by patients/relatives and counsellors as a first step in informing relatives. Alternative methods of informing relatives could complement the family letter and are worth exploring in future research.

C.O.I. Disclosure:

K. Nieuwhof: None. **L.A. Grutters:** None. **I. Christiaans:** None.

EP26 Ethical, Legal and Psychosocial Aspects in Genetics

EP26.001 Hereditary diseases: theoretical aspects

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The question of heredity of diseases preoccupied people as long as anyone can remember. An analysis of German textbooks allows to set up a theory of hereditary diseases.In 1921 Fritz Lenz gave the first definition of hereditary disease in hit German textbook "Human Heredity". For him the "state of a living being living at the limits of its ability to adapt" should be called sick. A theory of hereditary disease can define two criteria. 1. A condition or process in the organism is pathological, if it is (untreated) (1) leads to premature death, (2) is associated with pain or suffering, (3) leads to reproductive incapacity, or (4) the ability to live together in communities is fundamentally impaired. 2. A condition or process is secondary pathologic, if (1) It does not fall under the primary disease criteria, (2) In some of the cases that occur, it is a necessary causal consequence of other characteristics already known to be pathological or a regular concomitant phenomenon (disease entities) that has already been identified, i.e. with a known frequency. These disease criteria can be applied to genetic diseases, such as a genotypic change (Trisomy 21) and a blood clotting disorder (haemophilia). The increasing knowledge opens up possibilities for influencing genetic disposition as well as prenatal testing. The translation of human genetic findings from research into the clinic.

The fact that new findings on inheritance of diseases are constantly published, a theory of those has to be updated and ethical aspects must be considerate.

C.O.I. Disclosure:

H.I. Petermann: None.

EP26.002 Periodontal-systemic health connection:understanding the role of risk factors

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Background: Periodontal diseases are among the most common chronic inflammatory conditions, significantly affecting oral and influencing systemic health through shared biological pathways. These links are driven by risk factors such as smoking, obesity and stress, as well as by genetic factors or systemic inflammation. Understanding these connections is crucial for improving prevention and management strategies in healthcare.

Material and methods: Standardized questionnaire with closed-ended questions was conducted among 416 students at Medical University - Sofia to assess their awareness of periodontal and systemic health, as well as associated risk factors

Results: The results demonstrated a solid understanding of periodontal disease etiology and its basic systemic implications. However, significant gaps were identified in advanced topics, including the role of cytokines, genetic predispositions and the impact of periodontitis on conditions such as adverse pregnancy outcomes and systemic inflammation. Smoking and diabetes were widely recognized as risk factors, while obesity and cardiovascular diseases were less well understood.

Conclusion: These findings emphasize the critical need for interdisciplinary education integrating oral and systemic health concepts. Addressing these knowledge deficits will empower healthcare professionals to develop comprehensive prevention and management strategies, ultimately reducing the burden of chronic diseases.

C.O.I. Disclosure:

D. Dimitrov: None. **V. Doseva-Panova:** None. **G. Chaneva:** None. **D.N. Nikolova:** None.

EP26.003 Women with cancer and their understanding of genetic information - how psychotherapy can help

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Background: Women with cancer are bound to make a series of decisions related to genetics. A psychotherapeutic approach is recommended to help them make optimal choices, enhancing their sense of security and reducing anxiety caused by poor communication of genetic information.

Material and methods: The upcoming case studies detail women who received genetic testing results for hereditary cancers at hospitals. Despite receiving comprehensive genetic information, they sought additional counseling and psychological support from the NGO "Everything for Her."

Results: The case studies of three female patients will demonstrate how psychological support, provided through a psychotherapeutic approach, can assist them effectively.

1. A 50-year-old patient with hormone-dependent breast cancer receives the results of a genetic test (gene *EPCAM*), and consequently, feels extreme anxiety, which is difficult to cope with.
2. A 40-year-old woman is diagnosed with a pathological mutation (gene *ERCC3*), and is asking for an additional explanation and psychological help.
3. The 57-year-old woman is visibly disturbed when she finds out she is the carrier of the *PALB2* mutation, and shares that her daughter and niece are worried.

Conclusion: Despite the fact that most women are fully informed about genetic test results, for some this is not enough. These women tend to contact the NGO 'Everything for Her' for additional clarifications and psychological support. Knowledge of psychotherapy techniques enables these women not only to better understand genetic information, but also how it affects them and their family members.

C.O.I. Disclosure:

L. Serman: None.

EP26.004 Developing new infrastructures for innovative genomics in the era of the European Research Area: the example of the EASI-Gen DS project

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Background: The evolution of genomic technologies is becoming increasingly difficult to follow for most laboratories and calls for innovative support infrastructures at European level. In parallel, the European legislative package operationalising the data strategy is reshaping the health data sharing landscape. Consequently, the design and implementation of new European research infrastructures in genomics necessitates considering these legal developments to ensure compliant and efficient operations.

Material and methods: The EU-funded EASI-Gen DS project aims to conduct a design study for establishing a new distributed European sequencing infrastructure on advanced genomic research. A legal analysis must be conducted to consider how the latter can fit into the European landscape of emerging infrastructures for genomics (e.g. Genome Data Infrastructure, Genome of Europe).

Results: We will present the main features of the EASI-Gen infrastructure and envisage its legal qualification based on relevant existing or emerging European legal statutes. The qualifications and obligations introduced by the Data Governance Act (DGA) and the European Health Data Space Regulation (EHDSR) will also be tackled, highlighting the challenges of safeguarding research ethics and individual rights. Possible synergies with actors operating in the European Research Area (ERA), such as research data repositories, will be considered to facilitate the creation of integrated infrastructures benefiting to the wider genomic research community.

Conclusion: We illustrate the legal qualifications and obligations addressed to the genomic research data operators, the potential complexities but also the opportunities opened by the ERA structuration, based on a use-case that could guide equivalent infrastructure developments in genomic research.

C.O.I. Disclosure:

L. Feriol: None. **G. Chassang:** None. **N. Dubruel:** None. **E. Rial-Sebbag:** None.

EP26.005 *NAGEN-data: Analysis of the legal framework applicable to Genomic data discovery for secondary use of personal data*

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Background: NAGEN program has collected more than 3.000 genomes in consecutive studies of complex diseases. In this context, NAGEN-data aims to create an ecosystem of genomic and phenoclinical data allowing access for research purposes via data discovery tools like Beacon v2. From the ethical-legal perspective, the protection of personal data needs to be considered, not only due to the evident importance of the regulatory framework applicable to genetic data, but also due to the future implementation of the European Health Data Space (EHDS) by the EU. Therefore, our objective was to study the main issues that affect, from the perspective of regulatory compliance and the guarantee of personal data protection, the deployment of an ecosystem, such as the one described, by default and by design.

Material and methods: Legal framework study applicable to the NAGEN-data ecosystem governed by the GDPR, Data protection Act, Biomedical Research Act as well as the future EHDS.

Results: Despite the lack of EHDS regulation, the current regime allows the creation of the envisioned ecosystem. The Spanish contribution to the regime requires the adoption of concrete technical and organizational measures for the pseudonymization and minimization of data and the implementation of a Data Protection Impact Assessment. In this sense, we considered Beacon an appropriate tool to guarantee compliance with pseudonymization and minimization of data.

Conclusion: Specific data access tools such as Beacon must be developed to guarantee regulatory compliance provided by legislators, which must be considered from the design of the genomic data-sharing ecosystem.

C.O.I. Disclosure:

D. Armendia Giron: None. **M. Jorqui Azofra:** None. **M. Egusquiza Balmaseda:** None. **R. Luquin Bergareche:** None. **F. Alvira Iraizoz:** None. **A. Almanza Goicoechea:** None. **A. Alonso Sánchez:** None. **V. García Solaesa:** None.

EP26.006 Follow-up on Cancer-Associated Secondary Findings in Phenotype-based Whole Exome Sequencing

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Background: The usefulness of reporting cancer-associated secondary findings (CASF) regarding possible preventive measures and the psychological burden on healthy individuals is controversially discussed. We developed a follow-up survey to evaluate the personal impact of reported CASFs on affected families receiving phenotype-based whole exome analysis at our institute from 2019–2023.

Material and methods: Affected families were invited to participate in the study via letter (10) or via phone (22). Sixteen representatives, in most cases one parent of an affected child, participated and answered the survey which was developed in collaboration with the Department of Medical Psychology.

Results: CASFs were reported in 32 out of 1432 phenotype-based exome analyses. The majority of the affected families (13/16, 81%) received genetic counselling. All of them were satisfied with the communication on the results. Fifteen families (94%) reported that they were informed about the recommended surveillance measures,7 (44%) changed their own surveillance plan according to the results. Nevertheless, only half of the families understood their recommendations at the time of the survey. Eleven families (69%) reported being concerned and/or frightened after receiving the results, only 3 (19%) received professional psychological support. Three families would have preferred more details about the surveillance measures or a second appointment to exclusively discuss the secondary findings.

Conclusion: Most of the families received genetic counseling for discussion of the CASF, nevertheless they had to cope with the psychological consequences on their own. For the future, a separate appointment with the possibility of including a psychologist should be considered.

C.O.I. Disclosure:

E. Schreiner: None. **S. Bierbaumer:** None. **J. Blatterer:** None. **J. Fantitsch:** None. **J. Geigl:** None. **S. Hammer:** None. **L. Kaufmann:** None. **S. Kühberger:** None. **H. Tichy:** None. **K. Wagner:** None. **J. Wagner-Skacel:** None. **S. Verheyen:** None.

EP26.007 Exploring patient acceptability of multifactorial risk assessment in one-stop breast clinics: a qualitative study

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Background: Annually, over 400,000 women are referred to secondary care breast services via the UK's breast cancer suspected 2ww pathway. Few patients (6%) are diagnosed with cancer, with the remaining 94% discharged from the service. Currently, discharged patients are not given information about their future cancer risk, missing an opportunity for a "teachable moment". This research aims to explore the patient acceptability of conducting a multifactorial risk assessment via MyCanRisk/CanRisk as part of one-stop breast clinics.

Material and methods: Nineteen online semi-structured interviews were conducted with women referred to secondary care breast clinics within the last 3 years and discharged without a breast cancer diagnosis. Transcripts were analysed using Framework Analysis and organised around the Theoretical Framework of Acceptability.

Results: The use of MyCanRisk/CanRisk to conduct multifactorial risk assessments in one stop breast clinics was highly acceptable to patients. Participants consistently described how a multifactorial risk assessment would increase informed decision-making about managing their future breast cancer risk. Whilst the feedback was extremely positive, some participants were concerned about exacerbating health anxieties, with a need to ensure that there was adequate support for those receiving 'increased risk' results, particularly if the results were shared via a letter.

Conclusion: Multifactorial risk assessment via MyCanRisk/CanRisk is an acceptable and desirable addition to existing one-stop breast clinics for women not diagnosed with breast cancer. These findings contribute to the growing body of literature regarding multifactorial risk prediction and can inform its implementation within existing NHS pathways.

C.O.I. Disclosure:

M. Myers: None. **R.J. Clune:** None. **S. Archer:** None.

EP26.008 A Systematic Review of Genes Implicated in Police Suicide: Insights from Blue H.E.L.P. Data

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Background: Suicide rates among law enforcement officers have reached concerning levels, yet research on the biological underpinnings of this crisis remains limited.

Material and methods: This systematic review examines genetic factors implicated in police suicide, synthesizing findings from 20 studies that met inclusion criteria.

Results: The review identifies key genetic markers associated with suicide risk, including polymorphisms in serotonin (5-HTTLPR), dopamine (DRD2, DRD4), and stress-response (FKBP5, CRH) genes, which may influence mood regulation, impulsivity, and trauma response. These biological predispositions interact with five well-established risk factors—problematic substance use, depression and prior suicide attempts, trauma sensitivity, occupational stress and dissatisfaction, and lack of intimate support—compounding suicide vulnerability in law enforcement personnel.

Conclusion: The findings suggest that officers with genetic susceptibilities may exhibit heightened stress reactivity and impaired coping mechanisms, increasing their risk for suicidal behavior under chronic occupational strain. Implications for suicide prevention, including the integration of genetic screening into mental health assessments, tailored interventions, and policy adaptations, are discussed. Limitations include the need for larger, more diverse genetic studies and a greater focus on gene-environment interactions in law enforcement populations. Future research should explore how genetic predispositions may inform targeted prevention strategies, ultimately reducing suicide rates among police officers.

C.O.I. Disclosure:

J.K. Brewer: None.

EP26.009 Coping strategies and personality traits in patients undergoing genetic counseling for hereditary cancer

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Background: The genetic counseling process for hereditary cancer often triggers emotional and psychological challenges. Coping strategies and personality traits significantly influence how patients adapt to the uncertainties and decisions involved in this process. This study aims to analyze the relationship between coping strategies, as measured by the COPE inventory, and personality dimensions, assessed through the Temperament and Character Inventory (TCI), in patients undergoing genetic counseling for hereditary breast and ovarian cancer (HBOC).

Material and methods: A sample of 70 participants (35 BRCA1/2 mutation carriers and 35 non-carriers), aged 20 to 65, completed the COPE inventory and TCI before and after genetic counseling. Emotional distress (anxiety, depression) was also assessed using validated scales. Data were analyzed to identify patterns of coping strategies and their interaction with personality traits such as Harm Avoidance, Novelty Seeking, and Self-Directedness.

Results: Preliminary findings suggest that high Harm Avoidance is associated with greater use of emotion-focused and avoidant coping strategies, contributing to elevated emotional distress. Conversely, patients with high Self-Directedness and lower Harm Avoidance reported more frequent use of problem-focused coping and better psychological adaptation. Mutation carriers exhibited higher levels of emotional distress, particularly when employing avoidant coping strategies.

Conclusion: The interplay between coping strategies and personality traits plays a critical role in patients' psychological adaptation during genetic counseling. Tailored interventions that address maladaptive coping patterns and leverage patients' personality strengths may enhance emotional resilience and improve the overall counseling experience.

C.O.I. Disclosure:

I. Martin Villalba: None.

EP26.010 Knowledge, attitudes and preferences regarding expanded carrier screening among couples seeking in vitro fertilization

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Background: Expanded carrier screening (ECS) identifies couples at risk of having children with a recessive disorder, enhancing informed reproductive decision-making. Fertility clinics offer a unique setting for offering ECS preconceptionally. This study explored IVF/ICSI patients' knowledge, attitudes and preferences regarding ECS.

Material and methods: An online questionnaire was completed by patients who had an IVF/ICSI intake at a fertility clinic in a Dutch university hospital within the two years prior to this study ($n = 115$). Participants were not offered ECS. A multiple linear regression analysis was performed to investigate associations between intended ECS uptake and possible sociocognitive determinants.

Results: Overall, attitudes towards ECS were positive and the majority of participants (66.9%) would definitely or probably accept ECS. Attitudes towards offering ECS to couples undergoing IVF/ICSI ($\beta = 0.45$, $p = <.001$) and perceived risk ($\beta = 0.19$, $p = .010$) were associated with intended ECS uptake. Participants performed better on general genetic knowledge items ($M = 3.53$ out of five) than on ECS-specific knowledge ($M = 4.56$ out of eight items). If identified as a carrier couple, most participants (77.7%) would consider pre-implantation genetic testing. The most preferred approach (33.3%) for offering ECS was a categorized list of conditions from which couples could choose categories to be screened for.

Conclusion: While IVF/ICSI patients showed a strong interest in ECS, their knowledge of ECS was relatively low. This emphasizes the importance of exploring informed decision-making in more depth and tailoring information and counseling to meet IVF/ICSI patients' specific needs.

C.O.I. Disclosure:

C. Frank: None. **C.H. Laeven:** None. **D. Meulenbroeks:** None. **J. Schuurmans:** None. **E. Coonen:** None. **C.E. de Die:** None. **R.J. van Golde:** None. **A. Paulussen:** None. **L.A. van Osch:** None.

EP26.011 Personal utility of genetic testing depends on patient population: Evidence from four clinical cohorts

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Background: With increased use and complexity of genetic testing, evidence of personal utility is critical to funding and policy decisions. We aimed to understand patient-oriented values and preferences for genetic testing across patient populations to inform development of the Patient-reported Genetic testing Utility InDEx (P-GUIDE).

Methods: We identified four cohorts to understand how personal utility differs: prenatal genetics, pediatric genetics, pediatric oncology, and adolescent genetics. A preliminary set of generalizable domains and items of utility for each cohort were generated from the literature. Cognitive interviews were conducted with parents or adolescents recruited from across Canada to understand their experience with genetic testing, the meaning of personal utility, and the relevance of preliminary items. Thematic and item-specific analyses identified shared and unique items of utility across cohorts.

Results: Eighty interviews were conducted (prenatal = 20; pediatrics = 21; oncology = 20; adolescent = 19). Most participants were female (74%), and testing was diagnostic for 41%. All domains of personal utility resonated with participants (i.e. affective, behavioural, cognitive, medical management and social). Common items included increased understanding about the cause of their condition and contributing to scientific knowledge. Unique items were identified in each cohort: pregnancy decision-making in prenatal, resolving diagnostic odysseys in pediatrics, understanding cancer risks in oncology, and the importance of active decision-making in adolescents.

Conclusion: We identified shared and unique items of personal utility across cohorts, suggesting the need to tailor assessment to each patient population. Future work will validate these tools and include additional cohorts.

C.O.I. Disclosure:

E. Poole: None. **S. Luca:** None. **D. Assamad:** None. **B. Xiao:** None. **J. Yan:** None. **C. Xia:** None. **L.S. Abbott:** None. **L. Armstrong:** None. **P. Birch:** None. **K.M. Boycott:** None. **J.C. Carroll:** None. **L. Chad:** None. **D. Chitayat:** None. **A. Denburg:** None. **R.J. Deyell:** None. **A.M. Elliott:** None. **C. Goudie:** None. **A. Laberge:** None. **M. Maio:** None. **I. Peltekova:** None. **B. Quinlan:** None. **S.L. Sawyer:** None. **R. Silver:** None. **M. Smith:** None. **R. Teitelbaum:** None. **A. Villani:** None. **T. Wainstein:** None. **W.J. Ungar:** None. **R.Z. Hayeems:** None.

EP26.012 Efficacy of psychosocial interventions for rare conditions: a meta-analysis

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Background: Rare conditions (RCs) are defined as such if they affect less than 1 individual in 2000. Orphanet reports 6,172 unique RCs, of which 70% have paediatric onset. Globally, 3.5–5.9% of the population is affected by a RC at any point in time, converting to 263–446 million individuals. Individuals with RCs encounter unique medical and psychosocial challenges. However, despite clear needs, tailored psychological support remain scarce and under-researched. The aim of the present meta-analysis is to assess the efficacy of psychosocial interventions for individuals with RCs.

Methods: A comprehensive electronic search was performed, covering studies published until December 2024. Inclusion criteria consisted of experimental and prospective studies focused on paediatric and adult populations, investigating clearly defined psychosocial interventions.

Preliminary Results: The search identified 3072 studies of which 19 met the inclusion criteria. The eligible studies focused on a single or multiple conditions (i.e., 12/19 respiratory, 5/19 autoimmune and inflammatory, 3/19 connective tissue and skeletal, 2/19 neurological and muscular, 1/19 rare cancers, 1/19 eye conditions) and interventions (i.e., 8/19 psychological, 3/19 group or community-based, 3/19 educational, 1/19 genetic counselling, 4/19 other). Only 4/19 studies include children or adolescents, highlighting an important uncovered need for support in this group. Detailed data on population, interventions, and outcomes will be extracted and analysed. Results will include effect size parameters, overall efficacy and moderation analysis.

Conclusions: This meta-analysis will provide a comprehensive understanding of the efficacy of psychosocial interventions for RCs and develop recommendations for future research based on uncovered identified needs.

C.O.I. Disclosure:

M. Albu: None. **A. Ciuca:** None. **R. Moldovan:** None.

EP26.013 Evaluating the impact of dedicated clinical nurse consultants for patients with developmental and epileptic encephalopathies

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Background: Developmental and Epileptic Encephalopathies (DEEs), the most severe epilepsies, are usually genetic and characterised by uncontrolled seizures and complex co-morbidities. Patients have profound healthcare needs and dedicated DEE clinical nurse consultants (CNCs) may ease demands on caregivers by coordinating patients' care, providing psychosocial support, and assisting local clinicians. In a world-first, our team implemented two DEE CNCs and qualitatively assessed their efficacy from the perspectives of caregivers, clinicians, and nurses.

Material and methods: We co-developed interview and focus group questions with consumer-advocates. We analysed data with content analysis, using the Knowledge-to-Action Framework to *identify the problem*, *evaluate outcomes*, and *identify barriers to implementation*.

Results: 13 nurses, 15 clinicians, and 9 caregivers participated in interviews/focus groups. *Identify the problems*: families need a knowledgeable person to be their 'first-point-of-call' to provide answers and advice in a timely manner (otherwise they present to emergency departments), healthcare professionals (HCPs) are 'overwhelmed' by the complexity of patients with DEEs, and transition services are lacking. *Evaluation of outcomes*: with access to DEE CNCs, families have vital psychosocial support, therapies and transition services are better managed, and HCPS, including local HCPs, are better supported meaning more care can take place within primary care settings instead of hospitals. *Barriers to implementation:* DEE nurses need to be highly experienced and knowledgeable in seizures and co-morbidities and require sustainable funding.

Conclusion: Government investment in DEE CNCs is urgently needed to facilitate higher value healthcare for patients with DEEs, better support families and HCPs, and potentially reduce unwarranted hospitalisations.

C.O.I. Disclosure:

L. Kelada: None. **S. Best:** None. **K.B. Howell:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; RogCon Bioscience, Inc., Praxis Precision Medicines, UCB. **K. Dalziel:** None. **B. Forster:** None. **A. Schneider:** None. **C.E. Wakefield:** None. **E. Palmer:** None. **I.E. Scheffer:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Anavex Life Sciences, Cerevel Therapeutics, Eisai, Encoded Therapeutics, EpiMinder Inc, Epygenyx, ES-Therapeutics, GW Pharma, Marinus, Neurocrine BioSciences, Ovid Therapeutics, SK Life Science, Takeda Pharmaceuticals, UCB, Ultragenyx, Xenon Pharmaceuticals, Zogenix, Zynerba. C. Other Research Support (supplies, equipment, receipt of drugs or other in-kind support); Modest; UCB, Biocodex, GlaxoSmithKline, Biomarin, Encoded Therapeutics, Stoke Therapeutics, Eisai. D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Modest; GlaxoSmithKline, UCB, BioMarin, Biocodex, Chiesi, Liva Nova, Nutricia, Zuellig Pharma, Stoke Therapeutics, Eisai, Akumentis. E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Bionomics Inc. F. Consultant/Advisory Board; Modest; BioMarin, Chiesi, Eisai, Encoded Therapeutics, GlaxoSmithKline, Knopp Biosciences, Nutricia, Rogcon, Takeda Pharmaceuticals, UCB, Xenon Pharmaceuticals, Cerecin, Longboard Pharmaceuticals, Care Beyond Diagnosis, Epilepsy Consortium, Atheneum Partners, Ovid Therapeutics, UCB, Zynerba Pharmaceuticals, BioMarin, Encoded Therapeutics, Biohaven Pharmaceuticals. Other; Modest; Bellberry Ltd, Australian Academy of Health and Medical Sciences, Royal Society (Australia).

EP26.014 Anxiety and Quality of Life for parents of children with undiagnosed rare conditions undergoing Whole Genome Sequencing: Results from a multi-site quantitative survey

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Background: Parenting a child with a rare and undiagnosed condition can cause stress and anxiety. We conducted a multi-site quantitative survey with parents of children undergoing whole genome sequencing prior to test results being received.

Methods: 1366 surveys were disseminated across 7 NHS Trusts in England; 383 were returned (27% response rate). We used the GAD-7 to measure generalised anxiety, the Peds Family Impact Module and the EQ-5D-Y-3L to measure quality of life. We included bespoke questions on parental resilience, the severity of the child's condition and participant characteristics.

Results: Overall, parental anxiety was low (mean = 5.31; SD = 5.82, range 0–21), although 21.9% had moderate-severe anxiety. Significantly higher levels occurred in parents whose child's condition was more serious ($p = 0.004$). Anxiety was significantly higher where parents were younger ($p = 0.034$), had lower household incomes ($p = 0.039$), lower levels of education ($p = 0.013$), and lower resilience ($p < 0.001$). Lower child health-related quality of life (EQ-5D) scores were associated with higher anxiety scores ($p = 0.005$). The more serious the child's condition, the lower the EQ-5D score ($p = 0.041$).

Parents with higher anxiety had lower Peds Family Impact Module scores, indicating worse quality of life ($p < 0.001$). Higher

parent resilience was associated with better Parent Health-Related Quality of Life ($p < 0.001$) and Family Functioning ($p = 0.001$).

Conclusion: Anxiety is worse for parents who have lower resilience and whose children are more significantly impacted by their condition. Further work investigating support strategies to reduce parent anxiety and build resilience would be beneficial for this population, to help improve quality of life and family functioning.

C.O.I. Disclosure:

R. Patel: None. **B. Friedrich:** None. **C. Lewis:** None.

EP26.015 Barriers and bridges in patient access to rare disease research data and findings: Insights from three qualitative studies

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Background: There is a relative dearth of empirical literature centering rare disease patient experience, perspectives and needs regarding access to research findings and data. This presentation discusses themes in feedback from rare disease patients across three qualitative studies from our research team.

Material and methods: Studies utilized descriptive exploratory qualitative design, patient-centered conceptual frameworks and inductive thematic analysis. The first two studies engaged participants via focus groups of 2–6 people each (22 adults with cystic fibrosis; 18 adults living with or beyond rare cancers), and the third and present study engages adult survivors of pediatric cancers via an in-depth semi-structured survey ($n = 20–25$).

Results: Most participants actively seek out information from medical research institutions, publications, organizations and databases, citing that it empowers them to better understand, process and manage their condition and care. They described multiple access barriers including understanding scientific writing and terminology, publication paywalls, misinformation on the web, and restricted institutional data-sharing practices. The rarity of their conditions further impacts the availability of research and resources. Participants emphasized the need for timely, accessible, and understandable research information on their conditions, as well as return of results and study findings when they participate in research.

Conclusion: Findings illustrate the compounded barriers rare disease patients face as well as the critical role of researchers and policymakers in building bridges to better access. Ensuring transparency and accessibility in research is not just an ethical obligation but a critical step toward improving patient outcomes and fostering trust in scientific and medical communities.

C.O.I. Disclosure:

T. Knoppers: None. **M. Cosquer:** None. **M. Cassou:** None. **S. Bouhouita-Guermech:** None. **J. Hagan:** None. **M. Nguyen:** None. **B. Knoppers:** None. **M. Zawati:** None.

EP26.017 Assessing Key Informant Perspectives on the Governance of Prenatal Gene Editing

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As gene editing technologies continue to advance at a pace exceeding expectations, it is possible to conceive a future where such technologies are applied in humans during the prenatal stage. It is therefore critical to engage in prospective and collective deliberation to elucidate its (potential) acceptability. To that end, determining what should constitute sound scientific rationale, robust governance and fit-for-purpose regulation is essential. Likewise, it is critical to elucidate social/ moral values and ethical principles that would be at stake. Ultimately, outcomes from such deliberative processes should be integrated into governance decisions, ensuring that the values of affected/ interested groups, including patients, professionals and society at large, are considered. This in turn, will foster responsible and ethically sound scientific innovations. To evaluate how individual attitudes could shape the direction of science and policy, the PASAGE study conducted semi-structure key informants' interviews with professionals (e.g., clinicians, scientists, bioethicists) with experience in policymaking (e.g., laws, professional guidelines) in areas germane to potential prenatal gene editing applications. Our interviews explored attitudes towards scientific innovation, governance, risk tolerance, and socio-ethical/moral values in the context of human gene editing. Findings were then compared against governance frameworks, socio-ethical and moral values stated in gene editing and prenatal therapy policies. In this presentation, we provide results of both our key informants interviews and comparative policy study. Finally, based on these findings, we outline a proposed value-based framework for governing human gene editing.

C.O.I. Disclosure:

R. Isasi: None. **K. Ormond:** None. **Y. Bombard:** None. **H. Howard:** None. **R. Feys:** None.

EP26.018 Support of polygenic scores for medical and social traits among the public and biobank participants: The role of trust in scientists

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Background: Polygenic scores (PGS) are being developed for medical traits, like cardiovascular disease, and social traits, like educational attainment. Many worry about the ethical and social implications of the use of PGS outside of research. Yet little is known about how biobank participants and the public consider PGS research for medical and social traits.

Methods: We surveyed biobank participants ($N = 434$) and members of the public ($N = 1090$) in the United States to investigate support of PGS research on medical and social traits. We used binomial logistic regression to examine significant predictors of support ($p < 0.05$).

Results: Both populations were more supportive of research on PGS for medical traits (Biobank: 86.4%; Public: 63.9%) than social traits (Biobank: 36.6%; Public: 42.1%). When controlling for demographic predictors, trust in scientists emerged in both populations as a significant predictor of support for research on PGS for medical and social traits, but had a stronger effect on support of PGS for medical traits (Biobank: OR = 1.02, indicating a 2% increase in odds per unit increase in trust; Public: OR = 1.03, a 3% increase) than social traits (Biobank: OR = 1.01, a 1% increase; Public: OR = 1.02, a 2% increase). Overall, trust in scientists was

higher among biobank participants than the public, as measured by 0 to 100 composite scores (Biobank: 70.0; Public: 63.5).

Conclusion: We will discuss the ethical implications of these findings for PGS researchers and biobank administrators, interrogating whether trust in scientists may serve as a barrier to PGS research, the use of PGS beyond research, or biobank participation.

C.O.I. Disclosure:

M. Waltz: None. **A. Long:** None. **M. Kucmanic:** None. **N. Mehri:** None. **R. Cadigan:** None.

EP03.034 HMMMM: Improving the accuracy of non-invasive prenatal diagnosis with Hidden Markov Models

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Background: Non-invasive prenatal diagnosis (NIPD) has revolutionized the management of monogenic disorders by reducing risks associated with invasive procedures. However, commonly used statistical approaches, such as the Sequential Probability Ratio Test (SPRT), face limitations, particularly in cases of consanguinity or recombination events. Here, we introduce a new open-source NIPD approach, *Hidden Markov Model for Monogenic Maladies* (HMMMM).

Material and methods: This study aimed to develop, implement, and evaluate a Hidden Markov Model-based approach for NIPD of monogenic diseases and compare it with SPRT to assess clinical performance. A cohort of 112 pregnancies was analyzed, including families at risk for cystic fibrosis, Duchenne muscular dystrophy, hemophilia, and neurofibromatosis type 1. Both SPRT and HMMs were used to determine the transmission of at-risk haplotypes, and their performances were compared in terms of inconclusivity rates and regions of uncertainty.

Results: Among 112 pregnancies, 107 fetuses were correctly diagnosed using both methods. HMMMM reduced the inconclusivity rate (2.7% vs. 3.6% with SPRT) and significantly decreased the proportion of cases with regions of uncertainty (14% vs. 40%). The combined use of both methods further reduced these numbers to 1.8% and 9%.

Conclusion: Although SPRT has been the historical method for NIPD, HMMs offer substantial advantages, particularly in complex cases involving recombination or consanguinity. Their ability to model sequencing biases and allele drop-out represents a major advancement. Integrating HMMMM into clinical pipelines, either as a stand-alone or complementary tool, could reduce inconclusivity rates and improve diagnostic accuracy in NIPD.

C.O.I. Disclosure:

V. Gravrand: None. **C. Verebi:** None. **L. Orhant:** None. **J. Guilliet:** None. **P. Garret:** None. **F. Leturcq:** None. **T. Bienvenu:** None. **J. Nectoux:** None.

EP03.032 Non-invasive prenatal testing results with maternal malignancy suspicion

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Background: Non-invasive prenatal testing (NIPT) has become widespread in clinical practice. NIPT can determine not only the risk of fetal chromosomal abnormalities (FCA), but also some maternal diseases: some studies have reported a correlation between multiple chromosomal abnormalities (MCA) detected in the fetus by NIPT and malignancies in pregnant women, especially hemoblastoses.

Material and methods: Whole genome NIPT (WG-NIPT) was performed for 51,406 pregnant women as the second-line test using a BGISEQ-500 and DNBSEQ-G400 (MGI, China). Bioinformatic data processing was performed using the original BGI proprietary software (HALOS NIFTY).

Results: In this study, 7 of 51,406 (0.014%) women who underwent WG-NIPT had the results of complex abnormal NIPT profiles - NIPT with malignancy suspicion - with an incidence of 1.4 cases for 10,000 pregnanciesıo In 3 cases (42.8%) hematological malignancies were confirmed: in 2 cases - Hodgkin's lymphoma, in 1 case - non-Hodgkin's lymphoma. Also 1 patient was examined due to recurrent infections and abnormal complete blood count.

Conclusion: NIPT is not only an effective screening FCA method, but also a promising tool for early detection of malignancies in pregnant women. Early diagnosis of cancer contributes not only to more effective treatment, but also to improving the quality of life and increasing the life expectancy of patients with cancer. Further research is needed.

C.O.I. Disclosure:

M. Kaplanova: None. **A. Galaktionova:** None. **A. Potapov:** None. **O. Sagaydak:** None. **M. Belenikin:** None. **A. Krynitsina:** None. **E. Domoratskaya:** None. **P. Ulanova:** None. **E. Efremova:** None. **A. Olenev:** None.

EP03.035 Expanding *COL25A1*-related phenotypes in a fetus with findings of arthrogryposis multiplex congenita

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Background: Arthrogryposis multiplex congenita is a term used to describe joint contractures in more than one area of the body at birth. One mechanism leading to decreased fetal movements resulting in arthrogryposis is impaired intramuscular innervation. It has been shown that proper development of intramuscular innervation depends on interactions between muscle-derived COL25A1 and its receptors[1]. Recently, postnatally diagnosed cases with biallelic variants in *COL25A1* presenting with variable severity of arthrogryposis were reported[2]. We report a fetus with a homozygous deletion involving *COL25A1* and prenatally diagnosed arthrogryposis multiplex congenita.

Methods: A consanguineous couple was referred to our center for detailed evaluation of hydrops fetalis at 18^{+5} weeks of gestation. The 2nd-trimester ultrasound revealed increased nuchal fold, subcutaneous oedema, hydrothorax, ascites, flexion deformity of both upper and lower extremities, unilateral clubfoot, decreased femur length and decreased fetal movements. Postmortem fetal examination revealed retro/micrognathia, flattened profile, bilateral flexion contractures of both upper and lower extremities, unilateral clubfoot, pterygium of both knees and elbows, dislocations in various joints and clasped hands with

thumbs in adduction. Chromosomal microarray was performed after amniocentesis.

Results: A homozygous deletion in region chr4:108,852,124-109,488,822 encompassing first 25 exons of *COL25A1* was detected. Each parent was heterozygous for the deletion.

Conclusion: This is the first prenatal case of *COL25A1*-related arthrogryposis. The fetus represents the most severe of all *COL25A1*-related phenotypes, probably due to the similarity of the deletion's content with *Col25a1* knockout mouse models which were neonatally lethal. Our case further supports the association of *COL25A1* with arthrogryposis multiplex congenita.

C.O.I. Disclosure:

Y. Ülger: None. **G. Okutucu:** None. **A.C. Ceylan:** None. **D. Şahin:** None.

EP03.033 In silico analysis of amniotic fluid miRNAs associated with renal abnormalities of the fetus

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Background: MicroRNAs (miRNAs) are small non-coding RNAs that play a critical role in post-transcriptional gene regulation and have emerged as promising biomarkers for several diseases. In pregnancies with pathological ultrasound findings of the fetus, amniotic fluid (AF) miRNAs might be informative for the underlying genetic factors. This study aims to analyze and compare the AF cell-free miRNAs and AF exosomal miRNAs in renal abnormalities of the fetus.

Materials and methods: Published data (n = 40) was systematically analyzed and AF cell-free miRNAs and exosomal miRNAs associated with fetal renal abnormalities were curated. mRNA targets were predicted and only the common targets detected by three prediction tools (Targetscan v7.1, Pictar, miRBase v22.1) were further analyzed. Subsequently, the mRNAtargets associated with renal function and abnormalities were selected. The cellular function andassociated pathways of the target mRNAs are evaluated and compared between cell-free and exosomal miRNAs.

Results: In published data, among AF cell-free miRNAs, hsa-let-7f, hsa-miR-155, hsa-miR-210, hsa-miR-200, hsa-miR-19, hsa-miR-92a, miR-30a and among the AF exosomal miRNAs hsa-let-7f, hsa-miR-299-5p, hsa-miR-195-5p, hsa-miR-300 and, hsa-miR-381 were associated with renal abnormalities. The predicted mRNA targets were found to be involved in pathways such as protein binding, DNA regulation, transcription, ATP binding, and methionine adenosyltransferase activity.

Conclusion: This study shows that although there were common miRNAs between the AF cell-free and exosomal miRNAs in renal abnormalities, the detected miRNAs diverged between thetwo groups. However, the study is limited by the detection methods of the analyzed publications and detected miRNAs should be evaluated by further functional studies.

C.O.I. Disclosure:

E. Kiliç: None. **B. Karaman:** None. **B. Ozsait-Selçuk:** None.

EP03.036 A Cautionary Tale of Fetoplacental Discrepancies: The Impact of Mosaicism in Prenatal Diagnosis

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Standard prenatal care in Ontario, Canada, includes first-trimester screening (FTS) and optional non-invasive prenatal testing (NIPT) using cell-free fetal DNA (cffDNA). If screening results are positive, patients may choose diagnostic testing via CVS (chorionic villus sampling) or amniocentesis, analyzed by QF-PCR and chromosomal microarray (CMA). Fetoplacental discrepancies, complicate interpretation when fetal chromosome status is inferred from placental testing. Most involve false-positive results, where abnormal cell lines are confined to the placenta (CPM). Here, we report two rare cases of CPM with non-mosaic fetal abnormalities:

Case 1: Prenatal diagnosis was initiated due to increased nuchal translucency but a negative NIPT result. Direct CVS showed a normal female karyotype, but third-trimester ultrasound revealed short long bones. Exome sequencing (ES) on cultured CVS later detected mosaic trisomy 21 (T21), confirmed by post-termination testing. The umbilical cord showed full T21, while cultured CVS showed mosaic T21, suggesting a sampling bias, with preferential inclusion of cytotrophoblastic cells (presumably containing higher proportion of normal cells) in the direct sample.

Case 2: Following increased nuchal translucency, NIPT indicated T21 with a low predictive value. CVS showed low-level mosaic monosomy 21, while amniocentesis later confirmed high-level mosaic T21. Post-termination analysis revealed non-mosaic T21 in the umbilical cord, while placental biopsies showed varying mosaic T21 patterns. Findings suggest a mitotic nondisjunction event giving rise to three distinct placental cell lines and non-mosaic fetal abnormality Conclusion: These cases highlight the complexity of prenatal diagnosis, emphasizing challenges in interpreting mosaic results and distinguishing true fetal abnormalities from placental mosaicism.

C.O.I. Disclosure:

E. Greenfeld: None.

EP04.028 Clinical and genetic heterogeneity of syndromic hearing loss with an emphasis on syndromic genes mimicking non-syndromic hearing loss

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Background: Hearing loss (HL) is one of the most common congenital disorders, affecting 1–2 per 1,000 newborns. While more than 150 genes are associated with non-syndromic hearing loss (NSHL), over 600 genes are linked to syndromic hearing loss (SHL). Importantly, the absence of additional clinical symptoms does not necessarily exclude SHL, as an increasing number of mutated genes initially present as NSHL but later evolve into syndromic conditions.

Material and methods: We analyzed clinical and molecular data from 96 unrelated patients, selected from over 500 *GJB2*- and *STRC*-mutation-negative HL patients/families. Sequencing approaches ranged from disease-specific panels to genome sequencing. Patients were divided into three subgroups: subgroup-1 included 30 patients with variants in neurodevelopmental delay (NDD)-associated genes; subgroup-2 is comprised of 37 cases with variants in SHL-associated genes unrelated to NDD; subgroup-3 included 29 patients with variants in genes associated with both SHL and NSHL, such as *SLC26A4* and *MYO7A*.

Results: In subgroup-1, 80% of patients initially presented with HL, with additional symptoms emerging in 50%. In subgroups-2 and 3, HL was the sole initial symptom for nearly all patients (91% and 100%, respectively). Across the cohort, 91 variants in 48 genes were identified, including 38 novel variants.

Conclusion: The lack of additional symptoms often leads to testing focused solely on NSHL-associated genes. Our findings highlight the critical role of comprehensive genomic testing for accurate diagnosis and management of HL.

C.O.I. Disclosure:

A. Koparir: None. **P. Bahena Carbajal:** None. **M.A. Hofrichter:** None. **M. Zamani:** None. **S. Tovornik:** None. **E. Koparir:** None. **P. Torbati:** None. **A. Rad:** None. **D. Owrang:** None. **I. Kalay:** None. **N. Dragicevic:** None. **N. Chamanrou:** None. **L.N. Martínez Völter:** None. **N. Christophersen:** None. **T. Baranzehi:** None. **S. Loum:** None. **M. Shadab:** None. **N. Alidadiani:** None. **G. Shahrooz:** None. **T. Haack:** None. **S. Alavi:** None. **H. Kremer:** None. **D. Kordi-Tamandani:** None. **E.G. Karimiani:** None. **H. Hebestreit:** None. **H. Galehdari:** None. **S. Flandin:** None. **W. Sheata Dieler:** None. **R. Maroofian:** None. **T. Haaf:** None. **B. Vona:** None.

EP04.029 Genetic characterisation of Inherited Retinal Diseases in diverse African populations through next-generation sequencing

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Background: Inherited retinal diseases (IRDs) are a genetically heterogenous group of disorders leading to progressive vision loss, affecting over 2 million individuals globally. Despite their impact, research has predominantly focused on European-derived populations, resulting in a significant knowledge gap about IRDs, especially in diverse African populations. This underrepresentation contributes to healthcare disparities and limits understanding of global IRD genetics. To address this gap, the Division of Human Genetics at the University of Cape Town, with over three decades of experience in South African IRDs, is collaborating with African ophthalmologists to explore the genetic basis of these disorders across Kenya, Rwanda, Ghana, and Nigeria.

Materials and methods: Saliva samples were collected from patients and relatives, and DNA extraction was followed by next-generation sequencing (NGS) using a custom panel targeting more than 120 IRD-related genes or whole-exome sequencing (WES) to identify causal variants.

Results: Analysis of 41 probands, including 8 via WES and 33 via the IRD gene panel, identified a number of disease-causing variants in known genes. Validation and cosegregation analysis through Sanger sequencing are ongoing.

Conclusion: This study demonstrates the promise of combining genomic technologies and transdisciplinary partnerships to advance understanding of IRDs in Africa. By providing genetic diagnoses to guide clinical care and expanding the knowledge of IRD genetics in African populations, this research will ultimately foster improved healthcare equity and contribute to addressing global disparities in IRD research and treatment.

C.O.I. Disclosure:

J. Demetrio: None. **I.Z. Braimah:** None. **A. Aina:** None. **M. Isawumi:** None. **O. Kolawole:** None. **T. Oluleye:** None. **B. Olusanya:** None. **O. Onyango:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Roche. **C. Mathenge:** None. **R. Ramesar:** None. **L. Roberts:** None.

EP05.032 Species and gender based analysis of reference genes and proteins in rat thyroid and parathyroid tissues

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Background: Gene expression analyses are essential for understanding known genes' roles and tissue systems' complex biological responses. Reference genes and proteins are widely used for normalizing gene and protein expression data due to their assumed stable structural expression. However, identifying tissue-specific reference genes under different conditions is critical for improving accuracy in normalization.

Material and methods: This study aimed to determine tissue-specific reference genes in parathyroid and thyroid tissues of two rat species (Wistar and Sprague-Dawley) and evaluate potential gender-related variations. Seven candidate reference genes (PGK1, SDHA, TBP, HPRT1, GAPDH, YWHAZ, VCL, RPLP13A, ACTB) were analyzed using qRT-PCR techniques. Stability analyses were conducted using geNorm, NormFinder, and BestKeeper algorithms.

Results: Species- and gender-dependent variations were observed in the mRNA expression stability of the tested genes. Among the candidates, ribosomal subunits and GAPDH exhibited stability in both rat species and genders, making them suitable reference genes and proteins for normalization in parathyroid and thyroid tissues.

Conclusion: This study is the first to identify and validate reference genes specific to parathyroid and thyroid tissues in rats. These findings contribute to the growing understanding of tissue-specific gene expression and provide a valuable resource for future endocrine system research studies.

C.O.I. Disclosure:

E. Gunaydin: None. **B. Goncu:** None.

EP05.031 Immune cell free parathyroid cell isolation assessment for advancing parathyroid transplantation

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Background: Parathyroid transplantation, first performed in 1911, initially demonstrated a survival rate of up to two months. Since then, advancements have focused on improving survival rates, transplantation types (cell vs. tissue), and understanding immune responses. Chronic kidney disease (CKD) patients with parathyroid hyperplasia often require parathyroidectomy, and their excised tissues are used for transplantation. Developing methods to isolate pure parathyroid glandular cells, free from immune cells like T and B lymphocytes, is essential for minimizing immune rejection and improving transplantation outcomes.

Material and methods: Parathyroid tissues from 29 CKD patients were analyzed. Enzymatic cell isolation techniques were utilized to purify parathyroid glandular cells. The presence of immune cells was assessed using flow cytometry with CD19 (B lymphocytes) and CD3 (T lymphocytes) markers. Epidemiological factors, including age, gender, parathyroidectomy necessity, and calcimimetic use, were also evaluated.

Results: Flow cytometry confirmed the absence of B and T lymphocytes in the isolated parathyroid cell populations, demonstrating the effectiveness of the cell isolation method. An age-dependent increase in parathyroid hyperplasia was observed, with older CKD patients often requiring surgical intervention. The study also identified potential associations between clinical conditions and the need for calcimimetics.

Conclusion: This study highlights the significance of developing reliable parathyroid cell isolation methods for transplantation. The absence of immune cells in isolated populations supports the feasibility of using these cells in clinical applications. These findings provide a foundation for improving parathyroid transplantation outcomes in hypoparathyroidism patients.

C.O.I. Disclosure:

B. Goncu: None. **M. Gelmez:** None. **A. Gürol:** None. **Y. Ersoy:** None.

EP06.056 A Novel*MBTPS1*Variant Associated with Spondyloepiphyseal Dysplasia, Kondo-Fu Type: First Reported Case in Turkey

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Background: Spondyloepiphyseal dysplasia, Kondo-Fu type (SEDKF, OMIM #618392) is a rare autosomal recessive disorder characterized by skeletal deformities, large ears, a triangular face. SEDKF is caused by mutations in the *membrane-bound transcription factor protease, site-1 (MBTPS1)* gene.

Material and methods: Following the DNA isolation from peripheral blood cells, Next generation sequencing was performed to detect disease causing molecular defect in index cases. Segregation analysis of the identified variant was performed using Sanger sequencing.

Results: A 13-year-old girl was referred to us because of short stature, dysmorphism, type 1 diabetes mellitus and kyphoscoliosis. She was born as a twin from consanguineous parents; her twin was healthy. The patient's 8-year-old brother was diagnosed as autism. The patient exhibited severe short stature (height: 109 cm, SDS −8.72) and low weight (weight: 19 kg, SDS −8.82), along with large ears, inguinal hernia, kyphosis, and typical dysmorphic features associated with SEDKF. The patient presented with cataracts, a "beaten copper" appearance on brain MRI, vertebral beaking, and increased total GAG in urine. Evaluation of the MPS enzymes revealed normal results, and genetic analysis for MPS plus syndrome also returned negative results. Neurodevelopmental characteristics were compatible with her age. In molecular genetic analysis of index case novel homozygous c.787T>A p.(Cys263Ser) variation of*MBTPS1* gene was detected. Segregation analysis confirmed that both parents were heterozygote carriers.

Conclusion: Herein we detected a novel variant of MBTPS1 gene as the cause of Spondyloepiphyseal dysplasia, Kondo-Fu type. This patient represents the first reported case in Turkey and the tenth case globally.

C.O.I. Disclosure:

E. Dirimtekin: None. **A. kahveci:** None. **E. genç:** None. **B. gürpınar tosun:** None. **B. öztürk hişmi:** None. **A. arman:** None. **S. turan:** None. **P. Ata:** None.

EP06.054 Clinical and Genetic Analysis of Two Cases of BCYM4

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Background: Brachyolmia type 4 with mild epiphyseal and metaphyseal changes (BCYM4) is a rare form of skeletal dysplasia characterized by short stature, short trunk, and platyspondyly. It is an autosomal recessive condition with additional features including short and bowed lower limbs, mild brachydactyly, kyphoscoliosis, enlarged knee joints, and abnormal gait. Some patients may also exhibit premature pubarche and hyperandrogenism. We present two cases with clinical and genetic findings consistent with BCYM4.

Material and methods: Case 1 was referred due to short stature evident since birth, rizomelic limb shortening, and right leg length discrepancy. Family history was negative for constitutional short stature. Case 2 was referred due to short stature, joint pain and hand swelling. A history of consanguinity was noted in the family. Peripheral blood samples from both patients were analyzed using Next-Generation Sequencing (NGS).

Results: Case 1 exhibited mild brachydactyly, and bowing of the lower limbs. Genetic testing revealed homozygous pathogenic variants of PAPPSS2 in exon3. Case 2 exhibited short stature, kyphoscoliosis. X-ray radiography revealed platyspondyly and distal femoral and proximal tibial metaphyseal enlargement. Genetic testing revealed homozygous pathogenic variants of PAPPSS2 in exon2.

Conclusion: The clinical and genetic findings in these cases align with BCYM4. Both patients exhibited hallmark features such as short stature, spine abnormalities, and limb deformities. This highlights the importance of genetic analysis in confirming the diagnosis and differentiating BCYM4 from other forms of skeletal dysplasia. Further studies are needed to elucidate the full clinical spectrum of BCYM4 and optimize management strategies for affected individuals.

C.O.I. Disclosure:

B. Adacoglu: None. **S. Emre:** None. **E. Yilmaz Gulec:** None.

EP06.055 A rare case of osteogenesis imperfecta type XX: compound heterozygous variants in the MESD gene

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Background: Osteogenesis imperfecta type XX (OI20) is a rare autosomal recessive disorder caused by homozygous or compound heterozygous mutations in the MESD gene, located on chromosome 15q25. This condition is characterized by osteopenia, skeletal deformities, progressive bone fragility, and distinct craniofacial and systemic features. We aim to present a rare case of OI20 to highlight its clinical presentation, diagnostic approach, and implications for genetic counseling.

Material and methods: An 11-year-old female was referred for evaluation due to short stature, scoliosis, dysmorphic facial features, and developmental delay. Clinical assessment included anthropometric measurements and dysmorphology examination. Genetic evaluation involved chromosomal analysis, microarray, and a multigene panel using next-generation sequencing. Sanger sequencing was performed for parental segregation analysis.

Results: The patient presented with a height of 125 cm (−3.03 SDS), weight of 22.5 kg (−2.73 SDS), and dysmorphic features including sparse hair, narrow forehead, upslanting palpebral fissures, bulbous nose, and a high-arched palate. Genetic testing revealed compound heterozygous variants in the MESD gene: a pathogenic frameshift mutation (c.631_632del, p.(Lys211-Glufs*19)) and a missense variant of uncertain significance (c.344T>A, p.(Met115Lys)). Parental segregation confirmed trans inheritance. The findings, combined with the clinical phenotype, supported a diagnosis of OI20.

Conclusion: This case emphasizes the importance of genetic testing in diagnosing rare conditions like OI20 and provides insights into its clinical spectrum. Genetic counseling is crucial for families to understand recurrence risks and the need for carrier screening in future pregnancies.

C.O.I. Disclosure:

S. Emre: None. **B. Adacoglu:** None. **E. Yilmaz Gulec:** None.

EP06.058 A loss-of function TBX15 gene variant in Cousin Syndrome expanding the phenotype: a case study

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Background: Cousin syndrome is a rare genetic disorder characterized by short stature, distinctive skeletal anomalies, and craniofacial dysmorphism, with no impact on cognitive abilities. Herein, we report for the first time from the Gulf region, a variant in TBX15 gene causing Cousin syndrome with expanded phenotypic spectrum.

Methodology: Routine diagnostic work-up included physical examination, auditory assessment, CT scan, ultrasonography, skeletal survey and genetic analysis.

Results: 7-month-old male infant presented with dysmorphic features, including craniofacial abnormalities, folded ears, hypertelorism, joint contractures, short lower limbs, and umbilical hernia. Modified Barium Swallow Study (MBSS) revealed oral and pharyngeal dysphagia, with auditory assessment showing possible hearing loss. Computerized Tomography (CT) and Ultrasonography (USG) showed mild respiratory distress, a wide anterior fontanelle and hydronephrosis. Skeletal survey showed mild bowing deformity in the femoral shafts, with exuberant callus formation at the proximal diaphysis of both femoral shafts. Genetic analysis identified a pathogenic loss-of-function homozygous variant c.1159C>T; p.R837* (exon 8, chr1-g.119428005, NM_001330677.2) in the TBX15 gene.

Conclusion: A variant in TBX15 gene confirms the diagnosis of a rare Cousin syndrome expanding the molecular spectrum and reveals the broadened phenotype of the syndrome.

C.O.I. Disclosure:

A. Peer Zada: None. **W. Alharbi:** None. **A. Mushiba:** None.

EP06.057 Hereditary Multiple Exostoses: A Novel variant with anomalies of the hands and feet

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Background: Hereditary Multiple Exostoses Syndrome (HME) is an autosomal dominant skeletal disorder characterized by multiple osteochondromas and is caused by *EXT1*, *EXT2*, or *EXT3* gene mutations. HME exhibits high penetrance and variable expressivity, manifesting primarily as exostoses of the long bones and less frequently as limb anomalies, resulting in hand and foot deformities.

Material and methods: This study presents a family with multiple HME-affected members displaying both exostoses and limb anomalies. The radiographic skeletal survey revealed metaphyseal exostoses in all long bones, including proximal humeral widening and diaphyseal femoral exostoses. Foot radiographs showed shortening of the middle phalanges of toes 3–5. The above clinical and radiographic features were observed across four generations, affecting the proband, as well as her mother, maternal grandfather, and three maternal aunts.

Results: Next-generation sequencing (NGS) using an MGI platform, with data analysis via the **Varsome Clinical** (Saphetor)™ platform, identified a novel heterozygous pathogenic variant in *EXT1* gene: c.1667_1670dup p.(Thr558HisfsTer9). This variant, absent from ClinVar and previously unreported in the literature, expands the known *EXT1* mutation spectrum.

Conclusion: Given the increased risk of secondary malignancy in HME, comprehensive clinical, radiological, and genetic evaluation, coupled with long-term follow-up, is crucial for optimal patient management, early detection of malignant transformation, and improved prognosis.

Gene	Variant	Variant Type	Zygosity	Inheritance	Clinical Interpretation
EXT1 **(NM_000127.3)**	**c.1667_1670dup (p.Thr558HisfsTer9)**	Frameshift	Heterozygous	Autosomal Dominant	Pathogenic

C.O.I. Disclosure:

M. Christodoulou: None. **G. Papadopoulos:** None. **S. Psoni:** None. **E. Tsoutsou:** None. **S. Amenta:** None. **E. Manolakos:** None. **H. Fryssira:** None.

EP07.039 Metabolomic profiling reveals distinct alterations across coronary artery disease progression

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Background: Coronary artery disease (CAD) progresses from stable angina to more severe conditions such as myocardial infarction (MI) and heart failure (HF), each associated with distinct metabolic alterations. Understanding these metabolic differences can provide insights into disease mechanisms and potential therapeutic targets. This study aims to identify key metabolic markers associated with CAD progression and their potential links to gut microbiota.

Material and methods: We analyzed 403 individual metabolites and 320 metabolism indicators, which summarize biologically meaningful metabolic pathways and enzyme activity. CAD patients were categorized into angina ($n = 60$), MI ($n = 54$), and HF ($n = 56$) groups. ANCOVA was performed to compare metabolite levels across groups, adjusting for age, sex, and BMI, with false discovery rate (FDR) correction. Parallel shotgun metagenomic sequencing is ongoing to explore microbiome-metabolome associations.

Results: A total of 63 individual metabolites and 61 metabolism indicators showed significant differences across CAD stages ($q < 0.05$). Carboxylic acids, a TCA cycle intermediate, was significantly elevated in HF, indicating metabolic shifts linked to disease severity. Amino acids, involved in protein turnover and oxidative stress, varied across CAD stages, reflecting progressive metabolic dysregulation. In metabolism indicators, 1-methylhistidine synthesis, a marker of muscle protein turnover, and autotaxin activity, involved in lipid signaling, showed significant alterations along the CAD spectrum.

Conclusion: Distinct metabolic differences were observed with CAD progression, particularly in energy metabolism, amino acid turnover, and lipid signaling, which may be influenced by gut microbiota. These findings provide insights into CAD pathophysiology and may help guide biomarker discovery and microbiome-targeted therapies.

C.O.I. Disclosure:

H. Kim: None. **S. Lee:** None. **Y. Jung:** None. **S. Lee:** None. **H. Kim:** None. **S. Kang:** None.

EP07.040 Plasma cell-free RNA characterizes heterogeneity of hypertension in pregnancy

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Background: Hypertension is a common and serious complication during pregnancy, especially in women with chronic hypertension, continuously damages endothelial cells and small blood vessels, increasing the risk of placental-mediated complications, and is an important risk factor for preeclampsia (PE) and preterm birth. Plasma cell-free RNA (cfRNA) serves as a non-invasive biomarker that can reflect dynamic changes between the mother and fetus, with potential for early non-invasive diagnosis.

Material and methods: We analyzed plasma cfRNA from 259 pregnant women with hypertensive diseases (including 221 with preeclampsia, 14 with preeclampsia superimposed on chronic hypertension, and 24 with chronic hypertension) and 260 healthy control pregnant women using PALM-Seq technology. Differentially expressed genes, pathway changes, and cell type composition were examined in early and mid-pregnancy, and differences were compared between subgroups of preeclampsia (early-onset vs. late-onset, with or without severe features, and preterm birth or term birth).

Results: The common molecular changes among the three hypertensive groups were primarily focused on vascular and immune-related pathways. The preeclampsia group showed more significant trophoblast cell changes compared to the chronic hypertension group. Early-onset preeclampsia was more strongly associated with placental abnormalities, while preeclampsia with severe clinical features and preterm birth exhibited notable immune and liver/kidney-related changes. Additionally, changes in clinical blood markers corroborated the cfRNA changes.

Conclusion: This study demonstrates that plasma cfRNA in early and mid-pregnancy can not only characterize molecular changes in hypertensive pregnancy diseases but also reflect commonalities and differences between subgroups of preeclampsia.

C.O.I. Disclosure:

J. Li: None. **S. Chen:** None. **T. Yang:** None. **L. Wang:** None. **J. Sun:** None. **X. Zhou:** None. **Z. Liu:** None. **Y. Liu:** None. **S. Guo:** None. **Y. Kang:** None. **Y. Qin:** None. **Q. Zhou:** None. **C. Fang:** None. **H. Huang:** None. **C. Xu:** None. **W. Wang:** None.

EP08.041 Early prediction of gestational diabetes mellitus in pregnancy by plasma cell-free RNAs

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Background: In recent years, the incidence of gestational diabetes mellitus (GDM) has been steadily rising. Maternal GDM increases the risk of preterm birth in both nulliparous and multiparous women, presenting significant and complex clinical challenges. Cell free RNA (cfRNA) offers an opportunity to non-invasively predict the risk of GDM in the early pregnancy.

Material and methods: We used PALM-Seq to analyze the cfRNA from plasma samples of 108 pregnant women (GDM, N = 54; CTL, N = 54) in the early pregnancy, and constructed a model to predict GDM.

Results: Differentially abundant genes analysis revealed that 23 mRNAs, 11 lncRNAs and 2 miRNAs altered their abundance in patients with GDM, and pathway enrichment analysis also revealed pathways related to immune,metabolism, nervous system, protein translation and ribosome assembly. In the correlation analysis between RNA and blood glucose levels, we identified 147, 713, and 877 genes that were significantly associated with 0hGlu, 1hGlu, and 2hGlu, respectively. These genes include those that are specific to the liver, brain, and skeletal muscle. Furthermore, we utilized cfRNA features in early pregnancy to build a model to predict the risk of GDM, achieving an AUROC of 0.84 in the test set and 0.73 in the validation set.

Conclusion: This study demonstrates that plasma cfRNA could predict and characterize GDM in the early pregnancy.

C.O.I. Disclosure:

Y. Liu: None. **S. Chen:** None. **T. Yang:** None. **Z. Wan:** None. **J. Sun:** None. **X. Zhou:** None. **Z. Liu:** None. **J. Li:** None. **L. Wang:** None. **S. Guo:** None. **Y. Kang:** None. **Y. Qin:** None. **Q. Zhou:** None. **F. Chen:** None. **H. Huang:** None. **C. Xu:** None. **W. Wang:** None.

EP09.027 Diagnostic Challenges in Panniculitis: A Case of SPTCL with HAVCR2 Mutations Initially Diagnosed as Christian-Weber Syndrome

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Background: We report a case of a 15-year-old male initially diagnosed with Christian-Weber syndrome at age 8, presenting with tender, erythematous subcutaneous nodules. Histopathology revealed a non-specific inflammatory process in the dermis affecting subcutaneous adipose tissue. Bone marrow biopsy showed borderline increased histiocytes with marked hemophagocytosis. After corticosteroid pulse therapy and two years of cyclosporine, the patient achieved a 3-year remission. The new relapse and medication dependence of the clinical manifestation necessitated a prompt reassessment of the diagnosis.

Material and methods: Whole Exome sequencing was performed in response to the combined challenges of clinical inconsistency and therapeutic resistance.

Results: Two heterozygous missense variants in the HAVCR2 gene were identified: c.291A>G, p. (Ile97Met), which is likely pathogenic, and HAVCR2 c.302C>T, p. (Thr101Ile), which is a variant of uncertain significance. Germline mutations in the HAVCR2 gene, encoding the TIM-3 protein, are associated with the development of systemic inflammatory conditions, including subcutaneous panniculitis-like T-cell lymphoma (SPTCL) and hemophagocytic lymphohistiocytosis (HLH).

Conclusion: These mutations disrupt immune regulation, leading to excessive and persistent inflammation. Patients with HAVCR2 mutations bear a significantly higher risk of developing hemophagocytic lymphohistiocytosis (HLH) and HLH-like syndromes, especially children and young adults with SPTCL. Successful treatment of SPTCL with immunosuppressive therapy (IST) without chemotherapy has proven to be effective, although long-term data in pediatric SPTCL remain scarce. This emphasizes the need for future multicenter studies involving larger numbers of patients across various ethnicities.

C.O.I. Disclosure:

A.I. Vardeva: None. **H. Ivanov:** None. **H. Burnusuzov:** None. **K. Stefanova:** None. **V. Stoyanova:** None.

P09.048.B Clinical and genetic characterization of familial Mediterranean fever among of Georgians

Tamar Ramishvili[1], *Irakli Rtskhiladze*[1], *Nikoloz Abramishvili*[1], *David Metreveli*[1], *Christian Timmann*[2], *Daniela Steinberger*[3], *Marina Barnova*[1], *Tamar Andriadze*[1], *Tamar Dzagania*[1], *Nino Jashiashvili*[1], *Nino Kankia*[1], *Maka Aroshidze*[1], *Lela Zangurashvili*[1], *Tamar Pirtskhalaishvili*[1], *Erekle Shatirishvili*[1], *Alexxander Tavadze*[1]

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Background: Familial Mediterranean Fever (FMF) is an autosomal recessive hereditary disease, primarily affects non-Ashkenasi Jews, Armenians, Arabs and Turks. Very few data are available on the presence and genetic spectrum of FMF in Georgians. The purpose of our study was to find FMF cases in Georgians through genetic testing; to investigate distribution of MEFV gene mutations and to compare mutation distribution in Georgians with population at risk (Jews, Armenians, Arabs and Turks).

Material and methods: 320 patients from ethnical Georgians, with clinically suspected FMF, mean age 23.19 year (0–73 year), 171 male, 149 female, underwent molecular genetic studies using polymerase chain reaction. Genetic study was performed in Bernhard-Nocht-Institut for Tropical Medicine and in diagnosticum Zentrum für Humangenetik. We also registered clinical manifestations, severity of disease, treatment and its efficacy (using standardized questionnaire) and correlated them with mutation.

Results: MEFV gene mutations were found in 224 patients. The M694V Mutation was predominant. Distribution of mutations are: M694V - 149 (66.5%), M68OJ/M694V - 13(5,8%), M694V/R761H - 7(3,1%), M694V/E148Q - 6(2,7%), V726A/E167D-3(1,3%), other mutations - 46 (20,5%). 107 patients (47,7%) were Homozygous M694V/M694V, 80 (35,8%) - Heterozygous,37 (16.5%) - compound heterozygous for M694V and other mutation. Frequency of clinical symptoms: fever in 203 patients (90,6%), abdominal pain in 187(83,5%), arthralgia in 130 (58,1%). Renal function was deteriorated 22(9,8%) cases, 1 patient (0,5%) was on hemodialysis.

Conclusion: FMF is present in Georgians. Most frequent mutation is M694V. Distribution of mutations is more similar to north African Jews and differs from Armenians and Turks.

C.O.I. Disclosure:

T. Ramishvili: None. **I. Rtskhiladze:** None. **N. Abramishvili:** None. **D. Metreveli:** None. **C. Timmann:** None. **D. Steinberger:** None. **M. Barnova:** None. **T. Andriadze:** None. **T. Dzagania:** None. **N. Jashiashvili:** None. **N. Kankia:** None. **M. Aroshidze:** None. **L. Zangurashvili:** None. **T. Pirtskhalaishvili:** None. **E. Shatirishvili:** None. **A. Tavadze:** None.

EP10.069 Distinct pathophysiological mechanisms in primary microcephaly-causative CEP152 variants

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Background: Centrosomal protein 152 (CEP152) is a key regulator of centriole structure and function, essential for establishing cell

polarity during replication. Pathogenic variants in the CEP152 gene cause primary microcephaly (MCPH9), though the underlying pathophysiological mechanisms remain poorly understood. This study investigates the mechanisms underlying MCPH9-causative CEP152 variants: compound heterozygous variants p.W105* and p.K897*, and the homozygous variant p.Q32P.

Material and methods: In vitro cell biological and biochemical analyses were performed to characterize the variants. Additionally, two disease model mice, $Cep152^{W105*/K897*}$ and $Cep152^{Q32P/Q32P}$, were generated to assess the pathogenicity in vivo.

Results: In vitro analyses showed that the p.K897* variant exhibited diffuse cytoplasmic localization in neuronal cells, the p.W105* variant led to protein degradation, and the p.Q32P variant localized normally to the centrosome. Mechanistically, both the p.K897* and p.Q32P variants disrupted the centrosomal localization of Polo-like kinase 4 (PLK4), a critical CEP152-binding partner, through distinct pathways. In vivo, both model mice displayed microcephalic primordial dwarfism. $Cep152^{Q32P/Q32P}$ mice additionally exhibited brain malformations, including ventricular dilation and abnormal neuronal positioning, closely resembling patient phenotypes. Cellular analyses revealed increased aberrant cell division and heightened apoptosis susceptibility in both models, with more pronounced effects in $Cep152^{Q32P/Q32P}$ mice. Electrophysiological analyses further confirmed distinct neuronal impairments associated with each variant, correlating with the severity of clinical symptoms.

Conclusion: These findings demonstrate that CEP152 variants impair protein function through variant-specific mechanisms, leading to neurological abnormalities and MCPH9.

C.O.I. Disclosure:

K. Nagata: None. **N. Hamada:** None. **T. Nishijo:** None.

EP10.072 Beyond Intellectual Disability: Exploring Cognitive Variability in Genetic Syndromes

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Background: Neurodevelopmental disorders (NDDs) arise from diverse genetic alterations, leading to variable cognitive outcomes. While many conditions are associated with intellectual disability (ID), some patients exhibit preserved cognitive function despite genetic diagnoses linked to ID. This study explores the neuropsychological profiles of genetically diagnosed individuals without ID, assessing cognitive variability and potential discrepancies from reported phenotypes.

Material and methods: We assessed 355 patients referred to our genetics center for evaluation due to NDDs, using neuropsychological tests including full-scale IQ (FSIQ) and adaptive behavior evaluations. ID was defined according to DSM-5 criteria. Referrals were made to clarify intellectual status, guide educational planning, and inform clinical management. Among the 355 patients, 178 (50.1%) were diagnosed without ID, including 111 (62.4%) with a confirmed genetic diagnosis and 29 (16.3%) with negative genetic tests. These patients were analyzed to explore syndrome-specific cognitive profiles, compared with existing literature.

Results: Patients without ID displayed significant cognitive variability, with distinct patterns for different syndromes. For instance, 22q11.2 deletion syndrome showed preserved verbal abilities but executive function deficits, while Williams syndrome demonstrated strong verbal reasoning and significant visuospatial impairments. Unexpectedly, preserved cognitive functions were observed in some monogenic disorders, such as SYNGAP1 and *CRE*BBP gene mutations.

Conclusion: These findings highlight the complexity of genotype-phenotype correlations in NDDs, revealing preserved cognitive function in conditions typically linked to ID. This underscores the need for personalized cognitive and adaptive care approaches.

C.O.I. Disclosure:

C. Peduto: None.

EP10.070 Bi-allelic splice variant in *ERGIC2* gene is associated with syndromic intellectual disability, accompanied by facial and skeletal features

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Background: The coat protein complex II (COPII) sorts and transports proteins after translation from the endoplasmic reticulum (ER) to the Golgi apparatus. Here, we investigated the genetic basis of a consanguineous family with distinctive developmental delay and skeletal dysplasia associated with homozygote bi-allelic splice variant in *ERGIC2* gene encoding a conserved protein in COPII complex

Material and methods: Sanger, exome and genome sequencing were performed for sequence detection. Gene and protein expression were measured by real-time PCR, and Western blot. Cellular pathology was tested by viability test, Electron microscopy of patients' fibroblasts and fibroblasts transduced with lentivirus expressing mutant *ERGIC2*.

Results: NGS did not identify pathogenic/likely pathogenic variants in known disease-associated genes, rather we observed a shared homozygous c.572+5 G > A splice variant in *ERGIC2*. By RNA sequencing we confirmed aberrant splicing associated with this alteration, causing skipping of 5 base pairs (c.573_577delGG-CAA), but no differences on protein levels. Electron microscopy showed a significantly larger ER width in patient's fibroblasts compared to control fibroblasts. To validate disease-gene association, a transduction of lentivirus encoding GFP-ERGIC2 variant to control fibroblasts recapitulated ER morphology of patient's cells. Expression of ER stress genes was elevated in patients and in control fibroblasts transduced with mutant ERGIC2 compared to controls.

Conclusion: Our findings support *ERGIC2* as a novel cause of intellectual disability, skeletal dysplasia and distinctive dysmorphic features, characterized by cellular and molecular ER pathology. Our findings expand the family of COPII associated disorders.

C.O.I. Disclosure:

N. Danial-Farran: None. **Y. Tatour:** None. **F. Zagairy:** None. **M. Khayat:** None. **N. Warwar:** None. **C. Gafni-Amsalem:** None. **E. Chervinsky:** None. **E. Cohen Barak:** None. **R. Spiegel:** None. **W. Newman:** None. **S. A. Shalev:** None.

EP10.068 Genetic analysis of Cohen syndrome in Pakistani and Iranian families

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Background: Cohen syndrome (CS) (MIM# 216550) is a rare autosomal recessive disorder caused by loss-of-function (LoF) variants in *VPS13B*, leading to progressive retinal dystrophy, postnatal microcephaly, intellectual disability, hypotonia, joint hypermobility, and neutropenia. An estimated 50,000 patients exist worldwide, with most variants being truncating LoF; of 1217 reported in ClinVar, only six are missense. This study aimed to elucidate the genetic basis of CS in 6 families from Pakistan and Iran.

Methods: Whole-exome sequencing was performed on probands, followed by genotyping in additional family members to confirm co-segregation of variants with the disease. Clinical information was collected, and variants were classified according to ACMG criteria.

Results: In Family-1, one affected male carried compound heterozygous *VPS13B*(NM_017890.5) variants (c.10240_10282del,-p.Leu3414ValfsTer27 and c.10156dupA,p.Thr3386AsnfsTer3). Family-2 involved two affected males with a homozygous variant (c.6879delT,p.(Phe2293LeufsTer24)). In Family-3, one male and one female patient harbored a homozygous splice-site variant (c.11570+1G > A). Family-4 (from Iran) included a female proband and her male uncle homozygous for c.412+1G > T. In another Iranian family (Family-5), one male proband carried c.10360dupC,-p.(Leu3454ProfsTer7). And Family-6 presented a male and female with a homozygous c.10156dupA,p.(Thr3386AsnfsTer3). All affected individuals displayed classic CS features but varied in developmental milestones, cataract onset, and skeletal anomalies.

Conclusion: These findings broaden the known *VPS13B* mutation spectrum in CS, highlighting the recurrent c.10081dupA variant reported in 14 patients, highlighting its significance for future gene-editing strategies. Although *VPS13B* localizes to the Golgi apparatus and its loss causes Golgi fragmentation, further research is needed to elucidate the precise function of this bridge-like lipid transport protein.

C.O.I. Disclosure:

A. Iqbal: None. **A. Rashid:** None. **H. Frederiksen:** None. **S. Oreshkov:** None. **F. Vacca:** None. **H. Baig:** None. **M. Hussain:** None. **M. Iqbal:** None. **S. Ahmad:** None. **A. Ejaz:** None. **S. Khan:** None. **I. Anwar:** None. **M. Khorrami:** None. **M. Kheirollahi:** None. **S. Khan:** None. **S. Antonarakis:** None. **M. Iqbal:** None. **M. Ansar:** None.

EP10.071 Validation of a zebrafish model for studying molecular mechanisms in Xia-Gibbs syndrome

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Xia-Gibbs Syndrome (XGS) is a rare neurodevelopmental disorder caused by heterozygous pathogenic variants in the *AHDC1* gene. Patients exhibit intellectual disability, developmental delay, speech impairment, and hypotonia. Despite the identification of *AHDC1* as the causative gene, the molecular mechanisms underlying XGS remain poorly understood.

Materials and methods: We developed a zebrafish model (*ahdc1*^{dsao1}) using CRISPR-Cas9. To begin characterizing this model, the following assays were performed: (1) controlled crossings followed by genotyping of the offspring using Sanger sequencing; (2) RT-qPCR to evaluate the *ahdc1* expression in whole larvae and isolated brain tissue samples from the three genotypes (wild-type, heterozygous, and homozygous for the *ahdc1* induced mutation); (3) time-lapse imaging to compare morphological development between the three genotypes during the first 96 hours post-fertilization.

Results: Heterozygous and homozygous are viable and fertile, and the offspring follow Mendelian genotypic ratios. Homozygous *ahdc1* mutant larvae exhibited delayed body and retinal pigmentation in early development, with partial recovery at later stages, while heterozygous larvae displayed intermediate pigmentation phenotypes. Additionally, we detected reduced *ahdc1* expression in the brains of mutant animals. Our results suggest potential defects in neural crest cell migration in XGS.

C.O.I. Disclosure:

D. Fialho: None. **S. de Oliveira Farias:** None. **S. Ferreira Pires:** None. **A. Natucci Mergulhão:** None. **C. Rosenberg:** None. **L. Machado Lara Carvalho:** None.

EP11.098 Identification of novel mTOR pathway variants in focal cortical dysplasia through ultra deep sequencing

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Background: Focal cortical dysplasia (FCD) is a major cause of drug-resistant epilepsy. Recent molecular studies have demonstrated the pathogenic role of somatic variants in mTOR pathway genes, with particular emphasis on the *MTOR* gene. Despite extensive investigation, the complete mutational landscape and the spectrum of pathogenic variants in FCD remain to be fully characterized.

Material and methods: We employed ultra-deep targeted sequencing utilizing a custom-designed panel on surgically resected brain tissue specimens and matched peripheral blood samples from 38 FCD patients. Somatic variant calling was performed using Mutect2 pipeline. Validation of the somatic variants identified was performed using custom-designed deletion-specific PCR primers followed by sanger sequencing.

Results: Our molecular analysis identified pathogenic somatic variants in *MTOR*, *RHEB*, *NAV2* and other genes in 21/38 cases (55.2% diagnostic yield). Notable findings included a novel in-

frame 12-base pair deletion in *MTOR* (p.Lys1452_Glu1455del) and three previously unreported missense variants, all predicted to be deleterious by multiple computational algorithms.

Conclusion: This comprehensive molecular analysis substantially expands the known mutational spectrum in FCD including extended gene associations. The identification of a previously unreported 12bp deletion in *MTOR*, along with novel missense variants, enhances our understanding of FCD pathogenesis. The validation strategy using deletion-specific primers provides a reliable method for detecting low-level mosaic variants. These findings have significant implications for both molecular diagnosis and the potential development of targeted therapeutic interventions in FCD.

C.O.I. Disclosure:

R. Sukrutha: None. **G. Udupi:** None. **A. Kotambail:** None. **R. M b:** None. **D. M:** None. **C. G k:** None. **S. Sinha:** None. **A. Mahadevan:** None. **A. A:** None. **R. C m:** None.

EP11.097 Unravelling the role of Tandem Repeats (TR) in regulatory regions in Autism Spectrum Disorders (ASD)

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Background: It is widely known that Autism Spectrum Disorders (ASD) have a very important genetic component (83% of heritability), being both de novo and inherited mutations, a major component having a role in autism. Tandem repeats (TR), from 1 to 20 pb motifs repeated sequentially, are one of the major sources of de novo mutations in humans.

Material and methods: In order to study for the first time the regulatory role of TR in ASD, we have carried out a targeted sequencing study of the non-coding genome. 926,535 human cCREs (cis-regulatory elements) from the ENCODE project in 200 ASD Spanish trios were selected and sequenced. Then, de novo TRs were detected using a specific bioinformatic pipeline adapted from Mitra et al (Nature, 2021).

Results: A total of 102 de novo TR mutations were found in 54 different cCREs, in 83 probands. Of these, 10 were related to a known gene described in SFARI using T-gene. We applied SISTR to evaluate whether the mutated loci were under selection, finding three TR mutations linked to a SFARI gene in the top 25% of pathogenicity scores (*CASZ1* and *NFE2L3*), and genes studied in ASD and NDDs (*NR4A1* and *GRASP*) in the top 10% of pathogenicity scores.

Conclusion: These findings underline the importance of de novo TR in regulatory regions as possible contributors to the etiology of autism.

C.O.I. Disclosure:

P. Carballo-Pacoret: None. **S. Dominguez-Alonso:** None. **J. González-Peñas:** None. **C. Arango:** None. **M. Parellada:** None. **Á. Carracedo:** None. **C. Rodriguez-Fontenla:** None.

EP11.099 Additions to the phenotypic spectrum of *CHD8*-associated form of syndromic autism spectrum disorder

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Background: *CHD8* encodes a chromodomain-helicase-DNA-binding protein that negatively regulates the Wnt-β-catenin signaling pathway, affecting neurogenesis. Dysfunction of *CHD8* is linked to one of the most frequent forms of syndromic autism - characterized by variable degrees of intellectual disability, epilepsy, movement disorders, and overgrowth.

Patients and methods: 11 patients (4–17 y/o, 2 girls, 9 boys) underwent whole genome sequencing and found to have likely causative variants in *CHD8* (5 - likely pathogenic variants, 6 - VUS as sole relevant findings - functional analysis underway). All underwent deep phenotyping.

Results: All presented with autism and had developmental delay (4 went on not to have intellectual disability, though 1 has low normal intelligence), 5 have epilepsy, 8 - movement disorders, 8 - overgrowth, 2 have structural brain abnormalities. Other notable features: signs of a connective tissue disorder (7 patients), abnormal vision (3), hepatomegaly (4) and hyperbilirubinemia (2) (familial hyperbilirubinemia was ruled out). Additionally, 4 patients have cardiovascular disorders (atypical for *CHD8*-associated autism): Wolff-Parkinson-White syndrome (surgically corrected) (1), repolarization disorder (2), sinus arrythmia - bradyarrhythmia in one and tachyarrhythmia in another. One of them also presented with left ventricle excessive trabecularity, local contractility impairment of the left ventricular myocardium and structural anomalies of the mitral, tricuspid and pulmonary valves.

Conclusion: Patients with *CHD8*-associated autism present with a range of clinical features, including those with an unclear pathogenesis - cardiac abnormalities, hepatomegaly, and hyperbilirubinemia - the underlying mechanisms of which require further research to improve understanding and management of this condition.

C.O.I. Disclosure:

M. Parfenenko: None. **I. Dantsev:** None. **O. Groznova:** None. **V. Voinova:** None.

EP13.099 Phenotypic overlap of Lymphatic malformation and Capillary malformation-arteriovenous malformation in a family with *EPHB4* variant

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Background: Monoallelic *EPHB4* variants have been reported in two distinct phenotypes: Capillary malformation-arteriovenous malformation 2 (CMAVM2) (OMIM 618196) and Lymphatic malformation 7 (LMPHM7) (OMIM 617300). We present a family with phenotypic overlap of CMAVM2 and LMPHM7.

Material and methods: The proband presented with antenatal pleural effusion requiring iterative thoraco-amniotic shunting. At birth, he required intensive care for chylothorax. Abdominal and brain imaging reveled hepatic and cerebral arteriovenous malformations. He died at 67 days of age. His older brother had prenatal hydrops spontaneously regressive with persistence of pleural effusion. He died at 52 days.

Results: Prenatal whole-exome sequencing identified the heterozygous variant NM_004444.5: c.2354G>A, p.(Arg785Gln) in *EPHB4* in the two brothers and their mother. This variant was previously reported de novo in a patient with prenatal manifestation of LMPHM7 and was therefore considered as responsible for the LMPHM7 phenotype. Arteriovenous malformations in the proband suggested an overlap with the CMAVM2 phenotype. Furthermore, careful examination of the mother revealed cutaneous signs of CMAVM2. The *EPHB4* variant was inherited from the grandmother who reported varicose veins and lower limb edema since adolescence. Mother and grandmother both had incidental discovery of ascending aorta dilatation.

Conclusion: The pathophysiology leading to LMPHM7 or CMAVM2 is not elucidated. Cases with overlapping phenotypes are very rare. *EPHB4* has until now not been implicated in aortic dilation. Our observation reinforces the hypothesis of an overlap between the two phenotypes linked to *EPHB4*, suggests an expanding phenotype and highlights the complexity of management and genetic counseling for these disorders.

C.O.I. Disclosure:

S. Grotto: None. **J. De Sainte Agathe:** None. **B. Keren:** None. **P. Maurice:** None. **J. Guilbert:** None. **P. Charron:** None. **A. Palmyre:** None. **D. Héron:** None. **F. Coulet:** None.

EP14.026 International nomenclature for genomics

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Background: The Standing Committee for International Human Cytogenomic Nomenclature (ISCN) is responsible for reviewing the nomenclature. With the advent of new technologies and deepening understanding of the nature of genomic aberrations and/or pathogenic variants, there is an increasing need for an international nomenclature to report complex findings. ISCN provides a standard approach to describe any genomic rearrangement identified by karyotyping, FISH, microarray, genomic mapping, various region-specific assays, and DNA sequencing. ISCN also incorporates, as applicable, whether the abnormality is inherited or de novo, the proportion of the sample with the aberration and the variant allele frequency (VAF).

Material and methods: The Committee considered feedback from the genomics community to provide a more user friendly and organised presentation of general rules, improved example descriptions and additional examples. A key achievement is the provision of standardised nomenclature to ensure consistency in the ISCN description of findings irrespective of the technology used.

Results: ISCN 2024 represents one of the most significant reviews. The main changes in the ISCN 2024 compared to previous editions will be presented and includes two new chapters: generic ISCN rules and genome mapping. The region-specific chapter is also expanded with nomenclature for targeted karyotyping, targeted arrays and imprinted genes.

Conclusion: The streamlining of the flow of information will enhance search capabilities of the nomenclature. The nomenclature is standardized across different genomic applications and technologies to minimise ambiguity, misinterpretation and provide consistency of the ISCN description. Online ISCN educational and competency tools available through the ISCN Forum and GenQA will also be presented.

C.O.I. Disclosure:

R. Hastings: None. **S. Moore:** None. **N. Chia:** None.

EP14.027 Short-read genome sequencing for structural variant detection in the era of genomic medicine

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Background: Structural variants (SVs) are genomic alterations greater than 50 base pairs that include deletions, duplications, inversions, insertions, and translocations. The identification of these anomalies has historically relied on cytogenetic techniques such as karyotyping and fluorescence in situ hybridization, later complemented by microarray, which improved diagnostic yield by 5 to 15%. Genome sequencing has emerged as a powerful alternative, particularly within the framework of the Plan France Médecine Génomique 2025.

Material and methods: We retrospectively analyzed 850 patients using short-read genome sequencing (srWGS) at the SeqOIA laboratory. The cohort included individuals with intellectual disability, malformation syndromes, and autism spectrum disorder/neurodevelopmental disorders. We assessed the ability of genome sequencing to detect a broad spectrum of SVs, including rearrangements below microarray detection thresholds, and evaluated its capacity to characterize complex genomic architecture.

Results: Genome sequencing enabled the detection of a diverse range of structural variants, including translocations, inversions, ring chromosomes, and novel recurrent chromosomal patterns. It provided a refined characterization of complex genomic rearrangements (CGRs) and revealed gene-level structural anomalies that were undetectable by conventional techniques. Despite its advantages, short-read sequencing has limitations in detecting CGRs. Additional studies using optical genome mapping are necessary for a comprehensive characterization of CGRs, while RNA sequencing will be required to investigate the functional impact of intronic copy number variations.

Conclusion: srWGS enhances the detection of structural variants and improves the characterization of chromosomal rearrangements. However, a multimodal genomic approach integrating optical genome mapping and RNA sequencing is essential to fully resolve CGRs and optimize molecular diagnosis.

C.O.I. Disclosure:

J. Levy: None. **X. Latypova:** None. **S. Drunat:** None. **N. Couque:** None. **Y. Capri:** None. **Y. Vial:** None. **D. Germanaud:** None. **L.**

Perrin: None. **C. Collet:** None. **A. Pouzet:** None. **L. Ruaud:** None. **A. Maruani:** None. **P. Blanc:** None. **C. Mignot:** None. **M. Heulin:** None. **A. Delahaye-Duriez:** None. **C. Dupont:** None. **B. Chaumette:** None. **A. Verloes:** None. **A. Tabet:** None.

EP15.129 Profiling known clinical short tandem repeats from exomes of Middle Easterns highlights importance of underrepresented populations in genome studies

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Background: Short tandem repeats (STRs) are prone to variation, and novel disease-causing STRs are being uncovered continuously. However, less is known about frequency of expansions of clinical STRs in underrepresented populations, mainly non-Europeans. We aimed to profile known disease-causing STRs in our Middle Eastern cohort, providing a deeper perspective on human genome structure.

Material and methods: Exome BAMs of 2,063 Middle Eastern individuals were taken as input to ExpansionHunter. Assembly hg38 and a catalogue of 20 clinical STRs were utilised to measure repeat lengths. Our cohort includes both control and patients, basically neurodevelopmental and neurological disorders.

Results: STR loci *ATXN1*, *HTT*, and *TBP* showing multiple hits (20, 9, and 6 individuals, respectively) after filtering for expansions greater than normal thresholds. 8 of them are exome negative patients harbouring expansions exceeding the pathogenic thresholds. Whereas, distribution of other expansions shows that *ZFHX3* and *GLS* loci in our cohort, in contrast to Europeans, have stable repeat sizes without any patients diagnosed with their over-expansions.

Conclusion: Detected in 1.7% of individuals, premutation and pathogenic expansions of *ATXN1*, *HTT*, and *TBP* are prevalent in our cohort. Considering these loci in Middle Eastern cases with genetic anticipation would be beneficial. Differences in STR profiles between the present cohort and Europeans triggering the hypothesis that there might be unknown clinical STRs which are more variable in underrepresented populations, but they are neglected due to concentrated genomic studies on European populations. Thus, a population wide screening of potential STRs to find novel phenotype correlations would be advantageous.

C.O.I. Disclosure:

S. Alavi: None. **R. Maroofian:** None. **Z. Hojati:** None. **H. Houlden:** None.

EP15.124 Further delineation of defects in *MRPS2* causing human OXPHOS deficiency and early developmental abnormalities in zebrafish

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Background: Mitochondrial ribosomal protein-small 2, MRPS2 encodes a structural protein essential for assembling mitoribosomal small subunit and thus mitochondrial translation. Bi-allelic variants in MRPS2 have been recently implicated in four families with combined oxidative phosphorylation deficiency-36 (MIM#617950).

Material and methods: Singleton exome sequencing (ES) was performed for two unrelated probands, P1 and P2, followed by validation and segregation by Sanger sequencing. Fibroblasts were obtained from P1 for transcript analysis, protein expression analysis and assays for mitochondrial function. A zebrafish model was used to investigate the phenotypic and functional consequences of MRPS2 loss.

Results: The probands presented with variable phenotypes of acute onset severe metabolic decompensation and symptomatic hypoglycemia. ES identified biallelic variants in MRPS2 (NM_016034.5) in the affected individuals: P1: c.490G>A p.(Glu164Lys); and P2:c.413G>A p.(Arg138His). Parents were heterozygous carriers. Further evaluation of the novel variant c.490G>A p.(Glu164Lys) in patient-derived skin fibroblasts revealed decreased expression of MRPS2 transcript and protein levels of MRPS2 and several subunits of complex I and IV. Global proteomic analysis revealed decreased expression of all small subunit proteins and increased expression of large subunit proteins of mitoribosomes. Also, reduced complex I and IV enzyme activities, mitochondrial respiration (OCR), and altered mitochondrial morphology on confocal imaging were observed. Additionally, Mrps2 knockout zebrafish larvae demonstrated an abnormal developmental phenotype, reduced expression of OXPHOS subunit genes and Complex IV activity.

Conclusion: With these findings, we identify additional families with variants in MRPS2, illustrate their clinical and genomic spectrum and validate the pathogenicity of defects in MRPS2 through in-vitro and in-vivo assays.

C.O.I. Disclosure:

A. Anand: None. **A. Kandettu:** None. **M. Yeole:** None. **H. Sekar:** None. **K. Garapati:** None. **N. Kaur:** None. **P. Hegde:** None. **K. Nair:** None. **R. Medishetti:** None. **V. Bhat:** None. **R. Periyasamy:** None. **S.C. Mundkur:** None. **S.A. Hebbar:** None. **A. Pandey:** None. **A. Sevilimedu:** None. **S. Chakrabarty:** None. **A. Shukla:** None.

EP15.130 Evaluation of a complex*MTMR2*variant (all introns deletion-all exons duplication) in a cohort of 1408 patients affected by neurological disorders

Rosangela Ferese[1], *Federica Sammarone*[1], *Rosa Campopiano*[1], *Maria Antonietta Chiaravalloti*[1], *Anna Maria Griguoli*[1], *Simona Scala*[1], *Gabriele Macari*[2], *Marianna Storto*[1], *Alessia Lombardi*[1], *Luana Di Pilla*[1], *Cristina Peconi*[3], *Fabio Buttari*[1], *Emiliano Giardina*[3], *Srefano Gambardella*[1,4]

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Background: *MTMR2* is a gene responsible for AR CMT4B1, a sensorimotor demyelinating neuropathy characterized by weakness, distal muscle atrophy, sensory loss and frequent cavus foot. We identified a genomic variant composed of a duplication of all 15 exons and a deletion of all 16 introns in *MTMR2*. Segregation analysis indicated the in-cis presence of deletion and duplication, suggesting the possibility of an extra copy of coding regions of

MTMR2.In this study, we evaluated the frequencies of this variation to understand its possible involvement in neurological diseases.

Methods: We selected 1408 patients affected by neurodegenerative disease (97 CMT, 231 AD, 115 PD,193 FTD, 103 MS, 95 SP, 54 ATX, 88 ALS, 79 CADASIL and 351 Complex Phenotypes) and 312 subjects without neurological signs, older than 75 yo. NGS sequencing, MLPA and gel electrophoresis were applied.

Results: The genetic analysis identified the heterozygote del/dup in *MTMR2 in* 17 patients and no variant was detected in controls (0.99% of patients, $p = 0.0175$).Among patients, the variant has been identified in 1 AD, 1 SP, 3 Ataxias, 3 FTD, 1 CMT, 3 ALS, 1 PD and 4 CADASIL.

Conclusions: The present study evaluated the possibility of involvement of the del/dup variant in neurological disorders. Frequencies seem to suggest a positive correlation between the variant and the susceptibility of some neurodegenerative phenotypes. These data need to be confirmed in larger populations. Studies are in progress to confirm the hypothesis that this del/dup is a retrotransposon made up of all exons of MTMR2, and ongoing cytogenetic analysis will try to evaluate the precise genomic location.

C.O.I. Disclosure:

R. Ferese: None. **F. Sammarone:** None. **R. Campopiano:** None. **M. Chiaravalloti:** None. **A. Griguoli:** None. **S. Scala:** None. **G. Macari:** None. **M. Storto:** None. **A. Lombardi:** None. **L. Di Pilla:** None. **C. Peconi:** None. **F. Buttari:** None. **E. Giardina:** None. **S. Gambardella:** None.

EP15.131 Evaluation of GAA repeat expansion in FGF14 (SCA27b) in 200 Italian patients affected by adult-onset cerebellar ataxia

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Background: Spinocerebellar ataxias (SCAs) are a heterogeneous group of progressive neurological disorders that are estimated to affect 1:33,000 individuals. SCAs are caused by pathogenic expansions of short tandem repeats (STRs, also called microsatellites) and deleterious point mutations. SCA27b, caused by an intronic (GAA) repeat expansion in fibroblast growth factor 14 (*FGF14*), is considered the primary cause of adult-onset cerebellar ataxias.

Methods: We evaluated the expansion in *FGF14* gene by capillary electrophoresis and agarose gel electrophoresis in a cohort of 200 patients from sud-central Italy, affected by adult-onset slowly progressive cerebellar ataxia with variable features. In addition, 70 subjects without neurological signs, older than 75 yo, were evaluated.

Results: Genetic analysis identified 8 patients (4%) with (GAA) > 300 (considered as disease-causing and fully penetrant). In one patient, segregation was carried out in two affected family members, and the full penetrance of these alleles was confirmed.- Three patients (1,50%) have a (GAA) > 250 (considered pathogenic with reduced penetrance). All control subjects have (GAA) < 200.

Conclusions: This study identified alleles with (GAA) > 250 in 5,5% of patients. These data need to be replied to more extensive population studies, and the evaluation of GAA repeat expansion in *FGF14* needs to be considered in the genetic routine for adult-onset cerebellar ataxia.

C.O.I. Disclosure:

F. Sammarone: None. **A. Griguoli:** None. **R. Campopiano:** None. **M. Chiaravalloti:** None. **S. Scala:** None. **M. Storto:** None. **A. Lombardi:** None. **L. Di Pilla:** None. **G. Macari:** None. **F. Buttari:** None. **M. Fanelli:** None. **S. Gambardella:** None. **R. Ferese:** None.

EP15.125 Identification of genetic variants in Thai patients withdisorders ofsexdifferentiation byshort-readgenomicsequencing

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Background: Disorders of Sexual Differentiation (DSD) encompass a heterogeneous group of conditions characterized by atypical development of the genitalia. Given the phenotypic variability of DSD, molecular diagnosis is essential for accurate classification and management. While targeted gene panels and exome sequencing (ES) are widely employed, the contribution of non-coding variants and structural variants to disease pathogenesis necessitates an evaluation of genome sequencing (GS) as a diagnostic tool.

Materials and methods: Patients diagnosed with DSD at King Chulalongkorn Memorial Hospital between 2020 and 2024 were recruited. After excluding cases with chromosomal abnormalities, short-read GS was performed to identify potential pathogenic variants.

Results: A total of 18 unrelated patients were included, with a median age at testing of 4.4 years. Among them, 15 (83.3%) had a 46,XY karyotype. GS identified six (likely) pathogenic variants in four genes—DYRK1A, NR2F2, SRD5A2 (three variants), and WFDC8—in six patients (33%). Additionally, nine variants of uncertain significance (VUS), including three in non-coding regions, were identified in seven genes—AR(two variants), CAMK2B, CREBBP, POU3F3, SETBP1, SGMS2, and SRD5A2 (two variants)—among the remaining 12 unresolved cases.

Conclusion: GS has the potential to enhance the diagnostic yield in patients with DSD, particularly in those harboring pathogenic variants in non-coding regions. These findings underscore the value of comprehensive genomic approaches in elucidating the genetic etiology of DSD, thereby facilitating improved diagnosis and management.

C.O.I. Disclosure:

T. Sathavornmanee: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Thailand's Health System Research Institute (HSRI 67-095). **W. Kamolvisit:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Thailand's Health System Research Institute (HSRI 67-095). **V. Shotlersuk:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Thailand's Health System Research Institute (HSRI 67-095).

EP15.126 Diagnosis of Rare Short Stature Disorders Using Genomics in Qatar

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Background: Short stature, defined as a height that is 2 standard deviations or more below the mean height for a given age, sex, or ethnic group, can arise from a range of underlying causes. These include endocrine disorders, rare genetic syndromes, and skeletal disorders. In Qatar, many children are referred to endocrine clinics for short stature, but the genetic etiology is not known.

Objective: This study aimed to investigate the molecular and genetic basis of short stature through whole genome sequencing (WGS).

Methods: WGS was performed on eight patients with short stature and their families (total = 26). Variant filtration was applied based on family pedigree, mendelian inheritance pattern, allele frequencies, location and quality using SeqR platform (v1.0) to identify variants from a manually curated list of over 800 genes known to be associated with short stature-related syndromes. Molecular docking using HADDOCK (v3.0) and simulation was additionally used to explore the functional impact of the variants.

Results: Genetic analysis helped the diagnosis of 6 out of 8 (75%) cases with variants in the following genes: *IGF1R* missense variant (n = 1), associated with short stature and growth failure; pathogenic *SHOX* variant (n = 3) linked to SHOX-related short stature; *GLI2* missense de novo variant (n = 1) causing Culler Jones syndrome; and *FLRT3* (n = 1) missense variant linked to hypogonadotropic hypogonadism. These findings highlight the genetic heterogeneity underlying short stature in this cohort.

Conclusion: Identifying genetic variants in patients with short stature allows for more accurate diagnosis, genetic counseling, and possible treatment options.

C.O.I. Disclosure:

F.K. Mohammed: None. **U. Ismail Umlai:** None. **Z. Jan:** None. **D. Velayutham:** None. **N. Hamed:** None. **H. Issa Dauleh:** None. **K. Hussain:** None. **P. Jithesh:** None.

EP15.127 Clinical and Genetic Insights From *MECP2* Variants

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Background: Childhood developmental delay and intellectual disability are common developmental disorders with high genetic and clinical heterogeneity. Over 1,500 genes are linked to the disorders. This study aimed to delineate the mutation spectrum of *MECP2* and provide a basis for improved clinical management and genetic counseling.

Material and methods: Singleton or trio-based whole exome sequencing was performed on the patients. Single-nucleotide variants (SNVs), insertions/deletions (indels) and copy number variants (CNVs) were concurrently analyzed and interpreted according to American College of Medical Genetics guideline. The variants were confirmed by Sanger sequencing, QPCR or MLPA accordingly. The clinical presentation and family history were collected.

Results: A total of 43 Chinese patients were identified, with 29 being female. Speech delay was most common, followed by motor delay, epilepsy, intellectual disability, autism, and facial dysmorphism. Besides, missense variants were most frequently identified, and then frameshift variants, nonsense variants, and CNVs. It was worth noting that intragenic CNVs were identified in three patients, including two boys with de novo deletions in exons 1–2 who exhibited severe clinical phenotypes. Rett syndrome predominantly affected females, mostly caused by de novo variants in exon 4. Missense variants were predominantly located in the methyl-CpG DNA-binding domain, with p.Arg133Cys and p.Thr158Met as most frequent. In contrast, truncating variants were concentrated in the transcriptional repression domain, with p.Gly269Alafs*20 and p.Arg255* being most common. Moreover, 11 novel variants were identified.

Conclusion: Our findings expanded the mutation spectrum of *MECP2*, providing valuable insights for patient care and reproductive planning.

C.O.I. Disclosure:

J.X. Tang: None. **N.S. Wu:** None.

EP15.128 The understanding the role of Mitochondria in Cilia and Ciliopathy

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Background: Cilia are not ubiquitous cellular organelles, but they are essential for human health due to their roles in developmental signaling, motility, and sensory functions. Although mitochondria are not found inside cilia, their energy production is likely crucial for ciliogenesis and intraflagellar transport (IFT) system, given the high ATP demand of IFT and ciliary motility. Powered by ATP, kinesin- and dynein-driven ciliary transport is essential for ciliogenesis. Both ciliopathies and mitochondrial disorders can lead to similar organ dysfunctions, like retinal dystrophy, cerebellar dysfunction, liver dysfunction, and cardiomyopathy. Mitochondrial dysfunction may exacerbate ciliopathies by disrupting the cilia-mitochondria balance, thereby affecting structure, function, and disease progression.

Material and methods: To study the relationship between mitochondria and cilia, we monitor kinesin and dynein-driven intraflagellar transport (IFT) dynamics using fluorescent microscopy upon ATP depletion. We took time lapse movies following ATP depleted and non-depleted C.elegans, followed by kymograph analysis. We also obtained mutants that display mitochondrial dysfunctions. We are about to recruit patients with mitochondrial disease following ethical board approval.

Results: Our preliminary findings strongly indicate a critical link between ATP levels, provided by mitochondria, and the proper functioning of the IFT system. The observed accumulation of IFT components at the ciliary tip upon ATP depletion suggests a disruption of the transport machinery, potentially impacting ciliogenesis and ciliary function.

Conclusion: Further work, including the analysis of mitochondrial dysfunction mutants in C. elegans and patient samples, will be crucial to elucidate the complex interplay between mitochondrial health and ciliary integrity, and to understand the implications for ciliopathies.

C.O.I. Disclosure:

F. Güzel: None. **G. Yilmaz:** None. **S. Cevik:** None. **S. Öner:** None. **A. Sezer:** None. **O. Kaplan:** None.

EP17.036 Advanced predictive healthcare through novel deep learning models using predict the genetic disorders dataset

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Background: Human genetics has seen remarkable progress, with machine learning (ML) and deep learning (DL) revolutionizing predictive healthcare. Despite these advancements, the application of ML/DL for extracting actionable insights from genomic datasets remains underdeveloped, particularly in rare disease diagnosis and personalized medicine. Utilizing open-access datasets like the Predict the Genetic Disorders Dataset from Kaggle presents an opportunity to develop computational models that address clinical and research challenges in human genetics.

Material and methods: This study used the Predict the Genetic Disorders Dataset from Kaggle, which includes genomic variations, patient demographics, phenotypic annotations, and health outcomes. Data preprocessing involved cleaning, normalization, and feature selection to ensure high-quality input for model training. Exploratory analysis was conducted using statistical and visual techniques to identify patterns. Model development incorporated deep learning architectures such as Convolutional Neural Networks (CNNs) and Long Short-Term Memory (LSTM) networks alongside ensemble ML methods like Gradient Boosting Machines. Cross-validation and external datasets ensured generalizability. Model interpretability was enhanced using SHAP (SHapley Additive exPlanations) and LIME (Local Interpretable Model-agnostic Explanations).

Results: Our CNN-LSTM hybrid model achieved 95% accuracy and 0.92 F1-score in predicting rare genetic disorders. Ensemble ML methods identified novel genomic markers associated with disease susceptibility, validated through ROC-AUC scores exceeding 0.90. A DL framework for personalized medicine reduced treatment prediction error rates by 15% compared to baseline models.

Conclusion: This study highlights the potential of ML/DL in genomic healthcare, improving disease prediction and biomarker identification. Future work will integrate multi-omics data and expand datasets for large-scale population genetics research.

C.O.I. Disclosure:

F. Veisi: None.

EP18.028 Multi-ancestry variance quantitive trait loci inquiry into the human proteome

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Background: Variance quantitative trait loci (vQTLs) influence trait variance in complex traits and can serve as proxies for gene-environment interactions. Their contribution towards the genetic architecture of complex traits has only recently been explored, albeit not exhaustively. Here, we investigate their role in the circulating human proteome across diverse ancestries.

Material and methods: We mapped vQTLs for 2,826 proteins in the UK Biobank (UKB) using the median-based Levene's test (for homogeneity of variances), implemented in OSCA. We identified population-specific and shared cis-acting protein vQTLs in unrelated participants of European (EUR; N = 45,486), African (AFR; N = 1,336), and South-Asian (SAS; N = 933) ancestries. Finally, we assessed whether protein vQTLs independently influence coronary artery disease (CAD) and Type 2 diabetes (T2D) risk using cis-multivariable MR (MVMR) implemented in Robust-PCGMM.

Results: We identified 2,042 independent cis-acting vQTLs (PEUR = 6.2E-11, Pnon-EUR = 5.0E-08), including EUR (1,885; 748 proteins), AFR (98; 83 proteins), and SAS (59; 53 proteins). Among these, 4.7%, 21%, and 6%, respectively, exhibited vQTL effects exclusively ($p > 0.05$ for main effects), with 29 proteins sharing cis-vQTLs across all ancestries. In the EUR, we identified 530 proteins with distinct causal variants for main and variance effects (colocalization H3). MVMR revealed that trait variance influenced CAD and T2D risk independently of main effects for some key proteins: LPA, PCSK9, VAMP5, and FN1 (CAD) and ANGPTL4 (T2D) ($p < 1E-03$, both effects).

Conclusion: Our findings provide insights into how vQTLs shape the genetic architecture of complex traits, highlighting trait variance as an independent causal risk factor for certain disease outcomes.

C.O.I. Disclosure:

C.R. Ben-Eghan: None. **S. Ritchie:** None. **E. Persyn:** None. **C. Foguet:** None. **S. Burgess:** None. **M. Inouye:** None.

EP18.029 Genetic Risk and Polygenic Risk Score assessment of Prediabetes and Progression to Type 2 Diabetes

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Background: Prediabetes, predisposes individuals to diabetes and heightened risks of vascular complications. Genetics offers unique potential for identifying long-term risk of progressing to T2D since birth. However, genetic studies focusing on prediabetes and its progression to T2D are limited.

Material and methods: A case-control GWAS was conducted using Qatar Biobank (QBB) participants with prediabetes ($n = 2{,}267$) and normoglycemia ($n = 8{,}665$). 142 previously derived T2D polygenic risk scores (PGSs) were evaluated for association with prediabetes and for predicting progression to T2D using logistic regression and cox regression analyses, respectively. All analyses were adjusted for age, gender, BMI and genetic principal components.

Results: GWAS identified two loci (7p13 and 10q22.1) associated with prediabetes ($P < 5 \times 10^{-8}$), mapping near *GCK* and *HK1*genes, respectively. Among 142 PGSs, PGS004838 showed the strongest association with prediabetes (OR/SD: 1.37, 95% CI: 1.29–1.45, *P*-value: $< 2.0 \times 10^{-16}$). Among, 2,143 individuals free of diabetes at baseline, 9.3% progressed to T2D during the 6.0 years of median follow-up. PGS004838 outperformed the other 141 PGSs in predicting progression to T2D (HR/SD: 1.79, 95% CI: 1.53–2.10, *P*-value: 2.08×10^{-13}), the strongest association reported to date. Individuals in the very high genetic risk quintile were younger and had a 2.4-fold increased risk of progressing to T2D compared to the intermediate quintile.

Conclusion: This study identified two genetic loci associated with prediabetes and reported the strongest clinical utility of PGS in predicting prediabetes and progression to T2D.

C.O.I. Disclosure:

U. Aliyu: None. **U. Ismail Umlai:** None. **N. M. Al-Thani:** None. **S. M. Toor:** None. **A. Abou−Samra:** None. **O. M. E. Albagha:** None.

EP18.026 Transcriptome Wide Association Study identifies *ADTRP* as a novel candidate gene for late rectal bleeding after radiotherapy in prostate cancer

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Background: Late rectal bleeding is one of the most concerning side effects of prostate cancer radiotherapy, greatly impacting patients' quality-of-life. Genome-wide association studies(GWAS) have identified biomarkers associated with radiotherapy side effects. However, most are located in non-coding regions, making the results difficult to interpret. Transcriptome-wide association studies(TWAS) address this by integrating genomic and transcriptomic data to estimate associations between genetically regulated gene expression and traits of interest. We aimed to identify candidate genes associated with late rectal bleeding in prostate cancer patients treated with radiotherapy (RT) using a TWAS approach.

Material and methods: The TWAS was performed by integrating genomic data (GWAS) from the RADIOGEN-prostate cohort with transcriptomic expression prediction models from GTEx v8 (predictDB).The GWAS included 140 patients (47 patients with rectal bleeding grade CTCAE ≥ 2 and 93 without CTCAE $= 0$), with age-at-radiotherapy and rectum-volume-65 as covariates. Single-tissue-TWAS analyses were performed for six tissues (sigmoid_colon, small_intestine_ileum, testicles, visceral_adipose_omentum, prostate, and whole_blood), followed by a multi-tissue analysis across these tissues. Bonferroni correction was applied(3.5×10^{-06}).

Results: Nine significantly associated genes were identified. Notably, *ADTRP*, which regulates the expression of the tissue factor pathway inhibitor (TFPI), a key coagulation inhibitor and vascular homeostasis modulator, exhibits a compelling mechanism that may influence rectal bleeding risk in RT-treated patients.

Conclusion: The identification of *ADTRP* and its role in coagulation offers a promising target for future research aimed at reducing RT-induced rectal bleeding. Extending TWAS approach to radiogenomics appears to be a promising strategy for identifying novel genes associated with toxicity, advancing personalized approaches to cancer therapy.

C.O.I. Disclosure:

O. Fuentes-Ríos: None. **M. Aguado-Barrera:** None. **C. López-Pleguezuelos:** None. **J. Galego Carro:** None. **A. Gómez-Caamaño:** None. **A. Vega-Gliemmo:** None.

EP18.027 The metagenomic medicine and the silent symphony of microbes toward human wellbeing

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Background: Metagenomic medicine is an emerging paradigm that leverages the analysis of the human microbiome to transform the prevention, diagnosis, and treatment of diseases. Unlike traditional medicine, which focuses on specific pathogens, metagenomic medicine aims to understand the broader interactions between the human host and its microbiota. This review explores the latest advancements in metagenomic medicine. The objective is to assess how metagenomic technologies, such as whole-metagenome sequencing, contribute to understanding human health and disease within a personalized, network-based framework.

Material and methods: A comprehensive literature review was conducted, analyzing recent studies and advancements in metagenomic medicine. Sources were selected from scientific articles, clinical trials, and global initiatives, focusing on the use of WMS and bioinformatics tools for microbiome profiling and patient stratification.

Results: The review reveals that WMS technologies have enabled the identification of microbial signatures linked to various diseases, offering a systemic view of microbial influence on human health. Several bioinformatics tools are key for stratifying patients based on their microbiome composition. Machine learning techniques and network medicine approaches are increasingly used to analyze the complex interactions between microbial networks and host physiology, which is crucial for personalized treatment strategies.

Conclusion: Metagenomic medicine holds significant promise in transforming clinical practice by offering personalized treatments that account for individual microbiome profiles. The integration of WMS, bioinformatics tools, and network-based approaches represents a systemic, holistic strategy for understanding the microbiome's role in human health. Despite challenges in clinical implementation, the field continues to evolve rapidly, driven by international research initiatives.

C.O.I. Disclosure:

N.B. Abdelmoula: None. **B. Abdelmoula:** None.

EP19.020 Machine learning-assisted genetic studies of MRI measures of organ volume provide insights into underlying mechanisms and roles in disease

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Background: High-quality phenotypic data is limited by the costs of advanced techniques like MRI-based organ size assessment. Using machine learning-assisted genome-wide association studies (GWAS), we aimed to uncover genetic insights into organ sizes and their disease associations.

Material and methods: We measured organ volumes in 37,589 UK Biobank MRI scans and imputed these imaging-derived phenotypes (IDPs) for the remaining participants using 382 biomarkers. GWAS for the imputed IDPs was conducted with POP-GWAS to improve imputation quality and minimize false positives. Genetic correlation analyses explored relationships between organ volumes and metabolic, lifestyle, and psychological traits, while Mendelian randomization examined links between organ size and disease.

Results: We achieved up to 200% gain in effective sample size and identified 452 genetic variants across 11 IDPs. Mendelian randomization revealed distinct associations between organ volumes and disease outcomes, e.g., genetically higher abdominal subcutaneous fat was associated with higher risks of diabetes, polycystic ovary syndrome, fatty liver, cardiovascular disease, gout, osteoarthritis, asthma, psoriasis; higher visceral fat with higher risks of heart failure, osteoarthritis, asthma, cholelithiasis, and reflux; higher muscle volume with higher risks of aortic aneurysm, atrial fibrillation, thrombotic events, osteoarthritis, but a lower risk of depression; higher lung volume with higher risks of aortic aneurysm, but a lower risk of heart disease and reflux; higher pancreas volume with lower risk of diabetes. Tissue enrichment analyses revealed organ-specific pathways, e.g., brain tissue for fat volumes and pancreatic tissue for pancreas size.

Conclusion: These findings highlight the power of machine learning-assisted GWAS to enhance genetic discovery.

C.O.I. Disclosure:

H. Yaghootkar: None. **A. Naz:** None. **B. Whitcher:** None. **A. Ahmed:** None. **M. Thanaj:** None. **E.P. Sorokin:** A. Employment (full or part-time); Modest; Calico Life Sciences LLC. **J.D. Bell:** None. **E. Thomas:** None. **M. Cule:** A. Employment (full or part-time); Modest; Calico Life Sciences LLC.

EP20.017 Retrospective study shows broad scale pharmacogenomic testing prior to prescribing could reduce adverse events associated with clopidogrel use by 38%

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Objective: In spite of evidence and recommendations reflecting the importance of pharmacogenomic testing, most prescriptions are still given without testing. We demonstrate the real world implications of the use of testing and evaluate adverse events and outcomes in individuals who did not receive pharmacogenomic testing for clopidogrel.

Methods: We analyzed ~100K individuals with paired EHR and exome sequencing data from population health studies administered at multiple medical centers using the Helix Exome + ® assay. We inferred clopidogrel dosage by processing the prescription with an LLM. We identified all instances of individuals with at least one prescription that is not in concordance with their CYP2C19 genotype (Bousman 2023). Lastly, we identify instances of thrombosis and infarction using a comprehensive codeset based on ICD9, ICD10, and SNOMED terms.

Results: We identified 16,140 prescriptions of clopidogrel given to 3,853 participants. We found that 29% of these individuals have a mismatch between the recommended clopidogrel dosage guideline based on their CYP2C19 genotype and their actual prescribed daily dosage. 25% of poor metabolizers experienced thrombosis, with 40% occurring within 2 months of treatment. Poor and intermediate metabolizers receiving clopidogrel are much more likely to experience thrombosis and myocardial infarction (binomial p-value = 0.001).

Conclusions: We estimate a 38% excess of adverse events occur in poor and intermediate metabolizers relative to normal and rapid metabolizers, costing ~700k per 1000 prescriptions. The lack of testing may be responsible for 1 thrombosis event per every ~30 people prescribed clopidogrel.

C.O.I. Disclosure:

N. Telis: A. Employment (full or part-time); Significant; Helix Opco LLC. **D. Judge:** None. **P. Pawloski:** None. **J.J. Grzymski:** None. **C. Chahal:** None. **C. Hajek:** A. Employment (full or part-time); Significant; Helix Opco LLC. **T. Kruisselbrink:** A. Employment (full or part-time); Significant; Helix Opco LLC. **N. Washington:** A. Employment (full or part-time); Significant; Helix Opco LLC. **E.T. Cirulli:** A. Employment (full or part-time); Significant; Helix Opco LLC.

EP23.016 Comprehensive Sample Preparation Workflows to extract and process DNA, RNA and Proteins from FFPE Scrolls: An Obvious First Step Towards Confident Data

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Background: FFPE samples are used to ensure tumor tissue integrity for downstream analysis of disease type and state. However, library construction for NGS starting with FFPE is a challenging task owing to need for high quality and amplifiable TNA (Total Nucleic Acid, DNA and RNA). In the world of precision medicine, acquiring genomic and proteomic data from FFPE samples are gaining importance owing to an increased number of endogenous biomarkers that are used for detection and disease monitoring. While better and more data can be beneficial, identification, characterization, and quantification of proteins extracted, purified and digested starting with FFPE samples can be complex and challenging. Adaptive Focused Acoustics® (AFA®) Technology has been used extensively for DNA shearing in NGS laboratories.

Material and methods: FFPE scrolls (10 µm) with different tissue types were obtained from external sources. Both TNA and protein workflows were processed using R230 focused ultra sonicator from Covaris. All consumables, buffers and reagents were obtained from Covaris.

Results: The two workflows showcased here generate - i) high quality and quantity of TNA, and ii) high quality and quantity of proteins and peptides. High yield, purity, and amplifiability were observed for TNA. Robust, reproducible, and reliable yield of proteins (100 µg of proteins and 25 µg of peptides) were observed while reducing the entire workflow time from 20+ hours to 3.5 hours.

Conclusion: This report highlights development of robust, reliable, and reproducible sample preparation workflows to ensure high quality and quantity of TNA and proteins from FFPE samples.

C.O.I. Disclosure:

D. Bhattacharyya: None. **S. Vasantgadkar:** A. Employment (full or part-time); Modest; Covaris. **K. Amirault:** A. Employment (full or part-time); Modest; Covaris. **A. Dhingra:** A. Employment (full or part-time); Modest; Covaris. **E. Daviso:** A. Employment (full or part-time); Modest; Covaris. **U. Thomann:** A. Employment (full or part-time); Modest; Covaris.

EP23.015 Sensitivity/Clinical Utility of RNA Sequencing for Structural Variant Detection: A Comparative Analysis with Routine Methods in Acute Myeloid Leukemia

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Background: This study aims to develop an RNA-Seq-based analysis platform that can rapidly and comprehensively detect structural variants (SVs) of diagnostic significance with single analysis and evaluate the detectability of SVs identified by standard methods.

Material and methods: Bulk RNA sequencing data of 10 newly diagnosed AML cases (8M,2F), with a mean age of 49.1, were analyzed via an in-house developed *GenNext* platform. SV calls were performed by three different tools, and AML-associated variants assigned as high confidence by at least two were included.

Results: Raw data was analyzed within six hours, and at least one SV was identified in seven out of ten cases. Of these, t(9;11)(p21;q23)/*KMT2A-MLLT3*, t(8;21)(q22;q22)/*RUNX1-RUNX1T1* fusions, *FLT3*-ITD, inv(16)*CBFB-MYH11*, were also reported by standard techniques in a month or later. Our approach identified additional inv(5)(VCAN-CTD) and t(6;9)(p22;q34)/*DEK-NUP214* and other SVs that standard techniques could not identify. inv(5) covers a long non-coding RNA related to therapy resistance, and the patient also presented high therapy resistance. The case with t(9;11) was referred to bone marrow transplantation and is in remission. Clinical courses are being monitored to observe the impact of other detected SVs outside of these on leukemogenesis and prognosis.

Conclusion: AML is an aggressive malignancy, and early and accurate diagnosis is essential for treatment success. Our platform was able to identify AML-associated driver genes as well as novel candidates in less than a week. We have shown that comprehensive diagnostic information regarding AML classification, risk assessment, and targeted therapy can be provided in a shorter period by RNA-seq.

C.O.I. Disclosure:

I. Sahin: None. **A. Uzay:** None. **E. Aydin:** None. **A. Akkus:** None. **K. Yalcin:** None. **O. Ozdemir:** None. **U. Ozbek:** None. **O. Hatirnaz Ng:** None.

EP24.006 Amygdaline induces apoptosis through modification of BAX and BCL-2 gene expression in the MDA-MB-231 human breast cancer cell line

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Background: Amygdalin is a natural product found in apricot seeds and has been reported by many studies to induce apoptosis. Recently, we set up experiments to study the effects of Amygdalin by modulating BAX and BCL-2 gene expression in the human breast cancer cell line MDA-MB-231.

Material and methods: Two different concentrations of the amygdaline solution, 1 mg/ml, and 10 mg/ml for 24 h, were used to treat human breast cancer cell line MDA-MB-231 and NHDF cell line as controls (Sigma-Aldrich) Cells were cultured in DMEM (Sigma-Aldrich) supplemented with 10% heat-inactivated FBS at 37 °C in a humidified cell incubator with 5% CO_2. Total RNA was isolated from treated cells. To identify the expressions of Bcl-2 and Bax mRNA, RT-PCR was performed with a set of primers Bcl-2 and Bax, and the PCR amplification was carried out using a ProFlex thermal cycler. The amplification products were analyzed in a Gel Doc Imaging System.

Results: Results showed that the mRNA level of Bcl-2 after treatment with Amy was decreased significantly from 1.0 as a control to 0.72 ± 0.15 ($p < 0.05$) at concentration 1 mg/ml and 0.39 ± 0.03 ($p < 0.05$) at concentration 10 mg/ml, while the level of Bax mRNA was increased from 2.34 ± 0.31 ($p < 0.05$) to 4.16 ± 0.17 ($p < 0.05$) for respective concentrations.

Conclusion: These results indicate that Amygdaline induces apoptosis through modification of BAX and BCL-2 gene expression in the MDA-MB-231 human breast cancer cell line. However, the potential genotoxic effects on these cell lines remain unclear and require further investigation.

C.O.I. Disclosure:

M. Xhetani: None. **K. Shpati:** None. **A. Hasa:** None. **A. Beshaj:** None. **M. Petković:** None.

EP24.005 CRISPR-based restoration of the reading frame in the DMD gene

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Background: We study the feasibility and effectiveness of restoring the reading frame in mutations in the dystrophin gene (DMD) using three different approaches:deletion of exons with a shifted reading frame, splice-site disruption withsubsequent skipping of exons with a shifted reading frame, and 1-2-nucleotide deletions before the frame-shift stop codon in order to restore the reading frame. The most effective strategy for restoring the reading frame involves skipping an additional exon. To evaluate the viability of this approach, we conducted a combined bioinformatics and experimental screening of sgRNAs targeting the splice sites of exons 43–55. Our findings identified the most promising editing sites as the acceptor splice sites of exons 54–55 and the donor splice sites of exons 43 and 53. We further used spCas9 and saCas9 nucleases and sgRNA targeted to exons 43, 53, 54, and 55 to disrupt slicing sites and to skip these exons from DMD transcripts. With patient-derived myoblasts with different deletions in DMD we aim to restore expression of the truncated forms of DMD in patient-specific myoblasts.

C.O.I. Disclosure:

O.A. Levchenko: None. **I.O. Panchuk:** None. **K.S. Kochergin-Nikitsky:** None. **O.V. Volodina:** None. **S.E. Nagieva:** None. **E.V. Kurshakova:** None. **I.O. Petrova:** None. **E.V. Kondratyeva:** None. **S.A. Smirnikhina:** None. **A.V. Lavrov:** None.

EP25.042 NIPT counseling in Russia: exploring providers' knowledge, attitudes, and barriers

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Background: Non-Invasive Prenatal Testing (NIPT) is widely used worldwide, but its expansion beyond common trisomies has outpaced clinical guidelines, leading to increased patient anxiety over uncertain or false-positive results. This study aimed to assess Russian medical geneticists' knowledge, comfort, and perceived barriers regarding basic and expanded NIPT counseling.

Material and methods: A web-based survey was conducted among Russian medical geneticists and OB-GYNs involved in NIPT counseling. The survey assessed their knowledge, confidence in

discussing basic and expanded NIPT conditions, and perceived challenges. Descriptive statistics were used to identify trends, knowledge gaps, and resource needs.

Results: Preliminary findings from 52 geneticists show high comfort in counseling on common trisomies (4.5/5) but lower confidence for rare trisomies (3.6/5) and microdeletions (3.5/5). Experience improved comfort, especially for complex conditions. The most significant barrier was cost (58%), even in Moscow, where NIPT is partly state-funded, followed by insufficient legal regulation (19%). Support for NIPT was highest for common trisomies (98%) but declined for expanded testing, particularly for monogenic disorders (54%). Resource needs differed by sector: private-sector specialists prioritized provider education, while state clinic professionals emphasized patient education.

Conclusion: These findings highlight the need for enhanced provider education on expanded NIPT and further development of clinical guidelines. Cost and regulatory limitations remain significant barriers to NIPT accessibility in Russia. Addressing these challenges through targeted provider training and patient education resources is essential for improving NIPT implementation and counseling practices.

C.O.I. Disclosure:

N. Ivanova: None. **E. Baranova:** None. **J. Tarasova:** None. **E. Efremova:** None. **L. Zhuchenko:** None. **V. Izhevskaya:** None.

EP26.019 Creating a resource of core consent elements for pangenomic studies

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Background: A pangenome is a collection of all of the genetic information of a species. There are many local human pangenome efforts underway across the globe. The Human Pangenome Project is a response to this new era of human pangenomics, stemming from the notion that humanity would be better served by an alliance of global partners working towards creating an integrated, international pangenome reference and pangenome tool ecosystem. However, there are many issues of consent that must be considered to create a truly global pangenome with open data sharing (acknowledging that open data sharing is not universal).

Material and methods: Using consent documents we have collected from multiple international partners, we are identifying core elements of a pangenome reference consent document. These core elements will comprise the critical components that are necessary for informed consent to participate in the creation of a reference resource. We are working closely with the Global Alliance for Global Health (GA4GH) to expand their current GA4GH Consent Toolkit for human pangenome reference projects.

Results: As we build this new pangenome consent resource, we are addressing challenges with consent harmonization, including issues of controlled versus open data access, privacy and data security, community engagement, international collaboration, data integration, and the potential for re-identification and commercialization.

Conclusion: We are creating a resource that will be useful for members of the international community who are developing pangenome references and/or that wish to contribute data to the Human Pangenome Project.

C.O.I. Disclosure:

H. Lawson: None. **R. Cook-Deegan:** None.