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Novel Insights from Clinical Practice

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A Novel Truncating Variant in Sandestig-Stefanova Syndrome with Hydrocephalus

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Established Facts

- Bi-allelic loss-of-function variants of *NUP188* are associated with Sandestig-Stefanova syndrome, which is characterized by pre- and postnatal microcephaly, trigonocephaly, congenital cataracts, periventricular white matter loss, thin corpus callosum, delayed myelination, microphthalmia, camptodactyly, congenital heart disease, and central respiratory failure.
- Sandestig-Stefanova syndrome has been described in 9 patients in three studies to date.

Novel Insights

- This study reports a novel nonsense (truncating) homozygous variant of the *NUP188* gene.
- This study defines Sandestig-Stefanova syndrome with hydrocephalus, liver and kidney malformations, and tetralogy of Fallot for the first time.

Keywords
NUP188 · Hydrocephalus · Whole exome sequencing · Autosomal recessive · Novel mutation

Abstract
Introduction: Sandestig-Stefanova syndrome (MIM: 618804) is characterized by pre- and postnatal microcephaly, trigonocephaly, bilateral congenital cataracts, microphthalmia, cleft lip and palate or high-arched palate, camptodactyly, rocker-bottom feet, heart anomalies, periventricular white matter loss, thin corpus callosum, and delayed myelination. Bi-allelic loss-of-function variants in the *Nucleoporin 188 (NUP188)* (MIM:615587) gene are implicated in the etiology. ***Case Presentation:*** Our patient, born to consanguineous parents, presented with tetralogy of Fallot, bilateral congenital cataracts, hydrocephalus, a bifid uvula, a right pelvic kidney, hepatomegaly, facial

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feature findings, and a history of a similarly affected exsibling. Whole exome sequence analysis in the index case revealed a novel homozygous variant NM_015354.2: c.124C>T/p.(Arg42Ter) in the *NUP188* gene. ***Conclusion:*** This study describes a new patient with Sandestig-Stefanova syndrome harboring a novel pathogenic variant in the *NUP188* gene.

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Introduction

Bi-allelic loss-of-function variants of *Nucleoporin 188 (NUP188)* (MIM:615587) are associated with Sandestig-Stefanova syndrome (MIM:618804), which is characterized by pre- and postnatal microcephaly, trigonocephaly, congenital cataracts, periventricular white matter loss, thin corpus callosum, delayed myelination, microphthalmia, camptodactyly, congenital heart disease, and central respiratory failure.

The *NUP188* gene is a component of the large nuclear pore complex (NPC). The NPC is located on the nuclear envelope and forms a gateway that regulates the flow of proteins and RNAs between the cytoplasm and nucleoplasm. Comprising approximately 30 distinct proteins, collectively known as nucleoporins, the NPC forms an octagonal channel across the nuclear envelope. It functions in nucleocytoplasmic transport, maintains the nuclear framework, and regulates gene expression. Molecular trafficking between the cytoplasm and nucleus is controlled by NPCs composed of multiprotein structures embedded in the membrane [1]. Although the complete loss of nuclear pore function is assumed to be incompatible with cell viability, pathogenic variants causing partial loss have been associated with numerous inherited diseases, including nephrotic syndromes, fetal akinesia deformation sequence, and progressive neurological disorders [2]. *NUP188* is part of the *NUP93* subcomplex, the second major structural unit of the NPC, which controls the passage of membrane or transmembrane proteins through NPC [3]. It has also been implicated in various cellular functions and its role in nuclear transport, including ciliogenesis, chromatin organization, transcriptional regulation, and chromosome segregation [4].

Overall, only 9 cases of Sandestig-Stefanova syndrome have been reported to date. We hereby present a new patient with a novel bi-allelic variant in *NUP188*.

Case Report

The index patient (III-3) was born to healthy second-degree cousin parents as their third child. One older brother was healthy, whereas another sibling had been similarly affected and died on the 42nd day of life due to respiratory failure (III-1) (shown in Fig. 1). Fetal ultrasound at 23 weeks of gestation revealed intrauterine growth restriction, tetralogy of Fallot (ToF), mild ventriculomegaly, a right pelvic kidney, hyperechogenic bowel (grade III), and an echogenic intracardiac focus in the left ventricle. As the parents did not want to pursue invasive testing, the pregnancy continued uninterrupted. The infant was born at 36 weeks of gestation via cesarean section with a birth weight of 1,900 g and was immediately admitted to the neonatal intensive care unit. She received treatment for respiratory distress and congenital heart disease, but her condition deteriorated as she developed sepsis. Postnatal MRI showed progression of ventriculomegaly, along with a thin corpus callosum. On the 38th day of life, a shunt was placed due to tetraventricular hydrocephalus. Bilateral cataracts were detected on eye examination, and hepatomegaly was observed on abdominal ultrasound. The patient died of respiratory failure on the postnatal 72nd day. Following her demise, she was referred for a genetic evaluation and underwent postmortem examination.

On postmortem examination, the 72-day-old female (corrected age: 40 days) measured 53 cm in length (−0.04 SD), weighed 3,900 g (−0.36 SD), and had a head circumference of 35 cm (−1.72 SD). She exhibited a coarsened facial appearance with bitemporal narrowing, flared eyebrows with prominence in the medial region, abundant facial hair confined to the forehead, a depressed nasal bridge, a short nose with a bulbous tip, a long philtrum, a thin upper lip, a small mouth, and micrognathia. Her ears were low-set and posteriorly rotated, displaying prominent antihelices, hypoplastic tragi, and anterior lobular creases. Intraoral examination revealed a narrow and high palate, hypertrophic alveolar ridge, multiple gingival cysts, and a bifid uvula. She had a short neck, bilateral hypoplastic nipples, accessory flexion creases on the forearms, proximally placed thumbs with extra phalangeal creases, overlapping toes (T3,4), and toes widening toward the tip with proximal placement of halluces. Significant skin edema was present, particularly pronounced around the external genitalia (shown in Fig. 2). Skeletal survey was unremarkable. The family did not opt for an autopsy.

Similarly affected older brother of the proband, also presented with cleft palate and complex heart anomalies detected in the second-trimester ultrasound scan (III-1). Following full-term delivery, he developed respiratory distress and feeding difficulties, necessitating admittance. He died due to respiratory complications on the 42nd day after birth.

Informed consent was obtained from the family for genetic investigations and the use of photographs for publication. This study was approved by the Local Ethics Committee of the Istanbul Faculty of Medicine.

DNA was isolated from skin fibroblasts from index case, and peripheral blood samples with sterile 2 ml K3EDTA from family members following the manufacturer's protocols (Qiagen, Germantown, MD, USA). A microarray (Agilent Sure-Print G3Hmm CGH+SNP, 4×180K) study was performed as the first step. Whole exome sequencing (WES) analysis was conducted in a research project setting to screen for disease-causing gene variants by the

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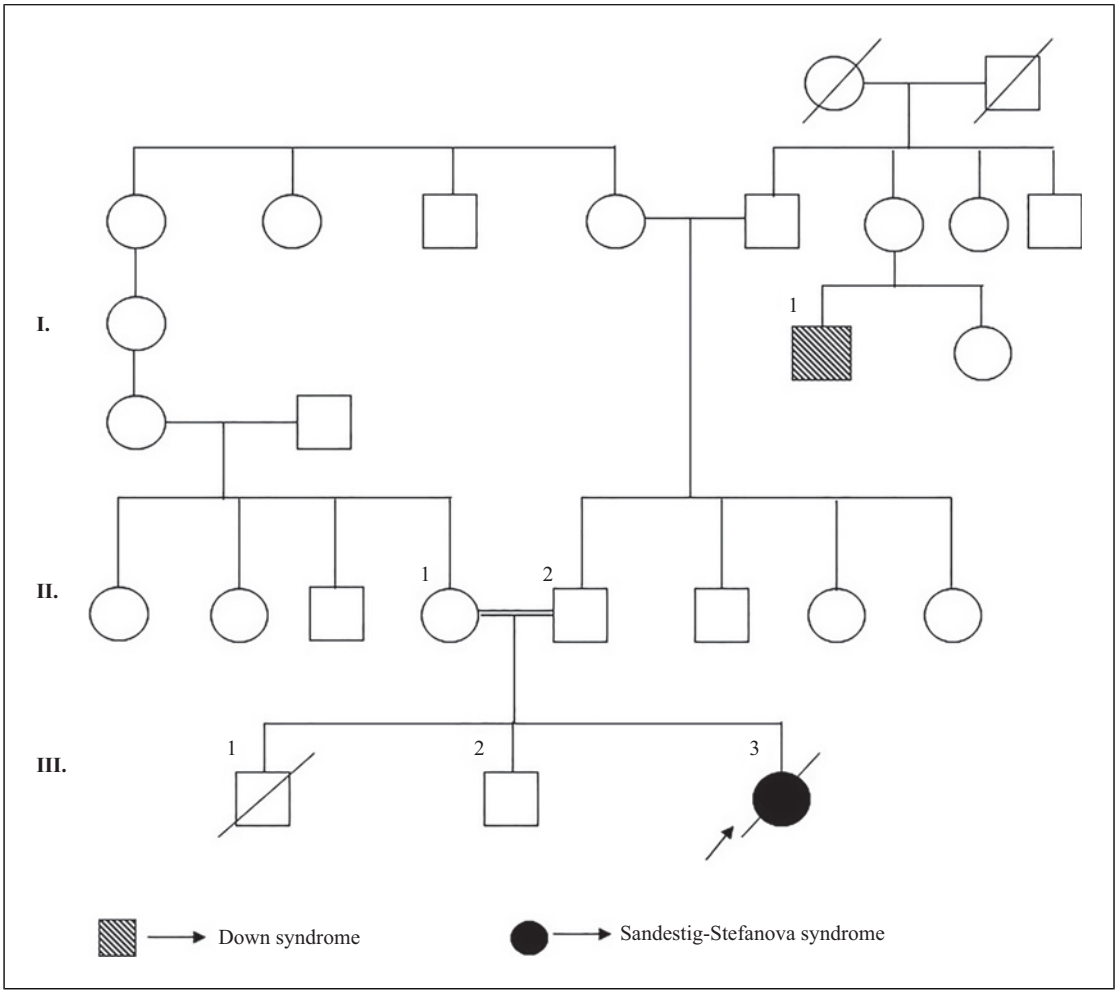


Fig. 1. Pedigree of the family. I-1: affected by trisomy 21, II-1: mother, II-2: father, III-1: similar clinical affected ex-sibling, III-2: healthy brother, III-3: index case affected by Sandestig-Stefanova syndrome (MIM:618804).

İstanbul University, İstanbul Faculty of Medicine, Department of Medical Genetics. DNA samples were used for library preparation for sequencing using the IonAmpliSeq Exome Kit (Thermo Fisher Scientific). Template preparation with emulsion PCR and chip loading was performed using a fully automated template device, IonChef (Thermo Fisher Scientific). Sequencing was performed using an Ion S5XL System (Thermo Fisher Scientific). Torrent Suite Software v5.12 (Thermo Fisher Scientific) evaluated quality, mapping, and run performance. The final sequence files were loaded into IonReporter v5.18.2 (Thermo Fisher Scientific). Sanger sequencing (ABI3500) confirmed candidate variants and their zygosity with specifically designed primers.

WES analysis revealed a novel homozygous c.124C>T/p.(Arg42Ter) variant in exon 3 of the *NUP188* gene in the patient (shown in online suppl. Fig. S1; for all online suppl. material, see https://doi.org/10.1159/000540314). Moreover, variant details are included in a supplementary file (shown in online suppl. Table S1). This variant introduces a premature termination codon in exon 3 of 44 of *NUP188*, likely resulting in nonsense-mediated decay of the mRNA product. Familial segregation analysis using Sanger sequencing revealed that both the healthy brother and the parents carried this variant in a heterozygous state (shown in Fig. 3). This variant has not been reported in clinically affected subjects. This variant is pathogenic (PVS1, PM3, PM2) according to the American College of Medical Genetics (ACMG) classification. Previously reported *NUP188* variants and corresponding clinical findings are summarized in Table 1. Neurological and cardiac examinations could not be performed on the parents.

Discussion

Here, we describe a new multiplex family affected by Sandestig-Stefanova syndrome caused by a novel bi-allelic truncating variant located in *NUP188*. *NUP188*

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Fig. 2. Postmortem examination of the case. **a** Short neck, bilateral hypoplastic nipples, major edema of the female labium. **b** Increased hair growth on the forehead, scattered eyebrows on the medial side, flat nasal root, short nose, bulbous nasal tip, short columella, long philtrum, fish-mouth appearance. **c** Positional anomaly of the right foot (4th finger located below).

and *NUP93* control the organization of subcompartments of the nuclear envelope, such as the outer and inner nuclear membranes, which are contiguous with the endoplasmic reticulum [6]. NUP188 also regulates chromosome segregation by localizing its C-terminal region to spindle poles during mitosis. Thus, in NUP188-depleted mitotic cells, chromosomes fail to align to the metaphase plate, which activates the spindle assembly checkpoint and causes subsequent mitotic arrest. Although NUPs are typically associated with NPC-related roles, they have also been found in other subcellular components, including the kinetochore, centrosome, cilia base, and chromatin. Notably, specific NUPs at cilia bases regulate transport between the cilia and cytoplasm. Moreover, mutations in some NUPs have been shown to contribute to ciliopathies [7]. Morpholino-based knockdown of Nup188 or its binding partner Nup93 in *Xenopus* leads to the loss of cilia during embryonic development, while the function of the NPC remains largely intact [8]. Cilia dysfunction contributes to various ciliopathies including hydrocephalus, polycystic kidney disease, retinal dystrophy, and congenital heart disease. This may be attributed to the crucial role that most NUPs play in maintaining fundamental life activities, making them susceptible to loss-of-function mutations [7].

The finding of a duplication affecting *NUP188* and five neighboring genes in a cohort of 262 individuals with congenital heart disease and heterotaxy resulting from abnormalities in left-right body patterning prompted functional studies [4]. Haskell et al. [9] reported a heterozygous splice donor variant (c.4737+ 1G>T) in *NUP188* in an adult patient with mitral valve prolapse from an independent cohort of 55 cases. In another study, Strauss et al. [10] reported one missense (c.313C>T; p.Arg105Trp) and one intronic variant (c.3429+5G>A), residing in *NUP188* in a 3-year-old child with motor delay, cervical vertebra (C1) dysplasia, hearing loss, tracheomalacia, and cryptorchidism. These studies failed to show a clear association between the *NUP188* gene variant and the corresponding phenotype. These findings suggest that the *NUP188* protein may have, apart from its involvement in the NPC, other cellular localizations and functions [11].

For the first time, Sandestig et al. [3] (2019) presented two unrelated individuals with bi-allelic truncating variants of *NUP188*, who exhibited overlapping features including premature birth, pre- and postnatal microcephaly, trigonocephaly, bilateral congenital cataracts, microphthalmia, ventricular septal defect, camptodactyly, clinodactyly, bilateral single transverse crease, rocker-bottom feet, and central nervous involvement comprising enlarged ventricles, loss of periventricular white matter, and thin corpus callosum. Infants were hypotonic, required oxygen supplementation and tube feeding, and succumbed to central respiratory failure at 67 days and 140 days of life, respectively. Using WES analysis, both patients were identified with homozygous nonsense *NUP188* variants: c.287dupA; p.(Tyr96Ter) in patient 1;

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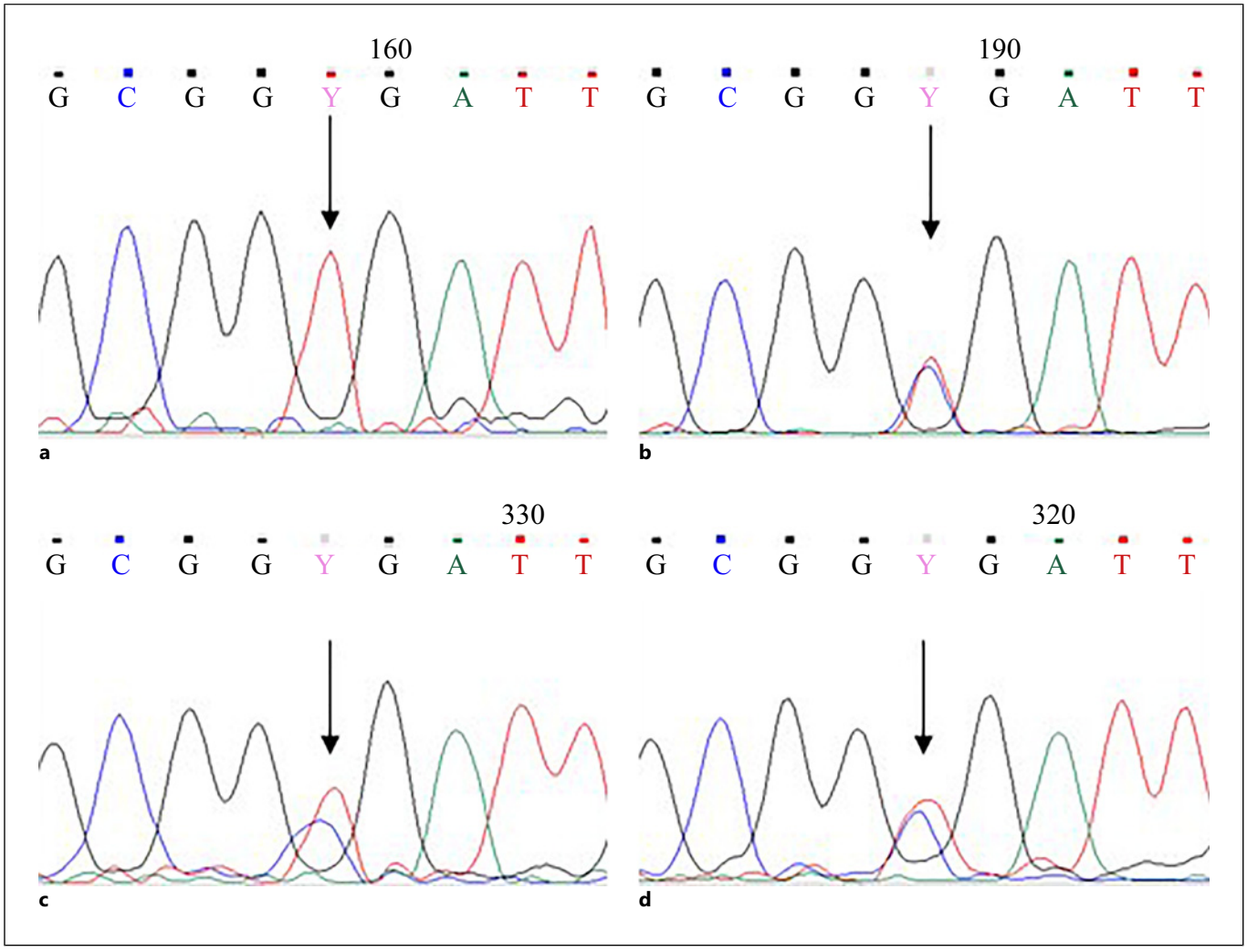


Fig. 3. Electropherogram images of segregation analysis using Sanger sequencing. **a** Proband, homozygous. **b** Healthy brothers, heterozygous. **c** Mother, heterozygous. **d** Father, heterozygous.

and c.337C>T; p.(Gln113Ter) in patient 2. Both sets of parents were found to be heterozygous for the respective alterations. This study is the first to define the clinical condition caused by homozygous nonsense variants in *NUP188*, termed Nucleoporin 188 insufficiency syndrome, also known as Sandestig-Stefanova syndrome.

Muir et al. [2] (2020) presented six individuals afflicted with Sandestig-Stefanova syndrome. Three individuals from two different families were of Ashkenazi Jewish descent, and all three individuals shared the same two variants in compound heterozygous state, c.904_907delATTT; p.(Ile302Valfs*7) and c.3144C>G; p.(Tyr1048Ter), in *NUP188*. The third family, with two siblings, had a homozygous variant predicted to cause a frameshift and premature truncation: c.5032dupC; p.(Arg1678Profs*13). Individual six had compound heterozygous nonsense variants c.1890G>A; p.(Trp630Ter) and c.4078C>T; p.(Gln1360Ter). Immunoblot and immunofluorescence analyses of fibroblasts from individuals carrying p.Ile302-Valfs*7 and p.Tyr1048Ter variants showed that these variants resulted in either complete loss of *NUP188* or a non-functional stump protein product. Functional studies have demonstrated an important role for *NUP188* in dendrite tiling and, potentially, neural circuit formation [2]. Korulmaz et al. [5] (2021) presented the first male patient with a homozygous c.1087C>T; p.(Gln363Ter) variant in *NUP188*. In addition to previously described findings of Sandestig-Stefanova syndrome, he displayed ambiguous genitalia, hypospadias, undescended testes, immunodeficiency, and hypothyroidism.

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Table 1. Comparison of the clinical findings of case with the literature

Features	Sandestig et al. [3] (2019)		Muir et al. [2] (2020)						Korulmaz et al. [5] (2022)	Present case
Individual	1	2	3	4	5	6	7	8	9	10
Variants (NM_015354)	c.287dupA; p.Tyr96* (hmz)	c.337C>T; p.Gln113* (hmz)	c.904_907delATTT; p.Ile302Valfs*7 c.3144C>G; p.Trp1048* (compound het)	c.904_907delATTT ;p.Ile302Valfs*7 c.3144C>G; p.Trp1048* (compound het)	p.Ile302Valfs*7 p.Trp1048* (compound het)	c.5032dupC; p.Arg1678Profs*13 (hmz)	c.5032dupC; p.Arg1678Profs*13 (hmz)	c.1890G>A; p.Trp630* c.4078C>T; p.Gln1360* (compound het)	c.1087C>T; p.Gln363* hmz	c.124C>T; p.Arg42* (hmz)
Gender	F	F	F	F	F	F	F	F	M	F
Age at death	67 days	147 days	5 months	5 weeks	7 months	2.7 years	2.5 months	1 month	6 months	72 days
Cause of death	Respiratory failure	Respiratory failure	Respiratory failure	Respiratory failure	Respiratory failure	Respiratory failure	Respiratory failure	Respiratory failure	Respiratory failure	Respiratory failure
Small for gestational age (SGA)	+	+	+	+	+	+	+	+	+	+
Bilateral congenital cataracts	+	+	+	+	+	−	−	+	+	+
Congenital heart defects	+	+	+	+	+	Not tested	+	+	+	+
Respiratory abnormalities	+	+	+	+	+	+	+	+	+	+
Narrow lateral forehead	+	+	+	+	+	+	+	+	+	−
Abundant facial hair confined to the forehead	−	−	−	−	−	−	−	−	−	+
Wide nasal bridge	+	+	+	+	+	+	+	+	+	+
Long philtrum	−	−	−	−	−	−	−	−	−	+
Low-set ears	+	+	+	+	+	+	+	+	+	+
Micrognathia	+	+	+	+	+	+	+	+	+	+
Cleft lip/plate	+	−	−	−	−	−	−	−	−	+
Short neck	+	+	+	+	+	+	+	+	+	+
Camptodactyly	+	+	+	+	+	+	+	+	+	−
Overlapping toes	+	+	+	+	+	+	+	+	+	+
Hypotonia	+	+	+	+	+	+	−	+	+	+
Thin corpus callosum	+	+	+	+	+	+	+	−	+	+
Ventriculomegaly	+	+	+	+	+	+	−	+	+	+
Hydrocephalus	−	−	−	−	−	−	−	−	−	+

*, stop codon; F, female; M, male; hmz, homozygous; compound het, compound heterozygous.

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Although genetic testing could not be performed due to a lack of DNA, the older similarly affected brother of the proband reported herein indirectly provided evidence of the second male patient afflicted with Sandestig-Stefanova syndrome, also originating from Türkiye. In terms of the cardiac phenotype, various defects have been reported in association with *NUP188* loss-of-function variants. Heterozygous truncating variants have been linked to mitral valve prolapse, while patients with bi-allelic variants were reported to have a ventricular septal defect, bicuspid aortic valve, patent ductus arteriosus, partial anomalous pulmonary venous return, and left ventricular hypertrophy [8]. Our patients presented with ToF, expanding the cardiac spectrum of *NUP188*. Additionally, the lack of a detectable cardiac phenotype in the parents and carrier sibling reinforces the idea that distinct pathogenic mechanisms are triggered depending on the type of variant affecting *NUP188*.

Similar to previous reports, prenatal-onset mild ventriculomegaly was identified in our case during the 2nd trimester. However, unlike in those reports, the ventriculomegaly in our patient progressed to overt hydrocephalus, necessitating shunt placement. It is uncertain whether this progression is a new insight into the clinical spectrum or if it was caused by neonatal sepsis exacerbating the ventriculomegaly. Additional novel clinical findings include hepatomegaly and a pelvic ectopic kidney; however, further reports are required to confirm these associations within the disease spectrum.

This study identified a novel truncating variant in the *NUP188* gene, bringing the total number of patients with Sandestig-Stefanova syndrome up to ten. Our patient exhibited clinical features similar to those reported in previous studies, with the additional findings of hydrocephalus, hepatomegaly, ectopic kidney, and ToF, expanding the clinical spectrum of this rare disease.

Acknowledgment

The authors would like to thank the patient's family for their participation and approval of our submission of this case report.

Statement of Ethics

Written informed consent was obtained from the patient's family to publish this case report and accompanying images. The study protocol was reviewed and approved by the Local Ethics Committee of Istanbul University, Istanbul Faculty of Medicine, Approval No. 1453 (date: October 31, 2018).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

B.K. projected the study. U.A. and G.T.T. made physical examinations and gathered clinical data and biological samples. A.D.A. made a clinical evaluation. G.B. and G.T. performed WES and Sanger sequencing. G.T., G.B., and Z.O.U. performed bioinformatics analyses. G.B. and B.K. wrote the paper.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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