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Diagnostic Utility of Whole Exome Sequencing in patients with suspected mitochondrial disease: the single center experience in Turkish population

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BACKGROUND: Mitochondrial diseases are the most common form of IEMs associated with nuclear and mitochondrial (mtDNA) pathogenic variants. Next-generation sequencing technologies have given important support to elucidating the genetic etiology of such diseases, which are difficult to diagnose. This study aims to determine the molecular base of undiagnosed IEMs using whole-exome sequencing (WES).

MATERIAL AND METHODS: Twenty-five affected patients from 20 unrelated families which were suspected of mitochondrial disease were included. Thermo Scientific™ IonGeneStudioS5 System was used for WES and mtDNA sequencing. Transcript studies were performed from cultured muscle/blood cells to confirm the novel variants.

RESULTS: Eighteen different pathogenic/likely pathogenic variants were shown in *TK2, POLG, EARS2, AARS2, MICOS13, NDUFAF6, FBXL4, ECHS1, OXCT1, CAPN3, DYSF, TCAP* genes in seventeen affected cases from thirteen families. All of the diagnosed variants confirmed by Sanger sequencing and segregation in families. Altogether, three novel and five ultra-rare variants first time associated with disease in this study; *EARS2* c.1283delC ((p.Pro428Leufs*)) and c.319C>T (p.Arg107Cys) **(Figure 1),** *AARS2* c.277C>T (p.Arg93Ter) and c.845C>G (p.Ser282Cys), *ECHS1* c.202G>A (p.Glu68Lys), *NDUFAF6* c.479delA (p.(N162Ifs*27)) **(Figure 2.)**, *OXCT1* c.1370C>T(p.Thr457Ile), c.1173-139G>T(p.?).

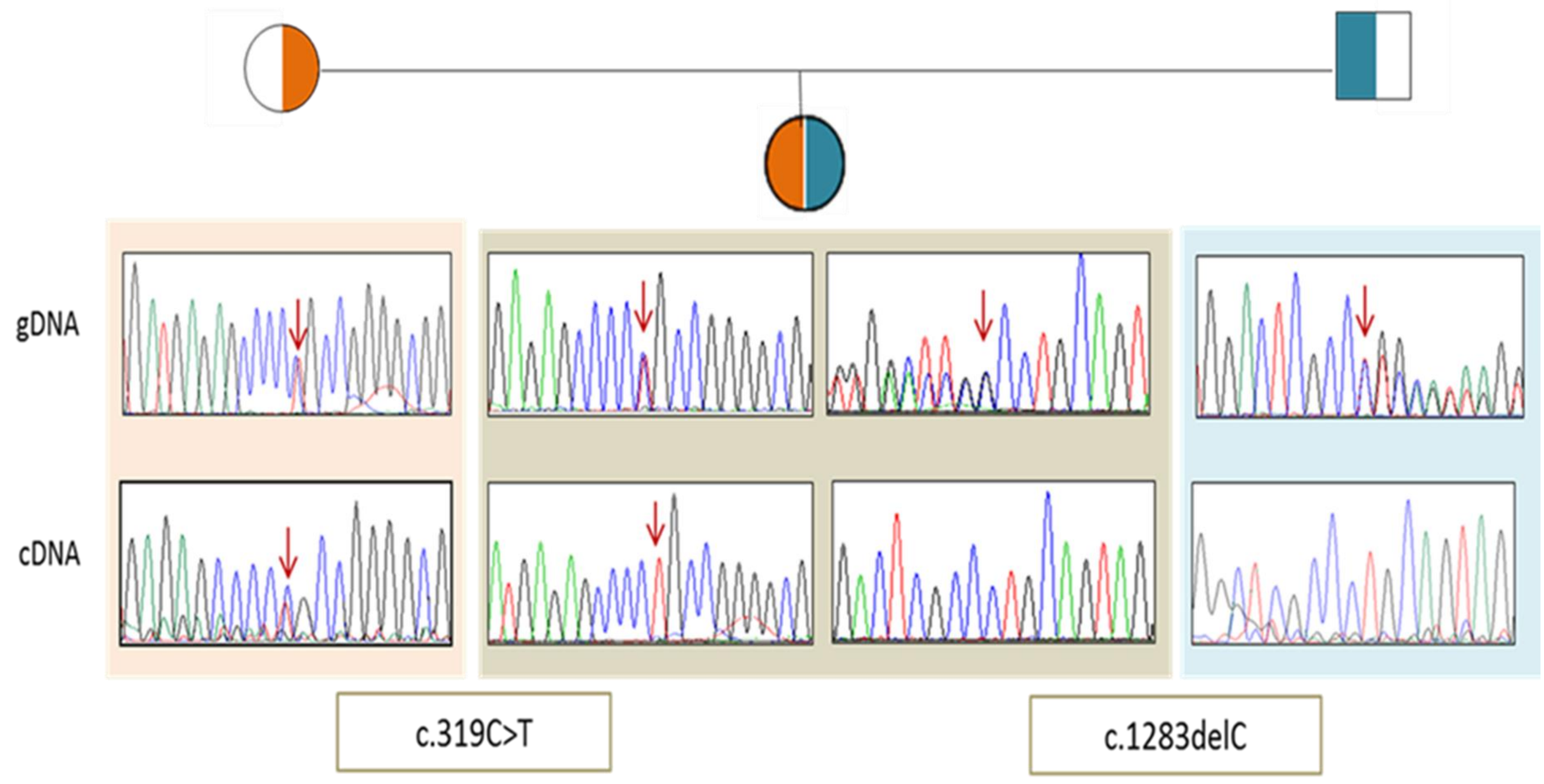


Figure 1. Novel variants detected in the EARS2 gene. The genomic DNA sequence shows variants in the compound heterozygous form. Electropherograms in cDNA synthesized with primers in the 'random' hexameric sequence from total RNA obtained from a peripheral blood sample. The presence of only the allele carrying the missense mutation in the case and only the normal allele in the father indicates that the deletion type mutation is subject to mRNA decay.

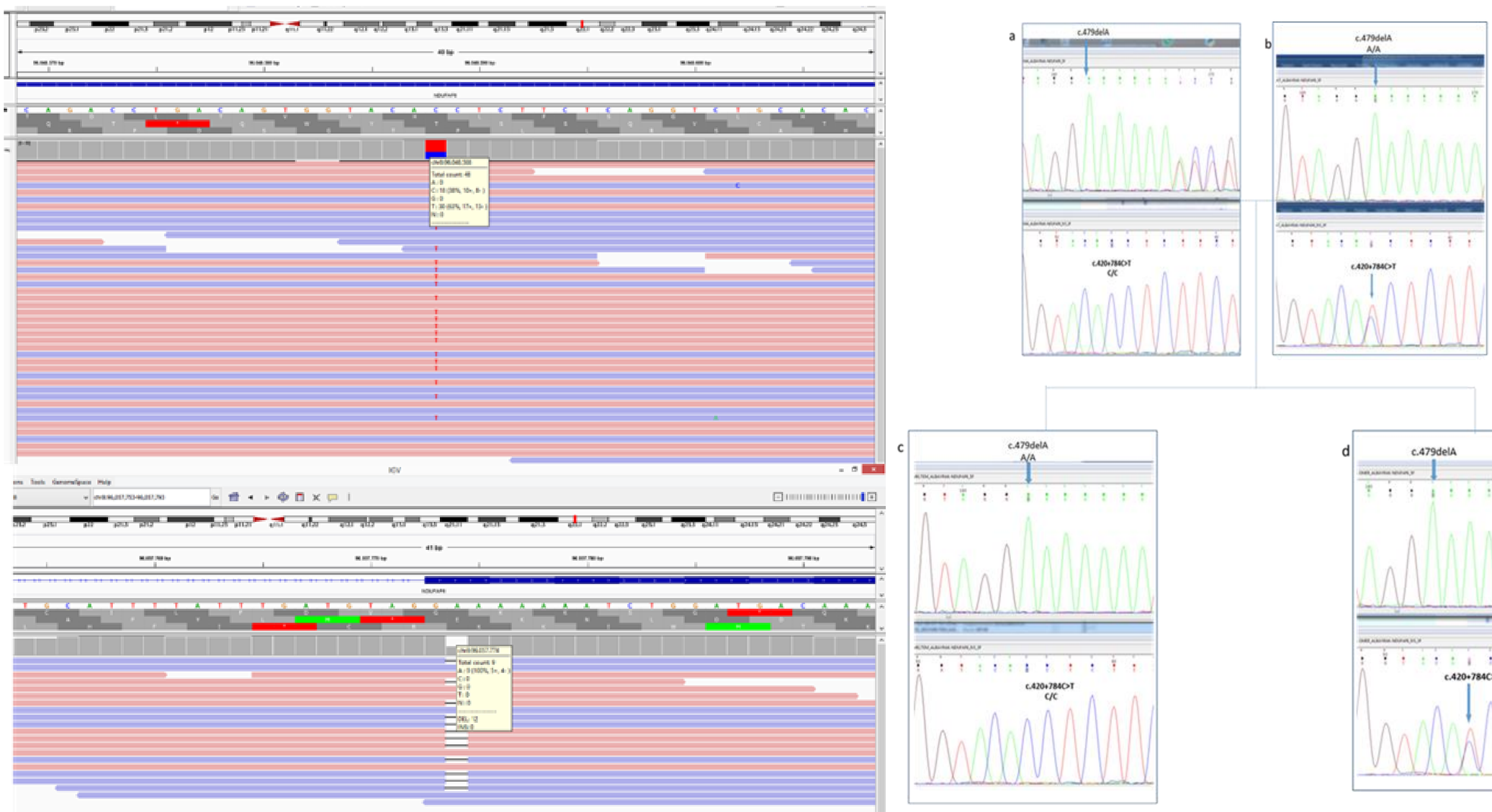


Figure 2: The WES analysis performed on the index case, heterozygous c.479delA in exon 5 (p.(Arg162Ilefs*27)) and heterozygous c.420+784C> in intron 3 in the NDUFAF6 gene (NADH Dehydrogenase (Ubiquinone) Complex I, Assembly Factor 6) was detected. These changes were confirmed by Sanger sequence analysis and parental study was performed for segregation. Sanger sequencing electropherogram images of mother heterozygous for c.479delA (a), father heterozygous for c.420+784C>T (a), sister homozygous normal (c), and index case in composite heterozygous form.

CONCLUSION: The clinical, radiological, biochemical and histopathology results of the cases supported the gene and variant associations. Five cases included in this study had neuromuscular findings and pathogenic variants were identified in nonmitochondrial genes. The molecular genetic diagnosis was achieved at a rate of 65% for all groups. The genetic result allowed two patients to receive early access to the medicine for *TK2* deficiency. Genetic counselling is made available for all families. Seven of the families remain undiagnosed and are planned for whole-genome sequencing.