Received: 27 January 2023 | Revised: 4 April 2023 | Accepted: 17 April 2023

DOI: 10.1002/ccr3.7308

CASE REPORT

Clinical Case Reports Open Access WILEY

Molecular analysis of gene variants in an Iranian family with psychomotor retardation mitochondrial disorder patient

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Key Clinical Message

In 1-year-old girl presenting with neurodegenerative mitochondrial disease (Leigh syndrome), mutation analysis was performed by whole exome sequencing. Pathogenic variants were then analyzed in parents and relatives by Sanger sequencing. We identified a point mutation c.G484A in *NDUFS8* gene which was homozygous in patient and heterozygous in parents.

KEYWORDS

Leigh syndrome, mitochondrial disease, whole exome sequencing

1 | INTRODUCTION

Mitochondrial disorders are a devastating group of inherited diseases that result from dysfunction of the mitochondrial respiratory chain.[1] Mitochondrial respiratory chain disorders are probably the most common group of metabolic disorders, affecting at least 1 in 5000 individuals worldwide.[2] They can present during childhood or adulthood. There is an extreme diversity of clinical presentations, involved multiple organs in mitochondrial diseases. They have many nonspecific symptoms that overlap with other diagnoses.[3] Predominant presentations express in muscles and nerves. Common clinical features of mitochondrial disorders include ptosis, external ophthalmoplegia, proximal myopathy and exercise intolerance, cardiomyopathy, fluctuating encephalopathy, seizures, dementia, migraine, stroke-like episodes, ataxia.[4]

Mitochondrial disorders caused by mutations in nuclear DNA or mitochondrial DNA.[3] More than 30 mtDNA genes and more than 30 nuclear genes have been identified to have mutations causing mitochondrial diseases.[3] Diseases caused by mtDNA mutations are maternally inherited, while those caused by mutations in nuclear genes are inherited in a Mendelian fashion.[5] In some cases, the clinical, pathological, and biochemical results for a patient may suggest a limited number of genes to investigate.[3]

Leigh syndrome is a neurodegenerative mitochondrial disease usually presents within the first months of life with psychomotor retardation.[6] The symptoms of Leigh syndrome include hypotonia, ataxia, dystonia, ophthalmoplegia, and lactic acidemia.[6] Complex I deficiency is the most frequent oxidative phosphorylation defect in children with Leigh syndrome.[7,8] Nicotinamide adenine dinucleotide (NADH): ubiquinone oxidoreductase (complex I) is the largest multimeric enzyme complex of the respiratory chain, which is composed of seven mtDNA encoded subunits and at least 38 nuclear DNA subunits.[7]

The nuclear-encoded NDUFS8 is an assembly factor of complex I, which is highly conserved among eukaryotes and prokaryotes.[9,10] This subunit of complex I is encoded

by nuclear gene *NDUFS8* [OMIM 602141], which is located on chromosome 11q13.2 and includes 7 exons and 6 introns.[11] This gene is translated into a 210-amino acid protein with molecular weight of 23.0 kDa.[11]

Variants in *NDUFS8* gene have been identified as a cause of severe multisystem mitochondrial disease.[11]

In a patient presenting with Leigh syndrome, we identified a homozygous variant of *NDUFS8* gene with whole exome sequence and confirmed it in parents by Sanger sequencing.

2 | CASE PRESENTATION

Proband was a 1-year-old girl, the second child of consanguineous parents (first cousins). She was born at a gestational age of 37 weeks with a birth weight of 3000 g.

At the age of 3 months, she followed objects with her eyes; she started rolling over at 4 months, sat unsupported and was normal completely. At 5 months of age, a weak seizure with limbs tremor was seen and followed by frequent vomiting, continuous crying, irritability and dysphagia, horizontal nystagmus, muscle weakness, and ataxia. Treatment with thiamin, diazepam, and CoenzymeQ10 was recommended.

Plasma amino acids and urine organic acids were normal except for an increase in 3-hydroxy butyric acid in urine (28.3 mMol/molcrt, normal is <5 mMol/molcrt). Blood and urine carnitines were in the normal range. Measurements of lactate and pyruvate concentrations in blood, urine, were also normal. Head MRI and other laboratory investigations including blood cell counts, serum urea, serum electrolytes, and liver function tests were normal.

At 10 months of age, she lost her appetite completely, and developed respiratory problems which lead to impairment of respiratory function and required breathing via ventilator. Eventually, she lost her consciousness and died at 1 year old due to cardiorespiratory failure following an episode of aspiration.

3 | METHODS

3.1 | Samples and DNA extraction

Genomic DNA was isolated from EDTA peripheral blood samples using salting out method.[12] DNA sample concentration was measured using Nanodrop spectrophotometry (Nanodrop 2000) and electrophoresed on 1% agarose gel.

Parents provided and signed a written informed consent for molecular analysis, clinical data usage, and the use of DNA samples from the tested individuals for both research and diagnosis purposes.

3.2 | Whole exome sequencing and data analysis

For proband, whole exome sequencing was carried out using SureSelect Human All Exon V6 Kit (Agilent Technologies, Inc.) according to manufacturer's instructions. Sequencing was done by the NovaSeq6000 sequencing system (Illumina Inc.). Color space reads were iteratively mapped to the hg19 reference genome, and the variants were annotated using an in-house annotation pipeline. Calls with variant quality <20 were filtered out to obtain clean reads, and at least 95% of the targeted nucleotides were covered sufficiently to pass the thresholds for calling single-nucleotide mutations (SNPs) and small insertion/deletion (InDel) sequences. Variants associated with mitochondrial disorders were selected.

3.3 | Variant amplification and Sanger sequencing

Based on a recessive inheritance model only a single candidate gene discovered, which was validated by Sanger sequencing in patient and her parents and relatives. Oligonucleotides were designed from the *NDUFS8* gene sequence (*Gene ID: 4728*). Primers to amplify and sequence exon 6 were *NDF*: 5′-ggcttctggcagaccccagg-3′ and *NDR*: 5′-aactcaaagttggggccctg-3′. PCR reaction was done employing a Biorad thermal cycler at thermal profile include: 5 min at 95°C (one cycle), 30 at 95°C, 30 at 62°C, 35 at 72°C (35 cycle), and 5 min at 72°C (one cycle) for final extension. PCR products were analyzed by 1.5% agarose gel electrophoresis.

Cycle sequencing reactions were performed and separated on an ABI 3130-XL DNA sequencer according to the manufacturer (Perkin-Elmer-Applied Biosystems). Pedigree analysis was done using the Genopro 2020 software.

3.4 | In silico analysis

After Sanger sequencing, variants were aligned to the RefSeq NM_002496.4 and they were evaluated by different on-line tools such as Mutation Taster and SIFT (Sorting Intolerant From Tolerant).

4 | RESULTS

4.1 | WES analysis

All variants with a minor allele frequency (MAF) of less than 1% in the gnomAD database, as well as disease-causing

variants reported in HGMD®, ClinVar, or CentoMD® are considered. Due to the large number of protein affecting variants detected in WES, we decided to focus our attention on mitochondrial disease-associated genes, hypothesizing an autosomal recessive manner. We thus detected one homozygous missense variant (NM_002496: c.G484A, p.Val162Met) in the exon six of *NDUFS8* gene, inherited from the unaffected parents (Table 1).

The variant is known in dbSNP (rs1277027467). Each parent of the patient carried this variant heterozygously. Alignment of the homologous proteins to the human NDUFS8 showed that mutant amino acid position is extremely well conserved (Figure 1).

As we showed in Table 1, WES results revealed variants in *ZEB2*, *ITGA7*, and *MC2R* genes in the patient. The patient carried these variants heterozygously (Table 1).

4.2 | Variant confirmation by Sanger sequencing

Amplified *NDUFS8* gene was cycle sequenced and variants were aligned to the RefSeq NM_002496.4 in proband, parents and her older brother. In the patient this confirmed a homozygous transition, G > A at position 484, resulted in an amino acid change of a valine into a methionine at amino acid position 162. Each parent and brother of the patient carried this variant heterozygously (Figure 2). According to the mutation taster, this variant is a disease causing one, and its PHRED score is 21.0. SIFT predicts whether an amino acid substitution affects protein function based on sequence homology and the physical properties of amino acids.

To further evaluation, we counseled with some patient's relatives and searched identified variant in their amplified genes using Sanger sequencing. Finally, we found mutant variant in proband's two grandmothers and some her relatives in the heterozygote state (Figure 3).

5 | DISCUSSION

Clinical diagnosis of multi systemic mitochondrial diseases is difficult due to their extremely genetic diversity. In the last years, next generation sequencing (NGS) techniques, such as exome sequencing, have made possible the simultaneous analysis of all known coding genes in a single reaction, greatly facilitating, and accelerating the

TABLE 1 Variations have been identified by WES in the proband.

Gene	Gnome position	Mutation	Associated disease	Inheritance	Genotype
NDUFS8	Chr 11/67803831	G > A	Mitochondrial complex 1 deficiency	AR	Homozygous
ZEB2	Chr 2/145147310	G > C	Mowat-Wilson syndrome	AD	Heterozygous
ITGA7	Chr 12/56092271	del C	Muscular dystrophy	AR	Heterozygous
MC2R	Chr 18/13885199	C > T	Glucocorticoid deficiency	AR	Heterozygous

Abbreviations: AD, autosomal dominant; AR, autosomal recessive.

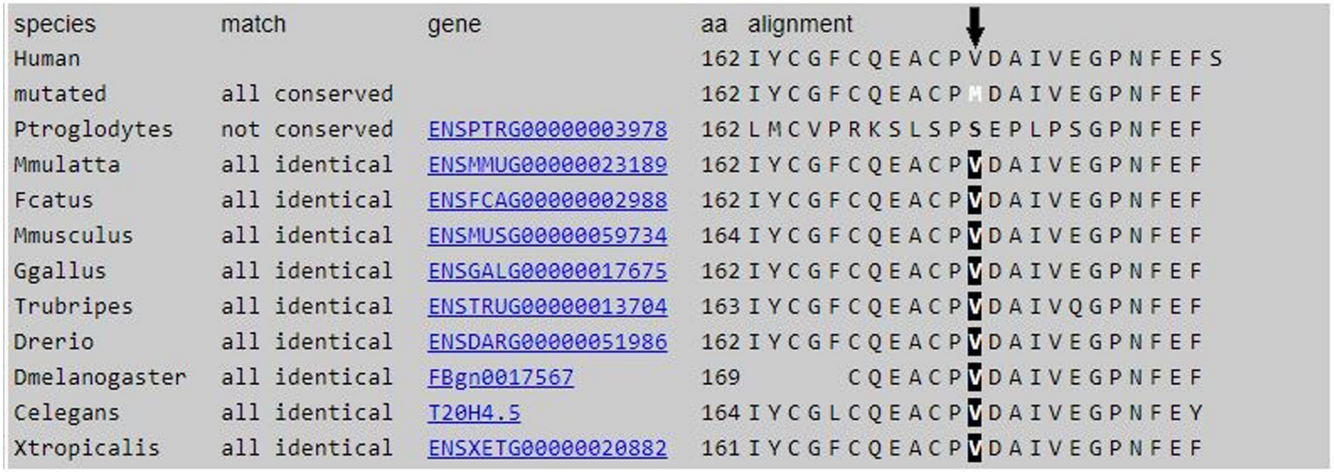

FIGURE 1 Sequence conservation of NDUFS8 between different species. Alignment of amino acid sequences from human to bacteria. Arrow indicates the mutation at the position V162M, strictly conserved in all species down through bacteria, except the Pan troglodytes (Chimpanzee).

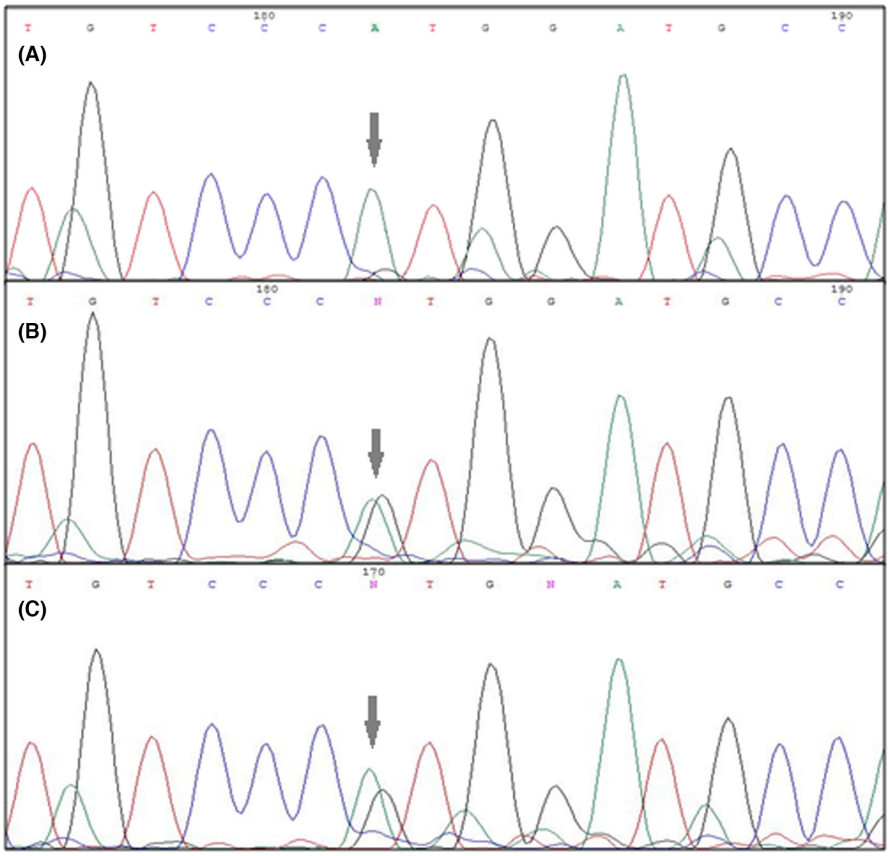

FIGURE 2 Sanger sequencing chromatograms of the *NDUFS8* mutated region in the proband (A), her father (B) and her mother (C). Arrows show that the proband carries the mutation homozygously and her parents carry it heterozygously.

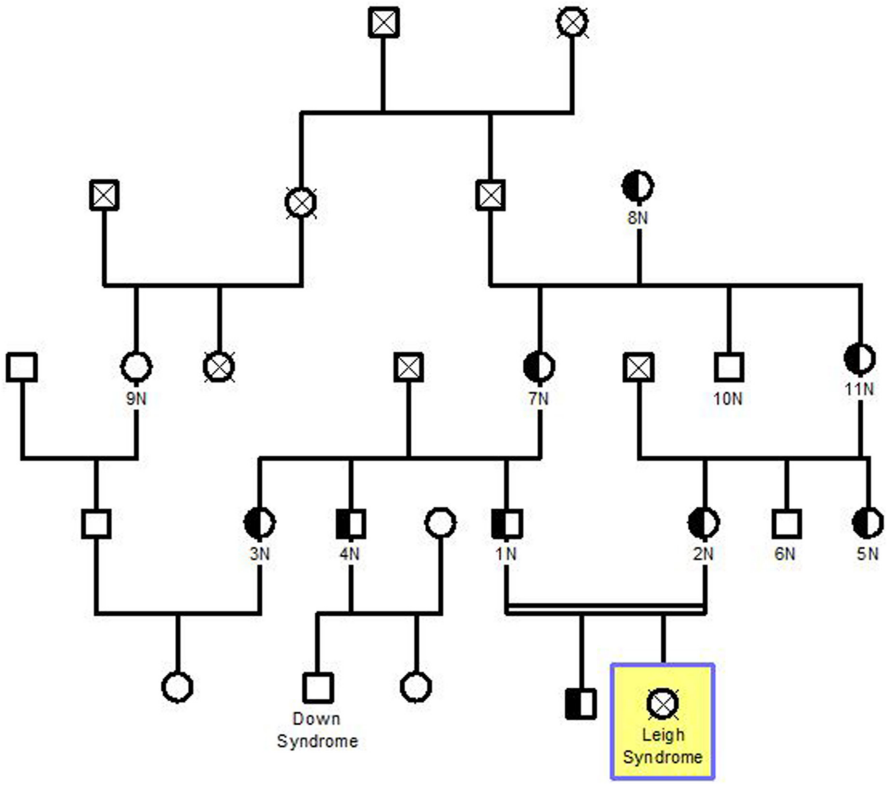

FIGURE 3 Family's pedigree of the patient. The proband is represented by the yellow margine. (Squares and circles indicate men and women, respectively. Half-filled symbols indicate carriers in the family, white symbols indicate unaffected individuals, a cross through a symbol means a deceased person and numbers represent the studied individuals in the family).

discovery of new disease genes.[13] This makes NGS approach useful to study disorders, which are difficult to diagnose on a clinical basis.[14]

WES analysis in the patient described in our study was shown a c.484G > A missense variant in *NDUFS8* gene. Sanger sequencing confirmed the homozygous state in

patient and heterozygous in her parents. The nuclear-encoded NDUFS8 subunit of complex I respiratory chain is highly conserved among eukaryotes and prokaryotes.[11]

Mutation in NDUFS8 has been identified the first time by Loeffen et al. in a patient with Leigh syndrome.[15] Variants in *NDUFS8* gene were reported in previous studies and molecular genetic relation between this gene and mitochondrial complex I deficiency like Leigh syndrome has been described. Procaccio et al. revealed that one patient with late-onset Leigh syndrome and partial complex I defect was a compound heterozygote for two mutations in *NDUFS8* gene.[16] Haack et al. discovered rare variants in *NDUFS8* in two unrelated individuals with mitochondrial complex I deficiency using exome sequencing.[11]

Mutation in nuclear gene encoded complex I subunits reported in several studies. Hangyan Bi et al. found compound heterozygous mutations in *NDUFAF5* gene by whole exome sequencing.[17]

Gholamipour et al. identified a novel homozygous missense variation in *NDUFA9* gene by exome sequencing in an Iranian Leigh syndrome patient.[18] Kamalzadeh et al. found a novel homozygous missense mutation in *NDUFAF6* gene of a 3 year old affected girl suspicious to Leigh syndrome.[19]

Some mutations we found in other genes were (*ZEB2*, *ITGA7*, and *MC2R*). These mutations were inherited heterozygously in the proband and were in secondary degree in our study because all of them were associated with non-mitochondrial diseases.

6 | CONCLUSION

Nuclear encoded complex I deficiency generally has a devastating clinical disease course, characterized by a severe neurological phenotype and most patients die before the age of 1 year. These findings contribute to possibilities for genetic counseling and prenatal diagnosis of psychomotor retardation mitochondrial diseases.

AUTHOR CONTRIBUTIONS

Forough Shabannejadian: Investigation; writing – original draft. **Seyedeh Zahra Masoomizadeh:** Investigation; supervision; validation. **Behnaz Andashti:** Methodology; project administration; resources; supervision; validation; visualization; writing – review and editing.

ACKNOWLEDGMENTS

The authors thank the patient and her family for participation in this study.

CONFLICT OF INTEREST STATEMENT

None.

FUNDING INFORMATION

This research was done with personal funding of authors.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

CONSENT

Written informed consent was obtained from the parents of patient to publish this report in accordance with the journal's patient consent policy.

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How to cite this article: Shabannejadian F, Masoomizadeh SZ, Andashti B. Molecular analysis of gene variants in an Iranian family with psychomotor retardation mitochondrial disorder patient. *Clin Case Rep*. 2023;11:e7308. doi:10.1002/ccr3.7308