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Investigation of *TMEM70* Gene Mutations Involved in Mitochondrial ATP Synthesis Pathway in Two Khuzestan Families

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Abstract

Background: Mitochondrial complex V deficiency refers to a shortage (deficiency) of a protein complex called complex V or a loss of its function. The *TMEM70* gene provides the blueprint for mitochondrial ATP synthase, a protein essential for cellular energy production through oxidative phosphorylation. The diagnostic method based on whole-exome sequencing (WES) provides more appropriate genetic counseling by saving time and money.

Methods: This study investigated three patients presenting with similar clinical symptoms using WES. After DNA extraction, WES was performed, and a novel mutation (c.311T > G: p.V104G) in the *TMEM70* gene was identified. Sanger sequencing was used to confirm the variant in the parents.

Results: The novel *TMEM70* mutation (c.311T > G: p.V104G) was identified as a potential cause of the disease in these patients.

Conclusions: This study highlights the utility of WES in the rapid and cost-effective identification of pathogenic variants associated with mitochondrial disorders. The identification of a novel *TMEM70* mutation underscores the importance of this gene in mitochondrial function and further emphasizes the need for comprehensive genetic screening in individuals presenting with clinical symptoms consistent with mitochondrial ATP synthase deficiency. Further research is needed to elucidate the specific functional consequences of this mutation and to investigate the prevalence of *TMEM70* mutations in various populations.

Keywords: *TMEM70* Gene, ATP Synthase, Oxidative Phosphorylation, Whole-Exome Sequencing

1. Background

Mitochondrial complex V deficiency, nuclear type 2, is a rare, inherited condition that disrupts the production of ATP, the cell's energy currency, by impairing the function of mitochondrial ATP synthase (1). This crucial process is essential for energy generation in all cells, particularly in tissues with high energy demands such as the brain, heart, and skeletal muscles. The nuclear type 2 designation indicates that the genetic defect responsible for the deficiency resides within the nuclear genome, rather than the mitochondrial genome (2).

While the exact prevalence of mitochondrial complex V deficiency, nuclear type 2, is unknown, it is estimated to be extremely low (1). This disorder can lead to a wide range of symptoms, from mild developmental delays to severe, multi-systemic complications that can be fatal. The clinical presentation is highly variable and depends on the severity of the ATP synthase deficiency, the specific tissues affected, and the underlying genetic mutation (3).

The *TMEM70* gene, located on chromosome 19, encodes a transmembrane protein that has garnered increasing attention in the field of mitochondrial research (4). Despite its enigmatic function, accumulating evidence suggests a critical role for *TMEM70* in maintaining mitochondrial integrity and function, with implications for a range of human health conditions (5). *TMEM70* exhibits a predominantly mitochondrial localization, with its protein product residing primarily within the inner mitochondrial membrane. This strategic positioning within the heart of cellular energy production, oxidative phosphorylation (OXPHOS), suggests a potential involvement in crucial aspects of mitochondrial

biogenesis, dynamics, or the regulation of critical metabolic pathways (6, 7). However, the precise molecular mechanisms by which *TMEM70* exerts its influence remain to be fully elucidated.

Mutations in the *TMEM70* gene have been linked to a spectrum of human disorders, primarily characterized by disruptions in mitochondrial function and cellular energy production (8). These include a range of clinically diverse phenotypes, encompassing: Mitochondrial complex I deficiency: Mutations in *TMEM70* have been implicated in deficiencies of mitochondrial complex I, a critical enzyme in the electron transport chain (ETC) responsible for ATP synthesis (9). Leigh Syndrome: Mutations have been reported in patients with Leigh syndrome, a severe neurodegenerative disorder characterized by mitochondrial dysfunction (10, 11). Other mitochondrial disorders: *TMEM70* mutations have also been associated with various other mitochondrial disorders, including cardiomyopathy, encephalopathy, and myopathy (12).

Exome sequencing offers a powerful tool for elucidating the genetic underpinnings of mitochondrial disorders. Through comprehensive exome analysis, we can identify pathogenic mutations responsible for these debilitating conditions. Furthermore, carrier screening through exome testing allows for the identification of individuals harboring these mutations, facilitating genetic counseling and preventative measures to mitigate the transmission of these deleterious alleles to subsequent generations. Extensive research has demonstrated the efficacy of exome sequencing panels encompassing genes associated with mitochondrial diseases, enabling accurate, expeditious, and cost-effective molecular diagnoses. This advancement represents a significant step forward in our understanding of the genetic complexities of sensory-neural disorders, facilitating more precise and timely diagnoses with greater accuracy.

2. Methods

This cross-sectional study investigated a family with mitochondrial disorders who were referred to the Noorgen Medical Genetics Laboratory in Ahvaz during the period of 1401 - 1402. The criteria for inclusion in the study included symptoms of mitochondrial complex V deficiency, such as weakness and cardiopulmonary distress, which were confirmed by a specialist physician. The patient's consent to participate in the research was also obtained.

2.1. Case Presentation

In this study, three patients from different families were investigated. The first patient was a 1-month-old boy presenting with various symptoms, including weakness, lethargy, heart problems, lung issues, and suspected metabolic diseases. Genealogical examination revealed that the patient's uncle had hearing problems, and his grandmother was also suffering from leukemia.

The second patient was a 12-month-old boy from a consanguineous marriage, exhibiting symptoms such as heart and lung problems. His older brother also suffered from similar symptoms, and the patient's uncle displayed the same disorder (Figure 1).

2.2. Participant Recruitment and Genetic Analysis

Study participants were recruited based on clinically confirmed manifestations and imaging findings, as determined by a specialist physician. Informed consent was obtained from all participants prior to enrollment.

2.3. Sample Collection and DNA Extraction

Genomic DNA was extracted from 5 ml peripheral blood samples using the salting-out method. The quality and quantity of the extracted DNA were assessed using Nanodrop and agarose gel electrophoresis.

2.4. Whole-Exome Sequencing and Variant Analysis

Whole-exome sequencing (WES) was performed using the Illumina HiSeq 2500 platform. Sequencing data were analyzed using standard bioinformatic pipelines (Figure 2). The minimum quality score and minimum read depth were set at 30 and 10x, respectively, with the minor allele frequency (MAF) kept below 0.01 to ensure the variants were rare. In this study, a depth of 32 and a coverage of 35 were considered. Sanger sequencing was then used to validate the identified variants. The primers used for Sanger sequencing are listed in Table 1.

2.5. PCR and Sequencing Confirmation

PCR amplification was conducted in a 20 µL reaction volume, using Red Mix solution, forward and reverse primers, double-distilled water, and extracted DNA. The amplified products were visualized using

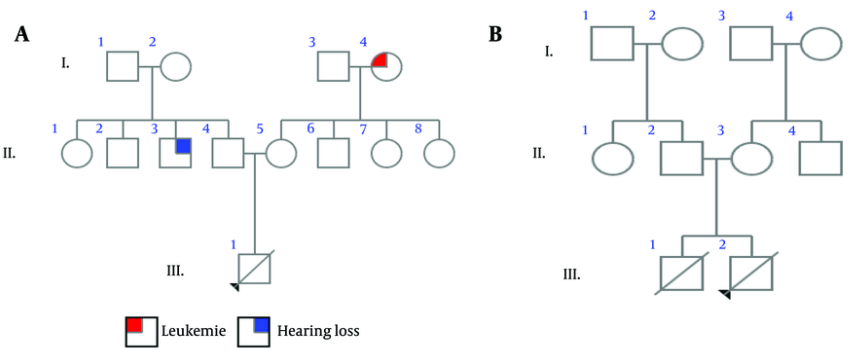

Figure 1. Family trees related to *TMEM70* gene

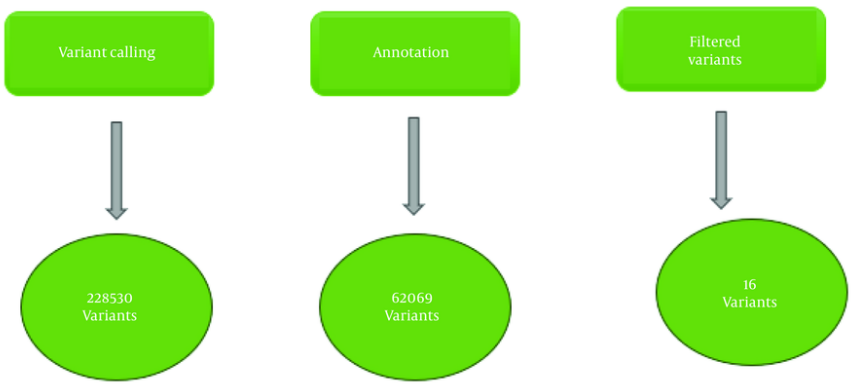

Figure 2. All stages of variant call, annotation and filtering and the number of variants in each step

electrophoresis (Figure 2) and confirmed using the ABI 3130XI sequencer and Chromas software.

2.6. Variant Annotation and Pathogenicity

Identified variants were cross-referenced against the ENSEMBL and Blast NCBI databases. The potential pathogenic effects of the identified mutations were further evaluated using PolyPhen2, MutationTaster, SIFT, and PredictSNP software.

3. Results

This research focused on two families from Khuzestan province whose children presented with mitochondrial disorders. After genetic counseling and reviewing the family history, the identified genetic variants were investigated (Figure 1). Electrophoretic analysis of the PCR product generated from the amplification of exon 2 of the *TMEM70* gene (approximately 500 bp in length) was performed on a 1.7% agarose gel, using a 100 bp DNA ladder as a size marker (Figure 3).

Through a comprehensive analysis of whole exome sequencing data, including primary, secondary, and tertiary analyses, a deleterious variant, NM_001040613: Exon2: c.T311G: p.V104G, was identified on chromosome 8, locus q21.11. This variant was identified based on its association with known candidate genes for bone genetic disorders. This change could be caused by a

Table 1. Sequence of Primers Used for PCR Reaction of *TMEM70* Gene

Primer	Sequence	Tm (°C)	Length (bp)
Forward	5' GTTGCAATGGTGAGCTGAGA 3'	60.0	20
Reverse	5' GGAGGCGAAGAGTATGGTGA 3'	60.2	20

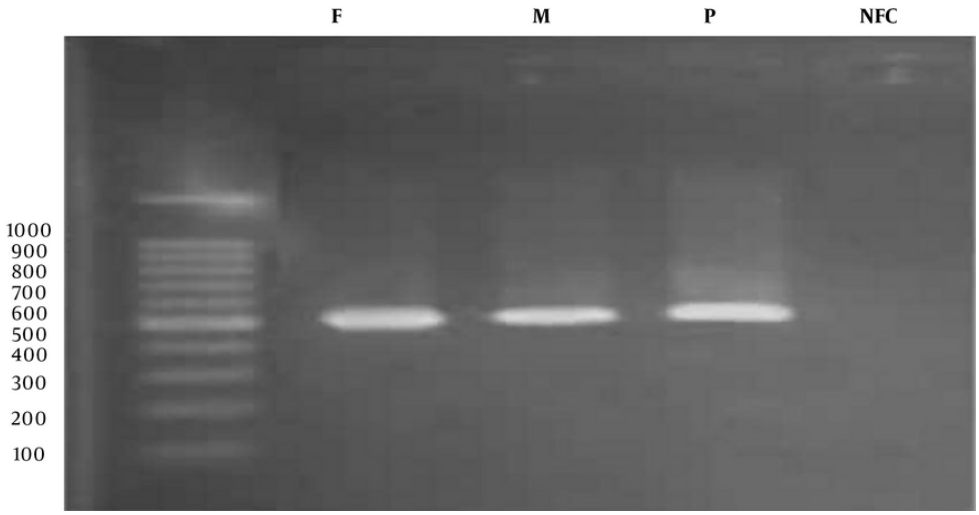

Figure 3. The results of the bands obtained from the electrophoresis of the PCR product resulting from the amplification of exon number 2 of the *TMEM70* gene with a length of 500 bp

homozygous or heterozygous mutation in the *TMEM70* gene on chromosome 8q21.11.

Sequence analysis revealed consistent nucleotide sequences across all samples, with variations observed solely at codon 311. The wild-type gene sequence at this position harbors a T nucleotide, while the variant gene sequence exhibits a G nucleotide. Genotype determination was based on the nucleotide composition at codon 311: Homozygous wild-type (GG): Both haplotypes (maternal and paternal alleles) contain a T nucleotide at codon 311. Homozygous variant (TT): Both haplotypes contain a G nucleotide at codon 311. Heterozygous (TG): One haplotype contains a T nucleotide, and the other contains a G nucleotide.

Two families were identified with heterozygous parents (carriers) and homozygous affected children. This observation, supported by Sanger sequencing results, demonstrates the inheritance pattern of the *TMEM70* variant, with heterozygous parents transmitting the homozygous variant to their offspring (Figure 4).

4. Discussion

This study highlights the utility of WES in unraveling the genetic underpinnings of complex mitochondrial disorders, specifically focusing on mitochondrial complex V deficiency, nuclear type 2 (ATP synthase deficiency, nuclear type 2). *TMEM70* is an essential factor in the biogenesis and stabilization of ATP synthase (13, 14).

Our findings underscore the critical role of the *TMEM70* gene in the pathogenesis of this debilitating condition. The identification of a novel homozygous mutation in the *TMEM70* gene in two families, resulting in affected offspring, underscores the significant role of this gene in mitochondrial function and its potential implications for human health. The observed mutation, a T-to-G substitution at codon 311, has previously been linked to mitochondrial complex I deficiency,

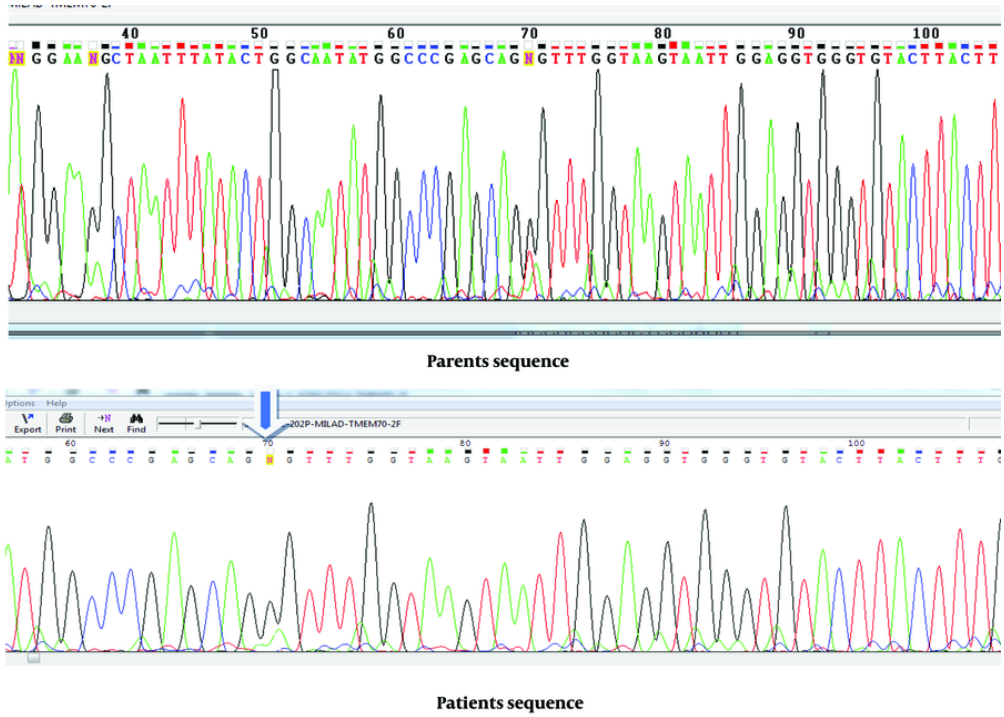

Figure 4. Sequencing results revealed a single nucleotide difference at codon 311, responsible for the observed heterozygosity in parents and homozygosity in affected children. All other regions and codon numbers were consistent across samples.

suggesting a complex interplay between *TMEM70* and the intricate machinery of OXPHOS. Further investigation into the functional consequences of this mutation is warranted to clarify its precise impact on ATP synthase function. The observation of consistent sequence variations exclusively at codon 311 across all samples highlights the potential significance of this specific region in the *TMEM70* gene and its susceptibility to mutation.

The utilization of WES as a diagnostic tool for mitochondrial disorders has proven to be highly effective in this study. The ability to identify pathogenic mutations rapidly and cost-effectively has significant implications for the clinical management of these diseases. Early diagnosis allows for informed genetic counseling and the potential implementation of preventative measures to mitigate disease transmission. Furthermore, the availability of comprehensive exome data provides a valuable resource for ongoing research efforts aimed at elucidating the underlying mechanisms of these disorders and developing novel therapeutic strategies.

Hirono et al. reported that multiple heterozygous mutations in the *TMEM70* gene were identified in a Japanese patient exhibiting a constellation of symptoms, including hyperlactatemia, metabolic acidosis, hyperalaninemia, growth delay, undescended testis, and left ventricular decompensation. Further analysis, including urine organic acid profiling, BN-PAGE/Western blotting, and ETC activity assays, confirmed a deficiency in mitochondrial complex V. These findings align with current research, reinforcing the crucial role of the *TMEM70* gene in disrupting mitochondrial complex V function (15).

Mackay et al. investigated *TMEM70* deficiency, a known cause of mitochondrial complex V deficiency, nuclear type 2 (MIM: 614052). This nuclear-encoded defect in ATP synthase is a common cause of a spectrum of clinical manifestations, often presenting with neonatal-onset symptoms such as poor feeding, hypotonia, lethargy, respiratory distress, heart failure,

lactic acidosis, and developmental abnormalities, including microcephaly and facial dysmorphisms. These findings further support the crucial role of the *TMEM70* gene in the pathogenesis of mitochondrial complex V deficiency and highlight the diverse clinical spectrum associated with this disorder (16).

This study contributes to the growing body of literature highlighting the importance of *TMEM70* in mitochondrial function and human disease. Further research is essential to fully elucidate the precise role of *TMEM70* in mitochondrial biogenesis, dynamics, and the regulation of OXPHOS. This includes investigating the specific impact of the identified mutation on ATP synthase function and exploring potential therapeutic interventions targeting this gene or associated pathways. The increasing availability of WES and the growing understanding of the genetic basis of mitochondrial disorders hold great promise for the development of personalized medicine approaches to diagnose and manage these debilitating conditions.

4.1. Conclusions

The successful application of WES as a diagnostic tool for this rare condition demonstrates its efficacy in uncovering the genetic basis of complex mitochondrial diseases. The ability to rapidly identify pathogenic mutations through WES enables timely diagnoses, facilitates informed genetic counseling, and opens opportunities for preventative measures to mitigate disease transmission.

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Footnotes

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