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Joubert syndrome caused by a *TMEM67* mutation: Genotype-phenotype analysis

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Abstract

Joubert syndrome (JS), a rare neurodevelopmental disorder, is characterized by a unique midbrain-hindbrain malformation known as the molar tooth sign, a distinctive radiological feature. Among the myriad manifestations associated with JS, the most prevalent features encompass hypotonia, ataxia, developmental delay, intellectual disability, abnormal eye movements, and neonatal breathing abnormalities. To date, 29 genes have been identified as contributors to the development of JS. This study aimed to pinpoint a pathogenic variant in JS within an Iranian consanguineous pedigree. We employed whole-exome sequencing (WES) to pinpoint a potential pathogenic variant likely responsible for the condition in an 8-year-old male patient. The WES analysis successfully revealed a novel homozygous missense mutation (c.725A>T; p.Asn242Ile) within exon 8 (NM_153704.6) of the *TMEM67* gene. Subsequent validation through Sanger sequencing confirmed the presence of the chr8-93780603A>T mutation. This study elucidated JS within an Iranian family, uncovering a new *TMEM67* gene mutation through comprehensive genetic analysis. The identification of this mutation enhances our understanding of the genetic landscape of JS, contributing valuable insights for both clinical diagnosis and future research endeavors.

Keywords: Joubert syndrome, *TMEM67* gene, mutation

INTRODUCTION

Joubert syndrome (JS, MIM 213300) stands as a rare and intricately heterogeneous neurodevelopmental condition, exhibiting patterns of autosomal recessive or X-linked inheritance. This syndrome is attributed to mutations identified in a comprehensive set of at least 29 genes, each contributing to the encoding of structural or functional elements crucial to the primary cilium.[1] JS is characterized by a distinctive cerebellar and brainstem malformation, identifiable on brain imaging as the distinct molar tooth sign, which serves as its diagnostic hallmark.[1-3] Neurological symptoms manifest during the neonatal period and encompass a spectrum from hypotonia evolving into ataxia, global developmental delay, to eye movement anomalies such as nystagmus and ocular motor apraxia. Additionally, individuals with JS may experience breathing dysregulation.[2-6] The spectrum of JS-related disorders is further characterized by variable features, including but not limited to retinal dystrophy, hepatic fibrosis, polycystic kidneys, and polydactyly. These additional clinical manifestations contribute to the heterogeneity observed within the JS phenotype, making it challenging to establish precise and universally applicable clinical diagnostic criteria for the syndrome.[1,4-6]

JS is thought to have a prevalence ranging from 1 in 80,000 to 1 in 100,000 live births on a global scale. Despite the extensive understanding of this prevalence on a worldwide scale, the occurrence of JS-related disorders remains less defined in several Middle Eastern countries, including Iran. The unique demographic and genetic landscape of the Middle East, characterized by a higher prevalence

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of consanguineous marriages, contributes to the likelihood of an increased incidence of JS in these populations.[7]

TMEM67 stands out as one of the most frequently mutated genes associated with JS in patients of northern European and Japanese descent. Nevertheless, a notable divergence emerges when considering the Middle Eastern and North African Arab populations, where, as per existing data, *TMEM67* does not emerge as the predominant causative factor for JS.[8-11] *TMEM67* (MKS3) mutations give rise to a spectrum of human ciliopathies, encompassing conditions such as COACH syndrome (MIM 216360), JS (MIM 610688), Meckel-Gruber syndrome (MIM 607361), and nephronophthisis (NPHP, MIM 613550). Additionally, *TMEM67* serves a noteworthy role in Bardet-Biedl syndrome, functioning as a modifier gene (MIM 615991).[12-16] MKS3, JS6, COACH, and NPHP11 collectively constitute a broad spectrum of allelic disorders arising from biallelic mutations in *TMEM67*. Notably, there exists no discernible correlation between the specific mutations in *TMEM67* and the resultant clinical outcomes across these diverse disorders.[17] However, mutations in the *TMEM67* subtype of JS are commonly linked to liver fibrosis.[12]

TMEM67, a critical genetic determinant, plays a pivotal role in the synthesis of meckelin, an intricate transmembrane protein 67. This protein, with a considerable length of 995 amino acids, exhibits a sophisticated structural arrangement. Meckelin's configuration involves a transmembrane orientation, where its extracellular N-terminus stands out for its complexity, incorporating a signal peptide and a cysteine-rich domain. This elaborate molecular architecture highlights the intricacies involved in the formation of meckelin, underscoring the significance of *TMEM67* in governing the synthesis of this multifaceted transmembrane protein.[18] This protein is essential for the structural integrity and proper functioning of cilia. In this study, we present the discovery of a previously unidentified mutation (chr8-93780603A>T) in the *TMEM67* gene within an Iranian family affected by JS.

CASE REPORT

An 8-year-old male patient (Figure 1) was referred to the Noor-Gene Genetic Laboratory (Ahvaz, Iran), seeking evaluation and guidance regarding visual impairment and physical weakness. The patient's birth history was unremarkable, with no reported complications during pregnancy or delivery. Neonatal symptoms, however, included hypotonia, which was noted soon after birth. Developmental milestones were significantly delayed, with psychomotor skills lagging behind age-appropriate expectations. The patient exhibited hypotonia persisting into childhood, along with challenges in controlling voluntary muscle movements. Notably, ataxia was evident, affecting activities such as walking, picking objects, and speaking. Speech development was notably delayed, contributing to communication challenges. Physical weakness was observed, impacting the patient's ability to engage in age-appropriate activities. Growth parameters were within normal limits for age, but the overall growth trajectory was affected due to

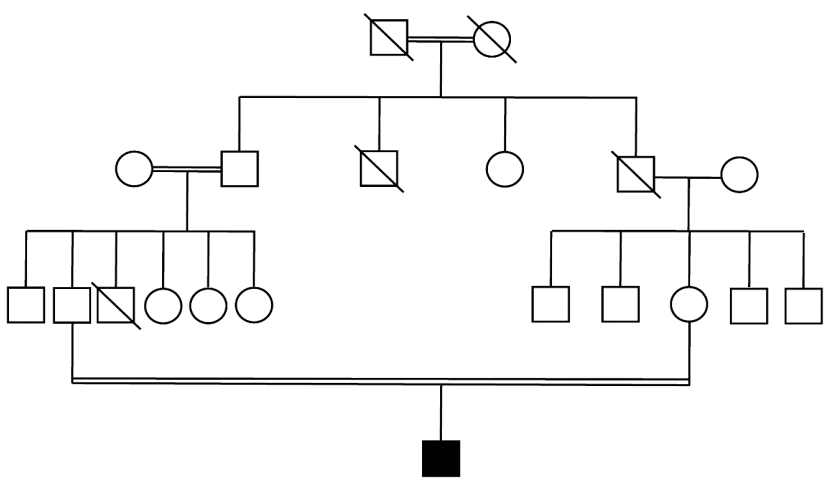

Figure 1. The pedigree of the studied family. Symbols marked with a slash indicate deceased individuals. Squares represent males, circles represent females, and the blackened symbol denotes the proband.

the limitations imposed by motor challenges. Hepatic dysfunction was noted, prompting a comprehensive investigation into liver function. Further evaluation included liver function tests, which revealed abnormalities consistent with the patient's hepatic challenges. Given the complexity of the presentation, a brain magnetic resonance imaging (MRI) was conducted, revealing features consistent with JS, as evidenced by the presence of the characteristic MTS (Figure 2A).

JS is a rare genetic disorder characterized by cerebellar vermis hypoplasia, leading to neurological and developmental abnormalities. The patient's clinical progression has been marked by ongoing challenges in motor coordination, speech development, and hepatic function. Despite these difficulties, the child's parents maintain good health, emphasizing the likely genetic nature of the condition.

Blood samples were collected from the patient and his parents, following the acquisition of informed consent. Subsequently, in adherence to established protocols, DNA extraction was carried out from the buffy coat utilizing the FAVORGEN kit (Biotech Corp, Cat. No.: FABGK 001, Taiwan).

Comprehensive whole-exome sequencing by Macrogen (Seoul, South Korea) was undertaken with a specific focus on genes associated with JS. The deliberate emphasis on JS genes in the WES aimed to uncover potential genetic variations and mutations contributing to the manifestation of this neurodevelopmental disorder. The analysis revealed a novel single homozygous mutation in the affected son, specifically identified as a missense *TMEM67* gene mutation (c.725A>T; p.Asn242Ile) located in exon 8 (NM_153704.6) of chromosome 8q. Furthermore, through the application of advanced in silico pathogenicity prediction tools, as outlined in Table 1, a meticulous examination of the identified mutation suggests its likely pathogenic nature in this disease. According to the American College of Medical Genetics and Genomics (ACMG) guidelines, the N242I variant is classified as likely pathogenic.

Finally, the Sanger sequencing (ABI 3130 Genetic Analyzer, California, USA) of coding exons confirmed the presence of this mutation, predicting a consequential alteration in codon translation, resulting in the conversion of asparagine to isoleucine. This transformation was identified as homozygous in the patient and heterozygous in his parents (Figure 2B). Importantly, the control specimen analysis did not reveal any nonspecific bands or contamination, ensuring the reliability of the identified mutation.

Asparagine and isoleucine differ significantly in their chemical properties. Asparagine is a polar, hydrophilic amino acid, while isoleucine is nonpolar and hydrophobic. This change in polarity and hydrophobicity can disrupt the normal folding of the *TMEM67* protein. Protein folding is a critical process that determines the three-dimensional structure of the protein, and this structure is essential for the protein to carry out its biological functions. Furthermore, according to information documented in the Human Gene Mutation Database (HGMD, https://www.hgmd.cf.ac.uk/ac/index.php), with specific mutations highlighted in Table 2, there is a discernible pattern indicating a higher prevalence of JS in connection with mutations in the *TMEM67* gene.

DISCUSSION

JS, akin to numerous other ciliopathies, presents a significant spectrum of clinical features and molecular complexities. Notably, manifestations such as retinal dystrophy, renal disease, and hepatic fibrosis may not become apparent until the second or third decades of life. The application of genetic testing proves invaluable in achieving a precise diagnosis, eliminating the need for unnecessary and costly examinations. Securing a molecular diagnosis of JS assumes critical importance for genetic counseling and gaining a comprehensive understanding of the disease's prognosis. In our study, we employed homozygosity mapping in a consanguineous family affected by JS. The 8-year-old male patient exhibited eye movement abnormalities, hepatic dysfunction, hypotonia, psychomotor delay, and impaired language skills. Given the extensive heterogeneity of genes and mutations

Table 1: Pathogenicity assessment of the new variant identified by WES

Gene	Variant	Polyphen-2 HDIV score	CADD score	SIFT score	LRT score	MutPred score	FATHMM-MKL score	MVP score	M-CAP score
TMEM67	N242I	1.000 (probably damaging)	26.0 (deleterious)	0 (pathogenic supporting)	0 (pathogenic supporting)	0.628 (pathogenic supporting)	0.9981 (pathogenic moderate)	0.9996 (pathogenic moderate)	0.6819 (pathogenic moderate)

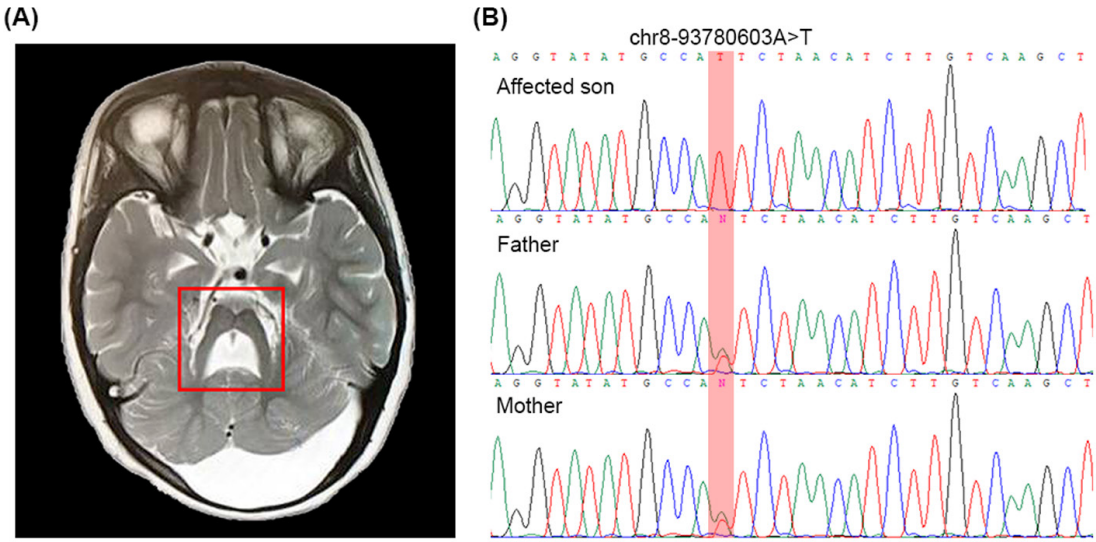

Figure 2. (A) MRI displaying the characteristic MTS, a diagnostic hallmark seen in conditions like JS. (B) The genetic analysis reveals results for the proband and his parents. Sanger sequencing for the proband uncovered a novel homozygous c.725A>T; p.Asn242Ile mutation in exon 8 of the *TMEM67* gene and the detected mutation is present in a heterozygous state in both of his parents.

Table 2: Reported mutations in the *TMEM67* gene

HGVS coding	HGVS Protein	Location	Coding impact	Phenotype
c.42G>A	p.Trp14Ter	Exon 1	Nonsense	Joubert syndrome
c.130C>T	p.Gln44Ter	Exon 1	Nonsense	Joubert syndrome
c.270T>G	p.Asn90Lys	Exon 2	Missense	Joubert syndrome
c.329A>G	p.Asp110Gly	Exon 3	Missense	Joubert syndrome
c.370G>A	p.Glu124Lys	Exon 3	Missense	Joubert syndrome
c.395G>C	p.Gly132Ala	Exon 3	Missense	Joubert syndrome
c.442G>T	p.Ala148Ser	Exon 4	Missense	Joubert syndrome
c.475T>C	p.Ser159Pro	Exon 4	Missense	Joubert syndrome
c.637C>T	p.Arg213Cys	Exon 6	Missense	Joubert syndrome
c.722C>G	p.Ala241Gly	Exon 8	Missense	Joubert syndrome
c.730A>G	p.Thr244Ala	Exon 8	Missense	Joubert syndrome
c.739C>G	p.Gln247Glu	Exon 8	Missense	Joubert syndrome
c.797A>C	p.Asp266Ala	Exon 8	Missense	Joubert syndrome
c.903C>G	p.Asp301Glu	Exon 9	Missense	Joubert syndrome
c.950C>G	p.Thr317Arg	Exon 9	Missense	Joubert syndrome
c.986A>C	p.Lys329Thr	Exon 10	Missense	Joubert syndrome
c.1285C>T	p.Gln429Ter	Exon 12	Nonsense	Joubert syndrome
c.1387C>T	p.Arg463Ter	Exon 13	Nonsense	Joubert syndrome
c.1538A>G	p.Tyr513Cys	Exon 15	Missense	Joubert syndrome
c.1634G>A	p.Gly545Glu	Exon 16	Missense	Joubert syndrome
c.1706G>A	p.Gly569Asp	Exon 17	Missense	Joubert syndrome
c.1847C>T	p.Ala616Val	Exon 18	Missense	Joubert syndrome
c.1888T>C	p.Ser630Pro	Exon 19	Missense	Joubert syndrome

associated with JS-related disorders, initiating next-generation sequencing, encompassing whole-exome and gene panel methodologies, becomes imperative for a thorough molecular diagnosis. It is noteworthy that homozygosity mapping emerges as an exceptionally potent and cost-effective tool for pinpointing disease-causing genes, particularly within the context of genetically heterogeneous conditions observed in consanguineous families.[19,20]

It's important to note that JS is a complex disorder with multiple genetic contributors, and mutations in other genes may also be involved in its development.[1] However, understanding the specific impact of the *TMEM67* gene mutation, such as the conversion of asparagine to isoleucine, (c.725A>T; p.Asn242Ile) provides insights into the molecular mechanisms underlying this rare neurological condition.

There is a notable association between mutations occurring in specific genes and distinct subtypes of JS. The most pertinent correlation between genotype and phenotype has been established with *TMEM67*, particularly in relation to JS subtypes that manifest with liver disease. Significantly, pathogenic variations in *TMEM67* contribute to around 80% of all JS-related disorders characterized by liver involvement.[12] The patient in this study exhibited hepatic dysfunction. Although hepatic fibrosis typically advances progressively, it seldom presents symptoms at birth. Consequently, annual monitoring of hepatic function is advisable for this patient. Considering the strong correlation between NPHP and *TMEM67*-related JS, it is imperative for regular assessments of kidney function to be conducted on an annual basis for this individual.[19]

TMEM67 sequence variations exhibit a broad clinical spectrum, with the distribution of sequence variants spanning the entire coding region. Notably, the anticipation of specific phenotypes is possible, particularly when considering *TMEM67* missense variants occurring in exons 8 to 15. The presence of these missense variants, especially in conjunction with a truncating variant, is indicative of a potential association with Meckel-Gruber syndrome. Furthermore, a significant concentration of *TMEM67* sequence variants is observed in 8 specific exons out of the total 28 (2, 6, 8, 11, 13, 15, 18, 24).[21,22] Upon reviewing previously documented *TMEM67* sequence variations in the medical literature, our report identifies a specific mutational hotspot (*TMEM67*: c.725A>T; p.Asn242Ile) consistent with the findings reported by Lannicelli *et al.*[23] exon 8 emerges as the most frequently mutated hotspot, followed by exons 24, 18, 6, 13, 11, 2, and 15. This underscores the recurrent nature of mutations within the *TMEM67* gene, providing valuable insights into its mutational landscape.

In the course of our extensive patient assessment, MRI emerged as a crucial diagnostic tool, unveiling a significant and distinctive finding in the form of the pathognomonic molar tooth sign. This particular radiological manifestation, characterized by a unique configuration resembling a molar tooth, stands out as a key identifier. Its presence not only underscores the complexity of the diagnostic process but, more importantly, serves as a conclusive and irrefutable confirmation of the underlying JS diagnosis.[2,3]

Lastly, our patient's clinical presentation aligns with a confirmed diagnosis of JS, characterized by the distinctive molar tooth sign evident in imaging studies. The observed clinical features encompass a spectrum of abnormalities, including eye movement irregularities, hepatic dysfunction, hypotonia, psychomotor delay, and impaired language skills, indicative of the multisystemic nature of JS. Notably, our genetic analysis uncovered a novel chr8-93780603A>T mutation in the *TMEM67* gene, shedding light on the underlying genetic basis of the condition. This discovery not only expands our understanding of the genetic landscape associated with JS but also underscores the importance of genetic testing in elucidating specific causative factors.

In conclusion, our identification of a novel *TMEM67* gene mutation (c.725A>T; p.Asn242Ile) in an Iranian patient diagnosed with JS holds pivotal implications. The ancestral inheritance of this mutation offers essential insights for genetic counseling, guiding decisions in subsequent pregnancies within affected families. Beyond its immediate clinical impact, this discovery contributes significantly to our comprehension of the genetic landscape of JS, fostering advancements in both diagnosis and future research endeavors.

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DISCLOSURE

Ethics: Written informed consent was obtained from the family members for this publication.

Date availability: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Conflict of interest: None

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