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# Clinical and genetic findings in an Iraqi family with *TMEM67*-associated Joubert syndrome

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### *Abstract*

Joubert syndrome is a rare, genetically complex disorder that significantly affects brain development, characterized by hallmark features such as hypotonia, developmental and motor delays, and the distinctive molar tooth sign on brain MRI. The disorder frequently arises from mutations in genes essential for ciliary function, crucial for cellular signaling and development across multiple organ systems. *TMEM67*, one of these genes, is particularly associated with cases involving multi-organ dysfunction, including kidney and liver abnormalities. We report a novel homozygous mutation in *TMEM67* (c.797A>T; p.Asp266Val) in a 7-year-old male from a consanguineous Iraqi family, who presented with classic neurological symptoms of Joubert syndrome and mild hepatic dysfunction. The mutation, located in exon 8 (NM_153704.6), substitutes aspartic acid with valine, likely disrupting protein structural integrity and function through the loss of polar charge. Using exome and Sanger sequencing, we confirmed this pathogenic variant, emphasizing the necessity of comprehensive genetic analysis for accurate diagnosis and management, especially in populations with high consanguinity rates. Our findings expand the mutational spectrum of *TMEM67* and highlight potential mutation hotspots related to severe multi-organ involvement. This study enriches the *TMEM67* variant database and stresses the importance of genetic counseling for guiding patient care and reproductive decisions.

*Keywords:* exome-sequencing, Joubert syndrome, mutation, *TMEM67* gene

## INTRODUCTION

Joubert syndrome (JS) is a rare, genetically complex disorder that primarily impacts brain development, specifically affecting the cerebellum and brainstem, which play central roles in motor control, balance, and cognitive function. The condition was first described in 1969, and one of its hallmark features is a characteristic molar tooth sign (MTS) seen on brain MRI scans, resulting from abnormalities in the brainstem and cerebellar peduncles.[1,2] Clinically, JS presents with a range of symptoms, including low muscle tone, irregular breathing patterns, abnormal eye movements, and developmental delays. However, the syndrome can extend beyond neurological symptoms to involve multiple organs, including the kidneys, liver, and retina, making it a highly variable disorder.[3,4]

The clinical presentation of JS is often classified based on which organs are involved in addition to the brain. These classifications—such as JS with renal involvement, hepatic involvement, or retinal defects—help clinicians manage and monitor associated risks specific to each type. For example, individuals with JS who also have retinal involvement may experience progressive vision loss, while those with renal involvement are at risk for kidney disease, which may necessitate regular kidney function monitoring. This clinical diversity

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underscores the importance of personalized care and highlights the complex genetic underpinnings of the disorder.[1,4]

Genetically, JS is primarily caused by mutations in genes responsible for the development and function of cilia, hair-like structures on the surface of cells that play crucial roles in cellular signaling and tissue formation. These cilia are integral to multiple signaling pathways during development, particularly in tissues where cell signaling and movement are essential, such as in the brain, liver, kidneys, and retina. Disruptions in ciliopathy genes—genes that contribute to cilia formation and function—are at the core of JS. Over 30 genes are now associated with the syndrome, with specific genes such as *AHI1, CEP290, CC2D2A, and TMEM67* playing significant roles in cases with particular organ system involvements. For example, *CEP290* mutations are often found in cases with both severe neurological symptoms and renal complications, illustrating how certain genes are linked to specific symptom patterns.[1,3,5,6]

Among these key genes, *TMEM67* has emerged as especially significant for its association with JS cases involving both neurological features and multi-organ complications, particularly those affecting the kidneys and liver. *TMEM67* encodes a protein crucial for maintaining the structure and function of cilia, especially in organs impacted by JS.[3] Mutations in *TMEM67* often correlate with a more extensive and severe presentation of the syndrome, including higher risks of hepatic and renal complications alongside the classic neurological signs. By further examining the role of *TMEM67* and identifying specific mutations within this gene, researchers can better understand how these variations contribute to the broad range of symptoms and clinical types within JS.[3,6]

Exome-sequencing has emerged as a powerful tool for detecting mutations associated with genetic disorders, particularly in consanguineous families, where the likelihood of autosomal recessive conditions is heightened due to shared genetic background. By focusing on the coding regions of the genome, exome-sequencing allows for the efficient identification of pathogenic variants that may contribute to complex phenotypes, facilitating accurate diagnosis and management strategies. Previous studies have demonstrated the effectiveness of exome-sequencing in uncovering novel mutations in genetically heterogeneous conditions like JS, highlighting its role in refining the phenotypic spectrum and aiding in targeted genetic counseling for affected families.[3,8,9] In light of these findings, our study utilized exome-sequencing to investigate the genetic basis of JS in a consanguineous family, aiming to identify potentially pathogenic variants and contribute to the growing understanding of the genotype-phenotype correlation in this complex disorder.

## METHODS

### *Sample collection and DNA extraction*

With informed consent, blood samples (5 ml) were collected from the patient and his parents using EDTA tubes. Genomic DNA was extracted using the FAVORGEN DNA Extraction Kit (Biotech Corp, Cat. No.: FABGK 001, Taiwan), and DNA quality was confirmed via 1.5% agarose gel electrophoresis. Concentration and purity were measured using a Nanodrop ND-1000 spectrophotometer to ensure the DNA was suitable for further analysis.

### *Exome-sequencing*

Given the clinical suspicion of JS, exome-sequencing was performed to identify potential pathogenic variants. exome-sequencing was carried out at Macrogen (Seoul, South Korea) with a focus on JS-related genes. Pathogenicity of identified variants was assessed using bioinformatics tools, including including MutationTaster, CADD, PolyPhen-2, SIFT, FATHMM, and additional tools such as LRT, BayesDel, REVEL, MetaLR, MetaRNN, and MetaSVM.

### *Polymerase Chain Reaction (PCR)*

To validate the identified mutation, a PCR assay was optimized with primers flanking the variant site. The forward primer (F: TGGAACAGACCTGCACAAAG) and reverse primer (R: GCCAAGGAAGATTCTGTCTCC) were used to amplify a targeted DNA fragment. PCR was carried out in 25 µl volumes with 1× reaction buffer, 0.25 mM of each dNTP, 2 pmol primers, 0.4 µg DNA, and 1.5 U Taq DNA polymerase. The reaction included an initial denaturation at 95 °C for 5 minutes, followed by 35 cycles at 95 °C for 30 seconds, annealing at 60 °C for 30 seconds, and extension at 72 °C for 30 seconds, with a final extension at 72 °C for 2 minutes. Successful amplification was confirmed on a 2% agarose gel stained with ethidium bromide.

*Sanger sequencing*

Sanger sequencing was performed to confirm the identified mutation and assess its segregation within the family, including the proband and both parents. The same primers used for PCR amplification were employed for sequencing. Sequencing reactions were prepared using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The thermal cycling process consisted of an initial denaturation followed by multiple cycles of denaturation, annealing, and extension. After purification through ethanol precipitation, the sequencing products were analyzed using capillary electrophoresis on an Applied Biosystems 3130 Genetic Analyzer. The resulting sequences were aligned with the reference human genome using Chromas software (Technelysium Pty Ltd, South Brisbane, Australia) to evaluate the inheritance pattern of the mutation in the family.

## RESULTS

*Clinical finding*

A 7-year-old male from an Iraqi family (Figure 1) was referred to the Noor-Gene Genetic Laboratory in Ahvaz, Iran, due to developmental delays, motor coordination issues, and low muscle tone (hypotonia). The patient's parents are consanguineous, which increases the likelihood of an autosomal recessive genetic condition such as JS. Pregnancy and delivery history were unremarkable; however, hypotonia was noted in the neonatal period and persisted into childhood.

Developmental milestones were significantly delayed across motor and psychomotor domains. The patient achieved independent sitting at approximately 20 months, walking with assistance at 3 years, and unassisted ambulation by 4 years. However, his gait remains unsteady with wide-based and ataxic movements, which limits his ability to walk long distances without support. His highest functional level includes performing basic self-care tasks with assistance. Fine motor skills are markedly impaired; he struggles with tasks requiring precise hand movements, such as grasping small objects.

Liver function tests indicated mildly elevated serum transaminases (AST: 70 U/L, ALT: 85 U/L) and slightly elevated gamma-glutamyl transferase (GGT: 65 U/L), with normal bilirubin levels. An abdominal ultrasound revealed mild hepatomegaly with a coarse liver echotexture but no evidence of fibrosis or cirrhosis. These findings align with liver involvement sometimes observed in JS cases.

Ophthalmologic examination revealed strabismus (esotropia) and nystagmus, alongside photophobia. Fundoscopy was unremarkable, but visual tracking was impaired, and the patient had difficulty maintaining fixation on objects. Visual acuity testing indicated significant impairment, with reduced ability to follow moving targets, consistent with oculomotor apraxia often reported in JS.

The patient exhibited episodic hyperpnea (irregular breathing with rapid, shallow breaths) during infancy, a known feature in JS, though the frequency of these episodes has decreased over time. No central apneas were noted in recent

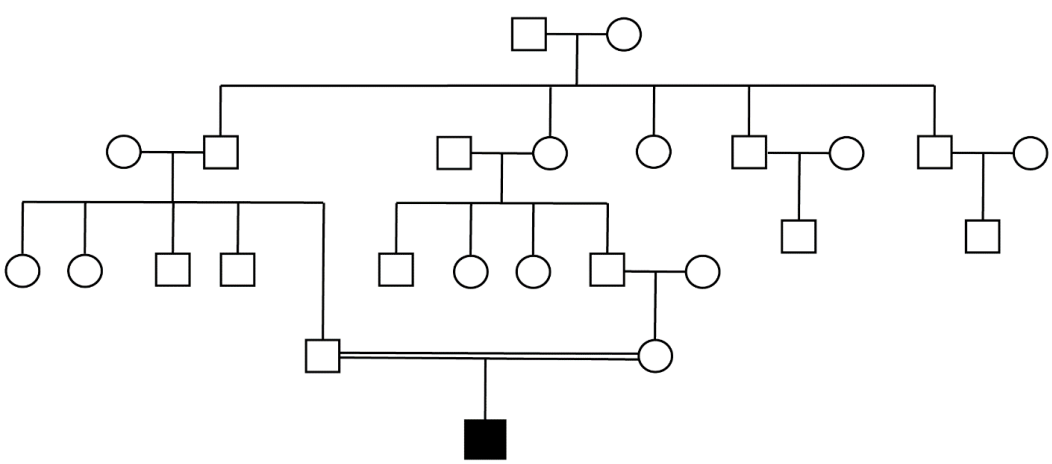

Figure 1. Pedigree of the studied family. Squares represent males, circles represent females, and the affected individual is shown with a filled symbol.

evaluations, and respiratory function tests were within normal limits for his age. Neurologically, deep tendon reflexes were generally hypoactive, particularly in the lower limbs. Examination also revealed truncal ataxia, dysmetria (inaccurate targeting of hand movements), and tremors during intentional movements.

Brain MRI findings revealed cerebellar vermis hypoplasia and the MTS, which is a hallmark radiologic feature of JS. These imaging results support the clinical diagnosis and provide insight into the extent of cerebellar involvement in this patient.

The patient's ongoing challenges include motor control difficulties, significant speech delays, visual impairment, and mild liver dysfunction. Despite the challenges, he has shown steady, though slow, progress in basic motor and self-care abilities. Both parents are in good health.

*Genetic study*

The genetic analysis revealed a novel single homozygous mutation in the affected patient, identified as c.797A>T; p.Asp266Val located in exon 8 (NM_153704.6) of the *TMEM67* gene on chromosome 8q, which has been associated with JS. In silico pathogenicity prediction tools were employed to assess the significance of this mutation, indicating its likely pathogenic nature, as detailed in Table 1. Following the guidelines set forth by the American College of Medical Genetics and Genomics (ACMG), the Asp266Val variant was classified as likely pathogenic.

Sanger sequencing confirmed the presence of this mutation, resulting in an aspartic acid-to-valine substitution at position 266. This alteration was homozygous in the patient, while both parents were heterozygous carriers (Figure 2). This variant has not been previously reported in other cases of JS.

*Functional impact of the Asp266Val Mutation in TMEM67*

Figure 4A presents a protein-protein interaction (PPI) network analysis of the *TMEM67* gene, sourced from the STRING database. The analysis shows that *TMEM67* interacts with other proteins essential for ciliary structure and function, such as MKS1, B9D1, CC2D2A, TCTN1, B9D2, NPHP1, TCTN2, TMEM216, TMEM231, and CEP290. *TMEM67* plays a key role in assembling and stabilizing the ciliary membrane, as well as in signal transduction within the ciliary complex. Disruptions in these interactions, as seen with mutations like Asp266Val, can impair the function of the entire ciliary apparatus, contributing to the clinical manifestations of JS.

The Asp266 residue is highly conserved across species (Figure 4B), emphasizing its functional importance in *TMEM67*. This residue is a charged, hydrophilic amino acid that likely contributes to the stability of *TMEM67* through electrostatic interactions with neighboring amino acids. The Asp266Val mutation substitutes this polar residue with valine, a nonpolar amino acid that lacks the capacity for electrostatic bonding. According to in silico predictions, this substitution may destabilize *TMEM67* by disrupting these crucial bonds, impairing its structural integrity and diminishing its interaction capability with binding partners in the ciliary protein complex.

**Table 1: Assessment of pathogenicity for chr8:93780675A>T variant identified by exome-sequencing.**

| Prediction Tool | Result | Score |
|---|---|---|
| PolyPhen-2 (HumDiv) | Probably damaging | 0.999 |
| SIFT | Damaging | 0.9125 |
| CADD | Damaging | 25.2 |
| FATHMM | Damaging | 0.9561 |
| LRT | Deleterious | 0.8433 |
| MutationTaster | Disease-causing | 0.99 |
| BayesDel addAF | Pathogenic strong | 0.5509 |
| BayesDel noAF | Pathogenic strong | 0.5536 |
| REVEL | Pathogenic strong | 0.978 |
| MetaLR | Pathogenic moderate | 0.9243 |
| MetaRNN | Pathogenic moderate | 0.9205 |
| MetaSVM | Pathogenic moderate | 1.0588 |

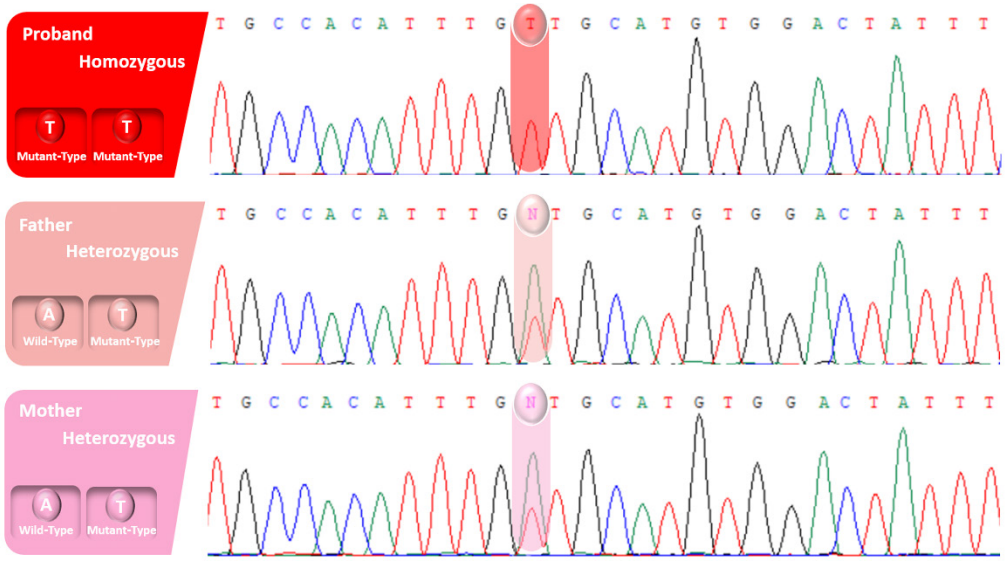


Figure 2. Sanger sequencing results validate the c.797A>T mutation in *TMEM67*, leading to a p.Asp266Val substitution. The mutation was found to be homozygous in the patient and heterozygous in both parents.

*Impact on TMEM67's interactions with ciliary proteins*

In the ciliary context, TMEM67 relies on a precise conformation to interact with other proteins involved in ciliogenesis and maintenance, such as MKS1 and CC2D2A. These proteins form part of a core ciliary signaling pathway, where TMEM67 acts as a structural and functional link. The Asp266Val mutation, through alterations in local charge and hydrophobicity, likely weakens TMEM67's ability to bind effectively with these partners. Reduced binding affinity may limit

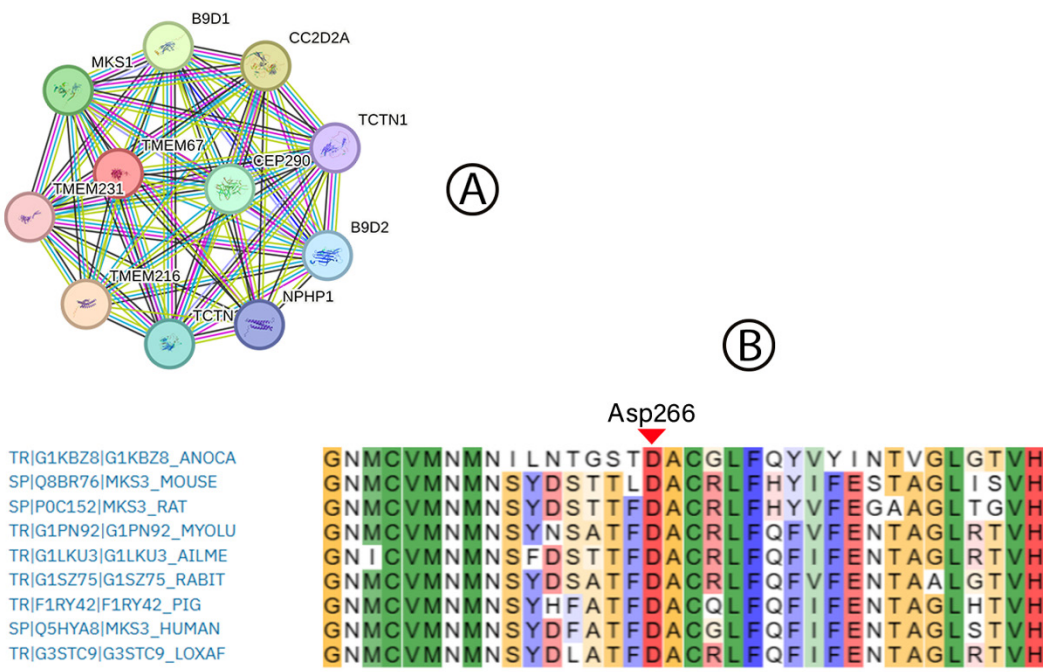


Figure 4. (A) PPI network analysis for the *TMEM67* gene, as determined using the STRING database, illustrating interactions with genes involved in ciliary function and signaling pathways. (B) Conservation analysis of the Asp266 residue across eight species, showing complete conservation, which underscores the functional importance of this amino acid position.

the assembly and function of the multiprotein complexes responsible for cilia formation and stability, ultimately affecting the integrity of the ciliary membrane and leading to ciliary dysfunction.

*Structural analysis of the Asp266Val mutation in TMEM67*

Figure 5 illustrates the predicted 3D structures of the wild-type (Asp266) and mutant (Val266) TMEM67 proteins. While the overall structure remains similar, the substitution of aspartic acid with valine introduces a nonpolar side chain in a region likely involved in stabilizing the ciliary protein interactions. This change is predicted to alter the binding surface, potentially disrupting *TMEM67*'s role as a scaffold protein within the ciliary complex. Reduced interaction stability within the ciliary network can compromise signaling and transport along the cilium, supporting the pathogenicity of the Asp266Val mutation as predicted by in silico analyses.

## DISCUSSION

In this study, we report a novel *TMEM67* mutation in a young male patient presenting with characteristic features of JS, emphasizing the importance of genetic assessment in consanguineous families with autosomal recessive conditions. This case highlights how the homozygous c.797A>T (p.Asp266Val) mutation in *TMEM67* contributes to the patient's clinical symptoms, such as motor coordination deficits, developmental delays, and visual impairment. *TMEM67* encodes a protein involved in ciliary function and cellular signaling pathways, and, as shown in Figure 5, from aspartic acid, a polar residue, to valine, a nonpolar residue, is likely to affect the structural stability and functional integrity of the TMEM67 protein. The loss of polar charge at this position may interfere with *TMEM67*'s role in cellular processes, particularly in ciliary function critical to neurological development.[10,11] This disruption aligns with the observed clinical manifestations in JS, including cerebellar hypoplasia, motor delays, and other neurological impairments.

Table 2 provides a summary of reported *TMEM67* variants associated with JS, revealing the wide range of genotype-phenotype correlations based on specific mutations. Our study identified the novel c.797A>T; p.Asp266Val variant, a missense mutation, which we found to be likely pathogenic based on in silico analysis and consistent clinical presentation. This variant aligns with similar *TMEM67* mutations, suggesting a broader pattern where changes at key residues lead to functional impairments that manifest in specific JS features. In our case, the Asp266Val substitution introduces a nonpolar valine in place of a polar aspartic acid, potentially altering local charge interactions within the protein and weakening its binding with other ciliary proteins. Such mutations have been linked to dysfunctions in ciliogenesis and signal transduction, contributing to JS's hallmark neurodevelopmental delays, motor coordination issues, and, in some cases,

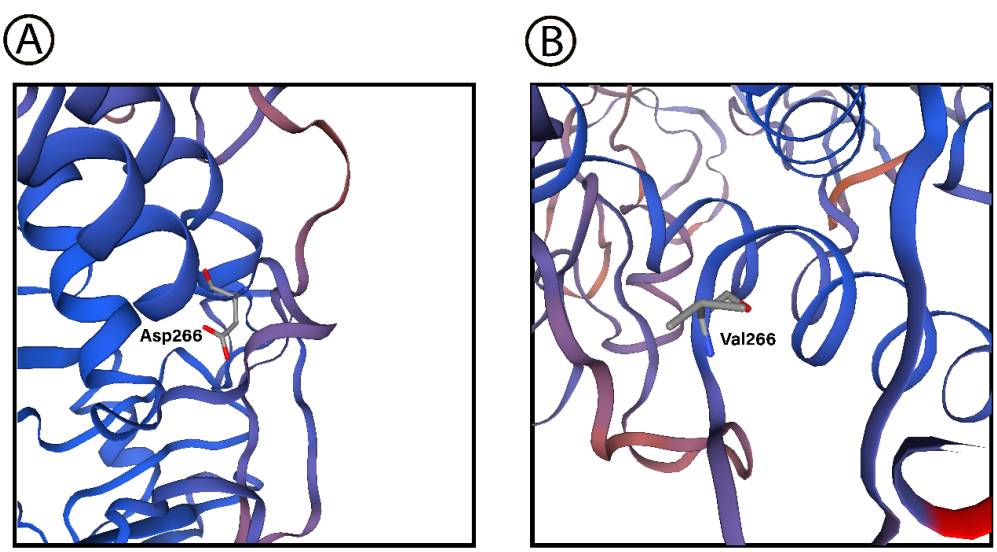


Figure 5. Predicted 3D structure of the TMEM67 protein for both wild-type (Asp266) and mutant (Val266) variants, modeled using the Swiss Model server.

**Table 2: Summary of reported *TMEM67* mutations**

| No. | Variant(s) | Protein Change(s) | Type of Mutation(s) | ACMG | Clinical Findings | Reference |
|---|---|---|---|---|---|---|
| 1 | c.725A>G | p.Asn242Ser | Missense | Pathogenic | MTS on MRI, hypotonia, nystagmus, retinal detachment, severe neurodevelopmental delay | Elsayed MEA *et al*.[13] |
| 2 | c.329A>G / c.2322+5delG | p.Asp110Gly / - | Compound heterozygous (Missense & splice site) | Likely pathogenic / VUS | MTS on MRI, neurological abnormalities, congenital hepatic fibrosis, hepatomegaly, portal hypertension | Tsurusaki Y *et al*.[16] |
| 3 | c.395G>C / c.312+5G>A | p.Gly132Ala / - | Compound heterozygous (Missense & splice site) | VUS / Likely pathogenic | MTS on MRI, moderate intellectual disability, severe developmental delay, hypotonia | Suzuki T *et al*.[17] |
| 4 | c.475T>C / c.755T>C | p.Ser159Pro; p.Met252Thr | Compound heterozygous (Missense & missense) | Likely pathogenic / Pathogenic | MTS on MRI, severe intellectual disability, severe developmental delay, hypotonia, ataxia | Suzuki T *et al*.[17] |
| 5 | c.517T>C / c.934T>C | p.Cys173Arg, p.Ser312Pro | Compound heterozygous (Missense & missense) | Likely pathogenic / VUS | MTS on MRI, developmental delay, hypotonia, hepatosplenomegaly, ocular colobomas, low platelet counts | Huynh JM *et al*.[15] |
| 6 | c.622A>T / c.1115C>A | p.Arg208* / p.Thr372Lys | Compound heterozygous (Nonsense & missense) | Pathogenic / Likely pathogenic | MTS on MRI, neurodevelopmental delay, liver involvement, eye abnormalities | Kroes HY *et al*.[12] |
| 7 | c.622A>T / c.2498T>C | p.Arg208* / p.Ile833Thr | Compound heterozygous (Nonsense & missense) | Pathogenic / Pathogenic | MTS on MRI, neurodevelopmental and liver abnormalities | Kroes HY *et al*.[12] |
| 8 | c.797A>C / c.2498T>C | p.Asp266Ala / p.Ile833Thr | Compound heterozygous (Missense & missense) | Likely pathogenic / Pathogenic | MTS on MRI, neurodevelopmental delay, ocular anomalies, liver involvement | Kroes HY *et al*.[12] |
| 9 | c.41G>A / c.2498T>C | p.Trp14Ter / p.Ile833Thr | Compound heterozygous (Nonsense & missense) | Likely pathogenic / Pathogenic | MTS on MRI, severe neurodevelopmental abnormalities, ocular anomalies, liver issues | Kroes HY *et al*.[12] |
| 10 | c.1843T>C / c.1927C>T | p.Cys615Arg / p.Arg643Ter | Compound heterozygous (Missense & nonsense) | Pathogenic / Pathogenic | cerebellar vermis agenesis, bat-wing fourth ventricle, MTS on MRI, developmental delay, hypotonia, visual impairment (rotatory nystagmus, strabismus) | Stembalska A *et al*.[14] |
| 11 | c.737G>A / c.1975C>T | p.Cys246Tyr / p.Arg659Ter | Compound heterozygous (Missense & missense) | Likely pathogenic / Pathogenic | Polycystic kidneys, occipital encephalocele, cardiac malposition, severe chest hypoplasia | Stembalska A *et al*.[14] |
| 12 | c.725A>G / c.313-3T>G | p.Asn242Ser / p.Lys105Valfs*16 | Compound heterozygous (Missense & splice site) | Pathogenic / VUS | MTS on MRI, developmental delay, renal hypoplasia | Bui TPH *et al*.[18] |

liver abnormalities. This finding is consistent with previously reported cases, such as Kroes HY' study of the p.Asp266Ala variant, where the change disrupts the stability of the ciliary complex, affecting motor development and leading to liver involvement.[12] Other variants in *TMEM67* show similar effects. For example, the c.725A>G; p.Asn242Ser mutation[13] is a missense mutation where the asparagine-to-serine substitution affects protein stability by altering hydrogen bonding within the ciliary membrane. Patients with this mutation typically present with hypotonia, nystagmus, and severe neurodevelopmental delays, along with the MTS on MRI, indicating that disruptions at this site compromise both ciliary function and neurodevelopmental processes. Similarly, Stembalska *et al*.[14] reported the c.1843T>C / c.1927C>T (p.Cys615Arg / p.Arg643Ter) mutations, where both changes impact ciliary protein function. The Cys615Arg substitution introduces a larger, charged arginine that disrupts the local structure, while Arg643Ter results in a truncated protein, likely impairing ciliary integrity. These variants are associated with more severe manifestations, including cerebellar vermis agenesis and developmental delays. Variants that introduce nonsense mutations, such as c.622A>T (p.Arg208*)[12], create premature stop codons, leading to truncated TMEM67 proteins that lose essential interaction domains, impacting neurodevelopment and liver function. This mechanism is further observed in compound heterozygous cases, such as p.Arg208* / p.Thr372Lys, which result in neurodevelopmental delays, liver abnormalities, and eye anomalies due to disrupted ciliary maintenance.[12] Missense variants that alter surface residues, such as the p.Cys173Arg mutation[15], introduce charged residues at hydrophobic sites, destabilizing protein conformation and affecting interactions with other ciliary proteins like MKS1 and CC2D2A. This variant is linked to developmental delay, hypotonia, and liver involvement, underscoring how disruptions in the ciliary complex directly affect multi-system development and function. These mutations in *TMEM67*, including our reported Asp266Val variant, consistently result in impaired ciliary function, particularly in neurodevelopment and organ systems such as the liver and eyes. The specific type of amino acid substitution often dictates the severity and range of clinical manifestations, with both missense and nonsense mutations affecting the stability, binding, and functional capability of the TMEM67 protein within the ciliary structure. This detailed genotype-phenotype correlation enriches our understanding of *TMEM67*'s role in JS, supporting its significance in maintaining ciliary integrity and underscoring how even subtle changes can lead to significant pathogenic outcomes.

Our study aligns with the growing body of evidence identifying *TMEM67* mutations as a crucial factor in JS, particularly in cases presenting with liver involvement and motor and developmental delays, as seen in previous studies. The strong association of *TMEM67* mutations with specific JS subtypes exhibiting liver disease, with nearly 80% of liver-involved JS cases attributed to *TMEM67* pathogenic.[19] In parallel, the importance of monitoring kidney function due to the correlation between *TMEM67* mutations and nephronophthisis (NPHP), suggesting the need for comprehensive management of JS with *TMEM67* mutations.[20] In a study by Neissi, M. *et al*., a novel *TMEM67* mutation was identified in an Iranian patient with the classic midbrain-hindbrain malformation (MTS) and similar developmental challenges, contributing to the broader understanding of *TMEM67*-related JS.[3] In the current report a newly discovered *TMEM67* mutation (c.797A>T; p.Asp266Val) in an Iraqi family, with the affected child displaying MTS, motor and psychomotor delays, hypotonia, and mild liver dysfunction—further expanding the phenotype-genotype spectrum of *TMEM67*-related JS. This novel variant is likely pathogenic and disrupts TMEM67's conserved amino acid position, highlighting its essential role in protein function. This cumulative data emphasizes the genetic and phenotypic heterogeneity in JS and the importance of detailed genetic analysis to inform diagnosis and targeted interventions.

The exploration of *TMEM67* mutations reveals a wide clinical spectrum of associated disorders, particularly highlighting the significance of specific phenotypic manifestations linked to distinct genetic variations. *TMEM67* sequence variations span the entire coding region, with a notable focus on missense variants located in exons 8 to 15. These missense variants may indicate a predisposition to particular clinical phenotypes, especially when present alongside truncating mutations, which can suggest an association with Meckel-Gruber syndrome. Furthermore, the identification of mutational hotspots within the *TMEM67* gene, particularly in exons 2, 6, 8, 11, 13, 15, 18, and 24, indicates that certain regions are more prone to mutations, highlighting the importance of these exons in clinical assessments.[21,22] In reviewing previously

documented *TMEM67* sequence variations, a specific mutational hotspot has been noted, particularly in exon 8, which is the most frequently mutated region, 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.[3,23] According to our results, we identified a novel homozygous mutation (c.797A>T; p.Asp266Val) located in exon 8 of the *TMEM67* gene in an Iraqi patient presenting with JS. This finding further supports the notion that exon 8 is a critical region for *TMEM67* mutations, aligning with the observed mutational hotspots and reinforcing the significance of genetic analysis in understanding the diverse clinical presentations associated with *TMEM67*-related disorders. The identification of this specific mutation contributes to the growing database of *TMEM67* sequence variations, enhancing our understanding of the gene's role in JS and its potential associations with other syndromes.

MRI plays a pivotal role in diagnosing JS by providing critical radiological evidence that fulfills the diagnostic criteria for the condition. Specifically, the presence of the MTS on MRI is essential for confirming the diagnosis, as it reflects a typical malformation of the cerebellum and brainstem associated with JS. In classic JS, the MTS is accompanied by developmental delays and abnormal ocular movements, leading to a comprehensive understanding of the syndrome's clinical presentation.[1-3,24] The diagnostic criteria delineate that patients must exhibit both the MTS and associated developmental impairments, such as hypotonia, as seen in the case of the 7-year-old male presented in this study, who displayed marked ataxia, speech delays, and visual impairments. This aligns with findings in the literature, which note that additional features like retinal dystrophy and hepatic dysfunction may also manifest in JS, further complicating the clinical picture.[25] Thus, MRI not only aids in confirming the diagnosis of JS but also provides valuable insights into the neurological anomalies that underpin the spectrum of symptoms experienced by affected individuals.

In this study, we observed a novel homozygous mutation in the *TMEM67* gene in a patient with JS, contributing to the existing body of knowledge surrounding genetic variations associated with this condition. Previous research has highlighted the significance of *TMEM67* mutations in JS, which is characterized by the MTS and a spectrum of clinical features including developmental delays, hypotonia, and visual impairments.[4,6] However, our findings underscore the potential for additional phenotypic manifestations such as mild hepatic dysfunction, which has not been universally emphasized in the literature. Similar cases have reported variations in the clinical spectrum of JS, suggesting that mutations in *TMEM67* may lead to diverse presentations beyond the classic features.[26] The identification of the c.797A>T (p.Asp266Val) mutation expands the known mutational landscape of *TMEM67*, reinforcing the necessity for comprehensive genetic screening in affected families. This mutation has not been previously reported in mutation databases, making it the first identification of c.797A>T (p.Asp266Val) in association with JS. Our study provides substantial evidence supporting the pathogenicity of this novel variant: 1) Exome-sequencing pinpointed the c.797A>T mutation as the primary genetic cause of JS in the affected patient, with no additional mutations identified to account for the phenotype. 2) Sanger sequencing, depicted in Figure 2, confirmed the mutation in a homozygous state in the proband and in a heterozygous state in both parents, aligning with the autosomal recessive inheritance pattern characteristic of *TMEM67*-associated JS. 3) In silico pathogenicity tools, including SIFT, PolyPhen-2, MutationTaster, CADD, and others, classified Asp266Val as damaging and disease-causing, with high confidence scores. (44) The substitution of aspartic acid with valine at position 266 is predicted to disrupt the function of TMEM67, potentially impairing its role in ciliary signaling and maintenance. This disruption likely affects the protein's interactions with other ciliary components, which are essential for maintaining the structural integrity and signaling functions of cilia. This novel mutation enhances our understanding of *TMEM67*'s role in JS and underlines the critical impact of genotype-phenotype correlations. Furthermore, our study emphasizes the need for ongoing research into how specific genetic mutations influence phenotypic variability, which is crucial for improving management strategies and offering informed genetic counseling to families affected by JS.

In conclusion, our study identifies a novel *TMEM67* mutation (c.797A>T; p.Asp266Val) in a young male with JS, presenting hallmark symptoms including motor coordination challenges, hypotonia, developmental delays, and the characteristic MTS on brain MRI. These findings highlight the value of genetic testing in consanguineous families for understanding

JS's genetic basis, enhancing diagnostic accuracy, and informing management strategies. Genetic counseling remains essential for at-risk families, facilitating informed decisions about future pregnancies and preparation for potential complications.

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## DISCLOSURE

Ethics: All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or compare ethical strand. Informed consent for publication was obtained from the family involved in this study.

Availability of data: The data supporting the findings of this study are available from the corresponding author upon request.

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