CASO CLÍNICO/CASE REPORT

Joubert Syndrome and the *AHI1* p.Gln981Glu Variant: A Molecular and Clinical Study

Síndrome de Joubert e a Variante *AHI1* p.Gln981Glu: Um Estudo Molecular e Clínico

Ridhab Ajeel Jasim [1], Ahmed Yaseen Abed [2], Mahmoud H. Khalaf AL-Fahdawi [3], Ayoob Radhi Al-Zaalan [4], Rusul Thabit Hamid [5], Raghad N. Shihab [5], Mostafa Neissi [6,7,*], Javad Mohammadi-Asl [8], Adnan Issa Al-Badran [9]

1-Department of Biology, College for pure Science, University of Anbar, Iraq
2-Department of Medical Laboratories Techniques, College of Health and Medical Technology, University of Al Maarif, Al Anbar,31001, Iraq
3-Collage of Biotechnology, Al-Nahrain University, Baghdad, Iraq
4-Department of Medical Lab Technology, College of Health and Medical Technology, Southern Technical University, Basrah, Iraq
5-Cancer Researches Department, Iraqi Center for Cancer and Medical Genetic Research, Mustansiriyah University, Baghdad-Iraq
6-Department of Genetics, Khuzestan Science and Research Branch, Islamic Azad University, Ahvaz, Iran
7-Department of Genetics, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran
8-Department of Medical Genetics, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
9-Department of Biology, College of Science, University of Basrah, Basrah, Iraq

DOI: https://doi.org/10.46531/sinapse/CC/141/2025

Informações/Informations:
Caso Clínico, publicado em Sinapse, Volume 25, Número 2, abril-junho 2025. Versão eletrónica em www.sinapse.pt; Case Report, published in Sinapse, Volume 25, Number 2, April-June 2025. Electronic version in www.sinapse.pt
© Autor (es) (ou seu (s) empregador (es)) e Sinapse 2025. Reutilização permitida de acordo com CC BY-NC 4.0. Nenhuma reutilização comercial.
© Author(s) (or their employer(s)) and Sinapse 2025. Re-use permitted under CC BY-NC 4.0. No commercial re-use.

Keywords:
Ciliary Motility Disorders/genetics;
Child;
Joubert Syndrome/diagnosis;
Joubert Syndrome/genetics.

Palavras-chave:
Criança;
Perturbações da Motilidade Ciliar/genética;
Síndrome de Joubert/diagnóstico;
Síndrome de Joubert/genética.

***Autor Correspondente / Corresponding Author:**
Mostafa Neissi
Department of Genetics, Khuzestan Science and Research Branch, Islamic Azad University, Ahvaz, Iran
iammostafaneissi@gmail.com

Recebido / Received: *2025-01-19*
Aceite / Accepted: *2025-03-27*
Ahead of Print: *2025-05-13*
Publicado / Published: *2025-07-05*

Abstract

Joubert syndrome (JS) is a rare autosomal recessive ciliopathy characterized by a distinctive molar tooth sign (MTS) on brain imaging and with variable multisystem involvement, including developmental delay, ataxia, and oculomotor abnormalities. The *AHI1* gene plays a critical role in ciliary function and neurodevelopment, with pathogenic variants implicated in JS and related ciliopathies.

A 3-year-old boy underwent comprehensive clinical evaluation, including developmental assessments, neurological and ophthalmological examinations, and systemic investigations. Magnetic resonance imaging (MRI) of the brain was performed to confirm the diagnosis. Genomic DNA was extracted, and exome-sequencing was employed to identify pathogenic variants, which was followed by in silico analyses, structural modeling, and protein-protein interaction (PPI) studies. Variant validation and segregation analysis were conducted using polymerase chain reaction (PCR) and Sanger sequencing. Evolutionary conservation of the variant residue was evaluated using comparative genomics.

The proband exhibited hallmark JS features, including generalized hypotonia, ataxia, developmental delay, and the MTS on MRI. Systemic evaluations revealed no extracerebral organ involvement. Exome-sequencing identified a novel *AHI1* variant, c.2941C>G; p.Gln981Glu, absent in public population databases. In silico prediction tools supported pathogenicity, with high conservation of the mutated residue across species. Structural modeling and PPI analysis revealed conformational changes and disrupted interactions in ciliary function-related pathways. Sanger sequencing confirmed autosomal recessive inheritance, with the proband homozygous for the variant, while both parents were heterozygous carriers.

This study expands the mutational spectrum of *AHI1* and demonstrates the clinical utility of exome-sequencing in diagnosing JS, especially in consanguineous populations. Findings emphasize the importance of genetic counseling, risk assessment, and advanced reproductive technologies to reduce the risk of recurrence in affected fami-

lies. The integration of clinical, genetic, and computational analyses enhances our understanding of JS pathogenesis and supports personalized care for affected families.

Resumo

A síndrome de Joubert (SJ) é uma ciliopatia autossómica recessiva rara, caracterizada pela presença do sinal do dente em mola (MTS) na neuroimagem e por um envolvimento multissistémico variável, incluindo atraso no desenvolvimento, ataxia e anomalias oculomotoras. O gene *AHI1* desempenha um papel crucial na função ciliar e no neurodesenvolvimento, sendo as suas variantes patogénicas associadas à SJ e a outras ciliopatias relacionadas.

Uma criança do sexo masculino, de 3 anos, foi submetido a uma avaliação clínica abrangente, incluindo exames do desenvolvimento, neurológicos e oftalmológicos, bem como investigações sistémicas. A ressonância magnética cerebral (RM) foi realizada para confirmação diagnóstica. O ADN genómico foi extraído e o exoma sequenciado, com subsequente análise in silico, modelação estrutural e estudo de interações proteína-proteína (PPI). A validação da variante e a análise de segregação foram efetuadas por reação em cadeia da polimerase (PCR) e sequenciação de Sanger. A conservação evolutiva do resíduo mutado foi avaliada através de genómica comparativa.

O probando apresentou as características típicas da SJ, incluindo hipotonias generalizada, ataxia, atraso do desenvolvimento e a presença do MTS na RM. As avaliações sistémicas não revelaram anomalias extracerebrais. A sequenciação do exoma identificou uma nova variante no gene *AHI1*, c.2941C>G; p.Gln981Glu, ausente nas bases de dados populacionais públicas. As ferramentas preditivas indicaram patogenicidade, com elevada conservação do resíduo mutado entre espécies. A modelação estrutural e a análise de PPI demonstraram alterações conformacionais e perturbações nas interações das vias relacionadas com a função ciliar. A sequenciação de Sanger confirmou um padrão de hereditariedade autossómica recessiva, com o probando homozigótico para a variante e os progenitores heterozigóticos portadores.

Este estudo expande o espectro mutacional do gene *AHI1* e demonstra a utilidade clínica da sequenciação do exoma no diagnóstico da SJ, especialmente em populações consanguíneas. Os achados enfatizam a importância do aconselhamento genético, da avaliação de risco e das tecnologias reprodutivas avançadas para reduzir o risco de recorrência. A integração de análises clínicas, genéticas e computacionais aprofunda a compreensão da patogénese da SJ e contribui para cuidados personalizados às famílias afetadas.

Introduction

Joubert syndrome (JS) is a rare autosomal recessive ciliopathy characterized by a distinctive malformation of the midbrain and cerebellum, which collectively form the molar tooth sign (MTS) on brain magnetic resonance imaging (MRI).[1] This anomaly is responsible for the hallmark clinical features of JS, which include hypotonia, ataxia, developmental delays, abnormal breathing patterns, and oculomotor apraxia.[2] As a ciliopathy, JS arises from defects in primary cilia, microtubule-based organelles essential for cellular signaling and development. The phenotypic spectrum of JS is broad and variable, with some individuals also exhibiting retinal dystrophy, renal anomalies, liver fibrosis, and polydactyly, reflecting the diverse roles of cilia in multiple organ systems.[3,4]

Genetic studies have elucidated a high degree of heterogeneity in JS, with variants in over 30 genes encoding proteins essential for ciliary and basal body structure and function of cilia and basal bodies.[5] Among these genes, *AHI1* (Abelson-helper integration site 1) is a key gene frequently implicated in JS. *AHI1* encodes the protein Jouberin, which plays a pivotal role in the proper locali-

zation and function of ciliary proteins, as well as in axonal decussation during brain development. Variants in *AHI1* are strongly associated with the neurological manifestations of JS, including the MTS, and may also predispose individuals to retinal and renal complications.[1-3]

In addition to *AHI1*, other genes frequently implicated in JS include *MKS1*, *CC2D2A*, *TCTN1*, *B9D2*, *NPHP1*, *TCTN2*, *TMEM216*, *B9D1*, *CEP290*, and *TMEM67*. These genes encode components of the ciliary transition zone or proteins that interact within ciliary networks to regulate signaling pathways.[6] For instance, variants in *CEP290* and *TMEM67* are frequently linked to JS with multisystem involvement, including severe renal and hepatic phenotypes. Deletions in *NPHP1* are often associated with milder forms of the disorder but frequently co-occur with nephronophthisis.[7,8]

Exome-sequencing is a powerful genomic tool that focuses on analyzing the protein-coding regions of the genome, which contain the majority of disease-causing variants.[9,10] Its efficiency and cost-effectiveness compared to genome-sequencing make it particularly advantageous for identifying rare genetic disorders.[11,12] Studies have demonstrated its high diagnostic yield, especially in consanguineous families, where the prevalence of homozygous variants is higher due to shared ancestry.[12,13] Building on these strengths, we utilized exome-sequencing to investigate the genetic basis of JS, a rare ciliopathy, in an Iraqi family.

Case Report

The proband, a 3-year-old boy from a consanguineous Iraqi family, presented with clinical features suggestive of JS. A pedigree analysis was consistent with autosomal recessive inheritance (**Fig. 1**). Comprehensive clinical and developmental assessments were conducted to characterize the phenotype and confirm the diagnosis. Neurological and ophthalmological evaluations assessed motor coordination, developmental milestones, and visual function. Laboratory investigations included liver function tests, and abdominal ultrasonography was performed to identify potential structural abnormalities. MRI was performed to confirm the hallmark structural anomalies associated with JS.

Sample Collection and Genomic DNA Extraction

Peripheral blood samples were collected from the patient and his biological parents after obtaining informed consent and in accordance with ethical guidelines. Genomic DNA was extracted using the Qiagen DNeasy Blood & Tissue Kit according to the manufacturer's protocol. The quality and concentration of the extracted DNA were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific) and Qubit 4 fluorometer (Thermo Fisher Scientific), to ensure suitability for downstream applications.

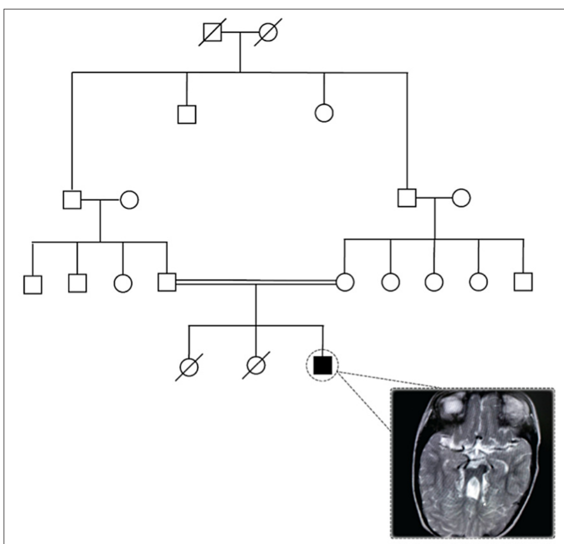

Figure 1. The pedigree of the studied family illustrates an autosomal recessive inheritance pattern. Circles represent females, and squares represent males. A diagonal slash through a symbol denotes deceased individuals, while the filled black square identifies the proband, the subject of clinical evaluation. The inset on the right features an axial T2-weighted MRI scan of the proband's brain, showing the characteristic MTS, a hallmark radiological feature of JS.

Exome-Sequencing and Variant Analysis

Exome-sequencing was performed to identify potential variants in the patient's genome. Library preparation was conducted using the Agilent SureSelect Human All Exon V7 kit, and sequencing was performed on an Illumina NovaSeq 6000 system to achieve an average coverage depth of approximately 120×. Raw sequencing data were processed using a standard bioinformatics pipeline, including read alignment to the human reference genome (GRCh38) using BWA-MEM, variant calling with GATK (Genome Analysis Toolkit), and annotation using ANNOVAR. Variants were filtered based on quality metrics and minor allele frequency (MAF $< 1\%$) in public databases such as the Genome Aggregation Database (gnomAD; https://gnomad.broadinstitute.org/) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/).

Impact of Variants

To assess the pathogenicity of the identified variants, functional impact predictions were conducted using tools such as MutationTaster (https://www.mutationtaster.org/), and Combined Annotation Dependent Depletion (CADD; https://cadd.gs.washington.edu/). Further evaluation was performed to prioritize variants based on gene-disease associations obtained from Online Mendelian Inheritance in Man (OMIM; https://www.omim.org/) and the Human Gene Mutation Database (HGMD; https://www.hgmd.cf.ac.uk/ac/index.php).

Structural 3D Protein Analysis and Protein Interactions

The structural impact of prioritized variants was modeled using homology-based 3D protein modeling by SWISS-MODEL (https://swissmodel.expasy.org/). The wild-type and variant protein structures were compared to evaluate conformational changes. Additionally, protein-protein interaction (PPI) analysis was performed using STRING (https://string-db.org/) to predict how the variant might disrupt molecular interactions relevant to the observed phenotype.

Evolutionary Conservation

Evolutionary conservation of the mutated residues was evaluated using Clustal Omega (https://www.ebi.ac.uk/jdispatcher/msa/clustalo), which calculates conservation scores to identify functionally important regions. Evolutionary conservation of the mutated residues was evaluated using Clustal Omega (https://www.ebi.ac.uk/jdispatcher/msa/clustalo) to calculate conservation scores and identify functionally important regions. Additional assessments of conservation and functional significance were performed using PhastCons100way, PhastCons17way (primate), and PhastCons100way (vertebrate). Similarly, PhyloP100way, PhyloP17way (primate), and PhyloP100way (vertebrate) were used to evaluate evolutionary constraints across different species. SiPhy29way was employed for site-specific phylogenetic analysis, providing further insights into evolutionary conservation. Fitness consequence-based metrics were assessed using fitCons-gm, fitCons H1 (H1-hESC), fitCons HU (HUVEC), and the Integrated fitCons framework to evaluate the functional significance across various cell types. Additional analyses included pi (π, nucleotide diversity) scores to examine population-level variation and bStatistic scores to assess evolutionary constraint in the genomic regions.

Polymerase Chain Reaction (PCR)

Targeted amplification of the genomic regions harboring the prioritized variants was carried out using polymerase chain reaction (PCR). Primers were designed using Primer3 software, ensuring specificity for the regions of interest. PCR amplification was performed using a Veriti Thermal Cycler (Applied Biosystems) under optimized conditions, with reaction components including Taq DNA polymerase (Thermo Fisher Scientific), dNTPs, MgCl2, and primers. Amplicons were confirmed by gel electrophoresis.

Sanger Sequencing

Segregation analysis of the prioritized variants was conducted using Sanger sequencing. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen) and sequenced on an Applied Biosystems 3730 DNA Analyzer. Chromatograms were analyzed using Chromas software to confirm the presence of the variants in the proband and determine their segregation in the parents.

Clinical Findings

The proband, a 3-year-old boy from a consanguineous family, presented with features suggestive of JS. He was born to consanguineous parents following an uneventful full-term pregnancy, weighing 3.2 kg at birth and no reported perinatal complications. Family history revealed two prior neonatal deaths, though no confirmed diagnoses were documented. The proband is the couple's third child and the only surviving offspring (**Fig. 1**).

On clinical examination, the child exhibited generalized hypotonia, pronounced head lag, and impaired truncal stability. His gait was broad-based and unsteady, consistent with ataxia. Dysmorphic features included a high-arched palate and low-set ears. Neurological evaluation revealed oculomotor apraxia and intermittent nystagmus, although retinal evaluation showed no signs of dystrophy. Cognitive assessment demonstrated moderate global developmental delay, with marked deficits in motor and language development. There was no evidence of clinically apparent seizures or significant feeding difficulties.

Neuroimaging findings were integral to establishing the diagnosis. Brain MRI demonstrated the classic MTS

(**Fig. 1**), characterized by hypoplasia of the cerebellar vermis and thickened, elongated superior cerebellar peduncles. Additional findings included a mildly enlarged fourth ventricle and a dysplastic corpus callosum. These structural anomalies are hallmark characteristics of JS.

Systemic evaluations were performed to assess extracerebral involvement. Renal ultrasonography revealed normal kidney size and morphology, with no evidence of nephronophthisis or cystic changes. Hepatic function tests and abdominal ultrasonography showed no abnormalities. Cardiologic assessment, including echocardiography, was within normal limits. Given the potential progressive nature of renal and retinal complications in JS, ongoing monitoring is recommended. Finally, after genetic counseling, genetic analysis was suggested to confirm the diagnosis and identify the underlying molecular defect.

Exome-Sequencing Findings

Exome-sequencing of the affected patient identified a novel homozygous variant in the *AHI1* gene (NM_001134831.2), located on chromosome 6q. The variant, designated as c.2941C>G (p.Gln981Glu), is situated in exon 21 of 29. Based on the GRCh38 (hg38) reference genome, this variant corresponds to the genomic coordinate chr6:g.135411368G>C. This alteration involves a nucleotide substitution at position 2941, where cytosine (C) is replaced by guanine (G). At the protein level, this nucleotide change results in the replacement of glutamine (Gln) with glutamic acid (Glu) at position 981. Population database analysis indicates that this variant is exceedingly rare, with an allele frequency of 0.0% reported in both the maximal founder and maximal non-founder subpopulations within gnomAD.

In Silico Analysis of *AHI1* c.2941C>G; p.Gln981Glu Variant

The novel variant identified in this study, *AHI1* (NM_001134831.2): c.2941C>G, resulting in the amino acid substitution p.Gln981Glu, underwent comprehensive in silico analysis, revealing its potential pathogenicity. Predictive tools indicated damaging effects, with FATHMM-MKL assigning a high score of 0.8525, MutationTaster classifying it as disease-causing with a score of 0.9323, and PolyPhen-2 evaluating it as probably damaging with a score of 0.969. Splice site analysis demonstrated significant changes, including an increased donor site score at gDNA position 86408, rising from 0.67 in the wild-type sequence (GTAAAACAGAGGCTT) to 0.99 in the mutant sequence (GTAAAAGAGAGGCTT), alongside the gain of a novel donor site at gDNA position 86403 with a score of 0.94 and the mutant sequence AGCTAGTAAAAGAGA at the exon-intron boundary (CTAG|taaa). Population frequency analysis confirmed the rarity of the variant, with an allele frequency below 0.001% across all gnomAD subpopulations, well below the recommended PM2 threshold of 0.082% for the *AHI1* gene, and it does not overlap with known pathogenic variants such as chr6:135467636:T>C, chr6:135466028:CTT>C, or chr6:135463145:G>GT. These findings collectively indicate the novel variant's likely pathogenic nature.

Structural Analysis of AHI1 Protein and Protein Interactions

The structural analysis of the AHI1 protein demonstrates critical differences between the wild-type and mutant models (**Fig. 2**). To explore the potential biological significance of this variant, we analyzed the PPI network of AHI1 using the STRING database. The resulting network (**Fig. 3A**) shows strong functional connections between AHI1 and several proteins known to play critical roles in ciliary function and structure, including CEP290, TMEM67, TMEM216, CC2D2A, and MKS1. These proteins are frequently associated with ciliopathies, including JS.

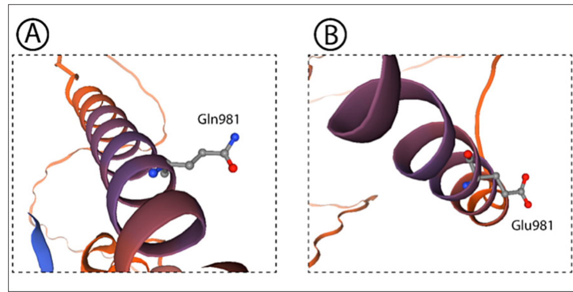

Figure 2. Structural comparison of (A) wild-type (Gln981) and (B) mutant (Glu981) AHI1 proteins.

Conserved Regions Across Species

The conservation scores and metrics for the identified variant are summarized in **Table 1**. These scores provide insights into the evolutionary conservation and functional importance of the affected region. In addition, to assess the evolutionary conservation of the region en-

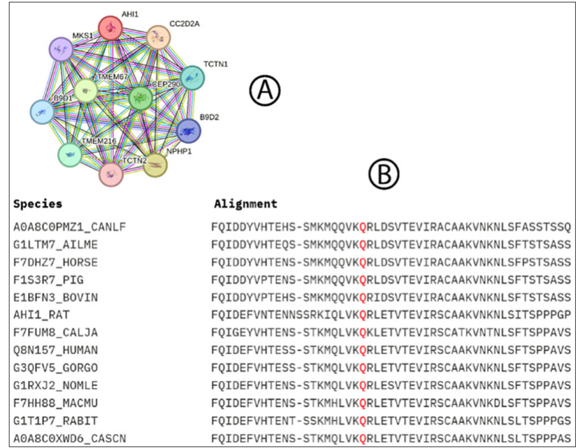

Figure 3. (A) PPI network of AHI1 and its interacting proteins. The PPI network highlights the functional relationships of AHI1 with other proteins, including MKS1, CC2D2A, TMEM67, CEP290, among others, which are commonly implicated in ciliopathy-related pathways. (B) Conservation of the AHI1 protein region encompassing the p.Gln981Glu variant across 12 species. The sequence alignment shows that the region surrounding the Glu981 residue is highly conserved across multiple species, including humans, primates (e.g., gorilla, macaque), and non-primate mammals (e.g., dog, pig, horse). The conserved Glu residue (highlighted in red) indicates functional significance, supporting its potential role in protein stability or interaction..

compassing the *AHI1* c.2941C>G; p.Gln981Glu variant, a multiple sequence alignment was performed across 12 species. The results (**Fig. 3B**) show a high degree of conservation in the protein sequence surrounding the variant site. The Glu residue at position 981 is conserved across all analyzed species, ranging from primates, including humans, gorillas, and macaques, to non-primate mammals such as dogs, pigs, and horses.

Validation and Segregation Analysis

The detected variant was validated, and segregation analysis was performed using Sanger sequencing. The analysis confirmed the presence of the c.2941C>G variant in the *AHI1* gene, which results in a substitution of glutamine with glutamic acid (p.Gln981Glu). The patient was found to be homozygous for the variant, while both parents were identified as heterozygous carriers. These findings are consistent with an autosomal recessive mode of inheritance (**Fig. 4**). Importantly, no deletion of exon 21 in the *AHI1* gene was detected, ruling out this possibility as an explanation for the presence of a single G signal in the chromatogram.

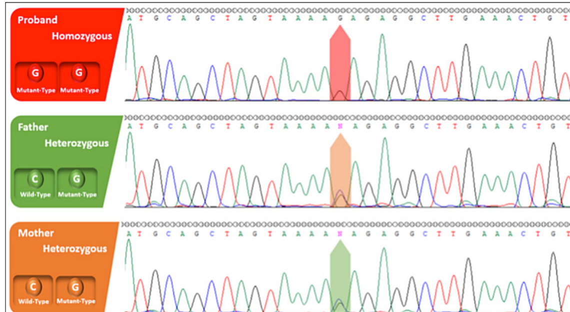

Figure 4. Sanger sequencing chromatograms depicting the segregation of the *AHI1* c.2941C>G (p.Gln981Glu) variant in the proband and his parents. The proband exhibits a homozygous variant (G/G), while both parents are heterozygous carriers (C/G).

Genetic Counseling and Risk Assessment

The identification of a novel variant in the *AHI1* gene highlights the essential role of genetic counseling in families affected by JS. In cases of consanguinity, the risk of autosomal recessive conditions is significantly elevated. Genetic counseling offers tailored risk assessment, education on inheritance patterns, and informed guidance

Table 1. Conservation scores of the novel variant.

Metric	Score	Interpretation
PhastCons100way	1.000	Indicates full conservation across 100 vertebrate species, suggesting strong evolutionary constraint.
PhyloP100way (version 14-Apr-2021)	3.563	A positive conservation score, highlighting strong evolutionary conservation across 100 vertebrates.
PhyloP100way (vertebrate)	3.5629	High conservation signal across vertebrate species, indicating functional significance.
PhastCons100way (vertebrate)	1.000	Fully conserved across 100 vertebrates, indicating essential functional roles.
fitCons HU (HUVEC)	0.7591	Suggests strong functional constraint in human vascular endothelial cells (HUVEC).
fitCons HU (HUVEC rankscore)	0.9976	Falls within the 99.76th percentile in HUVEC conservation, indicating high significance for functionality.
SiPhy29way	15.3115	High conservation across 29 mammalian species, consistent with evolutionary constraint.
SiPhy29way (rankscore)	0.7374	Falls within the 73.74th percentile in mammalian conservation, supporting functional importance.

for reproductive decision-making, enabling families to address potential challenges proactively.

Prenatal and Perinatal Management

Targeted prenatal diagnostic strategies are indispensable when a pathogenic variant, such as *AHI1*, c.2941C>G; p.Gln981Glu, is identified. Techniques like chorionic villus sampling (CVS) and amniocentesis allow for early genetic confirmation of fetal status. Early detection facilitates timely planning for specialized perinatal care, ensuring optimized outcomes and preparedness for managing potential complications.

Advanced Reproductive Options

The identification of the c.2941C>G; p.Gln981Glu variant enables the use of advanced reproductive technologies to minimize recurrence risks. In vitro fertilization (IVF) with preimplantation genetic testing (PGT) provides a precise method to screen embryos for pathogenic variants. This approach allows for the selection of unaffected embryos, offering a robust solution to break the cycle of autosomal recessive inheritance and ensure the health of future offspring.

Discussion

Identification of a Novel *AHI1* Variant in Joubert Syndrome (JS)

This study identifies a novel homozygous variant, c.2941C>G (p.Gln981Glu), in the *AHI1* gene, identified in a proband from a consanguineous Iraqi family with clinical and neuroimaging findings consistent with JS. The autosomal recessive inheritance observed in this case is consistent with the pedigree analysis and underscores the impact of consanguinity in increasing the risk of rare genetic disorders. The identified variant results in the substitution of glutamine with glutamic acid at position 981 within the AHI1 protein. This substitution alters the chemical properties of the protein by replacing a neutral, amide-containing side chain with a negatively charged, carboxyl-containing side chain.[13,14] This alteration likely disrupts the protein's structural stability or interaction with other cellular components, potentially impairing its function in ciliogenesis or neuronal development, processes which are key processes in the pathogenesis of JS. The rarity of this variant in population databases, along with its segregation with the phenotype in an autosomal recessive manner, strongly supports its pathogenicity.

Role of AHI1 in Wnt Signaling and Joubert Syndrome Pathogenesis

The *AHI1* gene encodes the jouberin protein, which serves as a key regulator of the Wnt signaling pathway by facilitating β-catenin nuclear translocation, a vital mechanism in cellular signaling and developmental processes.[15] Previous studies, as summarized in **Table 2**, have identified various *AHI1* gene variants that are proposed to encode impaired proteins, contributing to the understanding of JS pathogenesis. The missense variants listed in **Table 2** were selected based on their predicted functional impact, their presence in key functional domains of the *AHI1* gene, and supporting evidence from the literature. These variants are proposed to encode impaired proteins that may contribute to JS pathogenesis by disrupting the Wnt signaling pathway. Our study adds to this growing body of knowledge by identifying a novel missense variant in the *AHI1* gene. We propose that this newly detected variant encodes a dysfunctional jouberin protein, potentially disrupting its role in the Wnt pathway and contributing to the clinical manifestations of JS

Population Genetics and Founder Effect in the Arab Population

Research has shown that *AHI1* variants occur with higher frequency in the Arab population, likely due to the higher prevalence of consanguineous marriages, which increases the risk of autosomal recessive inheritance of genetic conditions.[25] In regions like Al-Basrah, southeastern Iraq, where many Arab families reside, such variants might have gone undetected, especially if they previously manifested in homozygous states. The early mortality of affected individuals, as observed in this family, could account for the lack of prior documentation of these cases. This pattern aligns with the concept of a founder effect, which likely underlies the prevalence of specific variants in JS patients, reflecting the shared genetic heritage and familial structures within this population.

Comparison with Other Joubert Syndrome-Associated Genetic Variants

Previous studies have identified variants in JS-associated genes, such as the *AHI1* variants found in several affected cohorts. For instance, variants in *CEP290* and *TMEM67* have been commonly implicated in JS due to their roles in ciliary function, as evidenced by Japanese cohorts, where the *CEP290* c.6012-12T>A vari-

Table 2. *AHI1* variations (NM_001134831.2) associated with a JS previously reported..

No.	Nucleotide Change	Protein Change	Type of Variant	ACMG[1]	Reference
1	c.662C>G	p.Ser221Ter	Nonsense	Pathogenic	Valente EM, *et al* [16]
2	c.1064T>G	p.Leu355Arg	Missense	VUS[2]	Neissi M, *et al* [1]
3	c.1213A>C	p.Thr405Pro			Neissi M, *et al* [2]
4	c.1267C>T	p.Gln423Ter	Nonsense	Pathogenic	Valente EM, *et al* [16]
5	c.1328T>A	p.Val443Asp	Missense		Dixon-Salazar T, *et al* [17]
6	c.1765C>T	p.Arg589Ter	Nonsense		Valente EM, *et al* [16]
7	c.1917T>A	p.Tyr639Ter			Parisi MA, *et al* [18]
8	c.1995T>G	p.Asp665Glu	Missense	VUS	
9	c.2012C>T	p.Thr671Ile		Likely Pathogenic	
10	c.2156A>G	p.Asp719Gly		Pathogenic	
11	c.2168G>A	p.Arg723Gln			Valente EM, *et al* [16]
12	c.2173T>C	p.Trp725Arg		Likely Pathogenic	Parisi MA, *et al* [18]
13	c.2212C>T	p.Arg738Ter	Nonsense	Pathogenic	Valente EM, *et al* [16]
14	c.2278T>G	p.Tyr760Asp	Missense	VUS	Suzuki T, *et al* [19]
15	c.2282C>T	p.Ser761Leu	Missense		Elsayed SM, *et al* [20]
16	c.2297G>A	p.Gly766Glu		Likely Pathogenic	Bachmann-Gagescu R, *et al* [21]
17	c.2361G>T	p.Trp787Cys		VUS	
18	c.2495T>G	p.Leu832Ter	Nonsense	Pathogenic	Parisi MA, *et al* [18]
19	c.2561G>T	p.Cys854Phe	Missense	VUS	Lee H, *et al* [22]
20	c.2671C>T	p.Arg891Ter	Nonsense	Pathogenic	Otto EA, *et al* [23]
21	c.2687A>G	p.His896Arg	Missense	Likely Pathogenic	Parisi MA, *et al* [18]
22	c.2705T>A	p.Val902Asp		VUS	Bachmann-Gagescu R, *et al* [21]
23	c.2950G>T	p.Glu984Ter	Nonsense	Likely Pathogenic	Bell CJ, *et al* [24]
24	c.3368C>T	p.Ser1123Phe	Missense	Benign	Lee H, *et al* [22]

ACMG[1]: American College of Medical Genetics and Genomics; VUS[2]: variant of uncertain significance.

ant predominated.[19] Similarly, our findings of the novel *AHI1* c.2941C>G (p.Gln981Glu) variant broaden the known genetic landscape of JS. While earlier variants like c.3263_3264delGG have been linked to truncation effects leading to severe phenotypes,[20] our identified variant suggests an alternative pathogenic mechanism, potentially disrupting protein interactions.. Both studies underline the significant heterogeneity within JS, but our variant's unique location and predicted effects provide novel insights into disease etiology.

Structural and Functional Impact of the *AHI1* p.Gln981Glu Variant

The pathogenicity of JS variants often arises from their interference with protein domains crucial for ciliary functions, as demonstrated by disrupted WD40-repeat structures in *AHI1*.[20] This aligns with our in silico findings, where the p.Gln981Glu variant in *AHI1* showed significant alterations in predicted splice sites and damaging protein-level effects. For example, the study of AHI1 truncating variants demonstrated that regions near the SH3 domain were dispensable, but N-terminal disruptions were deleterious due to their role in ciliary architecture.[20] Our study suggests that the p.Gln981Glu variant may uniquely disrupt WD40-repeat or SH3 interactions, providing a new dimension to understanding the structural integrity required for AHI1 functionality and its role in JS pathogenesis.

Genotype-Phenotype Correlation in Joubert Syndrome

Clinical manifestations of JS often correlate with specific genetic variants, with phenotypes ranging from classic JS to multiorgan involvement. In our case, the proband exhibited hallmark features of JS, including hypotonia, ataxia, and the characteristic MTS on brain MRI. In comparison, clinical cohorts with *TMEM67* variants often frequently present with additional features

such as liver fibrosis or coloboma,[19] while *CEP290* variants have been associated with retinal dystrophy.[21] Interestingly, despite severe developmental delays, our case lacked extracerebral involvement, highlighting the phenotypic variability even within *AHI1*-related JS. Such comparisons underscore the importance of genotype-phenotype correlation studies in guiding prognosis and clinical monitoring in JS.

Protein-Protein Interaction (PPI) Network and Pathogenic Mechanisms

The PPI network presented highlights the intricate interplay between key proteins implicated in the pathogenesis of JS. The identified *AHI1* c.2941C>G; p.Gln981Glu variant in the proband disrupts a critical node in this network, potentially altering interactions with partners such as CEP290, TMEM67, and other JS-associated proteins. AHI1 serves as a scaffold protein essential for the assembly and function of the primary cilium, a cellular organelle critical for signal transduction and development. Perturbation of AHI1 interactions could destabilize this network, impairing ciliary signaling pathways and contributing to the hallmark cerebellar and brainstem malformations observed in JS.[26] Our findings suggest that the c.2941C>G variant may alter protein binding affinity, disrupting the ciliary architecture and signaling cascade.

Future Directions

Finally, this study identifies a novel homozygous *AHI1* c.2941C>G; p.Gln981Glu variant and provides a detailed characterization of its potential pathogenic mechanisms. The integration of in silico predictive models, population database analysis, and structural and conservation data underscores the variant's likely disruptive effect on protein function and its role in the pathogenesis of JS. Although this variant is not present in population databases, its occurrence in an Arab family raises the possibility that it may be more frequent in this population, particularly in regions with high consanguinity. Future studies examining its prevalence in larger Arab cohorts could provide further insights into its contribution to JS and related disorders. Additionally, the findings reinforce the significant role of consanguinity and founder effects in shaping the genetic landscape of rare disorders in specific populations.

Conclusion

Identification of the novel *AHI1* p.Gln981Glu variant in a proband with JS underscores the importance of integrating genetic and clinical evaluations in the accurate diagnosis and management of rare neurodevelopmental disorders. This study expands the mutational spectrum of *AHI1* and reinforces the utility of exome-sequencing in consanguineous populations, where recessive inheritance patterns are more pronounced. The findings provide critical insights into genotype-phenotype correlations and highlight the importance of genetic counseling and advanced reproductive strategies to minimize recurrence risks. This work enhances our understanding of JS and offers a framework for improving diagnostic accuracy, genetic risk assessment, family planning, and personalized patient care in affected families. ■

Acknowledgments

We express our gratitude to the family members for their valuable participation in this study.

Contributorship Statement / Declaração de Contribuição

RAJ: Investigation; Writing – Original Draft.

AYA, RTH and RNS: Investigation; Writing – Review and Editing.

MHKA-F: Conceptualization; Investigation; Writing – Original Draft.

MN: Conceptualization; Investigation; Writing – Original Draft.

ARA-A, JM-A and AIA-B: Investigation.

All authors approved the final version to be published.

RAJ: Investigação; Redação - Rascunho Original.

AYA, RTH e RNS: Investigação; Redação - Revisão e Edição.

MHKA-F: Conceptualização; Investigação; Redação - Rascunho Original.

MN: Conceptualização; Investigação; Redação - Rascunho Original.

ARA-A, JM-A e AIA-B: Investigação.

Todos os autores aprovaram a versão final a ser publicada.

Responsabilidades Éticas

Conflitos de Interesse: Os autores declaram a inexistência de conflitos de interesse na realização do presente trabalho.

Fontes de Financiamento: Não existiram fontes externas de financiamento para a realização deste artigo.

Confidencialidade dos Dados: Os autores declaram ter seguido os protocolos da sua instituição acerca da publicação dos dados de doentes.

Consentimento: Consentimento do doente para publicação obtido.

Proveniência e Revisão por Pares: Não comissionado; revisão externa por pares.

Ethical Disclosures

Conflicts of Interest: The authors have no conflicts of interest to declare.

Financing Support: This work has not received any contribution, grant or scholarship.

Confidentiality of Data: The authors declare that they have followed the protocols of their work center on the publication

of patient data.

Patient Consent: Consent for publication was obtained.

Provenance and Peer Review: Not commissioned; externally peer-review.

References / Referências

1. Neissi M, Mabudi H, Mohammadi-Asl J. AHI1 gene mutation in a consanguineous Iranian family affected by Joubert syndrome: A case report. Clin Case Rep. 2021;9:e05002. doi: 10.1002/ccr3.5002.
2. Neissi M, Al-Badran AI, Mohammadi-Asl J. Homozygous AHI1 gene mutation (c.1213A>C; Thr405Pro) leads to Joubert syndrome in a consanguineous Iranian family: A case report. Neurol Asia. 2022;27. doi: 10.54029/2022anp
3. Morelli F, Toni F, Saligari E, D'Abrusco F, Serpieri V, Ballante E, et al. Visual function in children with Joubert syndrome. Dev Med Child Neurol. 2024;66:379-88. doi: 10.1111/dmcn.15732.
4. Neissi M, Mohammadi-Asl M, Roghani M, Al-Badran AI, Sheikh-Hosseini M, Mohammadi-Asl J. Joubert syndrome caused by a TMEM67 mutation: Genotype-phenotype analysis. NeurolAsia. 2024;29. doi: 10.54029/2024wir
5. Parisi MA. The molecular genetics of Joubert syndrome and related ciliopathies: The challenges of genetic and phenotypic heterogeneity. Transl Sci Rare Dis. 2019;4:25-49. doi: 10.3233/TRD-190041.
6. Hildebrandt F, Benzing T, Katsanis N. Ciliopathies. N Engl J Med. 2011;364:1533-43. doi: 10.1056/NEJMra1010172.
7. Garcia-Gonzalo FR, Corbit KC, Sirerol-Piquer MS, Ramaswami G, Otto EA, Noriega TR, et al. A transition zone complex regulates mammalian ciliogenesis and ciliary membrane composition. Nat Genet. 2011;43:776-84. doi: 10.1038/ng.891.
8. Baala L, Audollent S, Martinovic J, Ozilou C, Babron MC, Sivanandamoorthy S, et al. Pleiotropic effects of CEP290 (NPHP6) mutations extend to Meckel syndrome. Am J Hum Genet. 2007;81:170-9. doi: 10.1086/519494.
9. Neissi M, Sheikh-Hosseini M, Mohammadi-Asl M, Al-Badran AI, Roghani M, Mohammadi-Asl J, et al. Identification and characterization of NMNAT1 gene mutations in an Iranian patient with Leber congenital amaurosis 9. Clin Case Rep. 2024;12:e9506. doi: 10.1002/ccr3.9506.
10. Neissi M, Mohammadi-Asl J, Mohammadi-Asl M, Roghani M, Sheikh-Hosseini M, Issa Al-Badran A. Usher Syndrome Type 2 in An Iranian Family: A Novel Founder Variation in The USH2A Gene. Cell J. 2024;26:392-7. doi: 10.22074/cellj.2024.2024223.1
11. Runheim H, Pettersson M, Hammarsjö A, Nordgren A, Henriksson M, Lindstrand A, et al. The cost-effectiveness of whole genome sequencing in neurodevelopmental disorders. Sci Rep. 2023;13:6904. doi: 10.1038/s41598-023-33787-8.
12. Neissi M, Al-Badran AI, Mohammadi-Asl M, Al-Badran RA, Sheikh-Hosseini M, Roghani M, et al. Clinical and genetic analysis of Niemann-Pick disease type C with a novel NPC1 variant. J Rare Dis. 2024;3:19. doi: 10.1007/s44162-024-00043-0
13. León I, Alonso ER, Mata S, Cabezas C, Alonso JL. Unveiling the Neutral Forms of Glutamine. Angew Chem Int Ed Engl. 2019;58:16002-7. doi: 10.1002/anie.201907222.
14. Nikolaeva O, Takahashi Y, Moiseyev G, Ma JX. Negative charge of the glutamic acid 417 residue is crucial for isomerohydrolase activity of RPE65. Biochem Biophys Res Commun. 2010;391:1757-61. doi: 10.1016/j.bbrc.2009.12.149.
15. Lancaster MA, Schroth J, Gleeson JG. Subcellular spatial regulation of canonical Wnt signalling at the primary cilium. Nat Cell Biol. 2011;13:700-7. doi: 10.1038/ncb2259.
16. Valente EM, Brancati F, Silhavy JL, Castori M, Marsh SE, Barrano G, et al. AHI1 gene mutations cause specific forms of Joubert syndrome-related disorders. Ann Neurol. 2006;59:527-34. doi: 10.1002/ana.20749.
17. Dixon-Salazar T, Silhavy JL, Marsh SE, Louie CM, Scott LC, Gururaj A, et al. Mutations in the AHI1 gene, encoding jouberin, cause Joubert syndrome with cortical polymicrogyria. Am J Hum Genet. 2004;75:979-87. doi: 10.1086/425985.
18. Parisi MA, Doherty D, Eckert ML, Shaw DW, Ozyurek H, Aysun S, et al. AHI1 mutations cause both retinal dystrophy and renal cystic disease in Joubert syndrome. J Med Genet. 2006;43:334-9. doi: 10.1136/jmg.2005.036608.
19. Suzuki T, Miyake N, Tsurusaki Y, Okamoto N, Alkindy A, Inaba A, et al. Molecular genetic analysis of 30 families with Joubert syndrome. Clin Genet. 2016;90:526-35. doi: 10.1111/cge.12836.
20. Elsayed SM, Phillips JB, Heller R, Thoenes M, Elsobky E, Nürnberg G, et al. Non-manifesting AHI1 truncations indicate localized loss-of-function tolerance in a severe Mendelian disease gene. Hum Mol Genet. 2015;24:2594-603. doi: 10.1093/hmg/ddv022.
21. Bachmann-Gagescu R, Dempsey JC, Phelps IG, O'Roak BJ, Knutzen DM, Rue TC, et al. Joubert syndrome: a model for untangling recessive disorders with extreme genetic heterogeneity. J Med Genet. 2015;52:514-22. doi: 10.1136/jmedgenet-2015-103087.
22. Lee H, Deignan JL, Dorrani N, Strom SP, Kantarci S, Quintero-Rivera F, et al. Clinical exome sequencing for genetic identification of rare Mendelian disorders. JAMA. 2014;312:1880-7. doi: 10.1001/jama.2014.14604.
23. Otto EA, Ramaswami G, Janssen S, Chaki M, Allen SJ, Zhou W, et al. Mutation analysis of 18 nephronophthisis associated ciliopathy disease genes using a DNA pooling and next generation sequencing strategy. J Med Genet. 2011;48:105-16. doi: 10.1136/jmg.2010.082552. Erratum in: J Med Genet. 2015;52:866. doi: 10.1136/jmg-2010-082552corr1.
24. Bell CJ, Dinwiddie DL, Miller NA, Hateley SL, Ganusova EE, Mudge J, et al. Carrier testing for severe childhood recessive diseases by next-generation sequencing. Sci Transl Med. 2011;3:65ra4. doi: 10.1126/scitranslmed.3001756.
25. Ben-Salem S, Al-Shamsi AM, Gleeson JG, Ali BR, Al-Gazali L. Mutation spectrum of Joubert syndrome and related disorders among Arabs. Hum Genome Var. 2014;1:14020. doi: 10.1038/hgv.2014.20. Erratum in: Hum Genome Var. 2015;2:15001. doi: 10.1038/hgv.2015.1.
26. Hsiao YC, Tong ZJ, Westfall JE, Ault JG, Page-McCaw PS, Ferland RJ. Ahi1, whose human ortholog is mutated in Joubert syndrome, is required for Rab8a localization, ciliogenesis and vesicle trafficking. Hum Mol Genet. 2009;18:3926-41. doi: 10.1093/hmg/ddp335.