CASO CLÍNICO/CASE REPORT

Clinical and Molecular Delineation of *AHI1*-Associated Joubert Syndrome in a Consanguineous Pedigree: A Case Report

Delineação Clínica e Molecular da Síndrome de Joubert Associada ao Gene *AHI1* numa Linhagem Consanguínea: Caso Clínico

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Abstract

Joubert syndrome (JS) is a rare autosomal recessive neurodevelopmental disorder characterized by the molar tooth sign (MTS) on brain magnetic resonance imaging (MRI), resulting from cerebellar vermis hypoplasia and elongated superior cerebellar peduncles.

A 4-year-old female presenting with global developmental delay, hypotonia, oculomotor apraxia, and MTS on MRI underwent whole-exome sequencing (WES). Bioinformatic analysis, Sanger validation, and segregation studies were performed. Pathogenicity was assessed using ACMG/AMP guidelines and in silico tools.

WES revealed a novel homozygous nonsense variant in *AHI1* (c.2938A>T; p.Lys980Ter), absent in population databases. Segregation analysis confirmed autosomal recessive inheritance, with both parents as heterozygous carriers. Evolutionary conservation of Lys980 underscored its functional importance.

This study expands the mutational spectrum of *AHI1*-related JS and highlights the utility of WES in consanguineous populations. The findings facilitate precise diagnosis, genetic counseling, and informed reproductive planning for at-risk families.

Resumo

A síndrome de Joubert (SJ) é uma doença rara do neurodesenvolvimento, de modo de hereditariedade autossómico recessivo, caracterizada pelo sinal do dente molar (SDM) na ressonância magnética (RM) cerebral, resultante de hipoplasia do vermis cerebeloso e alongamento dos pedúnculos cerebelosos superiores.

Uma criança do sexo feminino, de 4 anos, com atraso global do desenvolvimento, hipotonia, apraxia oculomotora e SDM na RM, foi submetida a sequenciação do exoma completo (WES), seguida de análise bioinformática, validação por Sanger e estudo de segregação.

A WES identificou uma nova variante nonsense homozigótica no gene *AHI1* (c.2938A>T; p.Lys980Ter), ausente nas bases de dados populacionais. A análise de segregação confirmou um modo de hereditariedade autossómico recessivo, com ambos os progenitores heterozigóticos portadores. A conservação evolutiva do resíduo Lys980 reforça a sua importância funcional.

Este caso amplia o espectro mutacional do *AHI1* associado à SJ e reforça a utilidade da WES em populações consanguíneas, permitindo diagnóstico preciso, aconselhamento genético e planeamento reprodutivo informado para famílias em risco.

Introduction

Joubert syndrome (JS) is a rare autosomal recessive neurodevelopmental disorder that is primarily defined by a distinctive midbrain-hindbrain malformation seen on axial brain MRI, known as the molar tooth sign (MTS). This neuroradiological hallmark arises from cerebellar vermis hypoplasia or aplasia, thickened and elongated superior cerebellar peduncles, and an abnormally deep interpeduncular fossa.[1] First described in 1968 by Marie Joubert and colleagues, the syndrome presents with a heterogeneous constellation of clinical features that include hypotonia, ataxia, oculomotor apraxia, irregular breathing patterns (episodic tachypnea and/or apnea), global developmental delay, and variable intellectual disability.[2] Additional findings may include nystagmus, strabismus, retinal dystrophy, nephronophthisis, polydactyly, liver fibrosis, and endocrine dysfunction.[3] Craniofacial features—such as high and broad forehead, arched eyebrows, ptosis, hypertelorism, and an open-mouth appearance often with tongue protrusion—are frequently observed and may support early clinical recognition.[4,5]

JS is genetically and phenotypically diverse and is now recognized as part of the expanding group of ciliopathies, disorders resulting from defects in the structure or function of the primary cilium.[3,6] The primary cilium is a microtubule-based organelle present on nearly all mammalian cells, playing a crucial role in signal transduction pathways essential for neurodevelopment, organogenesis, and tissue homeostasis. Dysfunctional ciliary signaling disrupts critical developmental processes, particularly in the central nervous system, kidneys, retina, and liver, explaining the pleiotropic nature of JS.[7-9]

To date, more than 35 genes have been identified to cause JS or JS-related disorders, all of which encode proteins localized to the primary cilium or basal body. These include, but are not limited to, *TMEM67*, *CEP290*, *NPHP1*, *CC2D2A*, *ARL13B*, *RPGRIP1L*, *INPP5E*, *TCTN1*, and *TCTN2*.[1] The frequency of involvement of each gene varies depending on population genetics, ethnicity, and the presence of consanguinity. Among these, *AHI1* (Abelson-helper integration site 1) is a key causative gene, accounting for approximately 10%–20% of cases.[10]

The *AHI1* gene encodes Jouberin, a scaffold protein that localizes to the basal body and transition zone of the primary cilium.[1] It is involved in intracellular trafficking and modulating signaling pathways such as Wnt and Hedgehog, which are critical for cerebellar and cortical development.[5,8] *AHI1* is also expressed in developing neurons and has been implicated in axonal pathfinding and neuronal polarization. Pathogenic variants in *AHI1* disrupt normal ciliary function and neuronal development, leading to the structural and functional abnormalities observed in JS. Interestingly, *AHI1* mutations are more commonly associated with the "pure" neurological form of JS, although multisystem involvement has been reported.[1,8]

Advances in next-generation sequencing (NGS) technologies, particularly whole-exome sequencing (WES), have significantly enhanced the identification of disease-causing variants in genetically complex and heterogeneous disorders such as Joubert syndrome. WES focuses on the exonic regions of the genome, which constitute approximately 1%–2% of the genome but contain the vast majority of known pathogenic mutations.[11,12] Its diagnostic utility is especially pronounced in consanguineous families, where autosomal recessive disorders often arise from homozygous mutations due to shared ancestry and identity-by-descent.[12-14] In such settings, WES enables efficient detection of rare, homozygous variants that may otherwise remain undetected by conventional approaches. In this study, we applied WES to investigate the genetic cause of Joubert syndrome in a single affected individual from an Iranian consanguineous family. We aimed to identify the underlying pathogenic variant and to contribute to the growing body of evidence defining the molecular and clinical characteristics of *AHI1*-associated JS.

Case Report

Clinical Assessment and Methodology

A 4-year-old female from a consanguineous Iranian family (**Fig. 1A**) was evaluated due to developmental delay and abnormal neurological signs. She was referred to our genetics unit after exhibiting hypotonia, oculomotor difficulties, and delayed milestones since infancy. A multidisciplinary team including pediatric neurology, clinical genetics, and ophthalmology specialists conducted a thorough clinical and neurological examination.

As part of the diagnostic workup, brain magnetic resonance imaging (MRI) and detailed physical evaluation were performed to assess structural abnormalities and syndromic features. Clinical data, family history, and photographic documentation were obtained following parental consent. The family pedigree was also reviewed to evaluate any hereditary patterns or recurrence.

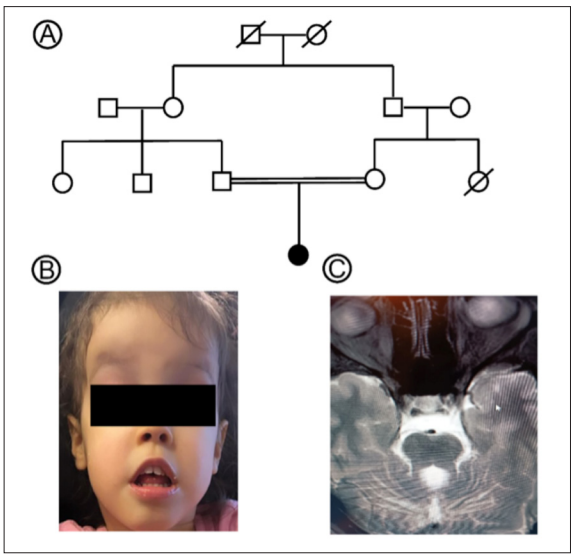

Figure 1. (A) Pedigree of the investigated family illustrating an inheritance pattern consistent with autosomal recessive transmission. Females are represented by circles and males by squares. The filled black circle indicates the proband, who is the subject of clinical evaluation. A diagonal slash through a symbol denotes deceased individuals. (B) Facial image of the patient showing arched eyebrows and an open mouth with hypotonic lips, consistent with craniofacial features observed in Joubert syndrome. (C) Axial T2-weighted brain MRI of the patient demonstrating the characteristic MTS with hypoplasia of the cerebellar vermis and thickened superior cerebellar peduncles.

Biological Sample Collection and DNA Preparation

Peripheral blood samples were collected from the proband and available first-degree relatives using standard venipuncture into EDTA tubes. Genomic DNA was extracted from leukocytes using the salting-out method. DNA quantity and purity were verified using NanoDrop spectrophotometry and 1% agarose gel electrophoresis. The DNA was then subjected to WES as part of the molecular diagnostic strategy.

Whole-Exome Sequencing and Bioinformatic Analysis

WES was performed to identify the potential causative variant underlying the patient's clinical phenotype. Genomic DNA from the proband was subjected to library preparation using the Agilent SureSelect Human All Exon V6 kit, followed by high-throughput sequencing on the Illumina platform. Paired-end reads (150 bp) were generated to ensure adequate coverage and depth across coding exons and flanking splice junctions.

The raw sequencing data were processed using a standard bioinformatics pipeline. Reads were aligned to the human reference genome (GRCh37/hg19) using the Burrows-Wheeler Aligner (BWA-MEM). Variant calling was carried out using the Genome Analysis Toolkit (GATK), with subsequent variant annotation performed using ANNOVAR and in-house scripts.

Variants were filtered to prioritize rare (minor allele frequency $<1\%$) coding and splice-site variants. Given the consanguineous background of the family and presumed autosomal recessive inheritance, the analysis focused on homozygous and compound heterozygous variants in genes previously implicated in ciliopathies, particularly Joubert syndrome and related disorders.

In silico prediction tools, including SIFT, MutationTaster, and CADD were used to evaluate the pathogenic potential of candidate variants. Population frequency data were obtained from gnomAD, and region-specific databases such as Iranome. Candidate variants were further evaluated for evolutionary conservation of the affected residues using NCBI resources and the Clustal Omega multiple sequence alignment tool (https://www.ebi.ac.uk/jdispatcher/msa/clustalo), which calculates conservation scores to identify functionally important regions. Variants were classified following the guidelines of the ACMG/AMP.

PCR Amplification and Sanger Sequencing Validation

To validate the candidate variant identified through exome sequencing and to assess segregation within the family, targeted PCR amplification followed by Sanger sequencing was performed. Specific primers flanking the region of interest were designed using Primer3 software to amplify the exon containing the putative variant. PCR was carried out in a total volume of 25 μL containing 50 ng of genomic DNA, 10 pmol of each primer, 1× PCR buffer, 1.5 mM $MgCl_2$, 200 μM dNTPs, and 1 unit of Taq DNA polymerase (Thermo Fisher Scientific, USA). Thermal cycling conditions included an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at a primer-specific temperature for 30 seconds, extension at 72°C for 30 seconds, and a final extension at 72°C for 5 minutes.

PCR products were visualized on a 1.5% agarose gel stained with ethidium bromide to confirm amplification specificity. The amplified fragments were then purified using a commercial PCR purification kit (Qiagen, Germany) and subjected to bidirectional Sanger sequencing using the ABI 3730xl DNA Analyzer (Applied Biosystems, USA). Sequencing chromatograms were analyzed using Chromas and aligned to the reference sequence

(GRCh37/hg19) using Sequencher or BLAST tools to confirm the presence of the variant and evaluate its segregation in available family members.

Clinical Phenotype Summary

The patient presented with hallmark features of Joubert syndrome, including global developmental delay, axial hypotonia, and episodic hyperpnea. Neurological examination revealed truncal ataxia and abnormal smooth pursuit with oculomotor apraxia. The patient did not experience seizures, and auditory responses were intact. Ophthalmological assessment showed mild retinal dystrophy, although visual evoked potentials were within normal limits.

Dysmorphic craniofacial features were noted, including a high anterior hairline, broad forehead, and distinctly arched eyebrows. The patient also exhibited a consistently open mouth posture and a tented upper lip, often associated with orofacial hypotonia (**Fig. 1B**).

Brain MRI revealed the pathognomonic MTS — reflecting cerebellar vermis hypoplasia, deepened interpeduncular fossa, and thickened, horizontally oriented superior cerebellar peduncles — further supporting the diagnosis of Joubert syndrome (**Fig. 1C**).

Family history revealed parental consanguinity, and there were no reports of similar conditions among siblings or extended family members. Systemic investigations, including renal ultrasonography, liver function tests, and echocardiography, showed no evidence of associated organ involvement at this stage.

Whole-Exome Sequencing Findings

WES of the proband identified a novel homozygous nonsense variant in the *AHI1* gene, located at chromosome 6:135411371A>T (hg38). This variant is designated c.2938A>T at the cDNA level and results in a premature stop codon at the protein level: p.(Lys980Ter). It is located in exon 21 of 29 of the *AHI1* transcript (NM_001134831.2, MANE Select: ENST00000265602.11), corresponding to position 174 of 197 within the coding portion of this exon.

The introduction of a premature termination codon at amino acid position 980 of 1197 is predicted to activate the nonsense-mediated mRNA decay (NMD) pathway, likely resulting in degradation of the mutant transcript and a consequent loss of functional AHI1 protein.

This variant is absent from major population databases, including gnomAD and Iranome, and has not been previously reported in the scientific literature or ClinVar, supporting its novelty. Based on the current ACMG/AMP guidelines, the variant is classified as Likely Pathogenic.

In Silico Functional Prediction of the *AHI1* (p.Lys980Ter) Variant

The c.2938A>T (p.Lys980Ter) variant in *AHI1* was subjected to comprehensive in silico analysis to assess its predicted pathogenicity. MutationTaster classified the variant as "disease-causing," with additional indications that it may marginally influence nearby donor splice sites; however, these effects were not scored as significant. Computational splice site predictions at gDNA positions 86408 and 86398 showed slight increases in donor scores between wild-type and mutant sequences, but remained below thresholds generally considered disruptive.

A Combined Annotation Dependent Depletion (CADD) score of 39 was observed for the variant, placing it well above the commonly used pathogenicity cutoff of 20 and suggesting a high likelihood of deleterious impact. The SIFT tool classified the variant as deleterious, consistent with its nature as a stop-gain mutation.

The variant introduces a premature stop codon at amino acid position 980, which is predicted to result in NMD. According to the ClinGen PVS1 decision framework, this qualifies as a very strong pathogenic criterion (PVS1). The loss-of-function mechanism is well-established for *AHI1*, and over 230 null variants have been reported as pathogenic in ClinVar, including multiple variants affecting exon 21, where this mutation is located. The variant is not located in the last exon nor within the last 50 base pairs of the penultimate exon, further supporting NMD prediction.

Population data from gnomAD confirm that this variant is absent across all subpopulations, meeting the PM2 (moderate) criterion for rarity. No homozygous occurrences were reported, which also supports pathogenicity. Other criteria (e.g., PS1, PM5, BP1–BP7, BS1–BS2) were unmet, primarily because this is a novel truncating variant with no previously reported substitutions at the same codon or region. Altogether, the in silico findings, in combination with ACMG/AMP classification criteria, support the designation of *AHI1* c.2938A>T; p.(Lys980Ter) as a Likely Pathogenic variant.

Sanger Validation, Familial Segregation, and Cross-Species Conservation Analysis

To confirm the presence and inheritance pattern of the *AHI1* c.2938A>T (p.Lys980Ter) variant identified through WES, Sanger sequencing was performed on the proband and both parents (**Fig. 2A**). Chromatogram analysis demonstrated that the proband is homozygous for the variant, whereas both the mother and father are heterozygous carriers. This segregation pattern is consistent with autosomal recessive inheritance and supports the likely pathogenic role of the variant in the proband's phenotype.

Furthermore, to assess the evolutionary conservation of the affected lysine residue (p.Lys980), a multiple sequence alignment was performed across 12 orthologous proteins from diverse vertebrate species, including human, bovine, murine, primate, and other mammals (**Fig. 2B**). The analysis revealed complete conservation of the lysine residue at this position across all examined species, indicating strong evolutionary constraint and suggesting functional importance. Substitution of this highly conserved residue with a premature termination codon further supports the deleterious impact of the c.2938A>T variant.

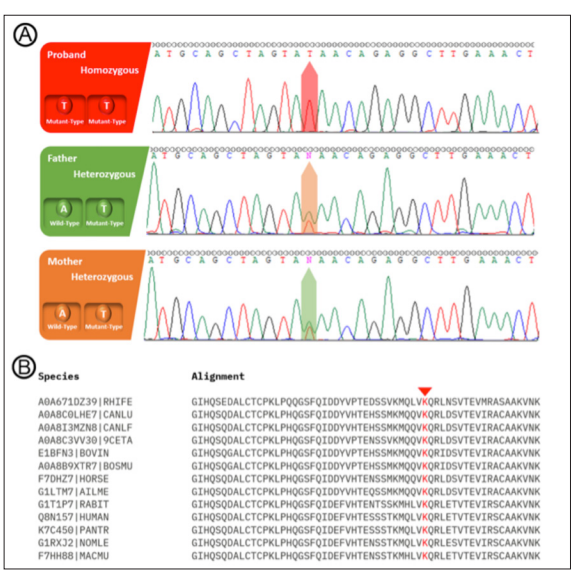

Figure 2. Sanger validation and evolutionary conservation of the *AHI1* c.2938A>T variant. (A) Electropherograms demonstrating the segregation of the *AHI1* c.2938A>T variant within the family. The proband carries the variant in homozygous state, while both parents harbor one wild-type and one mutant allele, confirming heterozygous carrier status. The altered nucleotide position is indicated by colored arrows. (B) Multiple sequence alignment across 12 vertebrate species showing high conservation of the lysine (K) residue at position 980 (highlighted in red), which is replaced by a premature stop codon (Ter) in the proband.

Follow-up

Long-Term Clinical and Genetic Management

Following the molecular diagnosis of JS due to a biallelic nonsense variant in *AHI1*, the patient was enrolled in structured long-term follow-up. Multidisciplinary surveillance was initiated to monitor neurological development, ocular function, and potential systemic involvement. Regular evaluations by pediatric neurology, ophthalmology, and developmental specialists were recommended to address evolving clinical needs.

Reproductive Risk Assessment and Counseling

Given the autosomal recessive inheritance pattern, the parents—confirmed heterozygous carriers—received targeted reproductive counseling. The couple was informed about the 25% recurrence risk in each pregnancy and the importance of extended carrier testing in consanguineous relatives. Counseling sessions emphasized informed reproductive planning, addressing both medical and ethical dimensions of reproductive risk in genetically at-risk populations.

Prospective Prenatal and Early-Life Planning

For future pregnancies, options for early fetal genetic assessment were presented, including chorionic villus sampling (CVS) and amniocentesis, enabling targeted testing for the known *AHI1* familial variant. Advanced fetal neuroimaging techniques, such as second-trimester fetal MRI, were recommended to detect hallmark cerebellar malformations associated with Joubert syndrome. Neonatal care planning focused on early identification of hypotonia, respiratory dysregulation, and oculomotor abnormalities to facilitate prompt intervention.

Assisted Reproductive Technologies and Preventive Strategies

The availability of assisted reproductive options was reviewed in detail. In vitro fertilization (IVF) paired with preimplantation genetic testing (PGT-M) was highlighted as a preventive strategy, allowing for selection of unaffected embryos before implantation. This approach is particularly valuable for carrier couples within consanguineous populations. Alternative reproductive options, including gamete donation, were also discussed, tailored to the couple's reproductive goals and cultural context.

Engagement in Research and Translational Advancements

The family was invited to participate in genetic research initiatives aimed at characterizing rare ciliopathies and refining genotype-phenotype correlations. Engagement in patient registries and rare disease networks provides opportunities for access to emerging therapeutic trials, including RNA-based interventions and future gene therapy prospects. Such participation not only benefits the family but also contributes to the broader understanding of Joubert syndrome and related disorders. Families were encouraged to remain engaged with research consortia and advocacy organizations to stay informed on developments in diagnosis and care.

Discussion

JS is a rare neurodevelopmental disorder inherited in an autosomal recessive manner and characterized by a distinctive mid-hindbrain malformation known as the MTS on axial brain MRI. Although precise epidemiological data are limited, estimates suggest a prevalence of approximately 1 in 90 000 live births.[1] However, underdiagnosis is likely, particularly in regions with limited access to neuroimaging and genetic testing, where MTS may go unrecognized during routine clinical evaluation. The disorder exhibits substantial genetic heterogeneity, with more than 35 genes implicated to date, most of which encode proteins involved in primary cilium structure and function. Among these, *AHI1* has been consistently associated with classical forms of JS, often presenting with neurological and oculomotor manifestations.[9,15] The identification and characterization of novel variants in these genes continue to expand our understanding of the molecular mechanisms underlying JS and inform diagnostic and reproductive strategies for affected families.

In this report, we describe a 4-year-old female from a consanguineous Iranian family who was clinically diagnosed with JS following a comprehensive neurological and genetic evaluation. The patient presented with global developmental delay, axial hypotonia, truncal ataxia, and oculomotor apraxia. Brain MRI demonstrated the MTS, confirming the clinical suspicion. WES identified a novel homozygous nonsense variant in the *AHI1* gene (c.2938A>T; p.Lys980Ter) located in exon 21 (NM_001134831.2), predicted to result in premature termination. Sanger sequencing validated the presence of this variant and confirmed its segregation within the family, with both parents shown to be heterozygous carriers.

The *AHI1* gene encodes the protein jouberin, which plays a crucial role in primary cilium function and is also involved in modulating the Wnt/β-catenin signaling pathway, where it promotes the nuclear translocation of β-catenin and contributes to neurodevelopmental signaling processes. Pathogenic variants in *AHI1* disrupt these cellular pathways, leading to ciliopathy-related phenotypes characteristic of JS.[15-17] Most reported *AHI1* mutations are protein-truncating variants, including nonsense and frameshift mutations, whereas missense mutations have been less frequently observed.[18,19] The nonsense variant identified in our study, c.2938A>T (p.Lys980Ter), expands the mutational spectrum of AHI1 and is predicted to introduce a premature stop codon that likely triggers NMD. As a result, either no protein or a severely truncated, nonfunctional product is produced, disrupting essential molecular processes during early brain development. Our findings support the role of loss-of-function *AHI1* mutations in the pathogenesis of Joubert syndrome and reinforce the importance of identifying such variants for precise diagnosis and genetic counseling.

The frequency of *AHI1* pathogenic variants appears to be elevated in populations with a high rate of consanguinity, such as those in certain Arab and Middle Eastern communities.[20,21] Given that Khuzestan province in Iran hosts a substantial Arab population with cultural norms that may include consanguineous marriage, it is plausible that *AHI1*-related Joubert syndrome is underdiagnosed in this region. Although our identified variant is novel, it is possible that the same mutation may exist in a homozygous state in other undiagnosed individuals within this population. Several factors may contribute to the lack of detection, including early mortality in severely affected infants, limited access to specialized genetic services, and sociocultural or economic barriers that prevent families from pursuing diagnostic evaluation. Furthermore, a general lack of awareness among healthcare providers regarding rare neurogenetic disorders such as JBTS may delay referral and genetic testing. These findings underscore the importance of expanding regional genetic screening programs and increasing clinical awareness in areas with high rates of consanguineous unions to improve early diagnosis and family planning strategies.

Previous studies on *AHI1* variants in JS have identified various types of pathogenic variants, including

nonsense (e.g., Q423X, K246X, R738X, W420X), frameshift (e.g., nt1898insGG, fsX648), splice-site (e.g., IVS11+5insTTAC, IVS8−2A>G), and missense mutations (e.g., W725R, D719G).[15,22] These variants commonly result in loss of function by affecting protein stability, splicing accuracy, or disrupting critical domains, which leads to JS-associated clinical features such as cerebellar vermis hypoplasia, oculomotor apraxia, episodic hyperpnea, and retinal dystrophy. Our patient carries a novel homozygous nonsense mutation (c.2938A>T; p.Lys980Ter), which introduces a premature stop codon in exon 21. Similar to previously reported truncating mutations, this variant is expected to impair *AHI1* function or protein stability, leading to the classic JS phenotype, including the MTS, hypotonia, and retinal involvement.

Although some individuals with *AHI1* variants may exhibit renal involvement resembling nephronophthisis or experience respiratory dysregulation in infancy,[22] no such systemic abnormalities were detected in our patient at the time of evaluation. Renal imaging, liver function testing, and cardiopulmonary assessments were all within normal limits, suggesting a milder or organ-limited clinical expression. Importantly, multiple lines of computational evidence support the deleterious nature of the identified *AHI1* variant. The nonsense mutation c.2938A>T (p.Lys980Ter) demonstrated a high CADD score, indicating strong predicted pathogenicity. Additionally, MutationTaster classified the variant as "disease-causing," and conservation analysis showed that the affected lysine residue is highly preserved across diverse vertebrate species, underscoring its functional relevance. Segregation analysis further reinforced its clinical significance, with both consanguineous parents confirmed as heterozygous carriers. Taken together, the genetic, computational, and segregation data provide compelling evidence that this previously unreported *AHI1* variant is likely pathogenic and causative of the proband's JS phenotype. These findings highlight the critical role of WES in identifying rare and novel variants in clinically complex or genetically heterogeneous disorders, particularly within consanguineous families.

Conclusion

We describe a novel homozygous nonsense variant in the *AHI1* gene (c.2938A>T; p.Lys980Ter) in a patient with clinically and radiologically confirmed JS. The variant is absent from population databases, segregates appropriately within the family, and affects a highly conserved residue, supporting its likely pathogenic classification. This case adds to the mutational landscape of *AHI1*-related JS and highlights the importance of early genetic diagnosis in populations with high consanguinity. Precise molecular characterization enables targeted counseling, informed reproductive planning, and consideration of preventive strategies in future pregnancies. ■

Availability of Data and Materials

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

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Contributorship Statement / Declaração de Contribuição

DHI: Conceptualization; Investigation; Writing – Original Draft.
AKH: Conceptualization; Investigation; Writing – Review and Editing.
SMS and EN: Investigation; Writing – Original Draft.
SKA-M and KJ: Investigation; Writing – Review and Editing.
MS-H: Investigation.
All authors approved the final version to be published.

DHI: Conceptualização; Investigação; Redação – Rascunho Original.
AKH: Conceptualização; Investigação; Redação – Revisão e Edição.
SMS e EN: Investigação; Redação – Rascunho Original.
SKA-M e KJ: Investigação; Redação – Revisão e Edição.
MS-H: Investigação.
Todos os autores aprovaram a versão final a ser publicada.

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