Molecular Evaluation of Joubert Syndrome and Hearing Impairment in a Patient with Ataxic Cerebral Palsy

N. Sreedevi 1  N. Swapna 2  Santosh Maruthy 1  T. Jayakumar 1  Charles Sylvester 3

1 Department of Speech-Language Sciences, All India Institute of Speech and Hearing, Mysore, India
2 Department of Speech-Language Pathology, All India Institute of Speech and Hearing, Mysore, India
3 Unit for Human Genetics, All India Institute of Speech and Hearing, Mysore, India


Abstract
Joubert syndrome (JBTS) is a rare autosomal recessive or X-linked congenital brain malformation with strong genetic heterogeneity. Other neurological features of JBTS include hypotonia, ataxia, developmental delay, and cognitive impairment. Hearing loss with JBTS has been reported in the literature. We present the case of a 3.5-year-old boy born to a healthy consanguineous South Indian couple who was presented with ataxic cerebral palsy (CP) and hearing impairment; medical reports confirmed typical brain malformations of JBTS. Hearing impairment was screened by audiological assessment, which confirmed the presence of severe-profound hearing loss with outer hair cell dysfunction. Whole-exome sequencing (WES) was performed to know the molecular aspects of the condition and to detect any novel mutations. The homozygous mutation AHI1 c.2023G > A associated with JBTS type 3 and GJB2 c.71G > A mutation associated with hearing impairment were identified. Sanger sequencing was performed to validate the result and it identified heterozygous AHI1 c.2023G > A and GJB2 c.71G > A in the patient’s parents. This study confirms the diagnosis of JBTS by WES helps identify the genetic causes of hereditary disorders that accelerate genetic evaluation and counseling for at-risk families.

Keywords
- Joubert syndrome
- hearing loss
- AHI1
- GJB2

Introduction
Joubert syndrome (JBTS) is a rare neurodevelopmental ciliopathy characterized by cerebellar and brainstem malformation, hypotonia, respiratory deficit, ataxia, developmental delay, and cognitive impairment with congenital onset. 1,2 Axial brain imaging of JBTS is a characteristic malformation that resembles a molar tooth sign. 3 Along with the neurological aspects, over two-thirds of individuals with JBTS have organ defects such as ocular dystrophy, renal disease, hepatic fibrosis, and skeletal changes, which are noticeable at different ages with varied severity. 4,5 JBTS and related disorders are associated with a high prevalence of strabismus. 6 The epidemiology of JBTS is estimated between 1/80,000 and 1/100,000 live births. 7 JBTS is an autosomal recessively or X-linked inherited syndrome with a strong genetic heterogeneity and consanguinity that has been frequently associated with JBTS. 2,8 Hearing loss with JBTS has been reported in the literature. 9,10 The significant phenotypic overlap and wide variability of the ciliopathy can be explained by molecular
and cellular etiology. To identify the underlying genetic defects in the proband, we performed whole-exome sequencing followed by direct sequencing. In this study, we show that mutations in AHI1, which encodes the Jouberin protein at the JBTS3 locus, cause JBTS and GJB2, which encodes Connexin 26 protein, causes deafness in a south Indian patient.

Clinical Report and Molecular Analysis

The proband, a 3.5-year-old boy, was born to a healthy consanguineous couple. He was born full term and was delivered through cesarean section weighing 3,500 g, with no birth asphyxia. He was hypotonic as an infant and presented with ataxia and global developmental delay. Delayed speech and language and severe mental retardation were noted (Table 1). Audiological evaluation revealed severe to profound hearing loss; distortion-product otoacoustic emission (DPOAE) was absent in both ears. Magnetic resonance imaging (MRI) has confirmed JBTS as per previous medical records. Blood samples were collected from the patient and his family members after obtaining written informed consent. Genomic DNA was extracted from peripheral blood by using PureLink Genomic DNA Mini Kit (Thermo Fisher Scientific, United States) according to the manufacturer’s instructions. Whole-exome sequencing was performed for the proband. The exome libraries were constructed using the Ion AmpliSeq Exome RDY kit (Thermo Fisher Scientific, United States) and sequenced on the Ion Proton sequencing platform (Life Technologies, United States). Variants were called using the Torrent server. Variants were annotated by Ion Reporter (Thermo Fisher Scientific, United States) using the human reference genome (hg19). Sanger sequencing was performed for variant validation of the proband and the proband’s parents’ samples by amplifying the AHI1 loci using the primers (forward: 5′-TTAATAACCCCTAACCCCATCTC-3′, reverse: 5′-TTTCTCTGTCGAAATGTCCT-3′) and the GJB2 loci were amplified using the primers (5′-TCTTTCACAGCACAACCCAA-3′, reverse: 5′-GACACGAAGATACGCTGCA-3′) for a total volume of 10 μL. An initial denaturation step at 95°C for 2 minutes was followed by 35 cycles of 98°C for 25 seconds, 65°C for the AHI1 variant, and 60.3°C for the GJB2 variant for 45 seconds for annealing, 72°C for 30 seconds for elongation, and final extension at 72°C for 7 minutes. The polymerase chain reaction (PCR) products were evaluated using a 2% agarose gel electrophoresis. PCR products were labeled with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, United States). The above-mentioned PCR primers (AHI1 forward and GJB2 forward) were used as sequencing primers and then analyzed by ABI 3500 Genetic Analyzer (Applied Biosystems, United States). Sequence data were analyzed with SeqScape v3 software (Applied Biosystems, United States).

Discussion

We present mutations in AHI1 and GJB2 genes in a patient clinically diagnosed with JBTS and hearing impairment. The AHI1 variant (NM_001134831.2) c.2023G > A.p.D675N and the GJB2 variant (NM_004004.6) c.71G > A.p.W24X, consistent with the clinical findings of Joubert syndrome 3 (JBTS3) and hearing impairment, respectively, were identified. The mutation AHI1 c.2023G > A.p.D675N is a classical feature of JBTS3 (OMIM #608629), heterozygous AHI1 c.2023G > A.p.D675N and heterozygous GJB2 c.71G > A.p.W24X were detected in the mother and father of the proband, which revealed the autosomal recessive mode of inheritance (Figs. 1 and 2). The AHI1 gene is located on chromosome 6q23.3, has 31 exons, and encodes Jouberin, a protein in the primary cilium with 1,196 amino acid residues containing a

<table>
<thead>
<tr>
<th>ID</th>
<th>CP_69A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
</tr>
<tr>
<td>Age</td>
<td>3.5 y</td>
</tr>
<tr>
<td>National origin</td>
<td>India</td>
</tr>
<tr>
<td>Variant 1</td>
<td>AHI1 c.2023G &gt; A.p.D675N</td>
</tr>
<tr>
<td>Variant 2</td>
<td>GJB2 c.71G &gt; A.p.W24X</td>
</tr>
<tr>
<td>Molar tooth sign</td>
<td>+</td>
</tr>
<tr>
<td>Hypotonia</td>
<td>+</td>
</tr>
<tr>
<td>Ataxia</td>
<td>+</td>
</tr>
<tr>
<td>Developmental delay</td>
<td>+</td>
</tr>
<tr>
<td>Mental retardation</td>
<td>+</td>
</tr>
<tr>
<td>Strabismus</td>
<td>+</td>
</tr>
<tr>
<td>Delayed speech and language</td>
<td>+</td>
</tr>
<tr>
<td>Hearing loss</td>
<td>+</td>
</tr>
<tr>
<td>Renal involvement</td>
<td>NA</td>
</tr>
<tr>
<td>Respiratory abnormalities</td>
<td>NA</td>
</tr>
<tr>
<td>Liver involvement</td>
<td>NA</td>
</tr>
<tr>
<td>Limb anomalies</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: +, present; NA, information not available.

Fig. 1 Pedigree of the family with genotypes of the variant AHI1 c.2023G > A shown in corresponding pedigree members. Parents are heterozygous for the mutation.
Molecular Evaluation of JJBTS and Hearing Impairment in a Patient with Ataxic CP  

Sreedevi et al.

Global Medical Genetics Vol. 10 No. 3/2023 © 2023. The Author(s).

The AHI1 c.2023G > A p.D675N mutation has been previously reported only once in the literature. To the best of our knowledge, this study is the second report of JJBTS and hearing loss in a patient by detecting the disease-causing mutations AHI1 c.2023G > A and GJB2 c.71G > A of JJBTS3 and hearing impairment, respectively. Despite its low incidence, JJBTS should be considered a differential diagnosis that can accelerate the genetic evaluation process and make an informed decision for at-risk families for accurate genetic evaluation and counseling.

Ethical Approval
The study was approved by the Ethics Committee of All India Institute of Speech and Hearing, Mysore, India.

Author Contribution
N. Sreedevi was responsible for conceptualization, supervision, and project administration. N Swapna contributed to conceptualization. Santosh Maruthy was responsible for supervision and project administration. T. Jayakumar contributed to supervision. Charles Sylvester was responsible for the methodology, molecular biology, bioinformatics, and writing the original manuscript.

Funding
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of Interest
None declared.

Acknowledgments
The authors thank the Director, All India Institute of Speech and Hearing, Mysore, and also acknowledge the subjects for their participation.

References
11 Huang L, Szymanska K, Jensen VL, et al. TMEM237 is mutated in individuals with a Joubert syndrome related disorder and expands the role of the TMEM family at the ciliary transition zone. Am J Hum Genet 2011;89(06):713–730
23 Dan B. How useful is the diagnosis of ataxic cerebral palsy? Dev Med Child Neurol 2020;62(03):264