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Homozygous *AHI1* gene mutation (c.1213A>C; Thr405Pro) leads to Joubert syndrome in a consanguineous Iranian family: A case report

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Abstract

Joubert syndrome (JS) is an autosomal recessive (AR) neurological disorder primarily characterized by aplasia/hypoplasia of the cerebellar vermis, retinal degeneration, and a molar tooth sign (MTS) can be seen on brain imaging. This study aimed to identify JS pathogenic variant in an Iranian consanguineous pedigree. We carried out whole-exome sequencing (WES) to identify likely causal pathogenic variant in the patient. The WES analysis identified a novel homozygous missense mutation (c.1213A>C; p.T405P) in the *AHI1* gene (in exon 10). The Sanger sequencing data has validated the c.1213A>C mutation. We postulated that the disease in our patient was caused by a novel homozygous missense mutation in the *AHI1* gene. To the best of our knowledge, this is the first report of the *AHI1* pathogenic variant causing JS phenotype in an Iranian family. Our data expand the spectrum of mutations in the *AHI1* gene in JS.

Keywords: Joubert syndrome, *AHI1* gene, mutation

INTRODUCTION

Joubert syndrome (JS) is an autosomal recessive (AR) neurodevelopmental disorder characterized by aplasia/hypoplasia of the cerebellar vermis and retinal degeneration. An exceptional case of X-linked recessive (XLR) transmission due to an OFD1 mutation (gene) in a patient affected by JS has been reported. Neuroradiologically, the syndrome is characterized by the molar tooth sign (MTS), brachium conjunctivum, and a deep interpeduncular cistern (Fossa interpeduncularis).[1,2]

Patients with JS are currently diagnosed by evidence of the MTS in their brains. Magnetic resonance imaging (MRI) of the brain usually reveals central nervous system (CNS) defects affecting mostly the tectum and midbrain. It has been demonstrated that MTS is a prominent feature of JS, but it is also seen in various disorders called Joubert syndrome-related disorders (JSRD), demonstrating the JS neurological aspects association with organ involvement that it can be related with more CNS deformities.[3]

The estimated frequency of JS is about 1 in 80,000 to 1 in 100,000. In Arab countries and Iran because of the rates of consanguinity, the prevalence of JS is higher than in other countries.[2,4]

Five candidate genes including *AHI1*, *NPHP1*, *CEP290*, *TMEM67*, and *RPGRIP1L* have been reported as being associated with JS.[5] Mutations in the Abelson helper integration-1 (*AHI1*) gene, which encodes a protein known as Jouberin, accounted for 10% to15% of cases of the AR JS.[1] *AHI1* gene is located on the long arm of chromosome 6 (6q23.3) and contains 31 exons, but two of the exons are alternatively spliced, and producing shorter isoforms.[6,7] In the current study, we aimed to screen the candidate causative gene mutant which could explain the JS phenotype in the family by using the whole exome sequencing (WES) followed by Sanger sequencing.

CASE REPORT

A consanguineous Iranian family referred to the Noor Gene Genetic lab (Ahvaz, Khuzestan, Iran)

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for molecular investigation (Figure 1). The parents have two children. Their first child was a girl who was dead at 3-months with a suspicious history of JS and her parents noted that she had polydactyly of the great toe and unusual eye movement. The proband was a 3-year-old boy who had hypotonia, psychomotor delay, and his speech was limited to unclear sounds. Clinical examination showed high arched eyebrows, open mouth (Figure 1B), and he could not control his voluntary motor movements (ataxia), in walking and speaking. The diagnosis of JS was confirmed by the findings of MTS in a brain MR image (Figure 1C). The father and mother were first cousins and showed no signs or symptoms of JS. A written and signed informed consent was obtained from the parents of the patient for publication.

A standard salting-out method was used to extract genomic DNA (gDNA) from lymphocytes in the peripheral blood of the patient and his parents. The samples were stored at -20°C until use. A Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE) was used to measure all samples' purity and concentration at wavelength ratios of 260/230 and 260/280 nm.

In our study, WES (Macrogen, Seoul, South Korea) with a focus on JS genes was performed. A homozygous potentially pathogenic mutation in *AHI1* gene (c.1213A>C; p.Thr405Pro), located in exon 10 (NM_001134831.2) was detected. This mutation was a novel mutation of the *AHI1* gene and results in a missense mutation with substitution of Threonine to Proline at position 405. This mutation has not been reported in the other patients with JS.

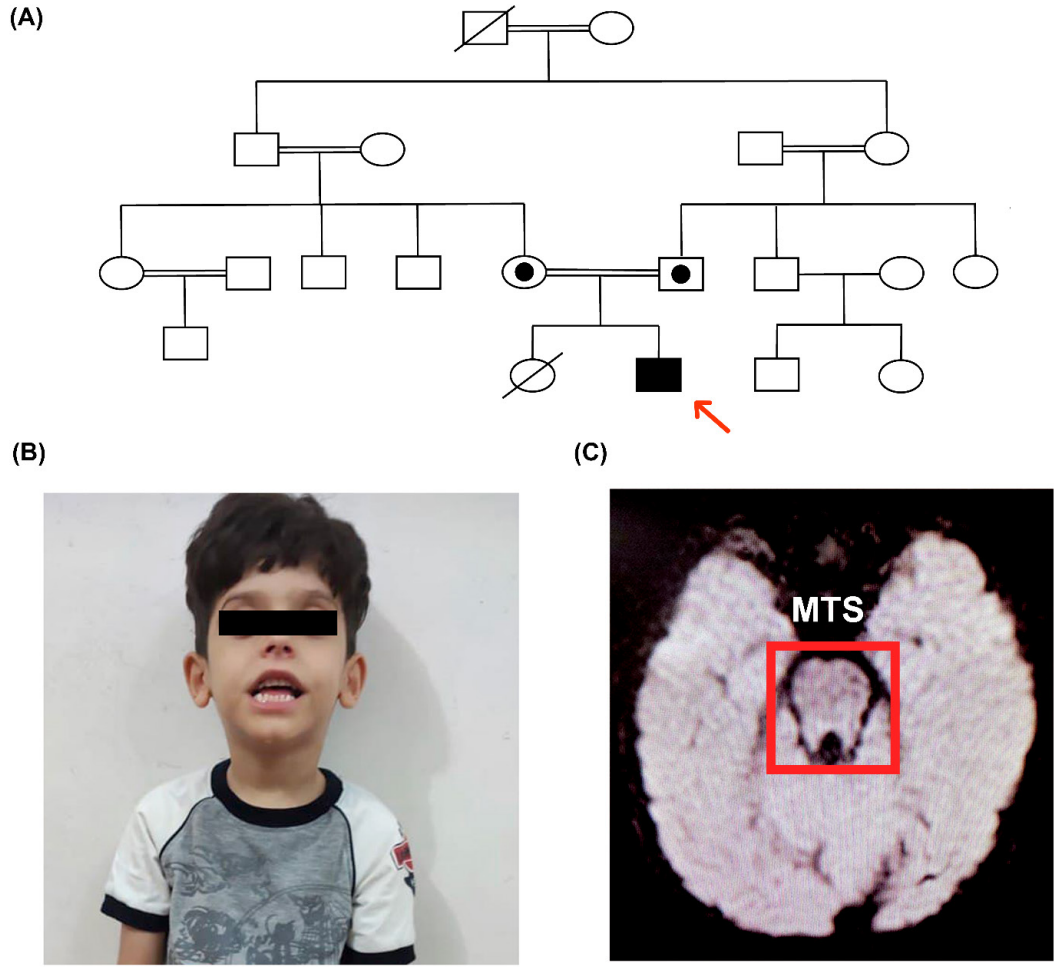

Figure 1. A. Pedigree of Iranian family with JS. Red arrow indicates the proband. Subjects indicated by a black dot within the symbol were heterozygous for the detected mutation but had no clinical manifestations. B. Photograph of the face showing typical facial features of JS including high arched eyebrows and wide open mouth. C. MTS on MR image.

Table 1: Results of in silico prediction tools for functional effect of the novel missense mutation

Gene/variant	Polyphen2 HDIV score	SIFT score	Mutation taster
ENST00000265602, T405P	0.891(possibly damaging)	0.002 (damaging)	Disease-causing

Public mutation and polymorphism databases such as 1000 Genomes Project (https://www.internationalgenome.org/), and ExAC Browser were used to analyze and interpret the sequencing data. Only variants with frequency of less than 1% were selected. in silico softwares such as SIFT, MutationTaster, and PolyPhen-2 were used to predict the variant's pathogenicity and was considered to be damaging and disease-causing (Table 1). Furthermore, this missense variant alter highly evolutionary conserved amino acid (Figure 2A). Thus, it is recommended the mutated residue shown in figure 2A (p.T405P) is necessary for proper function of the protein. Figure 2B shows the location of the novel *AHI1* variant of the Jouberin protein.

Finally, Sanger sequencing was carried outby Big Dye Terminators (ABI 3130 Genetic Analyzer; ABI, California, USA) to confirm the presence of potential pathogenic variant in the patient and family members. The findings showed true-positive of the novel homozygous missense variant within exon 10 in the patient and the normal parents are heterozygous for the identified mutation (Figure 3).

DISCUSSION

JS is a rare autosomal recessively inherited neurological disorder where accurate epidemiological data is still not available. WES in consanguine families is a valuable tool to identify genetic causes of the disease in patients with JS.[3] So, we used the WES technique to identify the impaired gene associated with JS in the family. To date, based on Human Gene Mutation Database (HGMD), 66 mutations responsible for JS have been recognized in the *AHI1* gene.

Hence, in this study, we have detected a novel mutation in the *AHI1* gene in the Iranian family. Their affected boy presents associated JS features such as high arched eyebrows, wide open mouth, ataxia, and MTS in MR image of the brain. But his father and mother showed no signs or symptoms of JS.

The human Jouberin protein encodes by the *AHI1* gene and expressed in high levels in the brain and kidney. This protein consists of a coiled-coil (CC) at the N-terminus, six WD40 domains, and an SRC Homology 3 (SH3) domain towards the C-terminus. Jouberin protein regulates Wnt signaling and promotes nuclear translocation of β-catenin. Previously have been described variation in the *AHI1* gene (encoding the disease-causing protein) in JS patients without any evidence of renal involvement. Since most *AHI1* gene mutations in humans can result in truncated *AHI1* proteins and there are few reported missense mutations[3,6,7], our findings add another a rare

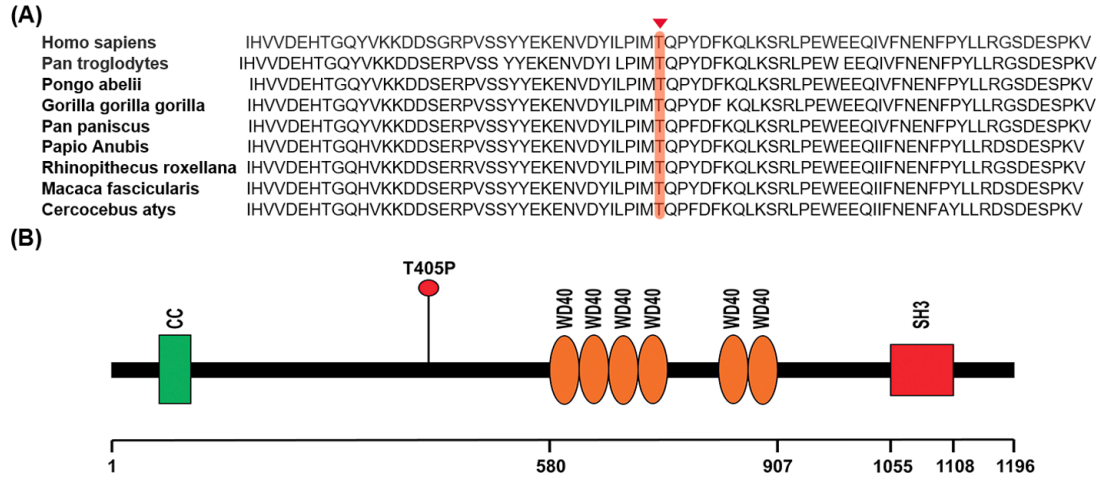

Figure 2. Conservation analysis. Protein alignment shows conservation of the amino acid sequence of *AHI1* at position 405 among different species. B. Molecular structure and location of the detected mutation (T405P).

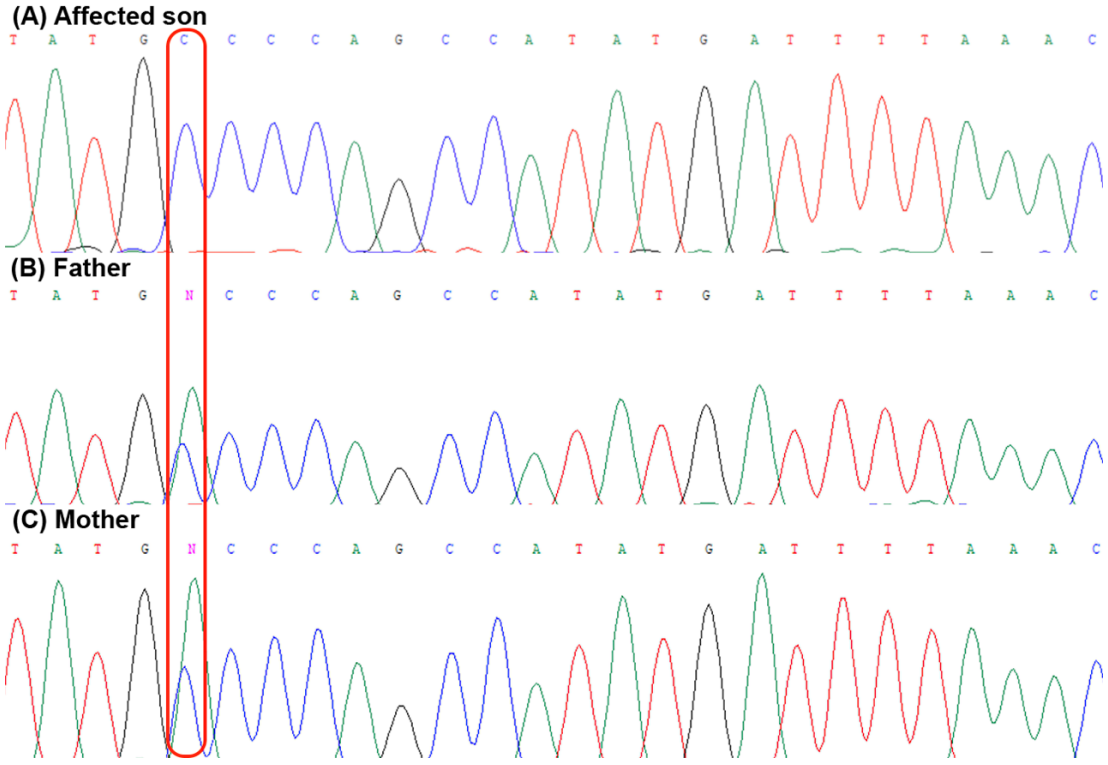

Figure 3. Electropherograms analysis of *AHI1* showing that homozygous c.1213A>C mutation (ACC>CCC) in the affected proband (A) and his healthy parents are heterozygous for the identified mutation (B, C).

novel missense mutation to exiting knowledge. We proposed that our identified Thr405Pro variant encodes an impaired protein that probably defects in function or stability of the protein, resulting in JS phenotype.

AHI1 gene mutations are more prevalent that cause specific forms of JSRD with different frequencies.[8] Neissi *et al*. reported that a novel *AHI1* gene mutation (c.1064 T>G; p.L355R) correlates with JS.[3] In line with these findings, Utsch *et al*. have identified three novel *AHI1* mutations (c.2369insT, c.444insA, c.365-688del) in patients affected by JS.[6] In another investigation in the area of molecular etiology in inherited JS demonstrated a frequency of 7.3% and with only one missense *AHI1* mutation.[8] The *AHI1* gene mutation that was recognized in our patient also cause a JS clinical manifestation.

Recently it has been reported that *NPHP1* mutations are responsible for 25% of NPHP and 1 to 2% of JS. Also, Tory *et al*. found out that NPHP patients with both homozygous *NPHP1* and heterozygous *AHI1* mutations had neurological symptoms. Additionally, some of the patients who were detected with homozygous *NPHP1* mutations were without neurological symptoms. These results, demonstrating a protein-protein interaction between Nephrocystin-1 and Jouberin, and patients with *NPHP1* mutations can have a different phenotype if *AHI1* mutations are present.[1,9] During our mutation screening of candidate genes in the patient with JS, only a homozygous c.1213A>C mutation was detected in the *AHI1* gene and no disease-causing variants have been identified in other genes such as *NPHP1*.

It was demonstrated that phenotype of JS is characterized by low-set ears, prominent forehead, wide open mouth, protrusion of tongue due to soft tissue swelling in the base of the mouth, and polydactyly. In addition, the pathognomonic finding in this syndrome is the unique MTS on MR image of the brain.[10-12] In our case, there was high arched eyebrows, wide open mouth, and MR image of the brain showing the characteristic MTS, although other signs or symptoms were absent.

In conclusion, in the current study, a novel single-nucleotide variant in the *AHI1* gene has been reported. This missense mutation in the exon 10 (c.1213A>C or p.Thr405Pro) makes substitution of Threonine by Proline which would be expected to affect the protein's function

resulting in this neurological condition. The results may be helpful during genetic counseling to the affected families with JS.

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