Joubert 증후군 소아에서 엑솜시퀀싱

Whole Exome Sequencing in a Korean Child with Joubert Syndrome-related Disorders

이종화1,5 · 오인경2 · 윤미진3 · 윤귀현4,5

Jong Hwa Lee, M.D.1,5, In Kyung Oh, M.D.2, Mi Jin Yoon, M.D.3, Kui Hyun Yoon, M.D.4,5

원광대학교 의과대학 산본병원 소아청소년과, 안과, 영상의학과, 진단검사의학과, 원광대학교 의과학연구소

Departments of Pediatrics¹, Ophthalmology², Radiology³ and Laboratory Medicine⁴, Wonkwang University Sanbon Hospital, Gunpo; Institute of Wonkwang Medical Science⁵, Iksan, Korea

Joubert syndrome and Joubert syndrome-related disorders (JSRDs) are rare autosomal recessive or X-linked disorders characterized by cerebellar vermis hypoplasia and a brain stem malformation, which presents as the “molar tooth sign” in magnetic resonance imaging (MRI). JSRDs are a group of clinically heterogeneous conditions that exhibit neurological manifestations and multiple organ involvement. JSRDs are also genetically heterogeneous, and approximately 20 causative genes that account for 45% of JSRDs have been identified. A 7-yr-old boy visited Wonkwang University Sanbon Hospital with the following presentations: no ocular fixation, ataxia, growth retardation, and hypotonia. Physical examination revealed facial dysmorphism, spindle shaped fingers, and height (99 cm) and weight (13 kg) below the third percentile. Ophthalmic examination revealed retinal dystrophy. A diagnosis of JSRDs was made based on clinical and brain MRI findings. We found two heterozygous variants c.2945G>T; p.Arg1325Trp (C>T) and c.2216dupA; p.Phe740Valfs*2 (dupA) in AHII, and a heterozygous c.3973C>T; p.Arg1325Trp (C>T) variant in KIF7 by whole exome sequencing (WES). Genetic analysis on the proband’s father revealed that he had both AHII variants, but did not have the KIF7 variant, which was inconsistent with autosomal recessive inheritance. Therefore, the G>T variant and C>T variant were presumed to be of “uncertain significance.” Furthermore, one novel dupA variant was interpreted as “pathogenic,” while the second allele was not detected. Caution should be exercised while interpreting the significance of variants detected by WES. In addition, the involvement of genes other than the 20 known ones will require further investigation to elucidate the pathogenesis of JSRDs.

Key Words: Joubert syndrome, Whole exome sequencing, AHII, KIF7

Joubert syndrome (JS) and Joubert syndrome-related disorders (JSRDs) are rare autosomal recessive or X-linked disorders. Their characteristic features include cerebellar vermis hypoplasia and a brain stem malformation that presents as the diagnostic marker “molar tooth sign” in magnetic resonance imaging (MRI). JSRDs are clinically heterogeneous, showing neurological manifestations and multiple organ involvement, particularly of the retina, kidney, liver, and skeleton. Therefore, they are classified into six subtypes: pure JS, JS with ocular defect, JS with renal defect, JS with urocerebral defects, JS with hepatic defect, and JS with orofaciocutaneous defects [1]. JSRDs are also genetically heterogeneous, and approximately 20 causative genes, accounting for 45% of JSRDs have been identified [2]. However, the number of identified genes is likely to increase with the discovery of novel genes [2, 3] (Table 1). More so since the diagnostic value of next generation sequencing in rare inheritance disorders has been recently reported [4-6].

The patient was a 7-yr-old boy, who visited Wonkwang University Sanbon Hospital with the principal presentations of lack of ocular fixation, ataxia, growth retardation, and hypotonia. Physical examination revealed facial dysmorphism and spindle shaped fingers, while ophthalmic examination revealed retinal dystrophy. Furthermore, his height (99 cm) and weight (13 kg) were below the third percentile. JSRDs was diagnosed based on clinical and
brain MRI findings (Fig. 1). Routine hematological and biochemical analyses were within normal limits (e.g., bilirubin, AST, ALT, blood urea nitrogen, creatinine), and his chest X-ray and abdominal CT findings were normal. His family history was unremarkable.

A few cases of JSRDs have been reported in Korea, diagnosed based on clinical and radiological findings, but without any molecular genetic studies [7-9]. To identify causative mutations, whole exome sequencing (WES) was performed with the patient’s DNA (with the written informed consent of the proband’s father) and the 20 known causative genes of JSRDs were included in the analysis [2]. Genomic DNA was enriched using the SureSelect all exon V4 (Agilent Technologies, Santa Clara, CA, USA), which targets 334,378 exons of a 51 Mb region spanning 20,965 genes. WES was performed using an Illumina HiSeq 2000 (Illumina Inc., San Diego, CA, USA) with the reference sequence UCSC assembly hg19 (http://genome.ucsc.edu/) and the BWA mapping program (http://bio-bwa.sourceforge.net/). SNPs and indels were detected using SAMTOOLS (http://samtools.sourceforge.net/). A mean coverage of 101.0X was achieved and 98.3% of targeted paired-end sequences were read more than 10 times by exome capture and sequencing. A total of 69,157 SNPs were identified and pathogenic variants were prioritized as follows [10]. Initially, the 20 known causative genes of JSRDs were selected as a target for analysis. Of the 23 exonic variants, 8 synonymous variants and 10 variants with allele frequency of ≥0.05 in the 1000 Genomes Project (http://1000genomes.org) were excluded, which left five candidate variants. These variants were not previously reported in the 1000 Genomes Project. The first variant was a heterozygous c.6860G>A; p.Ser2287Asn in C5orf42 (NM_023073.3), which was predicted to be tolerated and benign by SIFT and PolyPhen at Ensembl Genome Browser (http://ensembl.org). Its variant frequency was 0.0113 in the Korean Reference Genome Database (KRGDB) (http://152.99.75.168/KRGDB/menuPages/firstInfo.jsp), which corresponds to a polymorphism. In addition, a heterozygous c.501G>T; p.Lys167Asn variant in CC2D2A (NM_001080522.2) and a heterozygous c.3973C>T; p.Arg-1325Trp (C>T) variant in KIF7 (NM_198525.2) were predicted to be probably damaging by PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/index.shtml). The frequencies of these variants were 0.0129 and 0.0008, respectively, in the KRGDB. We excluded CC2D2A variant because of polymorphism. The heterozygous C>T variant in KIF7 was considered to be of uncertain significance rather than a primary pathogenic cause because JSRDs with this gene variant is inherited in an autosomal recessive manner and the other variant allele was not detected. Two heterozygous variants, that is, c.2945G>T; p.Arg982Met (G>T) and c.2216dupA;
p.Phe740Valfs*2 (dupA) in AHI1 (NM_017651.4) were considered possible pathogenic variants. The frequency of the G>T variant was 0.0040 in KRGDB, but the dupA variant was not present in the 1000 Genomes Project or the KRGDB. These variants occurring between exon 13 and exon 20 of AHI1 were expected to lose the WD 40 domain and SH3 domain at the C-terminus of the AHI1 protein thus damaging it [11, 12]. These candidate variants were confirmed by Sanger sequencing using an ABI PRISM 3730XL Analyzer (Applied Biosystems Inc., Foster, CA, USA) (Fig. 2). We found two heterozygous variants G>T and dupA in AHI1, and a heterozygous C>T variant in KIF7 in a JSRDs patient using WES. Although we were unable to perform genetic analysis on the proband's mother, his father had both AHI1 variants, but not the KIF7 variant, which was inconsistent with autosomal recessive inheritance. Therefore, the G>T variant in AHI1 and C>T variant in KIF7 were presumed to be of "uncertain significance." One novel dupA variant in AHI1 was interpreted "pathogenic," and its second allele may be located in noncoding regulatory or deep intronic regions that cannot be detected by WES. AHI1 variants in JSRDs are known to be associated with risks of developing retinal dystrophy and kidney disease [11]. KIF7 variants are implicated in craniofacial dysmorphism and epiphyseal dysplasia [13].

WES has the potential to become an effective tool for the diagnosis of rare heterogeneous genetic disorders because of its capacity to sequence several genes simultaneously. However, caution should be exercised when interpreting the significance of the variants identified by WES. In addition, genes other than the 20 known ones should be further investigated to fully elucidate the pathogenesis of JSRDs.

Fig. 2. Sequencing data from the variant alleles: (A) c.3973C>T (C>T) variant of KIF7 and (B) c.2945G>T (G>T) and c.2216dupA (dupA) variants of AHI1 were confirmed by Sanger sequencing in the proband and his father.

Abbreviations: w/t, wild type; NT, no test.

Joubert syndrome is a form of hereditary disease characterized by posterior fossa cystic anomalies, hypotonia, and other symptoms such as strabismus, ptosis, and nystagmus. The disease is caused by mutations in the AHI1 gene, which is located on chromosome Xp11.2. AHI1 encodes a protein that functions as a transcriptional repressor and is involved in the regulation of gene expression. Mutations in AHI1 have been shown to disrupt the normal development of the cerebellum and other brain structures, leading to the characteristic phenotypic features of the disease.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this article were reported.

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