단신
Lab Med Online
Vol. 7, No. 1: 45-48, January 2017
https://doi.org/10.3343/lmo.2017.7.1.45

진단유전학 LMO

Joubert 증후군 소아에서 엑솜시퀀싱

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

이종화[1,5] · 오인경[2] · 윤미진[3] · 윤귀현[4,5]
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[1], 안과[2], 영상의학과[3], 진단검사의학과[4], 원광대학교 의과학연구소[5]
Departments of Pediatrics[1], Ophthalmology[2], Radiology[3] and Laboratory Medicine[4], Wonkwang University Sanbon Hospital, Gunpo; Institute of Wonkwang Medical Science[5], 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.2945 G>T; p.Arg982Met (G>T) and c.2216dupA; p.Phe740Valfs*2 (dupA) in *AHI1*, 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 *AHI1* 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, *AHI1*, *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 oculorenal defects, JS with hepatic defect, and JS with orofaciodigital 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

Corresponding author: Kui Hyun Yoon
Department of Laboratory Medicine, Wonkwang University Sanbon Hospital, 321 Sanbon-ro, Gunpo 15865, Korea
Tel: +82-31-390-2658, Fax: +82-31- 391-2085, E-mail: wooju67@paran.com

Received: March 31, 2016
Revision received: June 16, 2016
Accepted: June 21, 2016

This article is available from http://www.labmedonline.org

Table 1. Joubert syndrome (JBTS)-related genes

Type	OMIM (Phenotype)	Gene	Locus	Inheritance
JBTS1	213300	*INPP5E*	9q34.3	Autosomal recessive
JBTS2	608091	*TMEM216*	11q12.2	Autosomal recessive
JBTS3	608629	*AHI1*	6q23.3	Autosomal recessive
JBTS4	609583	*NPHP1*	2q13	Autosomal recessive
JBTS5	610188	*CEP290*	12q21.32	Autosomal recessive
JBTS6	610688	*TMEM67*	8q22.1	Autosomal recessive
JBTS7	611560	*RPGRIP1L*	16q12.2	In progress
JBTS8	612291	*ARL13B*	3q11.1	In progress
JBTS9	612285	*CC2D2A*	4p15.32	Autosomal recessive
JBTS10	300804	*OFD1*	Xp22.2	X-linked recessive
JBTS11	613820	*TTC21B*	2q24.3	In progress
JBTS12	200990	*KIF7*	15q26.1	Autosomal recessive
JBTS13	614173	*TCTN1*	12q24.11	Autosomal recessive
JBTS14	614424	*TMEM237*	2q33.1	Autosomal recessive
JBTS15	614464	*CEP41*	7q32.2	Autosomal recessive
JBTS16	614465	*TMEM138*	11q12.2	Autosomal recessive
JBTS17	614615	*C5orf42*	5p13.2	Autosomal recessive
JBTS18	614815	*TCTN3*	10q24.1	Autosomal recessive
JBTS19	614844	*ZNF423*	16q12.1	Autosomal dominant
JBTS20	614970	*TMEM231*	16q23.1	Autosomal recessive
JBTS21	615636	*CSPP1*	8q13.2	Autosomal recessive

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

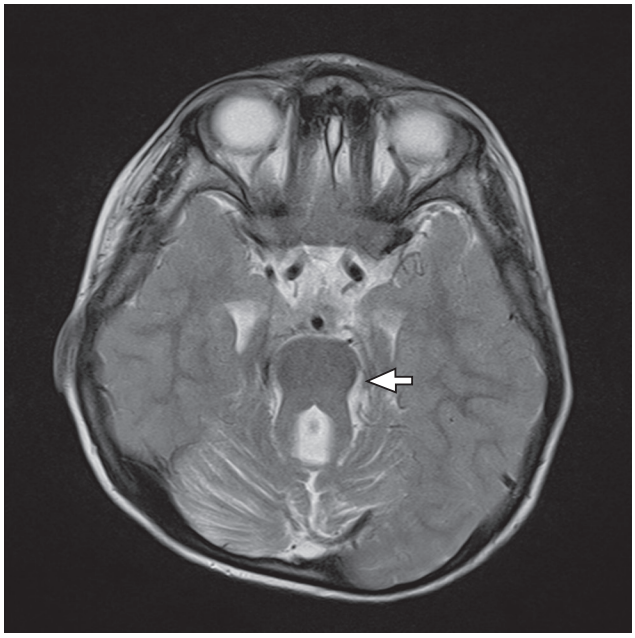

Fig. 1. Brain magnetic resonance imaging showing the typical "molar tooth sign" attributed to cerebellar vermis hypoplasia and brainstem malformation.

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 been 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.Arg1325Trp (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;

https://doi.org/10.3343/lmo.2017.7.1.45

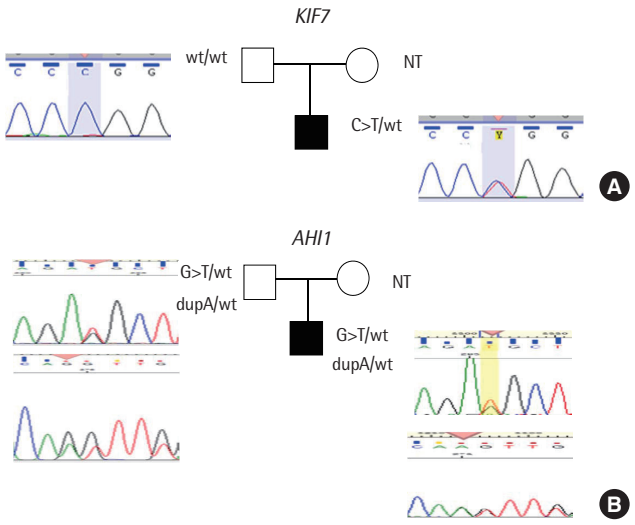

Fig. 2. Sequencing data from the variant alleles: (A) c.3973C>T (C>T) variant of *KIF7* and (B) c.2945G>T (G>T) and c.2216dupA (dupA) variants of *AHI1* were confirmed by Sanger sequencing in the proband and his father.
Abbreviations: w/t, wild type; NT, no test.

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.

요 약

Joubert 증후군은 소뇌충부 형성부전과 뇌간 기형을 특징으로 다양한 신경학적 징후를 보이면서 망막, 신장, 간, 골격 등 여러 조직을 침범하는 매우 드문 상염색체 열성, 또는 X염색체 유전질환으로 뇌 자기공명영상 소견으로 진단할 수 있다. 지금까지 약 45%의 환자에서 20여 개의 관련유전자가 보고되었다. 7세 남자 환아가 비정상 안구운동, 운동실조, 근 긴장저하, 발육지체를 주소로 내원하였다. 키와 몸무게는 99 cm와 13 kg으로 모두 3백분위수 이하였고, 안면기형과 단지증을 보였다. 안저검사에서 망막이형성을 보였으며, 뇌 자기공명영상과 임상소견으로 Joubert 증후군으로 진단되었다. 우리는 엑솜시퀀싱으로 *AHI1* 유전자에서 2개의 이형접합체변이 c.2945G>T; p.Arg982Met (G>T)와 c.2216dupA; p.Phe740Valfs*2 (dupA), 그리고 *KIF7* 유전자에서 하나의 이형접합체변이 c.3973C>T; p.Arg1325Trp (C>T)를 찾아 Sanger시퀀싱으로 확인하였다. 환아 어머니는 검사를 못했지만 아버지에서 시행한 검사결과가 *AHI1* 유전자의 2개의 이형접합체변이를 보여 상염색체 열성 유전으로 설명할 수 없었다. G>T와 C>T 변이는 불확실한 의미를 보이고, dupA는 원인유전자로 생각되지만 대립유전자를 발견하지 못하였다. 엑솜시퀀싱은 드문 유전질환의 진단에 유용하게 이용될 수 있지만 동시에 발견되는 유전자 변이들의 해석에 주의가 필요하다. 또한 아직 밝혀지지 않은 Joubert 증후군의 병인을 찾기 위해 알려진 20개의 원인유전자 이외의 다른 유전자에 대한 연구가 필요하다.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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

ACKNOWLEDGMENTS

This study was supported by the research fund of Wonkwang University (2016).

REFERENCES

1. Brancati F, Dallapiccola B, Valente EM. Joubert syndrome and related disorders. Orphanet J Rare Dis 2010;5:20.
2. Valente EM, Brancati F, Boltshauser E, Dallapiccola B. Clinical utility gene card for: Joubert syndrome – update 2013. Eur J Hum Genet 2013;21.
3. Akizu N, Silhavy JL, Rosti RO, Scott E, Fenstermaker AG, Schroth J, et al. Mutations in CSPP1 lead to classical Joubert syndrome. Am J Hum Genet 2014;94:80-6.
4. Tsurusaki Y, Kobayashi Y, Hisano M, Ito S, Doi H, Nakashima M, et al. The diagnostic utility of exome sequencing in Joubert syndrome and related disorders. J Hum Genet 2013;58:113-5.
5. Kaname T, Yanagi K, Naritomi K. A commentary on the diagnostic utility of exome sequencing in Joubert syndrome and related disorders. J Hum Genet 2013;58:57
6. Chen Z, Wang JL, Tang BS, Sun ZF, Shi YT, Shen L, et al. Using next-generation sequencing as a genetic diagnostic tool in rare autosomal recessive neurologic Mendelian disorders. Neurobiol Aging 2013;34:e11-7.
7. Eun MY, Seok HY, Kwon DY, Park MH, So-Hee E, Kang YS. Joubert syndrome presenting with young-age onset ischemic stroke: a possible etiologic association. J Child Neurol 2011;26:381-4.
8. Kim JT, Kim SJ, Joo CU, Cho SC, Lee DY. Joubert syndrome with peripheral dysostosis – a case report of long term follow-up. Korean J Pediatr 2007;50:315-8.
9. Jeong HB, Hwang SH, Kim KJ, Hwang YS, Kim SC, Kim IO. Clinical study of symptoms and various anomalies of patients with Joubert syndrome. Korean J Pediatr 1997;40:385-92.
10. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405-24.
11. 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.
12. Elsayed SM, Phillips JB, Heller R, Thoenes M, Elsobky E, Nurnberg 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.
13. Ali BR, Silhavy JL, Akawi NA, Gleeson JG, Al-Gazali L. A mutation in *KIF7* is responsible for the autosomal recessive syndrome of macrocephaly, multiple epiphyseal dysplasia and distinctive facial appearance. Orphanet J Rare Dis 2012;7:27.

https://doi.org/10.3343/lmo.2017.7.1.45