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Original Article

Exome sequencing identifies a compound heterozygote in *C5orf42* gene causing Joubert syndrome in a Chinese family

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Abstract: Purpose: This study is to present the diagnosis of a Chinese Joubert syndrome (JS) patient caused by compound heterozygote mutations in *C5orf42* gene. Methods: A 4 months old male child and was admitted to our hospital because of poor head control andcough at least for 10 days. Routine physical examination and auxiliary instrument inspection were undertaken. Whole exome sequencing was performed for the proband. Prioritized candidate genes based on clinics, pedigree, and mutation characters were selected and validated by Sanger sequencing. Functional analysis of JS genes was performed bioinformatics. Results: The proband, who suffered from molar tooth sign, global developmental delay, and cerebellar vermishypoplasia, was diagnosed as JS. Whole exome sequencing identified a compound heterozygote in *C5orf42* gene. Further Sanger sequencing confirmed the mutations included c.7570(exon37)delG from the proband's mother and c.8708-8709(exon46)delAA from his father. Bioinformatics findings suggested that genes caused JS might have a functional network, which is useful for prioritizing new JS genes. Conclusion: We firstly found compound heterozygote mutations in *C5orf42* gene that caused JS in Chinese population.

Keywords: Joubert syndrome, *C5orf42*, compound heterozygote, whole exome sequencing

Introduction

Joubert syndrome (JS) is a neurodevelopmental disorder characterized by hypoplasia of the cerebellar vermis and brainstem accompanied by shortness of breath, ocular dyskinesia, muscle reduce tension, ataxia, and mental retardation. In addition, JS may be associated with retinopathy, kidney, liver, and other multiple organ damage [1]. It is a relatively rare disease with the incidence in neonates about 1/8000 to 1/100000. In 1969, five cases of Joubert syndrome were first reported by Joubert, M [2]. To date, it has been found that JS is an autosomal recessive disorder and is genetic heterogeneity. About 20 genes are found to associate with JS, such as *INPP5E*, *TMEM216*, *AHI1*, *NPHP1*, *CEP290*, *TMEM67* (*MKS3*), *RPGRIP1L*, *ARL13B*, *CC2D2A*, *OFD1*, *TTC21B*, *KIF7*, *TCTN1*, *TMEM237*, *CEP41*, *TMEM138*, *C5orf42*, *TCTN3*, *TMEM231* and *TCTN2* [1], which caused JS 1-17, 18, 20, and 24, respectively. The genetic heterogeneity causes a variety of clinical manifestations, with only a few common symptoms found in all patients.

The mutations in *C5ORF42* gene can cause Oral-facial-digital syndrome type VI and JS 17. Mutations in this gene are the cause of JS in the original family was described by Joubert et al. and mainly occurs in French-Canadian families [2, 3]. The phenotypes include a global delay developmental, oculomotor apraxia and breathing abnormality, and molar tooth sign (MTS) which can be detected by MRI. None of the affected individuals has evidence of retinal involvement or renal impairment [3, 4].

Whole-exome sequencing (WES) is suggested for detecting novel mutations responsible for Mendelian diseases, especially for those with varying signs and symptoms among affected individuals [5]. It can capture all exons of protein-coding genes and is widely employed as a diagnostic method [6, 7]. In this study, we identified a compound heterozygote in *C5orf42*

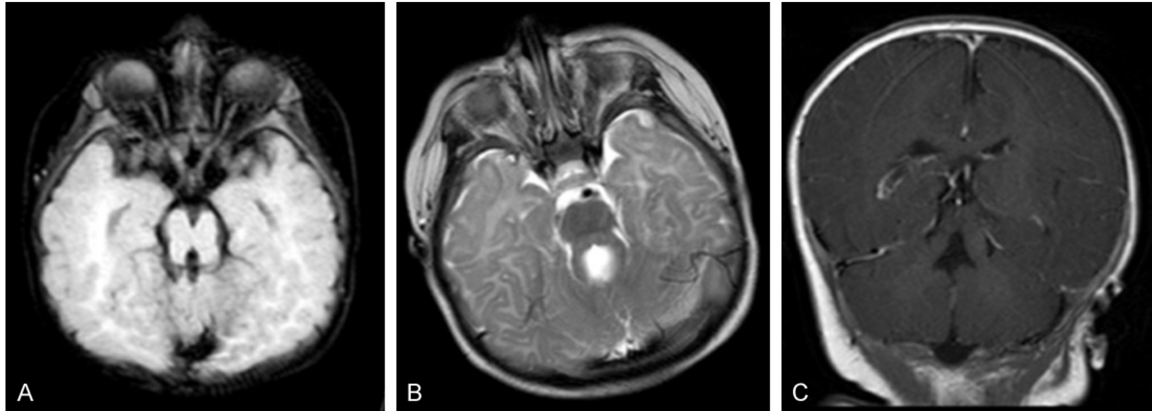

Figure 1. Molar tooth sign shown by MRI.

gene in a Chinese patient who suffered from global developmental delay, cerebellar vermis hypoplasia, and MTS via WES.

Patients and methods

Patient's data

The proband was a 4 months old male child and was admitted to our hospital because of poor head control andcough at least for 10 days. He was the second son of consanguineous parents and his parents and brother are healthy. He was pale after birth, with normal face and excessive nutrition. No lymphadenectasis was palpable in the superficial lymph node all over the body. No abnormality in the head, no edema in either eyelid, and no ocular proptosis or tremor was observed. His anterior fontanelle was 1.0 cm × 1.0 cm and flat. The bilateral pupils were equal and reflexive to light. His lips were without cyanosis and the tongue was in the middle. No tremor, hyperemia and herpes were observed in the pharynx. This study was approved by the ethics committee of Hunan Provincial People's Hospital, and informed consents were obtained from parents of the participant.

WES analysis

Genomic DNA was extracted from the patient and the exome DNA was captured using the Roche-NimbleGen Sequence Capture EZ Exome v2 kit (Roche NimbleGen, Madison, WI). The Illumina HiSeq 2500 platform (Illumina Inc., USA) was used to obtain sequence reads, which were aligned to human reference genome 19 (hg19) with BWA [8]. Picard (v1.67, (http://broadinstitute.github.io/picard/) and GATK Unified Genotyper (v2.3.6) were then used to call Single nucleotide variants (SNVs) and small indels [9]. The SNVs and indels were annotated with ANNOVAR tools [10]. Then variants and indels in the exons were reserved and those detected in the Chinese population of 1000 genome project and dbSNP137 were discarded. Then the rest variants in genes, which have been contained pathological sites in OMIM (http://www.omim.org/) or ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/), were given higher priority. In addition, the pathogenicity of variants were evaluated with SIFT [11], PolyPhen2 [12], MutationTaster2 [13] and CADD [14]. The genes with pathogenic variants were assessed based on patient-specific phenotype using Phenolyzer online tool [15]. Finally, the top ranked genes were selected for Sanger sequencing.

Sanger sequencing

Sanger sequencing was applied on all family members except the elder brother. Genomic DNA was extracted from each of the 3 family members. The sequence containing the variants in *C5orf42* gene (NM_023073.3) was amplified and PCR fragments were purified using the multiscreen Vacuum Manifold system (Millipore, Merck, France). Sequencing was performed by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). The primers were designed with PerlPrimer software (http://perlprimer.sourceforge.net/) [16]. The primers used were as following: C5orf42-1-F: 5'-TAGGAGAATGACCATCCCAG-3', reverse, C5orf42-1-R: 5'-TTCAGTCAGTCCTCAAGAGCA-3'; C5orf42-2-F: 5'-

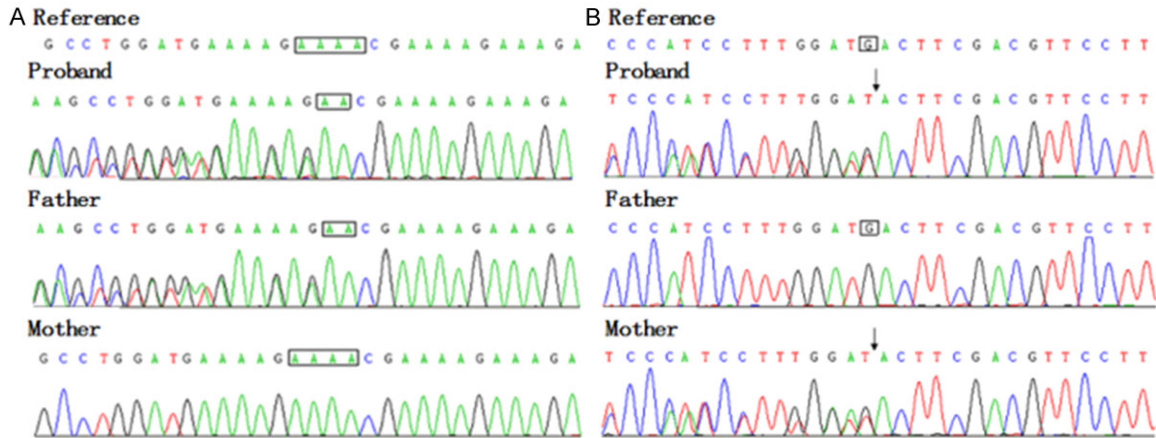

Figure 2. Pedigree of the family affected by hereditary JS and the mutations were confirmed by Sanger sequencing.

ATAAACTAAGTAAGGGAGCAGG-3', reverse, C5-orf42-2-R: 5'-CAGTGAGCAGAGAAGAAAGG-3'.

Functional analysis of JS genes

As for Protein-Protein interaction (PPI) network construction and GO enrichment, online server service string-db was applied (http://string-db.org/) [17]. Cytoscape was used for the visualization of PPI network [18].

Results

Clinical features of the patient

The probandwas 4 months old and was suspected as JS because of development delays, cerebellar vermis hypoplasia, and MTS shown by MRI (**Figure 1**). In addition to the above clinics, the proband also had obesity, poor eye movement, and enlarged fourth ventricle. No fetal chromosomal anomalies including trisomies 13, 18, and 21 were detected in prenatal screening. His parents and his 8 years old brother were healthy and had no signs of JS.

Characterization of C5orf42 mutation

To identify the genetic causing of the patient, WES was performed. Generally, WES achieved 80X average depth-of-coverage, with 99.1% and 95.3% of the exome sequences covering at 1X and 10X, respectively. A total of 11,224 variants identified in the index patient were subjected to a process designed to discover pathogenic mutations. After applying filtration process from WES result, we identified a compound heterozygote in *C5orf42* gene that could be implicated in the patient's phenotype. The compound heterozygote included c.7570(exon37) delG and c.8708-8709(exon46)delAA. The two deletions were both at heterozygous state and have not yet been reported.

Validated by Sanger sequencing

To eliminate false positives of WES and identify genetic inheritance, Sanger sequencing of the mutations was performed on the proband and his parents. Theresults showed that the proband inherited the c.7570(exon37)delG mutation from his mother and the c.8708-8709 (exon46)delAA mutation from his father (**Figure 2**). The two deletion variants both result in premature termination of the C5orf42 protein. In addition, the variants were both not found in our in-house Chinese control database, demonstrating its rarity. Additionally, a search of the Online Mendelian Inheritance in Man (http://www.omim.org/) confirmed the two deletions as novel mutations.

The functional analysis of JS genes

It is well documented that Joubert syndrome is genetic heterogeneity and about 20 genes are found to associate with JS [1]. To test the relationship of these reported genes, Protein-Protein interaction (PPI) analysis and GO enrichment analysis were performed. As shown in **Figure 3**, PPI results suggested that the genes had similar or related functions and could involve in the same or associated pathways. Therefore, GO enrichment analysis was imple-

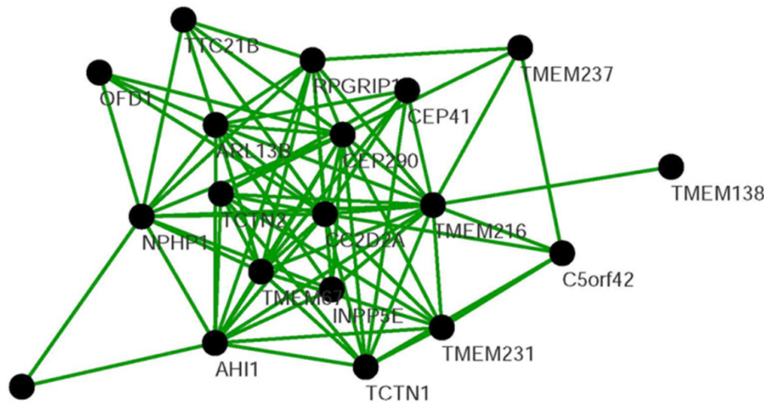

Figure 3. PPI network created with the 20 genes linked to Joubert syndrome.

mented for these 20 genes. The results showed that the genes located in cilium, centrosome, and basal body, which implies that the crucial roles of the genes focus on formation, development, morphology, and organelle functions. Not surprisingly, for GO biological process enrichment, the top terms with the most significant difference were cilium morphogenesis and cilium assembly (**Figure 4**). These findings suggested that genes caused JS might have a functional network, which is useful for prioritizing new JS genes.

Discussion

Here, we described one patient with clinical features of JS in a Chinese family. WES of the patient revealed a compound heterozygote in *C5orf42* gene including c.7570(exon37)delG and c.8708-8709(exon46)delAA. The mutations were further confirmed by Sanger sequencing. The sequencing revealed that these two mutations were inherited from his mother and father, respectively.

In 1997, Maria et al. proposed diagnostic criteria for JS: (1) MTS confirmed by MRI; (2) infants with low muscle tone; (3) varying degrees of developmental delay or mental retardation; (4) shortness of breath in infants and/or abnormal eye movement [19]. In addition to the performance, JS often associates with poor retinal development, kidney disease, congenital hepatic fibrosis, congenital heart disease, visceral inversion, Hirschsprung's disease, cleft lip and palate, and multiple fingers and toes deformity [19]. According to accompanying symptoms, Brancati et al. divide JS into six subgroups, including pure JS, JS with ocular defect, JS with renal defect, JS with oculorenal defects, JS with hepatic defect, and JS with orofaciodigital defects [20]. In the study, the proband infant showed MTS, cerebellar vermis hypoplasia, enlarged fourth ventricle, development delays, and low muscle tone suggested by poor head control. These features met the diagnostic criteria for JS. In addition, clinical features related to liver, kidney, multiple fingers, toes deformity, retina, and other vision abnormalitieshad been not identified. Therefore, the features of the proband were consistent with clinical diagnostic criteria for JS and the child was diagnosed as pure JS.

In this study, the proband caused by *C5ORF42* gene which belongs to the subtype 17 according to pathogenic genotype. In this study, compound heterozygote of c.7570(exon37)delG and c.8708-8709(exon46)delAA in *C5orf42* gene was identified using exome sequencing and was confirmed by Sanger sequencing. Additionally, Sanger sequencing showed that the two variants were inherited from the proband's mother and father respectively. To date, other 69 variants are detected in *C5orf42* gene and these variants caused JS 17 or OFDVI [21]. These findings suggested the important roles of *C5orf42* gene in development, though little has been known about its function. The experiments for *C5orf42* gene in cells or animal models are needed to clear the mechanism of the gene played in JS.

Disorders with phenotypic or genetic heterogeneity and diseases with complex symptoms are difficult to diagnose. Generally, de novo mutations and possible new disease genes should be taken into account. WES can capture and sequence all exons and thereby provide comprehensive and precise genotype information for specific phenotype. In the study, the patient described in the study had no family history, in addition, genes caused JS in Chinese population were not clear. As a result, WES is proper for the case to look for the cause as well as find possible new JS genes in Chinese population.

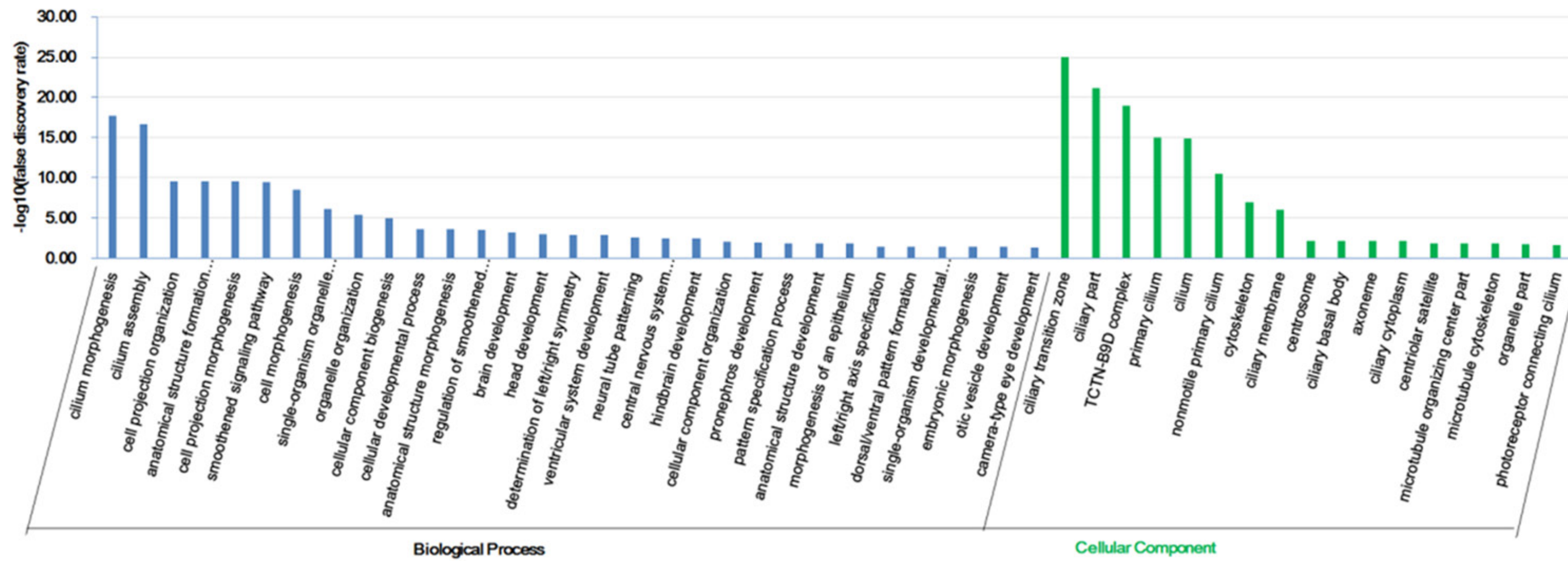

Figure 4. Functional enrichment of the 20 Joubert syndrome related genes.

In summary, a compound heterozygote of c.7570(exon37)delG and c.8708-8709(exon46) delAA in *C5orf42* gene was identified in a Chinese family affected by JS. Both the two variants had not been reported previously, which suggested that they were the new mutations in JS patients and *C5orf42* gene was the causative gene of JS. This study also showed that WES is a time and cost-effective method to identify causal mutations and had the potential to apply to disease diagnosis.

Disclosure of conflict of interest

None.

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