Novel biallelic loss-of-function variants in CEP290 cause Joubert syndrome in two siblings

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Abstract

Background: Joubert syndrome (JS) is a rare genetic disorder, which can be defined by brain stem malformation, cerebellar vermis hypoplasia, and consequent “molar tooth sign” (MTS). JS always shares variety of phenotypes in development defects. With the development of next-generation sequencing, dozens of causative genes have been identified to JS so far. Here, we investigated two male siblings with JS and uncovered a novel pathogenesis through combined methods.

Results: The siblings shared similar features of nystagmus, disorders of intellectual development, typical MTS, and abnormal morphology in fourth ventricle. Whole-exome sequencing (WES) and chromosome comparative genomic hybridization (CGH) were then performed on the proband. Strikingly, a maternal inherited nonsense variant (NM_025114.3: c.5953G>T [p.E1985*]) in CEP290 gene and a paternal inherited deletion in 12q21.32 including exons 1 to 10 of CEP290 gene were identified in the two affected siblings. We further confirmed the two variants by in vitro experiments: quantitative PCR and PCR sequencing.

Conclusions: In this study, we first reported a novel causative mechanism of Joubert syndrome: a copy number variation (CNV) combined with a single-nucleotide variant in CEP290 gene, which can be helpful in the genetic diagnosis of this disease.

Keywords: Joubert syndrome, Copy number variation, Compound heterozygous variants, WES, CGH
transcription factor associated with cAMP-dependent renal disease in JS patients [15]. Up to now, dozens of variants in CEP290 have been identified to cause JBTS5 (OMIM#610188) [16], a subtype of JS mainly associated with severe retinal and renal involvement in affected individuals [17]. However, almost all variants resulted in premature stop codon in CEP290 mRNA and truncated, nonfunctional CEP290 protein due to nonsense or frameshift variants, and the most frequent in which was c.5668G>T [p.G1890*] [15, 18, 19]. Interestingly, only one research reported a deletion from intron 43 of CEP290 to C12orf29 (chromosome 12 open reading frame 19) [20]; there were no other CNVs in CEP290 were reported in JS patients yet.

In this study, we presented clinical and molecular findings from a family with two JS affected siblings. Both of them carried a novel nonsense variant and a novel deletion in CEP290 inherited from their parents respectively. The pathogenesis is compound heterozygous variants lead to biallelic loss-of-function of CEP290, which has been associated with JS clearly.

Results
Clinical summary
An unrelated natural couple brought a 4-year-old boy with delayed development to the outpatient for genetic counseling (Fig. 1a). The proband exhibited features of moderate vision impairment, disorders of intellectual development, biparietal narrowing, and ataxia mainly on abnormal oculomotor (nystagmus) (Fig. 1b). Remarkably, the proband had a 10-year-old brother sharing the similar phenotype on vision impairment, intellectual development and oculomotor, and specially presenting gait disturbance (gait-limb incoordination) and abnormal form of the vertebral bodies (Fig. 1c). The axial brain magnetic resonance imaging (MRI) was then performed on these two siblings. Strikingly, hypoplastic cerebellar vermis, abnormal fourth ventricle, elongated superior cerebellar peduncles, and so-called “molar tooth sign” (MTS) were found on both of the two affected individuals (Fig. 1d, e). There was no functional and morphological abnormality of their kidney, lung, and liver so far. Thus, we initially diagnosed these two siblings with JS depending on the specific clinical characteristics. There was no phenotypic abnormality discovered in their parents.

Molecular genetic analysis
To elucidate the underlying genetic cause of JS in this family, we performed WES on all the family members. Importantly, a novel heterozygous nonsense variant

Fig. 1 Clinical summary for a JS family. a Family pedigree. An unrelated natural couple who gave birth to two affected siblings (black arrow denotes the proband). b–e The affected siblings and their axial brain MRI (Yellow arrowheads showed the thick and long superior cerebellar peduncles, forming the roots of the so-called “molar tooth sign”)

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(c.5953G>T [p.E1985*]) in CEP290 gene, a known causative gene of JS, was identified in the proband, his affected brother, and his mother but not the father. Furthermore, there was no report about this variation in any database, including ExAC browser, 1000 genome project, or GnomAD. In addition, this variant site is 100% highly conserved in many species (Fig. 2a). However, the nature of JS associated with CEP290 is an autosomal recessive inheritance, so we considered that there should be another variant in CEP290. We further analyzed the WES data, and strikingly, a suspected 65.97-kb deletion in 12q21.32 (chr12, 88523465–88589431) was found in the two affected individuals and their father but absent in the mother (Fig. 2b), which covered exons 1 to 10 of CEP290. Furthermore, this CEP290 deletion was confirmed on the proband by chromosome CGH analysis. As expected, a heterozygous 298.1-kb deletion in 12q21.32 (chr12, 88525732–88823847) was detected out, which, gives rise to exon 1 to exon 6 deletion of CEP290 (Fig. 2c). In view of the coding sequence (CDS) region of CEP290 started in exon 2, the deletion detected in this family causes a complete translation deficiency. These findings concluded that the biallelic loss-of-function of CEP290 variations was the genetic cause of JS in this family.

The identification and negative effects of CEP290 variants in the JS family
To understand the putative contribution of the single-nucleotide variant (c.5953G>T [p.E1985*]) to the proband’s phenotype, we performed Sanger sequencing on this family, and this variant was observed in the proband, his affected brother, and their mother, meanwhile which was absent in their father (Fig. 3a). This variation resulted in a premature stop codon in transcribed mRNA (NM_025114.3, exon 43) and thus a truncated CEP290 protein, which leads to the missing of CC (coiled-coils) domain XIII, P-loop (ATP/GTP-binding site motif A) domain, KID (RepA/Rep+ protein kinase interaction domain) IV-VI(15) resulting in the loss-of-function of CEP290 protein (Fig. 3b).

To verify the CNV, qPCR was carried out to analyze genome relative copy number of exon 1, exon 2, exon 6, exon 7, exon 10, and exon 11 of CEP290 in the family. Results showed that compared to the mother, the father and siblings only had relative half-fold copy for exon 1, exon 2, exon 6, exon 7, and exon 10, meanwhile exon 11 were equivalent (Fig. 3c). Furthermore, leukocyte mRNA levels of CEP290 also revealed significant decrease in the two siblings compared to their parents by qPCR (Fig. 3d). Thus, these novel biallelic loss-of-function variants in CEP290 could significantly reduce CEP290 expression and further caused JS in these two siblings.

Discussion
In this study, a novel genetic cause associated with a heterozygous nonsense variant combined a heterozygous deletion of a large fragment within CEP290 was identified in two JS patients, which uncovered a novel pathogenesis of JS and provide more clue for genetic diagnosis of this disease.

To date, variants of CEP290 have been demonstrated with several genetic disorders more than JBTS5, such as Leber congenital amaurosis (LCA10, OMIM#611755) [17], Meckel syndrome type 4 (MKS4, OMIM#611134) [21, 22], and Bardet-Biedl syndrome 14 (BBS14, OMIM#615991) [23, 24]. It is wondering that why variations in one gene could result in numbers of different syndromes. In 2010, Coppieters et al. reviewed over 100 distinct variants in CEP290 and indicated that no clear genotype-phenotype correlation could be established and the reason may be insufficient understanding of second-site modifiers alleles, such as variants in other genes encoding ciliary protein and interact with CEP290 [10]. In addition, we also hold the point that different variant subtypes in this gene may cause specific syndromes, as there are so many different domains with different vital functions in this large protein [14, 15]. In this study, we found two novel variants of CEP290, which may help to understand mechanisms of variation in CEP290. Currently, WES has been frequently used and is an effective scheme for genetic disorder diagnosis, especially rare or non-fully clear diseases. However, some CNVs, including deletions and duplications, cannot be readily detected by WES [25]. Thus so, missing detecting CNV may give rise to unexplained genotype-phenotype uncorrelation in some disease. In our study, initially, in view of the proband shared the typical JBTS5 phenotype, but their parents claimed just a heterozygous CEP290 nonsense variant was found in prior genetic diagnosis (details unknown). We therefore performed WES and chromosome CGH to focus on both SNV and CNV in CEP290. Expectedly, nonsense variant and deletion were detected out in the two patients. Our investigation of JS in this family showed it was very essential to use combined methods to study pathogenesis of genetic disorders.

Conclusions
In conclusion, in this study, we unveiled two new variants, a SNV and a CNV of CEP290, which caused a complete translation deficiency of CEP290 leading to JS in two siblings. Specially, such deletion over 100 kb in CEP290 detected in JS individuals was first reported. These findings may help to conclude and reveal specific variation types in CEP290, which is benefit for the diagnosis and then for the future precision treatment of JS.
Methods

Subjects
Peripheral blood samples were obtained from the proband and his family after informed consent was signed respectively. Experiment on human subjects was approved by the Ethical Review Board of West China Second University Hospital, Sichuan University. The two affected siblings’ brain MRI results were evaluated by two pediatric neuroradiologists.

Whole-exome sequencing and chromosome comparative genomic hybridization
Genomic DNA was extracted from peripheral blood leukocytes using whole blood DNA purification kit.
Fig. 3 Verification and negative effect of the variants in CEP290. a PCR sequencing confirmed a c.5953G>T [p.E1985*] mutation in this family. b c.5953G>T [p.E1985*] in CEP290 resulted in the loss-of-function of CEP290 protein due to lose numbers of functional domains. c Genomic qPCR revealed that father and the siblings only had relative half-fold copy for exon 1, exon 2, exon 6, exon 7, exon 10, and equivalent copy of exon 11 to mother. d qPCR showed significant decrease of CEP290 expression in the two siblings compared to their parents. All the values are means ± SEM from three independent experiments, and statistical analysis was performed by one-way ANOVA. **P < 0.01, ***P < 0.001
Table 1  Primers used in the current study

| Primers      | Forward                                     | Reversed                                  |
|--------------|---------------------------------------------|-------------------------------------------|
| CEP290 SNV   | 5′ CAACTTCCACAGGACCCGATAAA 3′               | 5′ ACACGCGGAAATGTGAGT 3′                 |
| CEP290 exon 1| 5′ GTCCACCCCTTCTCATC 3′                    | 5′ TGCCAGAGAAGCTACAGTT 3′               |
| CEP290 exon 2| 5′ AGGTGAGCCAGCTGAAAGAA 3′                 | 5′ TCTGACAGTTCTGTCGAG 3′                |
| CEP290 exon 6| 5′ ATGTCGAGGGAGGACGAGAT 3′                 | 5′ CCAACTCTTTTCCATGTC 3′                |
| CEP290 exon 7| 5′ CAGCCTAGCCGACAAGAATC 3′                 | 5′ CCTTTGTGAAACGACCAAA 3′               |
| CEP290 exon 10| 5′ GCACACTTATGTCGCTGTTT 3′                | 5′ CATACGTACATCTCCTACCC 3′              |
| CEP290 exon 11| 5′ CATCAGTGTGCAACAACCTTGA 3′              | 5′ TTTGCAATTTGACAGCTACCAC 3′            |
| CEP290 mRNA  | 5′ AAAGTGTGACCCAGATGCCT 3′                 | 5′ AAACCCAGATACCTCGTCAC 3′              |
| Human GAPDH  | 5′ ATGTTCGTCAATGGTGTGAA 3′                 | 5′ GTCTTCTGGGGTGACAGTGAT 3′             |

Genomic DNA sequencing and quantitative PCR
Genomic DNA was obtained from peripheral blood leukocytes using whole blood DNA purification kit (TIANGENE). PCR amplification was performed using primer pairs (Table 1) designed to cover variant identified by WES. Sequencing of PCR products was conducted on an ABI377A DNA sequencer (Applied Biosystems). Data were evaluated using the Chromas software.

Total RNA was extracted using TRIzol reagent (Invitrogen, 15596026), and then reversed to cDNA through RT reagent Kit (Takara, RR037A). Triplicate quantitative PCR for genomic DNA or cDNA was performed using SYBR Green qPCR Master Mix (Bimake, B21202) on an iCycler RT-PCR Detection System (Bio-Rad Laboratories). Delta-delta CT value analysis method was used to evaluate genome exon 1, exon 2, exon 6, exon 7, exon 10, and exon 11 relative copy number, or exon 3 to 5 mRNA expression for all samples. Target and internal control gene primer pairs were listed below (Table 1).
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