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 brainstem 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 a JS case in two male siblings aged 4 and 10 years old and uncovered a novel pathogenesis through combined methods.

Results The siblings shared similar features of nystagmus, delayed 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 mutation (NM_025114.3: c.5953G>T [p.E1985*]) in *CEP290* gene and a paternal inherited deletion in 12q21.32 including exon 1 to 10 of *CEP290* gene were identified in the two affected siblings. We further confirmed the two variants by in vitro experiments: qPCR, and PCR-sequencing.

Conclusions In this study, we first reported a novel causative mechanism of Joubert Syndrome: a copy number variation (CNV) compounding with a point mutation in *CEP290* gene, which can be helpful in the genetic diagnosis of this disease.

Background

Joubert Syndrome (JS) is a genetic ciliopathy disorder with multiple neurologic features of brain malformations, hypotonia, ataxia, and intellectual disability first described in 1969.(1, 2) With the medical use of magnetic resonance imaging (MRI), JS diagnostic criteria were importantly revised with “molar tooth sign” (MTS), an abnormally deep interpeduncular fossa in midbrain.(3–5) Sharing MTS has also been used to group any individual display additional non-neurological features to Joubert syndrome-related disorders (JSRD).(4) To date, more than 30 gene variants have been identified in JS, and which allowed to group JS into different subtypes (JBTS).(6–8) Most of these gene variants exhibit autosomal recessive inheritance, and the top three are C5ORF42, CC2D2A, and CEP290 in turn.(9, 10)

As with other genes associated with JS, CEP290 is a primary cilium related gene encodes centrosomal protein 290, a large essential protein expressed in almost all tissues and plays a critical role in cell motility and division through effects on centrosome and cilia development.(11–13) Disrupted CEP290 protein (also known as NPHP6) has been found to loss function binding to cellular membranes and microtubules,(14) and proved to activate ATF4, a 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 serve retinal and renal involvement in affected individuals.(17) Interestingly, almost all variants resulted in premature stop codon in CEP290 mRNA and truncated, nonfunctional CEP290 protein due to nonsense mutations or frameshift mutations,

and the most frequent in which was c.5668G > T[p.G1890*].(15, 18, 19) Except a 352 bp deletion of exon 38 and 39,(18) there were no CNVs in CEP290 were reported in JS patients yet.

In this study, we present clinical and molecular findings from a family with two JS affected siblings. Both of them carried a nonsense mutation and a CNV 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. To our knowledge, this is the first report about an CNV compounding with a point mutation in CEP290 causing JS.

Results

Clinical summary

An unrelated natural couple who gave birth to two affected siblings continuously (Fig. 1a) came to the outpatient for genetic counseling. The proband is a 4 years old boy with nystagmus and delayed intelligence development, and he has a 10 years old brother shares similar features (Fig.1b, c). Axial brain magnetic resonance imaging (MRI) was performed for him and hypoplastic cerebellar vermis, abnormal fourth ventricle, elongated superior cerebellar peduncles, and so-called “molar tooth sign” (MTS) were found (Fig.1d, e). Thus, we initially diagnosed this patient 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, we performed WES on this family. A heterozygous nonsense mutation (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 genomAD. In addition, this mutation 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 there should be another mutation of *CEP290*. We further analyzed the *CEP290* variants in the WES data, and strikingly, a suspected 65.97-kb deletion in 12q21.32 (88523465-88589431) was found in the two affected individuals and their father but absent in the mother (Fig. 2b), which covered exon 1 to 10 of *CEP290*. Furthermore, this *CEP290* deletion was confirmed by chromosome CGH analysis. Intriguingly, a heterozygous 29.8-kb deletion in 12q21.32 (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, deletions found through these two methods both cause a complete translation deficiency. These findings concluded that the biallelic loss-of-function of *CEP290* variations were the genetic cause of JS in this family.

The identification and negative effect of *CEP290* mutations in the JS family

To understand the putative contribution of the point mutation (c.5953G>T [p.E1985*]) to the proband's phenotype, this mutation was validated by Sanger sequencing in this family, and 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) which results in the loss of function of CEP290 protein (Fig. 3b).

To verify the CNV, quantitative PCR was performed to analysis 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 the siblings only had 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 RT-PCR (Fig. 3d). Thus, the novel biallelic loss-of-function variants in *CEP290* could reduce *CEP290* expression seriously and further caused JS in these two siblings.

Discussion

In this study, we combined WES, chromosome CGH, quantitative PCR and PCR-sequencing to identify a heterozygous nonsense mutation and a heterozygous deletion within CEP290 in two JS patients. Incredibly, the couples carried a different and rare heterozygous CEP290 variation respectively and then pass a biallelic CEP290 variation to their two affected children continuously. Therefore, the compound heterozygous variant in CEP290 is the genetic cause of JS in this family, which also uncovered a novel pathogenesis of JS.

To date, variants of CEP290 have been demonstrated with several genetic disorders more than JBST5, such as Leber Congenital Amaurosis (LCA10, OMIM#611755),(17) Meckel Syndrome Type 4 (MKS4, OMIM#611134),(20, 21) and Bardet-Biedl Syndrome 14 (BBS14, OMIM#615991).(22, 23) It is unknown that how different variations in CEP290 result in so many different syndromes, and all of which can affect the central nervous system (CNS).(10, 24) In 2010, Frauke Coppieters et.al reviewed over 100 distinct variants in CEP290 and indicated that there was no clear genotype-phenotype correlation could be established and the reason may be insufficient understanding of second-site modifiers, such as variants in other genes encoding ciliary protein and interact with CEP290.(10) In addition of this, we also hold the point that different mutation subtypes in this gene may cause specific syndromes, as there are so many different domains with different vital function 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, there's no effective specific method for JS treatment,(9, 13) nevertheless, several gene therapy targeted on CEP290 have been reported for LCA10,(25–27) and which might be used for the future treatment of JS. Therefore, genetic diagnosis becomes the first and pivotal step. WES has been frequently used and is an effective scheme for genetic disorders diagnosis, especially rare or non-fully clear diseases. WES is a relatively low-cost solution providing accurate base pair sequence for all coding region in genome, from which single nucleotide changes leaded to mutations (also called as single-nucleotide variants, SNV), such heterozygous or homozygous mutations can be detected out for variant mapping in complex disorders, discovery of Mendelian disorders, and prenatal screening. However, some CNVs, including deletions and duplications, cannot be readily detected by WES.(28) 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, which is an autosomal-recessive inherited disorder, but their parents claimed just a heterozygous CEP290 nonsense mutation was found in prior genetic diagnosis (details unknow). We therefore suggested to perform WES and chromosome CGH which may provide more precise results for chromosome aberration to focus on both SNV and CNV in CEP290. Unexpectedly, nonsense mutation and deletion were detected out. Due to different efficiency and specificity of probes,(29) WES and CGH gave different deletion results, but both of them indicated a complete translation deficiency of CEP290 gene. Moreover, subsequent qPCR for genome CEP290 exons copy number revealed a tendency result to WES. 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 identified and confirmed two new variants, a point mutation and a CNV of CEP290, which caused JS in two siblings. Specially, the CNV in JS individuals was first reported. These findings may help to conclude and reveal specific variation types in CEP290 causing JS.

Methods

Subjects

Peripheral blood samples were obtained from the proband and his family after informed consent was signed respectively. Experiment on human subjects were 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 (TIANGENE). For WES, exons were captured from 1 μg genomic DNA using Agilent SureSelect Human All Exon V6 Kit and then sequenced on Illumina HiSeq X system under instructions provided by the manufacturer. Functional annotation was performed through ANNOVAR and data were filtered by public database, such as ExAC, 1000 Genomes Project, and HGMD.

For chromosome comparative genomic hybridization, genomic DNA was digested with Nsp1 and ligated to adaptors. PCR amplicon were performed using primer pairs provided by the manufacturer, and the products were then purified and digested with DNase I to produce varying length DNA fragments. The fragments were labeled with biotinylated base and then hybridized with a pre-equilibrated Affymetrix chip Cytoscan HD chip. After washing and staining, the chip was scanned with the GeneChip Scanner and the signal intensity for each marker is assessed. The results were analyzed using the Chromosome Analysis Suite (version 3.3).

Genomic DNA sequencing and quantitative PCR

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

Primers		
	Forward	Reversed
CEP290 SNV	5' TAAATTCCACAGAGCCGATAAA 3'	5' ACAGCCCAAGAAATGAGGTT 3'
CEP290 exon 1	5' GTTCCACGCCTTCTCATCAT 3'	5' TGCCAGGAGAGCCTACAGTT 3'
CEP290 exon 2	5' AGGTGGAGCACAGTGAAAGAA 3'	5' TCTGCCAGTTCTTCTTGACG 3'
CEP290 exon 6	5' AGTCTGCAGGTGGACGAGAT 3'	5' CCAACTCCTTTTCCATGTCC 3'
CEP290 exon 7	5' CAGCCTAGGCGACAAAGACT 3'	5' CCTTTGTTGAACCACCACAA 3'
CEP290 exon 10	5' GGACACTTATGGCTGCGTTT 3'	5' CATCAGTCATCTTCTCCATTTCC 3'
CEP290 exon 11	5' CATCAGTTTGCAACAACTCTTGA 3'	5' TTTTGCATTGACAGCTACCAT 3'
CEP290 mRNA	5' AAAGTTGACCCAGATGACCT 3'	5' AAACCGAGTATCTCGTCCAC 3'
Human *GAPDH* (for exons copy number)	5' CCCACACACATGCACTTACC 3'	5' CCCACCCCTTCTCTAAGTCC 3'
Human *GAPDH* (for mRNA expression level)	5' ATGTTCGTCATGGGTGTGAA 3'	5' GTCTTCTGGGTGGCAGTGAT 3'

Table 1. Primers used in the current study.

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).

Abbreviations

JS: Joubert Syndrome

MRI: Magnetic resonance imaging

MTS: Molar tooth sign

JSRD: Joubert syndrome-related disorders

CNV: Copy number variation

WES: Whole exome sequencing

CGH: Comparative genomic hybridization

SNV: Single-nucleotide variants

Declarations

Ethics Approval and Consent to Participate

Experiment on human subjects were approved by the Ethical Review Board of West China Second University Hospital, Sichuan University.

Informed consent for participation to this study was obtained from all individuals.

Consent for Publication

Publication of data were informed consent to all individuals involved in this study.

Availability of Data and Materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare no conflict of interests.

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Authors' Contributions

Ying Shen designed the study experiments. Zhu Zhang collected data and conducted the clinical evaluations. Xueguang Zhang and Xiang Wang performed PCR-seq and qPCR. Xiang Wang wrote article.

Hongqian Liu supervised the study experiments. All authors revised and approved the article.

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Figures

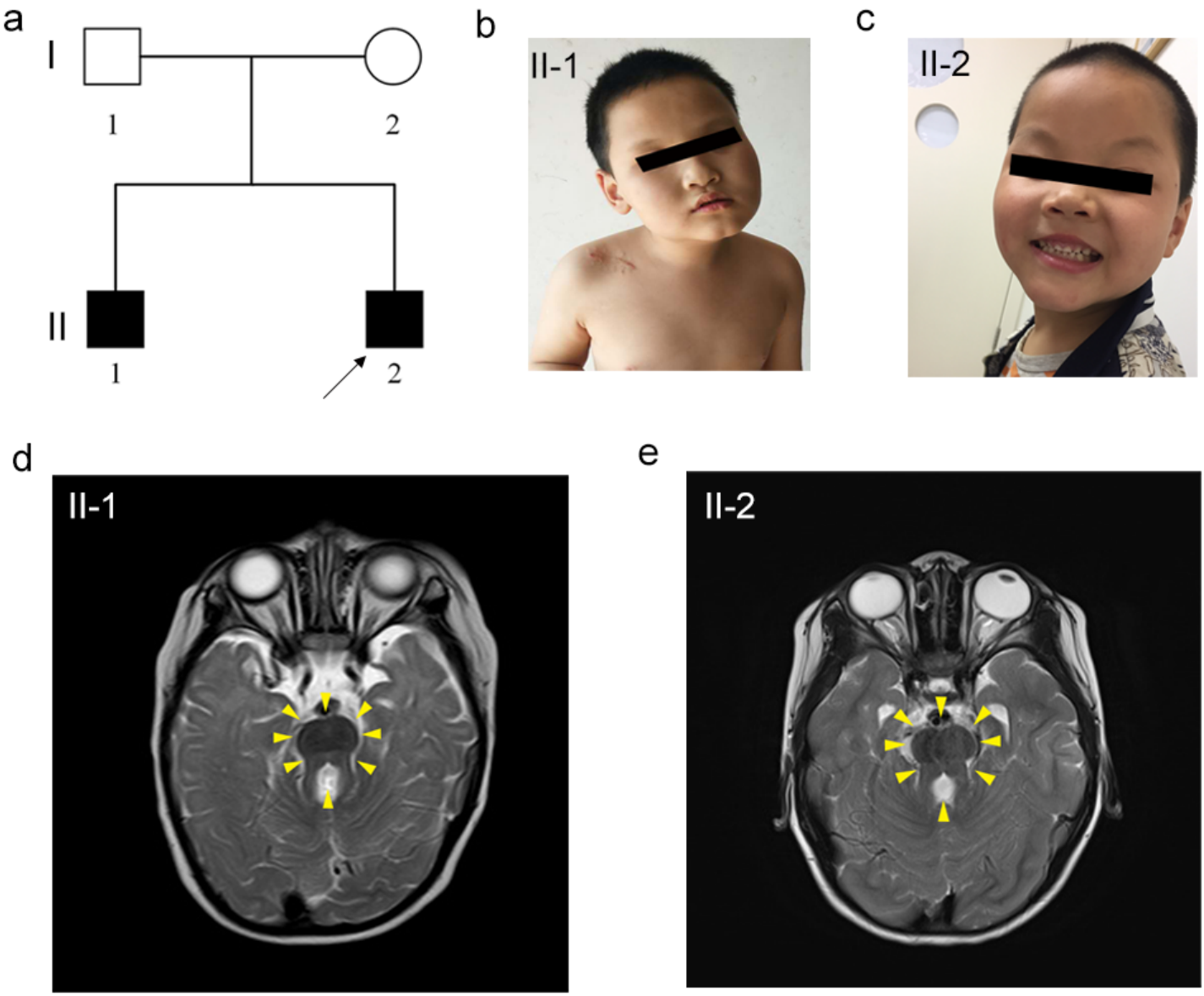

Figure 1

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”).

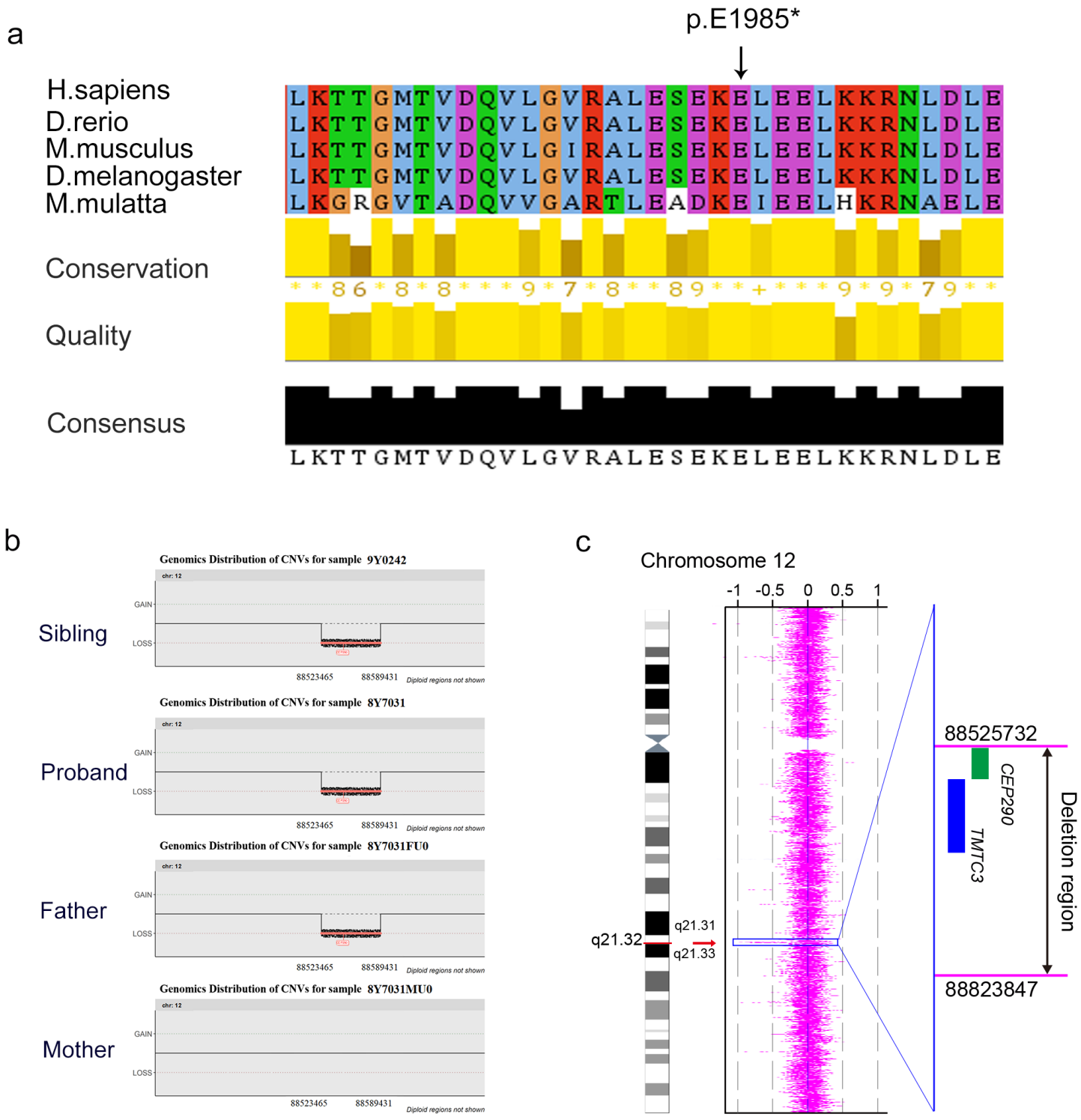

Figure 2

Variants identified from WES and chromosome CGH. (a) Multiple sequence alignment of the CEP290 protein for different species. (Black arrow denotes the position of the variant (c.5953G>T [p.E1985*]) identified in mother and siblings through WES. (b) WES identified a suspected 65.97-kb deletion in 12q21.32 which affected CEP290 in father and siblings (8Y7031: proband, 9Y0242: sibling, 8Y7031FU0:

father, 8Y7031MU0: mother). (c) Chromosome CGH identified a 29.8-kb deletion in 12q21.32 which affected CEP290 in the family.

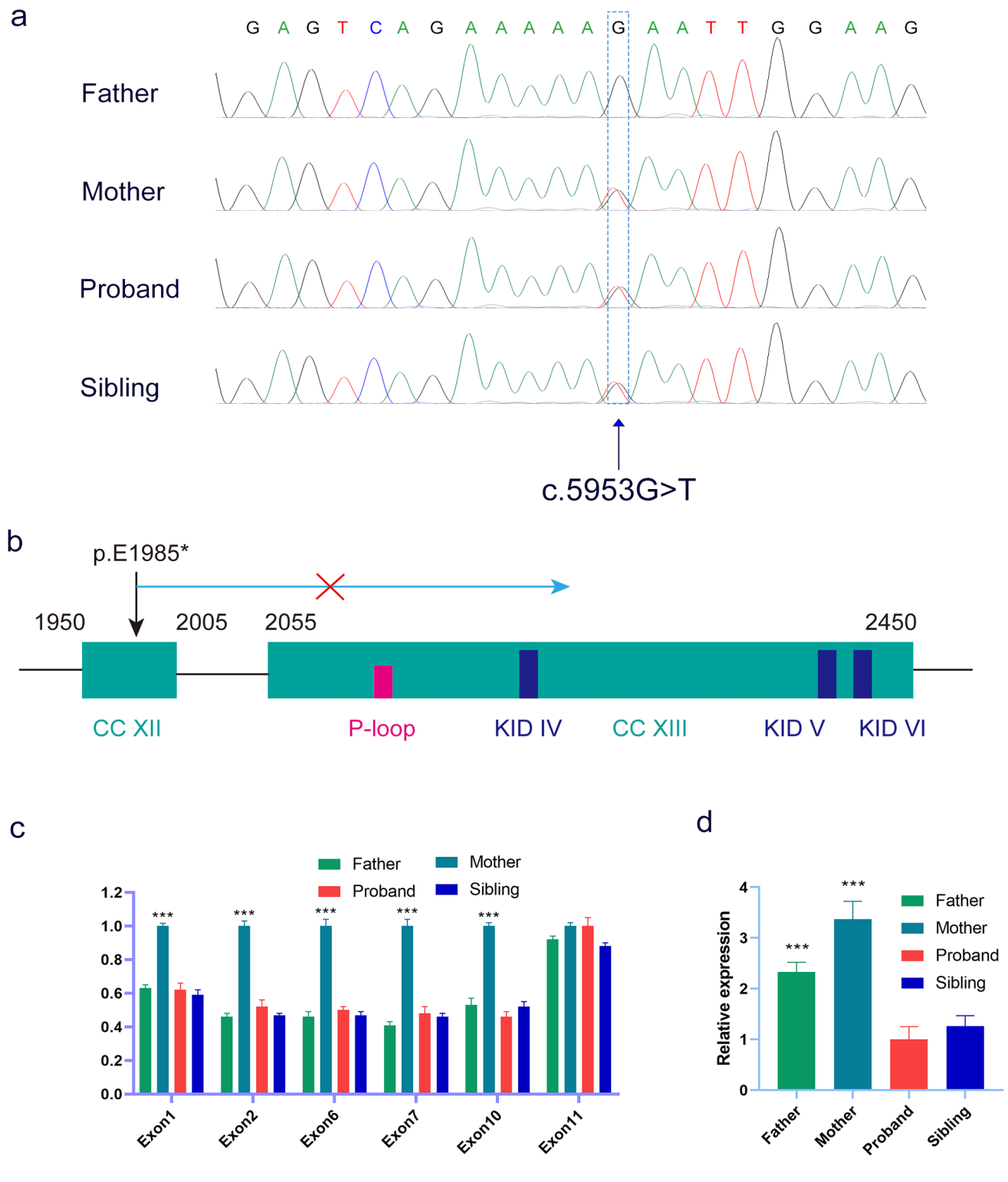

Figure 3

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 results in the loss-of-function of CEP290 protein due to lose numbers of functional domains. (c) Genomic quantitative PCR revealed that

father and the siblings only had half-fold copy for exon 1, exon 2, exon 6, exon 7, exon 10, and equivalent copy of exon 11 to mother. (d) RT-PCR 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.