Novel compound heterozygous CPLANE1 variants identified in a Chinese family with Joubert syndrome

Cheng Zhang1,2 | Zhenchao Sun1 | Lulu Xu3 | Fengyuan Che1 | Shiguo Liu2

1Department of Neurology, The Eleventh Clinical Medical College of Qingdao University, Linyi People’s Hospital, Linyi, China
2Medical Genetic Department, The Affiliated Hospital of Qingdao University, Qingdao, China
3Department of Geriatric Medicine, The Affiliated Hospital of Qingdao University, Qingdao, China

Correspondence
Fengyuan Che, Linyi People’s Hospital, 27 East Section of Jiefang Road, Lanshan District, Linyi, Shandong 276000, China.
Email: che1971@126.com
Shiguo Liu, Medical Genetic Department, The Affiliated Hospital of Qingdao University, Qingdao 266003, China.
Email: shiguo2006liu@163.com

Funding information
The work was supported by the National Natural Science Foundation (grant number: 81371499) and the National Key Research and Development Program of China (grant numbers: 2016YFC1000306, 2005DKA32408).

Abstract
Joubert syndrome (JS) and JS-related disorders (JSRD) are a group of neurodevelopmental diseases that share the “molar tooth sign” on axial brain magnetic resonance imaging (MRI), accompanied by cerebellar vermis hypoplasia, ataxia, hypotonia, and developmental delay. To identify variants responsible for the clinical symptoms of a Chinese family with JS and to explore the genotype–phenotype associations, we conducted a series of clinical examinations, including blood tests, brain MRI scans, ultrasound imaging, and ophthalmologic examination. Genomic DNA was extracted from the peripheral blood of the six-person family, and the pathogenic variants were detected by whole-exome sequencing (WES) and verified by Sanger sequencing. WES revealed two novel compound heterozygous variants in CPLANE1: c.1270C>T (p.Arg424*) in exon 10 and c.8901C>A (p.Tyr2967*) in exon 48 of one child, inherited from each parent. Both variants were absent in ethnically matched Chinese control individuals and were either absent or present at very low frequencies in public databases, suggesting that these variants could be the pathogenic triggers of the JS phenotype. Notably, these CPLANE1 sequence variants were related to the pathogenesis of autosomal recessive JS in this study. The newly discovered variants expand the mutation spectrum of CPLANE1, which assists in understanding the molecular mechanism underlying JS and improving the recognition of genetic counseling, particularly for families with a history of autosomal recessive JS.

KEYWORDS
C5ORF42, ciliopathy, CPLANE1, Joubert syndrome, variants

Abbreviations: 1000GP, 1,000 Genomes Project; ACMG, American College of Medical Genetics and Genomics; CVH, cerebellar vermis hypoplasia; gDNA, genomic DNA; HGMD, Human Gene Mutation Database; IF, interpeduncular fossa; JS, Joubert syndrome; JSOFD, JS with OFD; JSRD, JS-related disorders; MRI, magnetic resonance imaging; MTS, molar tooth sign; NGS, next-generation sequencing; OFD, oral-facial-digital syndrome; SCP, superior cerebellar peduncles; SIFT, Sorting Intolerant From Tolerant; SNPs, single-nucleotide polymorphisms; WES, whole-exome sequencing.

Fengyuan Che and Shiguo Liu contributed equally to this work.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2021 The Authors. International Journal of Developmental Neuroscience published by John Wiley & Sons Ltd on behalf of International Society for Developmental Neuroscience
Joubert syndrome (JS) is a rare, recessive congenital brain malformation with a prevalence between 1:80,000 and 1:100,000 (Brancati et al., 2010; Kros et al., 2007; Paprocka & Jamroz, 2012). Classic JS is a ciliopathy characterized by the three primary diagnostic criteria “molar tooth sign (MTS)” on axial brain magnetic resonance imaging (MRI; complex malformation of the cerebellar vermis and brainstem), hypotonia with the development of ataxia, and developmental delay; further symptoms include oculomotor apraxia, neonatal irregular respiratory pattern, and a spectrum of other clinically heterogeneous phenotypes, such as retinal dystrophy, congenital cardiac defects, liver and renal abnormalities, and craniofacial defects (Paprocka & Jamroz, 2012; Parisi, 2019; Parisi & Glass, 2003). Mutations in 35 candidate genes have been identified to cause JS; most are known to encode cilia proteins localized in the cilia transition zone or basal body, which explains approximately 60% of cases (Parisi, 2019; Parisi & Glass, 2003; Reiter & Leroux, 2017). While only oral-facial-digital syndrome type 1 (OFD I) pathogenic variants are inherited in an X-linked transmission, most causative genes are predominantly inherited in an autosomal recessive mode. In addition, digenetic inheritance has also been reported in some cases (Reiter & Leroux, 2017; Vilboux et al., 2017).

Mutations in CPLANE1 (ciliogenesis and planar polarity effector 1, previously CSF1R42/jbts17) can cause JS17 and OFD VI, which merely differ in symptom severity (Bachmann-Gagescu et al., 2015; Lopez et al., 2014). CPLANE1, located on chromosome 5p13.2, spans over 11,999 bp and contains 52 exons, encoding 3,197 amino acids with transmembrane structures and putative coiled-coil regions (Lopez et al., 2014). CPLANE1 interacts with p21-activating kinase 1 and small ubiquitin-like modifier 1, indicating roles in cellular signaling transduction, growth control, and neural differentiation (Bandyopadhyay et al., 2010; Ganesan et al., 2007). It is a part of the protein complex required for the assembly and maintenance of primary cilia located in the basal body of the cilia, explaining its significant role in ciliogenesis, body axis formation, and renal function (Hong et al., 2019; Toriyama et al., 2016). Mutations in CPLANE1 can disrupt the cilia transition zone, resulting in the perturbation of ciliogenesis and cilia-transduced sonic hedgehog signaling that underly the cerebellar defects in JS (Asadollahi et al., 2018). More than 60 CPLANE1 mutation loci have been found to be associated with JS and OFD, the most common types causing truncating variants, especially compound heterozygous mutations (Wentzensen et al., 2015). However, the correlation between genotype and phenotype in CPLANE1 mutations in JS and JSRD remains unknown.

In this study, coupling next-generation sequencing (NGS) with Sanger sequencing, we confirmed two novel compound heterozygous variants in CPLANE1 causing JS in a Chinese family. Our results provide novel insights into JS pathogenesis and may accelerate prenatal diagnosis of this disease.

2 | MATERIALS AND METHODS

2.1 | Subjects

A Chinese family (two parents, four siblings) without positive family history was recruited in our study. All members were physically examined and their medical history evaluated. Further diagnostic tests included blood tests, brain MRI, ultrasound imaging, and ophthalmologic examination. A pedigree was created and genetic testing of all available family members was performed. Control samples were randomly collected from patients who presented to our hospital in April 2020. The control group comprised 200 healthy individuals from the ethnic Han Chinese population in Shandong Province. The male-to-female ratio was 1:1. All participants signed informed consent forms. Parental consent was obtained for individuals under 18 years of age. This study was approved by the ethics committee of Linyi People's Hospital and complied with the Declaration of Helsinki.

2.2 | Genomic DNA preparation

From all six family members, genomic DNA (gDNA) was extracted from 200 µl of peripheral blood in the presence of ethylenediaminetetraacetic acid as an anticoagulant using a kit (Qiagen, Hilden, Germany). The gDNA was spectrophotometrically measured with a Sim-100 Ultra Micro Spectrophotometer (Thermo Scientific, New York, NY, USA) and prepared as Illumina sequencing libraries. Targeted sequencing was performed on the proband samples (II:4). The pathogenic variants were validated by Sanger sequencing in the proband and parental samples.

2.3 | Whole-exome sequencing (WES) for variant screening

The WES technology was used to screen for mutation sites in CPLANE1 (NM_023073, NP_075561). WES was performed according to the human exome capture protocol from Illumina’s TruSeq Exome Enrichment Guide (SureSelectXT Target Enrichment System for Illumina Paired-End Sequencing Library, Agilent Technologies, Santa Clara, CA, USA). Exome enrichment probe sets were constructed with the Agilent Human All Exon 50 Mb Exome Enrichment kit and sequenced on a HiSeq 4,000 NGS platform (Illumina, San Diego, CA, USA). The captured gDNA library was sequenced and 200 (2 × 100) bp were generated from the final library fragment using V2 Reagent 1.8 software (Illumina; data after June 22, 2011). The average depth of the target area was 257.15x; the target bases with coverage of at least 50x were 75.81%, 20 × 82.23%, 10 × 89.04%, 4 × 93.56%, and 1 × 96.09%. After
sequencing, the original data were stored in FASTAQ files, which were aligned with the human reference genome hg19 to obtain Bam files. Paired-end sequence reads were mapped and indexed with Burrows–Wheeler aligner (Li & Durbin, 2010) and converted into VCF files to obtain mutation information. Genome Analysis Toolkit software (McKenna et al., 2010) was used to identify single-nucleotide polymorphisms (SNPs) and insertions or deletions. All identified variants were submitted to ANNOVAR for functional annotation and genetic filtering. All information presented in this study was directly extracted from the reference data set or calculated in batches of all variants. Common variants were excluded by comparison with more than 1,000 exomes sequenced in our laboratory for unrelated conditions and subsequently filtered with the dbSNP v137, Human Gene Mutation Database (HGMD; professional version 2020.1), 1,000 Genomes Project (1000GP), National Heart, Lung, and Blood Institute, Exome Sequencing Project, and ExAC databases. Variant filtration was based on the following criteria: (1) excluding 3′- and 5′-untranslated region variants, non-coding RNA intron variants, and intron and synonymous variants; (2) excluding minor allele frequency >0.1 variants; (3) at least half of the harmful variants in the bioinformatics software (PolyPhen-2, Sorting Intolerant From Tolerant [SIFT], and Variant Taster) were retained.

2.4 | Sanger sequencing

The variations detected by WES were validated by Sanger sequencing. Two hundred healthy controls from the Han Chinese ethnic population in Shandong Province were randomly selected for validation. The male-to-female ratio was 1:1. The two primer pairs covering the variants were as follows: forward, 5′-GCTTGCTTTTTCATTGTTCTGTAC-3′; reverse, 3′-GATTTCCAAGAGCAAATGTATACTG-5′ for c.1270C>T (primer pair one); and forward, 5′-GCTGAAGAAAGAAAATGCTAAGTGAA-3′, reverse, 5′-CTGACGTATCTCTGGCAATGG-3′ for c.8901C>A (primer pair two). Amplification occurred in a total volume of 25 µl containing 250 nM dNTPs, 100 ng DNA, 0.5 mM of each primer, and 1.25 U AmpliTaq Gold DNA polymerase in 1× reaction buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl, 2.5 mM MgCl₂). Following a standard PCR protocol (60 s annealing at 55 and 58°C for primer pairs one and two, respectively), amplified products were purified and sequenced using the BigDye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) and evaluated on an ABI 7,500 automated sequencer (Applied Biosystems).

2.5 | Bioinformatics analysis

Functional impacts of the variants were predicted in silico. Multiple sequence alignment was used to analyze the conservation of gene sequences. The CPLANE1 amino acid sequences of multiple species were obtained from the UniProt website (http://www.uniprot.org), and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/www/SIFT_BLink_submit.html) software were used to analyze and predict the function of the mutations.

3 | RESULTS

3.1 | Clinical features

Two affected family members were identified and their clinical phenotypes matched with the primary diagnostic criteria of JS; the inheritance pattern was autosomal recessive. No carriers exhibited any clinical phenotypes (Figure 1).
The proband (II:4, patient 1), a 3-month-old male, was born to healthy, non-consanguineous parents with no perinatal risk factors. He came to our hospital for oculomotor apraxia, manifested strabismus, obvious horizontal nystagmus, gaze and gaze tracking impairment (eyes could not move flexibly with indicators), and abnormal vestibular eye reflex and light reflex. Further, the proband exhibited distinctive craniofacial abnormalities: prominent forehead with sharp skull, high, and round eyebrows with excessive eye distance, wide and flat nose, low ears, and sunken-in upper lip at the midline. Developmental delay and hypotonia manifested in instability of his head and difficulty in turning over and grasping objects, and the Gesell scale showed moderate retardation in all domains. He was unable to speak or laugh. Breathing was abnormal and short, with approximately 34 breaths/min, but no obvious facial cyanosis was observed. Ultrasound imaging revealed a patent foramen ovale and tricuspid insufficiency, whereas the liver and kidney were normal. Brain MRI showed mild MTS with cerebellar vermis hypoplasia (CVH), moderately elongated and straight superior cerebellar peduncles (SCP), and slightly deepened interpeduncular fossa (IF). The fourth ventricle was expanded and abnormally shaped, and long and line-like T1- and T2-weighted MRI signals were observed between cerebellar hemispheres showing a midline split sign (Figure 2a).

Patient 2 (II:2), a 7-year-old female, exhibited similar but more severe symptoms compared with the proband. She displayed severe nervous phenotypes with increasing age and obvious psychomotor retardation, including difficulties in independent crawling, sitting, standing, walking, and eating. She could not complete postural conversion and speech imitation or expression but could understand some short and simple instructions, replying intermittently using gestures. Heart and brain examinations revealed similar results compared with the proband (Figure 2b).

### 3.2 Genetic analysis

WES data were filtered to exclude non-genetic variants and compared with dbSNP and 1000GP databases. We identified two likely pathogenic compound heterozygous variants in CPLANE1 (NM_023073.3) (predicted by bioinformatics analysis tools) in the proband: the nonsense variants c.1270C>T (p.Arg424*) and c.8901C>A (p.Tyr2967*), inherited from his father and mother, respectively. The variants were analyzed according to the “Standards and Guidelines for Interpreting Sequence Variants” issued by the American College of Medical Genetics and Genomics (ACMG) and the Association of Molecular Pathology (Richards et al., 2015).

![Figure 2](image-url)

**Figure 2** Brain MRI images of patients with Joubert syndrome with CPLANE1 mutations. (a) Patient 1. T1-weighted axial MRI showed a small volume of the cerebellar vermis, elongated and straight superior cerebellar peduncles (SCP), and slightly deepened interpeduncular fossa (IF; red arrow), exhibiting a mild MTS (black arrow) and long and line-like T1-weighted signals between the cerebellar hemispheres showing a midline split sign (white arrow). (b) Patient 2. T1-weighted axial and midsagittal MRI showed a small volume of the cerebellar vermis, elongated and straight SCP, and deepened IF (red arrow), exhibiting a typical MTS (black arrow) and a midline split sign (white arrow).
Variant c.1270C>T (p.Arg424*) in exon 10, resulting in a change from C to T in the 2120th base, causes a substitution of arginine for a stop codon at the 424th amino acid of the encoded protein (Figure 3a); this may cause mRNA degradation or protein truncation, thereby affecting the function of the CPLANE1 protein (PVS1). This variant is present in population databases (rs755097302, ExAC 0.03%), and the frequency is $G = 0.0001$ (PM2). It has not been reported in the literature to be present in individuals, but the ClinVar database contains an entry for this mutation as a “pathogenic variant” (variation ID: 392,297) for OFD VI and JS17 (PM3_Strong).

The father of the proband carries a heterozygous mutation at this locus. According to the available evidence, it is defined as a pathogenic variant (PVS1+PM3_Strong+PM2) based on the 2015 ACMG guidelines (Richards et al., 2015).

The novel mutation c.8901C>A (p.Tyr2967*) in exon 48 of CPLANE1 results in a change from C to A at 8901bp, causing the 2967th amino acid to be changed from tyrosine to a stop codon (Figure 3b); this may cause loss of function, as the mutation is located within the last 10% of the coding region, leading to mRNA degradation or protein truncation (PVS1_PM4). This variant was not detected in the normal population.
database (PM2) but was identified as a pathogenic variant (PM3). According to the 2015 ACMG guidelines, it is defined as a likely pathogenic variant (PVS1_PM4+PM2+PM3). The probability of these variants occurring in the population is extremely low and neither variant was found in 200 healthy, unrelated controls. The identified mutations were co-segregated with JSOFD (JS with OFD) phenotypes in this family.

### 3.3 Multiple sequence alignment and molecular structure modeling

Multiple sequence alignment of the CPLANE1 protein across six species, including homo sapiens, monkey, chimpanzee, mouse, sheep, and cow, revealed that the arginine and tyrosine located at positions 424 and 2,967 of the CPLANE1 protein, respectively, are highly conserved (Figure 4).
DISCUSSION

JS is a type of ciliopathy, demonstrating substantial clinical phenotypic variability (Parisi, 2019). While pure JS only occurs in a small number of patients and most are diagnosed with pure JS in infancy, multiple systematic phenotypes gradually appear over time (JSRD). Once JS is diagnosed, a comprehensive examination should be carried out to assess whether other organs are involved.

In our study, a 3-month-old male and a 7-year-old female presented with clinical manifestations that met the clinical diagnostic criteria of JS (JSOFD). They also had congenital heart disease, which is rarely reported in JS patients. Using WES, we found two nonsense mutations in \textit{CPLANE1} (c.1270C>T [p.Arg424*] and c.8901C>A [p.Tyr2967*]) in the proband, which co-segregated with the JSOFD phenotypes in the family. Sanger sequencing verified that p.Arg424* was inherited from the father and p.Tyr2967* from the mother. In silico analysis identified these compound heterozygous variants as pathogenic according to ACMG guidelines. In addition, they were absent in 200 ethnically matched healthy controls, suggesting that they may be causative for the observed JS phenotype.

JS, as an allelic disorder, is highly genetically heterogeneous; that is, biallelic mutations in many different genes could cause a similar spectrum of diseases, albeit with different severity and clinical phenotypes (Lopez et al., 2014; Parisi, 2019). More than 35 causative genes have been identified to be related to JS, but there is no clear-cut correlation between genotypes and phenotypes. It was reported that the loss of \textit{CC2D2A} function is mostly associated with pure JS, whereas \textit{CPLANE1} is mostly related to polydactyly and Meckel syndrome; mutations in \textit{AH11} and \textit{CEP290} often lead to JSRD with retinal defects, while the most common pathogenic genes in the phenotype with renal defects are \textit{NPHP1} and \textit{RPGRIP1L} (Kroes et al., 2008, 2016). Interestingly, although mutations within the same gene might result in different clinical phenotypes, the same mutations in different variants of the same gene may also lead to different disease outcomes (Bonnard et al., 2018). In our study, using the identified mutations, \textit{CPLANE1} was confirmed as a pathogenic gene in JS. The protein encoded by \textit{CPLANE1} was predicted to have a transmembrane protein and putative coiled-coil domains (Srour et al., 2012) and is involved in ciliogenesis and establishment of cell polarity required for directed cell migration. Interestingly, most of the \textit{CPLANE1} variants identified in JS and OFD VI cases in previous studies were located outside of these predicted functional domains (Romani et al., 2015). More than 60 mutations in \textit{CPLANE1} have been associated with JS and OFD VI in HGMD (professional version 2020.1), including heterozygous and homozygous variants (Bayram et al., 2015; Kroes et al., 2016; Lopez et al., 2014; Romani et al., 2015; Srour et al., 2012; Wentzensen et al., 2015). In 2012, Srour et al. (2012) genotyped JS-related genes in French Canadians, demonstrating that \textit{CPLANE1} was the causative gene of JS in this population and had a complex founder effect. Lopez et al. (2014) described 14 novel mutations in \textit{CPLANE1} in 9/11 families that met the clinical diagnostic criteria of OFD VI, concluding that it is the major gene responsible for OFD VI. Romani et al. (2015) sequenced \textit{CPLANE1} in 313 JS probands and found causative mutations in 28 patients (8.9%) with pure
JS phenotype, while only 2/17 OFD VI patients (11.7%) carried CPLANE1 mutations. These findings revealed that CPLANE1 might not be the major pathogenic gene responsible for all OFD VI subtypes. Bonnard et al. (2018) compared 32 JS and 26 OFD VI patients with CPLANE1 mutations and concluded that OFD VI was more likely to be caused by such mutations than milder JS. In a more recent study, Liu et al. (2020) identified four novel compound heterozygous mutations in CPLANE1 in four Chinese families with JS, exhibiting pure JS clinical manifestations and mild neuroradiological features. Their findings indicate that CPLANE1 mutations generally result in a purely neurological JS phenotype and mild brain defects, rather than OFD VI. Our study provides evidence that mutations in CPLANE1 (c.1270C>T and c.8901C>A) mainly cause a neurological phenotype, accompanied by a mild OFD VI phenotype, which can be classified as a JSOFD subtype.

Due to the limitations of epidemiological surveys in China, more family studies have been conducted in European countries. A total of 207 cases with JS have been reported in China, including pathogenic variants of CPLANE1, TMEM67, MKS1, CC2D2A, AHI1, ARMC9, CEP290, NPHP1, ARL13B, B9D2, CSPP1, INPP5E, PIBF1, RPGRIP1L, and TCTN1 (Liu et al., 2020; Xiang et al., 2018). Eight JS cases caused by CPLANE1 mutations have been identified, covering more than 10 mutation sites (Table 1) (Liu et al., 2020; Xiang et al., 2018). Most of the pathogenic sites are located within the coding region, but there are no concentrated mutation hotspots. However, which mutations in CPLANE1 are related to JS in the Chinese population, and the correlation between genotype and phenotype in this syndrome has not been fully elucidated. Future studies with larger sample sizes and further molecular biological analyses are needed to verify the pathogenic genes and better understand the pathogenesis of JS.

| Genotype | Mutation type | Protein | Heter/homo |
|----------|---------------|---------|------------|
| c.1572del | Frameshift mutation | p.Leu524TyrfsTer16 | Heter |
| c.7978C>T | Missense mutation | p.Arg2660Ter | Heter |
| c.1819dup | Frameshift mutation | p.Tyr607LeufsTer12 | Heter |
| c.3676C>T | Missense mutation | p.R1226X | Heter |
| c.8310_8311insT | Frameshift mutation | p.L7770fsX5 | Heter |
| c.7351G>A | Missense mutation | p.V2451I | Heter |
| c.2315T>C | Missense mutation | p.L772P | Heter |
| c.8852_8855del | Frameshift mutation | p.R2952Cfs*17 | Heter |
| c.2876C>T | Missense mutation | p.Pro959Leu | Heter |
| c.3921+1G>A | Splicing mutation | p.Ser2333PhefsTer11 | Heter |
| c.6997_6998insT | Frameshift mutation | p.Arg2904Ter | Heter |
| c.2292-2delA | Splicing mutation | p.Pro1356Leu | Heter |
| c.4067delT | Missense mutation | p.Pro1356Leu | Heter |
| c.8710C>T | Nonsense mutation | p.Arg2904Ter | Heter |
| c.3981G>C | Missense mutation | p.Trp1327Cys | Heter |
| c.3599delT | Missense mutation | p.A1200V | Heter |
| c.3857G>A | Missense mutation | p.R1286H | Heter |

Note: The nucleotide and amino acid positions are based on reference sequence NM_023073.3.
Abbreviations: Heter, heterozygous; Homo, homozygous.

TABLE 1 Mutation loci in CPLANE1 have been identified in Chinese cases

JS phenotype, while only 2/17 OFD VI patients (11.7%) carried CPLANE1 mutations. These findings revealed that CPLANE1 might not be the major pathogenic gene responsible for all OFD VI subtypes. Bonnard et al. (2018) compared 32 JS and 26 OFD VI patients with CPLANE1 mutations and concluded that OFD VI was more likely to be caused by such mutations than milder JS. In a more recent study, Liu et al. (2020) identified four novel compound heterozygous mutations in CPLANE1 in four Chinese families with JS, exhibiting pure JS clinical manifestations and mild neuroradiological features. Their findings indicate that CPLANE1 mutations generally result in a purely neurological JS phenotype and mild brain defects, rather than OFD VI. Our study provides evidence that mutations in CPLANE1 (c.1270C>T and c.8901C>A) mainly cause a neurological phenotype, accompanied by a mild OFD VI phenotype, which can be classified as a JSOFD subtype.

Lopez et al., (2014) indicated that at least one truncating mutation is necessary to cause JS or OFD VI; in CPLANE1, a high rate of truncating mutations occurs, which is in contrast to most other pathogenic genes associated with JS. In our report, both c.1270C>T and c.8901C>A are nonsense mutations, causing truncation of the encoded protein. Generally, variants with biallelic truncating mutations often lead to severe or lethal phenotypes, which is in contrast to our own observations. A possible explanation could be that the function of CPLANE1 might be not principal or could be compensated by other ciliary proteins that act in the same pathway during embryonic development so that a complete loss of function would not lead to a lethal phenotype (Travaglini et al., 2013).

This would confirm the hypothesis that a total loss function of CPLANE1 may result in mild JS features, rather than a severe OFD VI phenotype (Bonnard et al., 2018; Enokizono et al., 2017; Kroes et al., 2016).

Due to the limitations of epidemiological surveys in China, more family studies have been conducted in European countries. A total of 207 cases with JS have been reported in China, including pathogenic variants of CPLANE1, TMEM67, MKS1, CC2D2A, AHI1, ARMC9, CEP290, NPHP1, ARL13B, B9D2, CSPP1, INPP5E, PIBF1, RPGRIP1L, and TCTN1 (Liu et al., 2020; Xiang et al., 2018). Eight JS cases caused by CPLANE1 mutations have been identified, covering more than 10 mutation sites (Table 1) (Liu et al., 2020; Xiang et al., 2018). Most of the pathogenic sites are located within the coding region, but there are no concentrated mutation hotspots. However, which mutations in CPLANE1 are related to JS in the Chinese population, and the correlation between genotype and phenotype in this syndrome has not been fully elucidated. Future studies with larger sample sizes and further molecular biological analyses are needed to verify the pathogenic genes and better understand the pathogenesis of JS.
In summary, we found two novel compound heterozygous mutations in CPLANE1 in a Chinese family with JS, although the underlying mechanism of action remains unknown. We examined and described the phenotypes associated with these CPLANE1 mutations and thus expanded the spectrum of genetic variation caused by this gene. Our findings may help clinicians and geneticists improve the accuracy of the clinical diagnosis of JS.

ACKNOWLEDGMENTS
We thank the proband and contributors for their participation.

CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

ETHICAL APPROVAL
This study was approved by the ethics committee of the Affiliated Hospital of Qingdao University (grant number: lyph20201619) and complied with the Declaration of Helsinki.

PATIENT CONSENT STATEMENT
The authors certify that all patients, patient’s parents, and individuals in the control group have signed appropriate consent forms for the publication of their images and other clinical information presented in this article. The participants understood that their names and initials would not be published.

PERMISSION TO REPRODUCE MATERIAL FROM OTHER SOURCES
No material from other sources has been used.

AUTHOR CONTRIBUTIONS
Data curation, C.Z. and L.X.; formal analysis, C.Z. and Z.S.; funding acquisition, S.L. and F.C.; investigation, Z.S. and L.X.; project administration, S.L. and F.C.; resources, C.Z., L.X., X.Z., Z.S., S.L., and F.C.; supervision, S.L. and F.C.; validation, C.Z. and L.X.; writing—original draft, C.Z.; writing—review & editing, C.Z., S.L., and F.C. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT
The datasets used and/or analyzed during the current study have been submitted to the ClinVar database (accession numbers: SUB9483757: SCV001571327, SUB9483777: SCV001571328). The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES
Asadollahi, R., Strauss, J. E., Zenker, M., Beuing, O., Edvardson, S., Elpeleg, O., Strom, T. M., Josef, P., Niedrist, D., Otte, C., Oneda, B., Boonsawat, P., Azzarello-Burri, S., Bartholdi, D., Papik, M., Zweier, M., Haas, C., Ekici, A. B., Baumer, A., … Rauch, A. (2018). Clinical and experimental evidence suggest a link between KIF7 and C5orf42-related ciliopathies through Sonic Hedgehog signaling. European Journal of Human Genetics: EJHG, 26(2), 197–209. https://doi.org/10.1038/s41431-017-0019-9

Bachmann-Gagescu, R., Dempsey, J. C., Phelps, I. G., O’Roak, B. J., Knutzen, D. M., Rue, T. C., Ishak, G. E., Isabella, C. R., Gorden, N., Adkins, J., Boyle, E. A., de Lacy, N., O’Day, D., Alsawid, A., Ramadevi A. R., Lingappa, L., Lourencó, C., Martorell, L., Garcia-Cazorla, À., … Doherty, D. (2015). Joubert syndrome: A model for untangling recessive disorders with extreme genetic heterogeneity. Journal of Medical Genetics, 52(8), 514–522. https://doi.org/10.1136/jmedgenet-2015-103087

Bandyopadhyay, S., Chiang, C. Y., Srivastava, J., Gersten, M., White, S., Bell, R., Kurschner, C., Martin, C., Smoot, M., Sahasrabudhe, S., Barber, D. L., Chanda, S. K., & Ideker, T. (2010). A human MAP kinase interactome. Nature Methods, 7(10), 801–805. https://doi.org/10.1038/nmeth.1506

Bayram, Y., Aydin, H., Gambin, T., Akdemir, Z. C., Atik, M. M., Karaca, E., Karaman, A., Pehlivan, D., Jiangiani, S. N., Gibbs, R. A., & Lupski, J. R. (2015). Exome sequencing identifies a homozygous C5orf42 variant in a Turkish kindred with oral-facial-digital syndrome type VI. American Journal of Medical Genetics. Part A, 167A(9), 2123–2137. https://doi.org/10.1002/ajmg.a.37092

Bonnard, C., Shboul, M., Tonekaboni, S. H., Ng, A. Y. J., Tohari, S., Ghosh, K., Lai, A., Lim, J. Y., Tan, E. C., Devisse, L., Stichelbaut, M., Alkindi, A., Banu, N., Yuökç, Z., Ghomjd, J., Elkhartoutf, N., Boutlaud, L., Micalizi, A., Brett, M. S., … Kariminejad, A. (2018). Novel mutations in the ciliopathy-associated gene CPLANE1 (C5orf42) cause OFD syndrome type VI rather than Joubert syndrome. European Journal of Medical Genetics, 61(10), 585–595. https://doi.org/10.1016/j.ejmg.2018.03.012

Brancati, F., Dallapiccola, B., & Valente, E. M. (2010). Joubert Syndrome and related disorders. Orphanet Journal of Rare Diseases, 5, 20. https://doi.org/10.1186/1750-1172-5-20

Enokizono, M., Aida, N., Niwa, T., Osaka, H., Naruto, T., Kurosaawa, K., Ohba, C., Suzuki, T., Saitsu, H., Goto, T., & Matsumoto, N. (2017). Neuroimaging findings in Joubert syndrome with C5orf42 gene mutations: A milder form of molar tooth sign and vermian hypoplasia. Journal of the Neurological Sciences, 376, 7–12. https://doi.org/10.1016/j.jns.2017.02.065

Ganesan, A. K., Kho, Y., Kim, S. C., Chen, Y., Zhao, Y., & White, M. A. (2007). Broad spectrum identification of SUMO substrates in melanoma cells. Proteomics, 7(13), 2216–2221. https://doi.org/10.1002/pmic.200600971

Hong, H., Joo, K., Park, S. M., Seo, J., Kim, M. H., Shin, E., Cheong, H. I., Lee, J. H., & Kim, J. (2019). Extracellular roles of the ciliopathy protein JBT17 in mitosis and neurogenesis. Annals of Neurology, 86(1), 99–115. https://doi.org/10.1002/ana.25491

Kroes, H. Y., Van der Zwaag, B., Duran, K. J., de Kovel, C. G., van Roosmalen, M. J., Harakalova, M., Nijman, I. J., Kloosterman, W. P., Giles, R. H., Knoers, N. V., & van Haften, G. (2016). Joubert syndrome: Genotyping a Northern European patient cohort. European Journal of Human Genetics: EJHG, 24(2), 214–220. https://doi.org/10.1038/ejhg.2015.84

Kroes, H. Y., Ravesloot, C., Fransen van de Putte, D., & Lindhout, D. (2007). The birth prevalence of Joubert syndrome: A population based study in the Netherlands. European Journal of Human Genetics, 15.
Kroes, H. Y., van Zon, P. H., Fransen van de Putte, D., Nelen, M. R., Nievelstein, R. J., Wittebol-Post, D., van Nieuwenhuizen, O., Mancini, G. M., van der Knaap, M. S., Kwee, M. L., Maas, S. M., Cobben, J. M., De Nef, J. E., Lindhout, D., & Sinke, R. J. (2008). DNA analysis of AH1, NPHP1 and CYCLIN D1 in Joubert syndrome patients from the Netherlands. European Journal of Medical Genetics, 51(1), 24–34. https://doi.org/10.1016/j.ejmg.2007.10.001

Li, H., & Durbin, R. (2010). Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics (Oxford, England), 26(5), 589–595. https://doi.org/10.1093/bioinformatics/btp968

Liu, Q., Wang, H., Zhao, J., Liu, Z., Sun, D., Yuan, A., Luo, G., Wei, W., & Hou, M. (2020). Four novel compound heterozygous mutations in C5orf42 gene in patients with pure and mild Joubert syndrome. International Journal of Developmental Neuroscience: The Official Journal of the International Society for Developmental Neuroscience, 80(6), 455–463. https://doi.org/10.1016/j.ijdevneu.2020.02.011

Lopez, E., Thauvin-Robinet, C., Reversade, B., Khartoufi, N. E., Devisse, L., Holder, M., Ansart-Franquet, H., Avila, M., Lacombe, D., Kleinfinger, P., Kaori, I., Takanashi, J., Le Merrer, M., Martinovic, J., Noël, C., Sbhoul, M., Ho, L., Güven, Y., Razavi, F., … Attié-Bitach, T. (2014). C5orf42 is the major gene responsible for OFD syndrome type VI. Human Genetics, 133(3), 367–377. https://doi.org/10.1007/s00431-013-1385-1

McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S., Daly, M., & DePristo, M. A. (2010). The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. Genome Research, 20(9), 1297–1303. https://doi.org/10.1101/gr.107524.110

Paprocka, J., & Jamroz, E. (2012). Joubert syndrome and related disorders. Neurologia i Neurochirurgia Polska, 46(4), 379–383. https://doi.org/10.5114/ninp.2012.30457

Parisi, M. A. (2019). The molecular genetics of Joubert syndrome and related ciliopathies: The challenges of genetic and phenotypic heterogeneity. Translational Science of Rare Diseases, 4(1–2), 25–49. https://doi.org/10.3233/TRD-190041

Parisi, M., & Glass, I. (2003). Joubert syndrome. In M. P. Adam (Eds.) et al., Joubert syndrome. In M. P. Adam (Eds.) GeneReviews®. University of Washington.

Reiter, J. F., & Leroux, M. R. (2017). Genes and molecular pathways underpinning ciliopathies. Nature Reviews Molecular Cell Biology, 18(9), 533–547. https://doi.org/10.1038/nrm.2017.60

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W. W., Hegde, M., Lyon, E., Spector, E., Voelkerding, K., & Rehm, H. L. (2015). 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. Genetics in Medicine, 17(5), 405–423. https://doi.org/10.1038/gim.2015.30

Romani, M., Mancini, F., Micallazzi, A., Poretti, A., Miccinilli, E., Accorsi, P., Avola, E., Bertini, E., Borgatti, R., Romaniello, R., Ceylaner, S., Coppola, G., D’Arrigo, S., Giordano, L., Janecke, A. R., Lituanis, M., Ludwig, K., Martorell, L., Mazza, T., … Valente, E. M. (2015). Oral-facial-digital syndrome type VI: Is C5orf42 really the major gene? Human Genetics, 134(1), 123–126. https://doi.org/10.1007/s00439-014-1508-3

Srou, M., Schwartzentruber, J., Hamdan, F. F., Ospina, L. H., Patry, L., Labuda, D., Massicotte, C., Dobrzeneiecka, S., Capo-Chichi, J.-M., Papillon-Cavanagh, S., Samuels, M. E., Boycott, K. M., Shevell, M. I., Laframboise, R., Désilets, V., Maranda, B., Rouleau, G. A., Majewski, J., & Michaud, J. L. (2012). Mutations in C5orf42 cause Joubert syndrome in the French Canadian population. American Journal of Human Genetics, 90(4), 693–700. https://doi.org/10.1016/j.ajhg.2012.02.011

Toriiyama, M., Lee, C., Taylor, S. P., Duran, I., Cohn, D. H., Bruel, A.-L., Tabler, J. M., Drew, K., Kelly, M. R., Kim, S., Park, T. J., Braun, D. A., Pierquin, G., Biver, A., Wagner, K., Malfroot, A., Panigrahi, I., Franco, B., Al-lami, H. A., … Wallingford, J. B. (2016). The ciliopathy-associated CPLANEx proteins direct basal body recruitment of intraflagellar transport machinery. Nature Genetics, 48(6), 648–656. https://doi.org/10.1038/ng.3558

Travaglini, L., Brancati, F., Silhavy, J., Iannicelli, M., Nickerson, E., Elkhartoufi, N., Scott, E., Spencer, E., Gabriel, S., Thomas, S., Ben-Zeev, B., Bertini, E., Bolthshauser, E., Chaouch, M., Roberta Cilio, M., de Jong, M. M., Kayserili, H., Ogur, G., Poretti, A., … Valente, E. M. (2013). Phenotypic spectrum and prevalence of INPP5E mutations in Joubert syndrome and related disorders. European Journal of Human Genetics: EJHG, 21(10), 1074–1078. https://doi.org/10.1038/ejhg.2012.305

Vilboux, T., Doherty, D. A., Glass, I. A., Parisi, M. A., Phelps, I. G., Cullinane, A. R., Zein, W., Brooks, B. P., Heller, T., Soldatos, A., Oden, N. L., Yildirimli, D., Vemulapalli, M., Mullikin, J. C., Program, N. C. S., Malidcan, M., Gahl, W. A., & Gunay-Aygun, M. (2017). Molecular genetic findings and clinical correlations in 100 patients with Joubert syndrome and related disorders prospectively evaluated at a single center. Genetics in Medicine: Official Journal of the American College of Medical Genetics, 19(8), 875–882. https://doi.org/10.1038/gim.2016.204

Wentzensen, I. M., Johnston, J. J., Keppler-Noreuil, K., Acrich, K., David, K., Johnson, K. D., Graham, J. M. Jr, Sapp, J. C., & Biesecker, L. G. (2015). Exome sequencing identifies novel mutations in C5orf42 in patients with Joubert syndrome with oral-facial-digital anomalies. Human Genome Variation, 2, 15045. https://doi.org/10.1038/hgv.2015.45

Xiang, J., Zhang, L., Jiang, W., Zhang, Q., Wang, T., Li, H., & Li, H. (2018). Prenatal diagnosis and genetic analysis of a fetus with Joubert syndrome. BioMed Research International, 2018, 7202168. https://doi.org/10.1155/2018/7202168

How to cite this article: Zhang, C., Sun, Z., Xu, L., Che, F., & Liu, S. (2021). Novel compound heterozygous CPLANEx variants identified in a Chinese family with Joubert syndrome. International Journal of Developmental Neuroscience, 00, 1–10. https://doi.org/10.1002/jdn.10135