

CLINICAL REPORT

Compound heterozygous splicing variants in *KIAA0586* cause fetal short-rib thoracic dysplasia and cerebellar malformation: Use of exome sequencing in prenatal diagnosis

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Abstract

Background: Short-rib thoracic dysplasia (SRTD) and Joubert syndrome (JS) are rare genetic ciliopathies, and individuals with either syndrome can manifest cerebellar malformation and variable developmental delays. However, neither of these conditions is easily diagnosed during pregnancy due to a limited fetal phenotype. Here, we investigated a fetus that was initially observed to have short limbs and polydactyly and discovered a compound heterozygous pathogenesis through exome sequencing (ES).

Methods: Simultaneous trio-ES and chromosome microarray analysis was provided for the fetus. The presence and effects of these variants on splicing were further validated at the DNA and RNA levels.

Results: Only short limbs and post-axial polydactyly of the fetus were detected during the second trimester. Two variants (c.3940+1G>A and c.3303G>A), affecting splicing of *KIAA0586*, were identified from amniocytes through ES and validated by Sanger sequencing. More intensive fetal monitoring was applied, and the fetus was also found to have deformed cerebellar malformation and a constricted thoracic cage.

Conclusions: Herein, we report the genetic pathogenesis of SRTD and/or JS associated with *KIAA0586* in a fetus. The novel splicing variants observed expand the spectrum of *KIAA0586* in SRTD and/or JS. Based on the genetic data and the distinct corresponding phenotypes discovered by imaging examination, a comprehensive diagnosis was made during pregnancy and more valuable prognostic information was provided for the parents.

KEYWORDS

exome sequencing, Joubert syndrome, *KIAA0586*, prenatal diagnosis, short-rib thoracic dysplasia, splicing variants

Mei Yang and Shanling Liu contributed equally to this study.

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1 | INTRODUCTION

Short-rib thoracic dysplasia (SRTD) syndromes are a group of autosomal or digenic recessive skeletal ciliopathies characterized by short ribs, short limbs and a constricted thoracic cage. Polydactyly is variably present, and non-skeletal involvement can include anomalies of major organs such as the brain, heart, kidneys, liver, pancreas, intestines and genitalia (Alby et al., 2015). Some types of SRTD are lethal in the neonatal period due to respiratory insufficiency secondary to a severely restricted thoracic cage, whereas others are compatible with life. SRTD displays genetic heterogeneity, and have been classified into 20 types (SRTD1~20), caused by variants in genes such as *IFT80*, *DYNC2H1*, *TTC21B*, *WDR19*, *NEK1*, *IFT140*, *CEP120*, *KIAA0586*, *DYNC2L1*, *IFT52*, *TCTEX1D2* and *INTU* (Chen et al., 2005; Chen, Chang, et al., 2012; Chen, Chern, et al., 2012).

Joubert syndrome (JS), a neurodevelopmental disorder characterized by a distinctive cerebellar and brainstem malformation that manifests as the molar tooth sign on magnetic resonance imaging (MRI), causes hypotonia and variable developmental delays. Additional findings in JS patients include retinal dystrophy, disturbances of respiratory control, renal disease, occipital encephalocele, hepatic fibrosis, polydactyly, oral hamartomas, and endocrine abnormalities (Brancati et al., 2010; Poretti et al., 2014). JS is generally inherited in an autosomal recessive manner, but it demonstrates extreme phenotypic variability and genetic heterogeneity (Bachmann-Gagescu, Dempsey, et al., 2015). Currently, pathogenic variants of more than 30 genes are known to cause JS. A molecular diagnosis can be established in approximately 62%–94% of individuals with a clinical diagnosis of JS (Parisi & Glass, 2003).

However, in fetuses SRTD and JS cannot easily be clinically diagnosed due to limited manifestations. Here, we present a prenatal case in which only short limbs and polydactyly were initially observed. A novel splicing and a known synonymous variants of *KIAA0586* (OMIM: 610178) were detected in amniocytes by exome sequencing (ES). Both variants affected splicing of *KIAA0586*. The compound heterozygous genetic pathogenesis contributed to the prenatal diagnosis of SRTD (type 14) or JS (type 23).

2 | METHODS

2.1 | Ethical compliance

The experiments on human subjects conducted in this study were approved by the Ethical Review Board of West China Second University Hospital, Sichuan University.

2.2 | Genetic testing

Genomic DNA (gDNA) was extracted from amniocytes and parents' peripheral blood leukocytes using a QIAamp DNA Blood Mini Kit (QIAGEN). To detect the genetic factors for the fetus, chromosome microarray analysis (CMA) and trio-ES were simultaneously performed using genomic DNA obtained from the family members.

gDNA obtained from the fetus and parents was analyzed using single nucleotide polymorphism (SNP) array with the CytoScan 750 K Array (Thermo Fisher Scientific), following the manufacturer's instructions. Molecular karyotype analysis was performed using Chromosome Analysis Suite v4.1 (Thermo Fisher Scientific). The GRCh38 (hg38) genome was used for annotation. Copy number variants (CNVs) larger than 100kb or those that affected more than 50 contiguous probes were considered. The genetic pathogenicity and their clinical significance of CNVs were systematically evaluated according to the American Society for Medical Genetics and Genomics (ACMG) guidelines (Riggs et al., 2020) and previous publication (Hu et al., 2019), respectively.

Exome capture sequencing was performed using the NanoWES Human Exome V1 (Berry Genomics) according to the protocol provided by the manufacturer. The enriched library was sequenced on the Illumina NovaSeq6000 platform with 150-bp paired-end reads. The Burrows-Wheeler Aligner software tool was used to align the sequencing reads with hg38. Local alignment and recalibration of base quality of the Burrows-Wheeler aligned reads were performed using the GATK Indel Realigner and the GATK Base Recalibrator, respectively (broadinstitute.org/). Single-nucleotide variants (SNVs) and small insertions or deletions (InDels) were identified by GATK Unified Genotyper (broadinstitute.org/). Finally, functional annotation was performed using ANNOVAR and the Enliven Variants Annotation Interpretation System (Berry Genomics). The public databases for filtering include gnomAD (<http://gnomad.broadinstitute.org/>), 1000 Genomes Project (1000G) (<http://browser.1000genomes.org/>), and etc. The pathogenicity of SNVs was evaluated based on the scientific medical literature and on disease databases such as PubMed (<https://www.ncbi.nlm.nih.gov/pubmed/>), ClinVar (<http://www.ncbi.nlm.nih.gov/clinvar/>), OMIM (<http://www.omim.org/>), the Human Gene Mutation Database (HGMD) (<http://www.hgmd.org/>), and the Human Genome Variation Society website (<http://www.hgvs.org/dblist/dblist.html>).

The candidate variants found in each independent gDNA sample were validated using Sanger sequencing: polymerase chain reaction (PCR) amplification was performed using primer pairs (Table 1) designed to cover the variants identified by ES. To figure out if the variants impact splicing, total RNA of was extracted from the parents' fresh peripheral

blood samples using an RNAPure Blood Kit (CW BIO, CW0582S). After reversed to cDNA using a synthesis Kit (Thermo Scientific, K1622), the cDNA target sequences were amplified by PCR, using primers designed using Primer 3 software Version 0.4.0 (<http://bioinfo.ut.ee/primer3-0.4.0/>) (Table 1). Sequencing of the PCR products on an ABI 3500 Genetic Analyzer (Thermo Fisher Scientific) was performed to verify the changes at the DNA and RNA levels. The data were evaluated using Chromas software (2.6.5).

3 | RESULTS

3.1 | Clinical summary

A primigravid woman was referred for genetic counseling during the second trimester because of fetal post-axial polydactyly, short limbs and persistent left superior vena cava (PLSVC) with a dilated coronary sinus. Ultrasound examination at 24⁺¹ weeks of gestation revealed post-axial polydactyly of the fetal right hand and a femur length (FL) of 3.9 cm (−1.7SD, Hadlock).

The woman and her husband were non-consanguineous, and there was no family history of congenital malformations or genetic diseases.

Additional distinct phenotypes of the fetus were identified through more frequent monitoring (Figure 1), and these features were consistent with the genetic results. Fetal MRI at 31⁺⁵ weeks of gestation showed deformed cerebellar vermis and characteristic molar tooth sign (Figure 1e). Strikingly, hypoplastic cerebellar vermis, an enlarged third ventricle, a relatively small thoracic cage and short humerus length (HL, 5.03 cm, −2.9SD) in addition to polydactyly were later found at 33 weeks of gestation by fetal sonography (Figure 1a–d). Ultimately, the couple chose to terminate the pregnancy after multidisciplinary consultation due to a potentially poor prognosis after birth.

3.2 | Molecular results

Amniocentesis was performed at 25⁺⁵ weeks of gestation, and DNA extracted from the amniocytes was tested by ES

and CMA simultaneously. No numerical abnormalities of chromosomes, CNVs or regions of homozygosity were discovered by CMA. However, using ES, we identified two variants of *KIAA0586* (NM_001244189.2), c.3940+1G>A and c.3303G>A (p.P1101P), that present in a compound heterozygous state. The presence of these variants was confirmed by Sanger sequencing (Figure 2).

Sanger sequencing of targeted transcribed products was performed on RNA obtained from the fetal parents' fresh blood specimens. The novel canonical splice site variant (c.3940+1G>A), which the fetus inherited paternally, has not been reported in any database, including the HGMD, the ClinVar database and the GnomAD database. Our study showed that the novel canonical splice site variant (c.3940+1G>A) resulted in deletion of exon 26 and introduction of a frameshift, producing a premature termination codon. The synonymous variant c.3303G>A (p.P1101P), which the fetus inherited maternally, was predicted to be deleterious by CADD, Splice AI and db-sSNV. The results indicated that the synonymous variant (c.3303G>A) led to deletion of 56 base pairs of exon 23, impacting splicing and resulting in a frameshift. This result is consistent with a previous study reported in a JS patient (Abou Tayoun et al., 2018) (Figure 3).

4 | DISCUSSION

Through DNA and RNA validation, we demonstrated that both the variants described here affect gene splicing and might lead to frameshift of *KIAA0586*. Therefore, the compound heterozygous variants were rated as likely pathogenic combined with low frequency in the general population, as is recommended to be reported for prenatal ES (Richards et al., 2015). It is worth noting that substitution at the last base pair of exon 23, although synonymous, leads to use of a cryptic splice site in exon 23 and the deletion of 56 base pairs, resulting in premature truncation of the *KIAA0586* protein (Bachmann-Gagescu et al., 2015). Therefore, disease databases such as ClinVar and HGMD and functional studies should be queried to identify putative pathogenic variants (Abou Tayoun et al., 2018; Bachmann-Gagescu et al., 2015).

TABLE 1 Primers used in the current study

Primers	Forward	Reversed
<i>KIAA0586</i> SNV (c.3940+1G>A)	5'ATGTCTGTGGCTAAGGATG 3'	5'AGGGTGGGATTATGAGGAA 3'
<i>KIAA0586</i> SNV (c.3303G>A)	5'AAGCTCTTGCTGAGACCATTGC 3'	5'AGCCCAGAACTTCAAGACCAGC 3'
<i>KIAA0586</i> mRNA (c.3940+1G>A)	5'CTACTACTACCTCCTCCA 3'	5'TCCAAGTCTCTGAATGATAG 3'
<i>KIAA0586</i> mRNA (c.3303G>A)	5'TTCCGCCAGTTGCTTCTA 3'	5'GGCAGGCATATCATCTCC 3'
β-actin	5'CTGGCACACCTTCTACAATG 3'	5'CCTCGTAGATGGGCACAGTGTG 3'

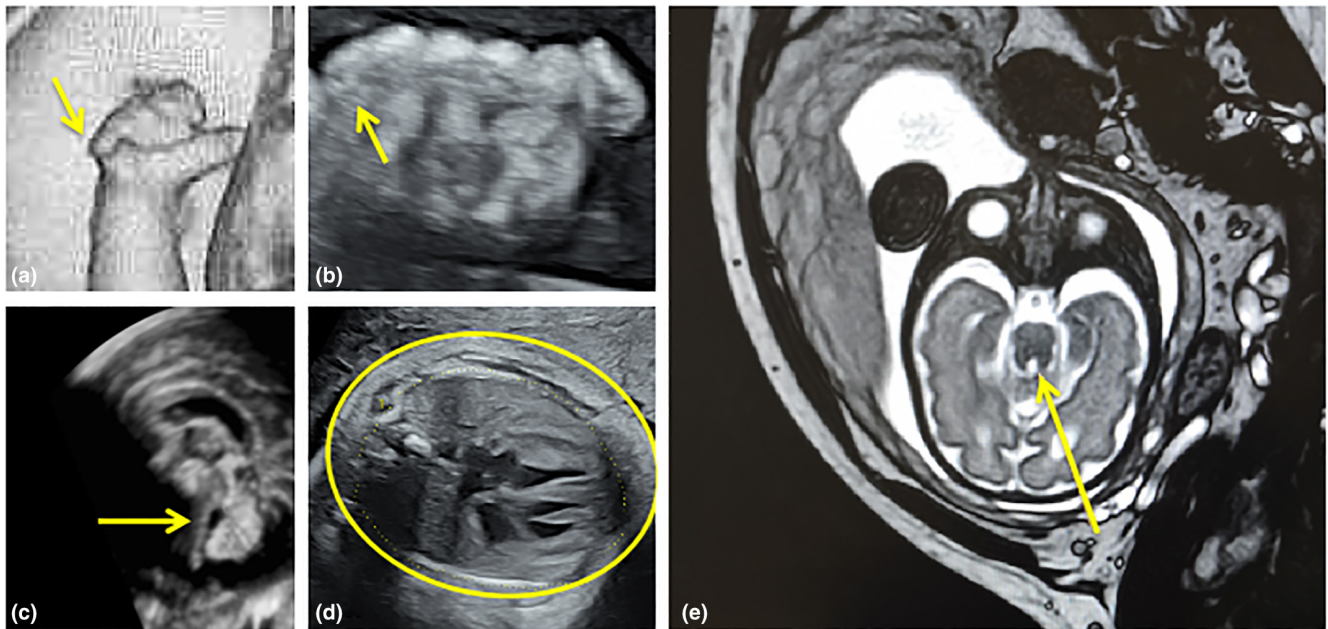


FIGURE 1 Prenatal imaging results. (a–d) The fetal ultrasound results (a, b post-axial polydactyly of the fetal right hand; c, the deformed cerebellar vermis; d, the constricted thoracic cage); e, the fetal MRI results (molar tooth sign due to thick and long superior cerebellar peduncles).

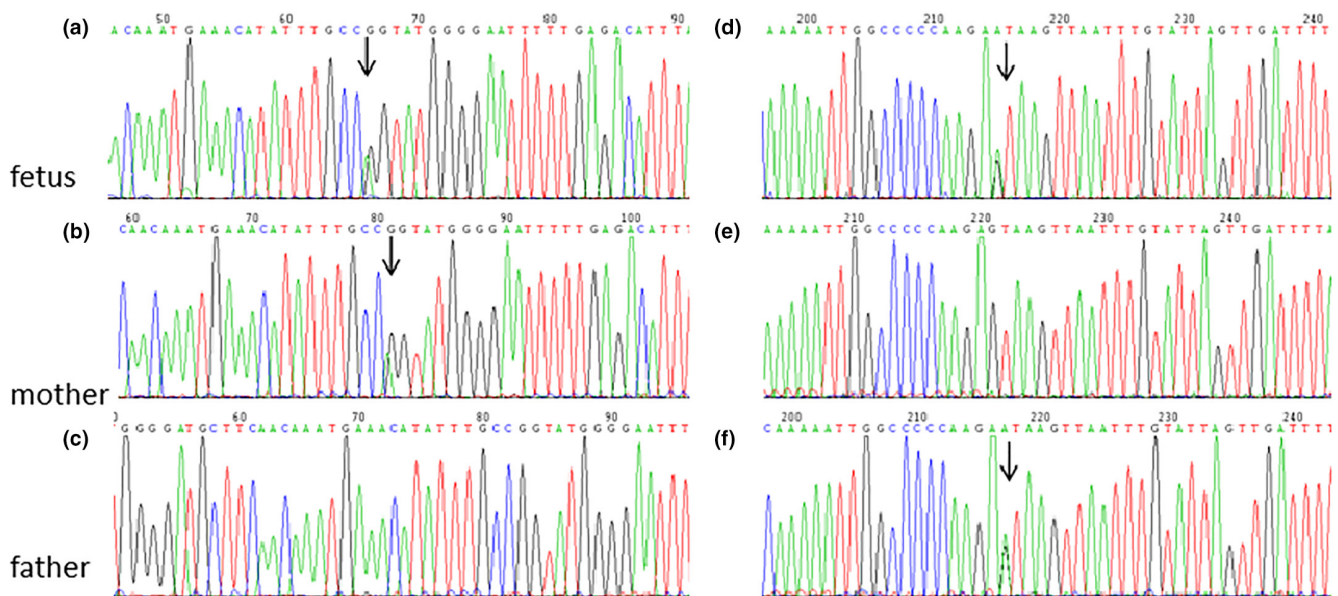


FIGURE 2 Sanger sequences of the *KIAA0586* variants. (a, d) compound heterozygous *KIAA0586* variants (c.3303G>A and c.3940+1G>A) in the fetus; (b, c) the synonymous variant (c.3303G>A) detected in the pregnant woman; (e, f) the novel canonical splice site variant (c.3940+1G>A) detected in the fetus' father.

In our study, the impact of splice-site variants was confirmed using RNA. RNA verification using the parents' blood samples made it possible to avoid another invasive operation on the fetus and resulted in rapid validation for the family.

According to recent research, ES may be considered for a fetus with ultrasound anomalies after standard CMA and karyotype analysis have failed to yield

a definitive diagnosis (Monaghan et al., 2020). ES is increasingly used in both pediatric and adult population in cases of multiple congenital anomalies and skeletal, neurodevelopmental and neuromuscular disorders (Posey et al., 2016; Retterer et al., 2016; Shen et al., 2015). Given the success in these patients, ES is currently applied prenatally. Studies reveal that ES increases the diagnostic yield in structurally abnormal

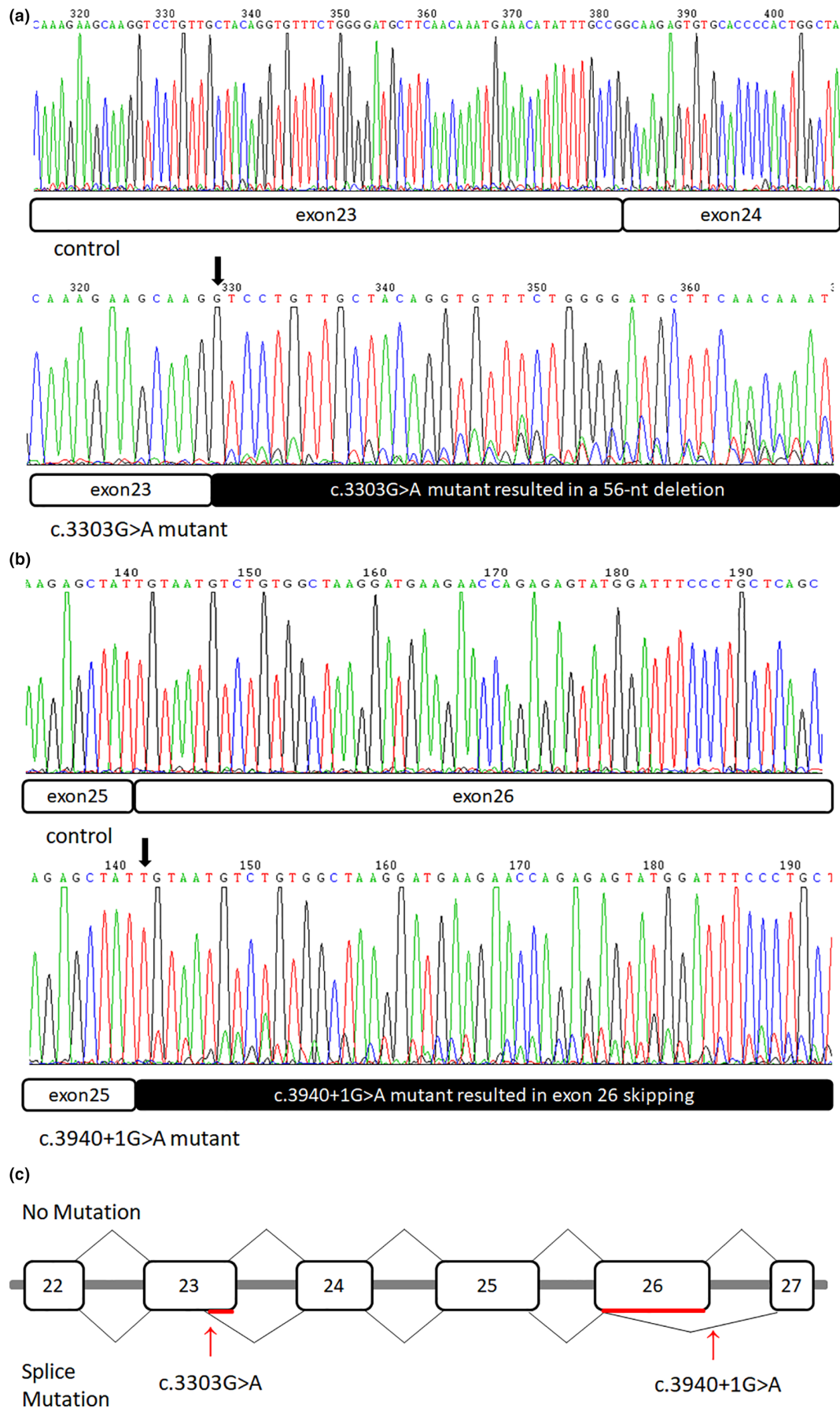


FIGURE 3 Molecular results on RNA using sanger sequencing. (a) c.3303G>A leads to a 56 bp deletion. (b) c.3940+1G>A leads to exon 26 skipping (black arrow indicates the starting position of the aberrant sequence). (c) schematic consequences of the variants in *K1AA0586* (red bars in exon 23 and 26 indicate the removed sequences by the splice site variants, respectively).

fetuses by approximately 8%–10% after normal karyotyping and CMA results are obtained, and the detection rate is strongly proportional to the severity of the phenotype (Lord et al., 2019; Petrovski et al., 2019). In the case reported here, the presence of multiple fetal anomalies provided a strong indication for implementing prenatal ES for our case. In contrast to sequential testing, simultaneous performance of CMA and ES leads to a timely and comprehensive genetic diagnosis for the fetus at already late gestation age.

Prenatal reporting of variants detected by ES is of a great challenge, due to limited and ambiguous fetal imaging findings. The accurate identification of fetal structural anomalies depends on gestational age, fetal position, the type of ultrasound equipment used, and the experience of the clinicians involved (Monaghan et al., 2020). The diagnostic yield rate of ES is strongly correlated with the number of fetal anomalies and the specificity of phenotypes (Zhang et al., 2017). In our case, the ES results indicated that the fetus was at risk of SRTD or JS, conditions in which molar tooth sign, hydrocephaly, polydactyly, and skeletal abnormalities may occur prenatally (Alby et al., 2015). Hence, serial ultrasound and brain MRI were additionally arranged. Markedly shorter (−2.9SD) fetal humerus length, small thoracic cage, molar tooth sign and cerebellar vermis were further found at 33 weeks of gestation.

Based on the genetic diagnosis made during this pregnancy, more distinct imaging findings were obtained. Consequently, multidisciplinary healthcare professionals collectively provided the couple with the clinical and genetic results. This type of information not only assists in determining the fetal prognosis, but can also indicate the risk of recurrence. According to authoritative databases such as OMIM, patients with *KIAA0586* variants may manifest JS (type 23) or SRTD (type 14). Specifically, neurodevelopmental disorders that are not detectable prenatally, may onset after birth. The couple's offspring have a 25% likelihood of possessing a compound heterozygous genotype that may lead to diseases. Subsequently, informed counseling, including preimplantation genetic testing or diagnostic prenatal testing, should be addressed.

5 | CONCLUSIONS

To sum up, we discovered a compound heterozygous pathogenesis of *KIAA0586* in a fetus that was initially observed to have short limbs and polydactyly. The novel *KIAA0586* splicing variant expands the spectrum of *KIAA0586* in SRTD and/or JS. Based on the validated ES results, additional distinct fetal phenotypes were timely discovered, leading to consistency of the genetic findings and the

clinical manifestations during pregnancy. Consequently, valuable prognostic information and future reproductive alternatives were provided for the parents.

AUTHOR CONTRIBUTIONS

Qianying Zhao and Mei Yang collected and analyzed the clinical and genetic data. Qianying Zhao was a major contributor in writing and formatting the manuscript. Mei Yang, Shanling Liu and He Wang edited the manuscript. Bocheng Xu, Qinqin Xiang, Yu Tan and Hanbing Xie conducted the experiments. Qianqian Gao performed the ultrasound for the fetus, while Linyi Wen performed the MRI.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

ETHICS APPROVAL

Experiment on human subjects was 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.

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