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# Prenatal diagnosis of fetal skeletal anomalies via whole-exome sequencing in a tertiary referral center

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**The accurate prenatal diagnosis of skeletal anomaly (SKA) using prenatal imaging alone remains challenging. We aimed to investigate the efficacy of whole-exome sequencing (WES) in the prenatal molecular genetic diagnosis of skeletal system abnormalities, with or without additional ultrasound anomalies. All fetuses with SKA were subjected to sequential genetic tests, and after excluding fetal chromosomal abnormalities and clinically significant copy number variations (CNVs) consistent with the observed phenotype, the affected fetuses were further subjected to WES. The clinical features of fetal SKA were collected, and the results of molecular genetic testing and perinatal outcomes were analyzed. Following negative routine genetic test results of the 78 fetuses, trio-WES was conducted for 73 fetuses, and fetus-only WES (single WES) was performed for five fetuses due to parental refusal. Fetal skeletal system abnormalities in our cohort were subdivided into seven groups: 39 (50%) had short long bones, 14 (17.9%) had abnormal limb morphology, 4 (5.1%) had polydactyly, 4 (5.1%) had the absence of the radius tibia or tibiofibula, 5 (6.4%) had spine anomalies, 6 (7.7%) had strephenopodia, and 6 (7.7%) had multiple deformities. In total, we identified the molecular diagnoses for 32/78 fetuses with SKAs, and confirmed 41 pathogenic/likely pathogenic variants in 28 genes, including nine novel variants in our cohort. The overall diagnostic rate was 41% (32/78). Our findings demonstrate that WES can greatly improve the genetic diagnostic rate of fetal SKAs following routine genetic testing, which can comprehensively guide perinatal management and help assess the risk of recurrence in future pregnancies. Our data also provide a basis for the association between the SKA phenotype and related genotypes and expand the spectrum of fetal SKA phenotypes and related genes.**

**Keywords** Whole-exome sequencing, Prenatal diagnosis, Skeletal anomaly, *TBX6*, Maternal uniparental disomy of chromosome 6

Skeletal anomaly (SKA) is a common fetal birth defect with an incidence of 1 per 500[1], and is characterized by the abnormal growth and development of bone and cartilage, with variable phenotypic and genetic heterogeneity.

SKA is caused by genetic and non-genetic factors. Non-genetic factors refer to exposure to environmental teratogenic factors, drugs, and maternal autoimmune diseases. Genetic causes include chromosomal aneuploidies,

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copy number variations (CNVs), and single-gene diseases. The 771 genetic skeletal disorders involve 552 bone dysplasia (BD)-related genes[2].

Currently, the prenatal diagnosis of fetal SKA mainly relies on detailed two- dimensional-ultrasound (US) examination[3,4], three-dimensional US, magnetic resonance imaging (MRI), and ultra-low-dose fetal computed tomography[5,6]. Because of the high clinical and genetic heterogeneity of SKA, ultrasonographic findings alone do not provide an accurate prenatal diagnosis of the specific type of BD, making comprehensive prenatal assessment of fetal prognosis and genetic counseling challenging[7]. Additionally, perinatal lethal BD is a major concern and worthy of attention. Thus, molecular genetic testing for fetal SKA may contribute to the accuracy of the differential diagnosis of various types of fetal BD.

When karyotyping and CNV results are negative, next-generation sequencing, particularly whole-exome sequencing (WES), has been increasingly applied in the prenatal genetic diagnosis of fetal SKAs[8–11]. Diagnostic yields of 64% (35/55) and 15% (10/65) and a pooled yield of 69.0% have been reported using WES in large sample sizes in prenatal settings[8,12,13].

However, reports on the prenatal molecular diagnosis of SKAs via WES remain limited, and the sample sizes of most studies are not sufficiently large. We collected 78 fetuses with SKA and with normal karyotypes and/or non-diagnostic CNV results related to the phenotypes, we performed WES and summarized the ultrasonographic phenotypes and molecular genetic testing results of the fetal SKAs to explore the correlation between fetal SKA phenotypes and causative genotypes. These findings will help comprehensively guide pregnancy management and accurately evaluate the risk of recurrence in future pregnancies.

## Materials and methods

### Ethics statement

The study complied with the principles set forth in the Declaration of Helsinki. It was approved by the Ethics Committee of the Fujian Maternity and Child Health Hospital (No.2021KLD09049). Written informed consent was obtained from each patient or from guardians.

### Subjects

A total of 78 fetuses with SKAs, with or without additional ultrasound anomalies (UAs), examined via ultrasound were recruited from December 2019 to December 2022 at Fujian Maternity and Child Health Hospital. Fetuses were excluded if they had \ a known infection or exposure to a known teratogenic drug, or a chromosomal abnormality and/or clinically significant CNV results related to the observed phenotype. Informed consent was obtained from all pregnant couples, and fetal samples were collected via invasive diagnostic procedures at different weeks of gestation. Peripheral blood was collected from the parents.

### Isolation of genomic DNA

Fetal samples, 15 mg of chorionic villi, 30–40 mL of amniotic fluid, or 2–5 mL of umbilical cord blood was obtained, and genomic DNA from the fetus and its parents were extracted using the QIAamp® DNA Blood Mini Kit (Qiagen Inc., Hilden, Germany) following the manufacturer's instructions. Maternal cell contamination was ruled out using a multiplex quantitative fluorescent polymerase chain reaction kit (Darui, Guangzhou, China), which tested 20 markers, including four short tandem repeats (STRs) from chromosome 13 (D13S634, D13S305, D13S628, and D13S742), four from chromosome 18 (D18S391, D18S1002, D18S535, and D18S386), six from chromosome 21 (D21S1411, D21S1445, D21S1414, D21S1412, D21S1433, and 21q11.2), and six from chromosome X and Y (AMXY, DXS1187, DXS8377, SRY, DXS6809, and DXS981).

### WES and bioinformatics analysis.

After excluding fetal chromosomal abnormalities and clinically significant CNVs related to the phenotype, potential recessive pathogenic variants were detected using WES, after obtaining parental consent. Seventy-three families underwent (fetus-mother-father) trio-WES, and five families performed fetus-only WES due to the parents' refusal. Exon capture was conducted using the Sure Select$^{XT}$ Human All Exon V6 kit (Agilent, Santa Clara, CA, USA), and samples were fragmented randomly, purified, and enriched to construct DNA libraries. Paired-end (150 bp × 2) sequencing was performed on a NovaSeq 6000 instrument (Illumina, San Diego, CA USA) according to the manufacturer's instructions.

For sequence alignment, variant calling, and annotation, the sequences were mapped to their location with the human genome reference sequence (hg19 build) using Burrows-Wheeler software (version 0.59)[14]. All SNVs and insertion/deletions (indels) were annotated using publicly available public population frequency databases, including NCBI dbSNP, 1000 Genomes Project, and the Exome Aggregation Consortium, as well as OMIM, Swiss-var, the Human Gene Mutation Database, ClinVar, and other disease databases. To identify possible effects on protein function, variants were evaluated using the Sorting Intolerant from Tolerant (SIFT) algorithm[15] and Polymorphism Phenotyping version 2 (PolyPhen-2)[16], and only variants that were clinically relevant or potentially relevant to the fetuses' phenotypes were reported. The annotation of mutations, prediction of protein function effects, and shear harmoniousness were conducted and the pathogenicity of the variants was assessed according to the American College of Medical Genetics and Genomics guidleines[17]. Sequencing data were reanalyzed 1 year later if necessary.

### Sanger sequencing

The clinically significant candidate variants were confirmed in our center using Sanger sequencing. The PCR primer sequences and protocols are available upon request. Amplified fragments were sequenced using a 96-capillary 3730xl system (Applied Biosystems, Waltham, MA, USA).

### Pregnancy outcome

For fetuses with SKAs, we collected basic information, imaging findings, routine diagnostic testing results of invasive diagnostic procedures, molecular genetic testing results, perinatal outcomes, and follow-up information. Perinatal outcomes were obtained from the delivery records at our hospital and/or via telephone calls.

### Statistical analysis

SPSS software version 22.0 (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Measurement data are expressed as mean ± standard deviation, statistical comparisons were performed using a chi-square test, and $p < 0.05$ was considered statistically significant.

## Results

### Patient characteristics

After excluding fetal chromosomal abnormalities and/or clinically significant (related to the fetal phenotypes) CNVs, we recruited 78 fetuses with SKAs examined via ultrasound, who underwent molecular genetic testing by WES. The demographic characteristics of the 78 fetuses with SKAs, with or without additional UAs, are shown in Table 1.

The number of weeks of gestation at the time of invasive prenatal diagnosis and maternal age for pregnancies with fetal SKAs were 21.2 ± 4.5 weeks and 33.0 ± 2.3 years, respectively. The clinically significant variants detected by WES, ultrasonographic findings, and the perinatal outcomes of 41 fetuses (Cases 1–41) with SKAs are summarized in Table 2, and the variables of unknown significance (VOUS) or incidental CNV results of four fetuses (Cases 42–45) with negative WES results are listed in Table 3.

### Clinical features

Seventy-eight fetuses with SKAs were divided into the following seven subgroups according to the main clinical features examined via ultrasound: 39 (50%) had short long bones, 14 (17.9%) exhibited abnormal limb morphology, 4 (5.1%) had polydactyly, 4 (5.1%) had the absence of a radius tibia or tibiofibula, 5 (6.4%) had spine anomalies, 6 (7.7%) had strephenopodia, and 6 (7.7%) had multiple deformities. Two families had one or more adverse pregnancy histories of fetal SKA. All fetal imaging findings with positive genetic testing results are shown in Table 2.

### WES results

WES was conducted in 78 fetuses with SKAs. Thirty-two cases with pathogenic/likely pathogenic LP(P/LP) variants associated with fetal phenotypes were observed, yielding a diagnostic rate of 41.0% (32/78) (Table 1). Nine fetuses carried VOUS related to fetal SKAs. The genetic cause was successfully determined for both families (Cases 1 and 31) with an adverse pregnancy history (G2 had similar findings to G1) was successfully detected. In addition, two cases with incidental findings were identified. In Case 26, an inherited paternal LP missense variant, c.427 C > T in *MYH7*, was identified, in addition to the diagnostic variant in *GL3*. In Case 46, the fetus presented with omphalocele and a missing left limb at 13 weeks of gestation; No diagnostic variant was detected, except for an inherited maternally pathogenic missense variant, c.725G > A in *SDHB*, which is associated with hereditary paraganglioma-pheochromocytoma syndromes.

In total, we identified the molecular diagnoses for 32/78 fetuses with SKAs, and confirmed 41 P/LP variants in 28 different genes, including nine novel variants, thus extending the spectrum of gene variants related to fetal SKAs. Of the 32 cases with diagnostic results identified by WES, 21 cases showed autosomal dominant (AD) inheritance (9 cases had variants inherited from one of the parents, 11 cases had de novo variants, and 1 case refused verification of the origin of the variant), 10 cases had compound heterozygotes or homozygotes variants presenting autosomal recessive (AR) inheritance, and 1 case showed X-linked recessive (XLR) inheritance. De novo variants were detected in 13 cases (40.6%, 13/32), of which 12 fetuses (37.5%, 12/32) had AD inheritance and 1 fetus had XLR inheritance. Familial co-segregation analysis of Case 18, which showed AD inheritance, was

| **Variant *n* (%)** | |
|---|---|
| Maternal age (mean ± SD) | (33.0 ± 2.3) |
| Gestation weeks at invasive PD (mean ± SD)<br>**WES type** | (21.2 ± 4.5)<br>n (%) |
| Trio-WES | 73 (93.6%) |
| Fetus only<br>**Specimens**<br>Chorionic villus<br>Amniotic fluid<br>Cord blood | 5 (6.4%)<br><br>2 (2.6%)<br>72(92.3%)<br>4(5.1%) |
| **Pregnancy outcome**<br>CTP<br>TOP | <br>25(31.6)<br>53(67.9) |
| Total molecular detection rate via WES | 32/78(41.0%) |

**Table 1**. Demographic characteristics of 78 fetuses with skeletal anomalies with or without additional ultrasound anomalies. CTP continuation of pregnancy; PD prenatal diagnosis; SD standard deviation; TOP termination of pregnancy.

| Case ID | Fetal skeletal anomaly with or without other UAs | Other indications for PD | Gene/RefSeq Mutation site/type | Origin | ACMG Classification | Inheritance pattern | Associated Disorder | Pregnancy Outcome |
|---|---|---|---|---|---|---|---|---|
| P/LP variants as diagnostic results | | | | | | | | |
| 1 | Fetal cleft palate, micrognathia, femur length and humerus length were less than the corresponding gestational weeks, pyelectasis, and hydramnios in the current pregnancy. | History of adverse pregnancy with PRS (the first fetus had cleft palate, micrognathia, the newborn presented falling back tongue, difficult feeding, causing pneumonia and dyspnea, and died a few days after birth) | *BMP2* (NM_001200) c.79delG p.E27Sfs*24 Frameshift | mat | LP (PVS1, PM2) | AD | SSFSC | TOP |
| 2 | Fetal short long bones, microcephaly, FGR | | *ASPM* (NM_018136.4) c.8214dupT p.Q2739fs Frameshift insertion<br>c.9541 C > T p.R3181X Stopgain | Pat<br>Mat | P(PVS1 + PM2 + PM3)<br>P(PVS1 + PM1 + PM2 + PM3) | AR | Primary Microcephaly 5 | TOP |
| 3§ | Fetal short long bones | | *SATB2* (NM_015265) c.1934T > A p.I645N Missense | Dn | LP(PS2, PM1, PM2, PP3) | AD | Glass syndrome | TB<br>The infant presents Intellectual disability, developmental delay at 1 year old. |
| 4 | Fetal short long bones, | The height of pregnant women is 122.6 cm(short stature) | *FGFR3* (NM_000142) c.1138G > A p.G380R Missense | Mat | P(PS2_VeryStrong, PS3, PM2_Supporting, PP3) | AD | Achondroplasia | TOP |
| 5§ | Fetal pes varus, thickened nuchal fold (6.6 mm), cystic hygroma, left ventriculomegaly, arrhythmia | Fetal SNP array: arr[GRCh37]15q11.2(22561772_23469867)x1 | *PEX1* (NM_000466) c.799_800del p.L267Nfs*2 Frameshift<br>c.782_783del p.Q261Rfs*8 Frameshift | Mat<br>Pat | LP(PVS1, PM2)<br>P(PVS1, PM2, PP5) | AR | Peroxisome biogenesis disorder 1 A/1B | TOP |
| 6 | Fetal limb undergrowth | | *FGFR3* (NM_000142) c.1138G > A p.G380R Missense | Dn | P(PS2, PS3, PM2, PP3, PP5) | AD | Achondroplasia | TOP |
| 7 | Fetal one femur is slightly curved and less long than the opposite and gestational month | | *ALPL* (NM_000478) c.979T > C p.F327L Missense<br>c.1166dupC p.T389fs Frameshift | Pat<br>Mat | P(PS3, PM1, PM2, PP3, PP5)<br>P(PVS1, PM1, PM2, PP5) | AR | Hypophosphatasia | TOP |
| 8§ | Ferl microcephaly | | *ZMYND11* (NM_006624) c.1718 A > C p.H573P Missense | Dn | LP (PS2 + PM1 + PM2 + PP3) | AD | Intellectual developmental disorder-30 with speech delay and behavioral abnormalities (MRD30) | TB<br>GDD, hypertrichosis, hypotonia after birth |
| 9§ | Fetal pes varus | | *PURA* (NM_005859) c.481G > T p.E161X Nonsense | Dn | P(PVS1, PS2, PM2_Supporting) | AD | NEDRIHF | TB<br>The infant presented hypotonia,<br>GDD after birth |
| 10 | Fetal long bones of the limbs were short and curved, clinical suspicion of achondroplasia, abnormal morphology of the fetal skull, enhanced intestinal echo (degree II), localized intestinal dilation, and no calcification of the placenta. The ultrasound at 25 weeks of gestation showed that the fetal BPD HC FL HL was smaller than the normal measurement value, small aortic diameter, right accessory renal artery, local enhanced intestinal echo, and oligohydramnios | | *FAM111A* (NM_001312909) c.1020_1022del p.S343del non-frameshift deletion | Dn | P(PS2_VeryStrong, PM4, PM2_Supporting) | AD | Kenny-Caffey syndrome, type 2 | TOP |
| Continued | | | | | | | | |

| Case ID | Fetal skeletal anomaly with or without other UAs | Other indications for PD | Gene/RefSeq Mutation site/type | Origin | ACMG Classification | Inheritance pattern | Associated Disorder | Pregnancy Outcome |
|---|---|---|---|---|---|---|---|---|
| 11 | Fetal bipedal hexadactylism. | The pregnant women with mild to moderate mental retardation, slurred speech, special features, nose bridge, low, wide nose, nostril ectropion, multiple nevus, the first knuckle of both hands retraction position, bipedal hexadactus deformity, specific facial appearance | *PTCH1* (NM_000264.5) c.452_464delinsTCTGTA p.A151Vfs*6 Frameshift | Mat | LP(PVS1 + PM2 ) | AD | BCNS | TOP |
| 12 | Fetal deformity of righthand finger, microcephaly | | *CASK* (NM_003688.3) c.1609 C > T p.Arg537* Frameshift | Dn | P(PVS1 + PM2 + PM6_Support + PS4-Support) | XLR | MICPCH | TB<br>Abnormal joint movement, jaundice, recurrent infection after birth, neonatal asphyxia. CNV-seq after birth: 46,XY, dup(1q41).seq[GRCh37/hg19] (220,079,872 – 220,415,340)×3 VOUS |
| 13 | Fetal congenital spinal deformity (suspicion of scoliosis) and atrial septal defect. | The father of the fetus also has short stature (163 cm), C3, L2 hemivertebra, deformity, T5, 9 butterfly vertebra, S1 level spina bifida with different degrees of scoliosis and C4-5, 6–7 disc herniation. | *TBX6* (NM_004608) c.1121_1122del p.P374Rfs*112 Frameshift<br>T-C-A haplotype<br>rs2289292(C > T)<br>rs3809624(T > C)<br>rs3809627(C > A) | Pat<br>Mat | LP(PVS1, PM2_Supporting) | similar to AR | SCDO5 | TB |
| 14 | Fetal thickened nuchal skin fold, pes varus, right pleural effusion, the spinal conus is located at the level of the upper margin of L4 | | *SNAP25* (NM_130811) c.593G > C p.R198P Missense | Dn | P(PS2_VeryStrong, PM1, PM2_Supporting) | AD | Myasthenic syndrome, congenital, 18 | TOP |
| 15 | Fetal bipedal cleft deformity, the third and fourth upper fingers of both hands may be syndactyly, the left hand with cleft hand deformity, tricuspid valve regurgitation, intra-cardiac echogenic foci, bilateral pyelectasis | | *TP63* (NM_003722.5) c.728G > A p.R243Q Missense | Mat | P(PS2 + PS3_P + PS4_M + PM1 + PM2 + PM5 + PP1 + PP3) | AD | SHFM4 | TOP |
| 16 | Fetal bilateral talipes equinovarus, FGR, subependymal cysts, cerebellar hypoplasia, bilateral ventriculomegaly | | *RARS2* (NM_020320) c.474_477del p.E159Lfs*2 Frameshift<br>c.1564G > A p.V522I (het) Missense | Mat<br>Pat | P(PVS1, PP1, PM2_Supporting)<br>LP((PM3_Strong, PP1,PM2_Supporting)) | AR | Pontocerebellar hypoplasia, type 6 | TOP |

Continued

| Case ID | Fetal skeletal anomaly with or without other UAs | Other indications for PD | Gene/RefSeq Mutation site/type | Origin | ACMG Classification | Inheritance pattern | Associated Disorder | Pregnancy Outcome |
|---|---|---|---|---|---|---|---|---|
| 17 | Fetal short long bones | | *KIF1A*(NM_001244008.2)<br>c.760 C>T p.R254W Missense | Dn | P(PS2_VeryStrong, PS4_Moderate, PM1, PM2_Supporting, PP3) | AD | NESCAV syndrome | TB<br>She presents accelerated skeletal maturation, talipes, intellectual disability, strabismus, astigmatism, cerebral palsy |
| 18 | Fetal short long bones | The pregnant woman presents enlargement of right wrists and ankles, large cafe-au-lait spot on the right waist, scattered subcutaneous nodules were found throughout the body | *NF1* (NM_001042492.3)<br>c.4331 A>G p.K1444R Missense | - | P(PS3, PS4_Moderate, PM1, PM2_Supporting, PP1) | AD | Neurofibromatosis, type 1 | TB |
| 19§ | Fetal fixed head retroflexion, fixed limbs, abnormal posturing, bilateral rocker-bottom foot, overlapping fingers in both hands, hydrops fetalis, a small amount of fluid in the right thoracic cavity, dilation of left lateral ventricles, cerebellar hypoplasia, and bilateral pyelectasis | | *COG5* (NM_006348)<br>c.463_467delinsCT p.S155_K156delinsL<br>Non-frameshift deletion<br>7q22.3(106882548_107037865) x1 deletion | Mat<br>Pat | LP(PM1, PM2, PM4)<br>LP(PVS1, PM2) | AR | Congenital disorder of glycosylation, type IIi | TOP |
| 20 | Fetal bilateral rocker-bottom foot, abnormally fixed finger posturing on both sides, thickened NF, right pyelectasis | | *KLHL40* (NM_152393.4)<br>c.602G>A p.W201* Nonsense<br>c.1516 A>C p.T506P Missense | Mat<br>Pat | P(PVS1+PM2+PM3)<br>LP(PM2+PM3_VS+PP1_M) | AR | NEM 8 | TOP |
| 21 | Fetal FL<-4.8SD, HL<-5.5SD at 31 weeks of gestation | | *OBSL1* (NM_015311.3)<br>c.458dupG p.L154Pfs*100 Frameshift<br>c.458dupG p.L154Pfs*100 Frameshift | Mat<br>Pat | P(PVS1, PM3_Strong, PM2_Supporting);<br>P(PVS1, PM3_Strong, PM2_Supporting) | AR | 3-M syndrome 2 | TOP |
| 22 | Fetal spinal conus is located at the lower margin level of L3, situs inversus totalis, dextrocardia. | Fetal SNP array:<br>arr[GRCh37] 4q21.21(79041013_79537221) x1 VOUS | *LRRC56* (NM_198075.4)<br>c.760G>T p.E254* Nonsense<br>c.1053dupC p.E352Rfs* Frameshift | Pat<br>Mat | P(PVS1, PM3, PM2_Supporting)<br>P(PVS1, PM3,PM2_Supporting) | AR | PCD 39 | TOP |
| 23§ | Fetal short long bones (<-5SD), bowing of the legs, genu varum. | The father of the fetus presents short stature (150 cm), bowing of the legs, genu varum, overweight (80 Kg) | *COMP* (NM_000095.3)<br>c.1127 A>C p.D376A Missense | Pat | LP(PM1, PM5, PM2_Supporting, PP3) | AD | Carpal tunnel syndrome 2; Epiphyseal dysplasia, multiple, 1; Pseudoachondroplasia | TB<br>He is significantly shorter in stature than that of the same age child |
| 24 | Fetal BPD was smaller than that of the corresponding gestation weeks, left ventriculomegaly. | MRI at three months after birth showed right choroid cyst, left ventriculomegaly, and part of the extracerebral space was slightly wider.<br>Fetal SNP array:<br>arr[GRCh37]14q13.1q24.2(34435418_72618432) x2 hmz VOUS | *COMP* (NM_000095)<br>c.1417_1419dup p.D473dup Non-frameshift insertion | Dn | P(PS2, PS4_Moderate, PM4, PP1, PM2_Supporting) | AD | Carpal tunnel syndrome 2; Epiphyseal dysplasia, multiple, 1; Pseudoachondroplasia | TB<br>5 months after birth, she cannot chase sight, chase objects, hypertonia, adducted thumb, poor head control. |

Continued

| Case ID | Fetal skeletal anomaly with or without other UAs | Other indications for PD | Gene/RefSeq Mutation site/type | Origin | ACMG Classification | Inheritance pattern | Associated Disorder | Pregnancy Outcome |
|---|---|---|---|---|---|---|---|---|
| 25 | Fetal cranial overlap, intervertebral space narrowing, vertebral fusion in cervical and coccygeal vertebra, ulna length <-4.7SD, flexion of both upper limbs, fixed posture, and talipes equinovarus in both feet (bone dysplasia) | Fetal SNP array: arr[GRCh37] 1p36.12p36.11(21908401_25676836)x3 VOUS | *FGFR2* (NM_000141.5) c.870G > T p.W290C Missense | Dn | P(PS2_VeryStrong, PS1, PS4_Moderate, PM1, PM2_ Supporting, PP3) | AD | Antley-Bixler syndrome without genital anomalies or disordered steroidogenesis | TOP |
| 26§ | Fetal postaxial polydactyly of both hands and feet, bilateral ventriculomegaly, bilateral choroid plexus cyst, flat face | | *GLI3* (NM_000168.6) c.4225_4229dupCTCAG p.D1411Sfs*10 Frameshift | Dn | P(PVS1_Strong, PS2, PM2_Supporting) | AD | Polydactyly, postaxial, types A1and B | TOP |
| 27 | Fetal FL <-3.4SD, HL<-2.9SD, bipedal varus, abnormal posturing of both hands, mild tricuspid regurgitation, echogenic intracardiac focus | | *B3GALT6* (NM_080605.4) c.694 C > T p.R232C Missense c.588dupG p.R197Afs*246 Frameshift | Mat Pat | LP(PM3_Strong, PS3_Supporting, PM2_Supporting) LP(PVS1_Strong, PM3, PM2_Supporting) | AR | Al-Gazali syndrome | TOP |
| 28 | Extremely short long bones and curved at angles | The pregnant woman presents short stature, recurrent fractures, blue sclerae, pes planus, and the father of the pregnant woman has a similar phenotype. | *COL1A1* (NM_000088) c.543 + 5G > A Splice-site variation | Mat | LP(PS3, PS4_Supporting, PM2_Supporting, PP3, PP4) | AD | OI, type I | TOP |
| 29 | Fetal left femur is slightly curved, FL<-2SD in the current pregnancy (G2). History of adverse pregnancy with suspected osteogenesis imperfecta in G1. | The father of the fetus has the history of recurrent fractures when he is young, and both legs are easy to fracture (when he takes off the pants and the legs are often fractured), and the symptoms become lighter at about 20 years old. | *COL1A1* (NM_000088.4) c.1615-1G > T Splice-site variation | Pat | P(PVS1, PS4_Supporting, PM2_Supporting) | AD | OI, type I | TOP |
| 30 | Fetal FL< -3SD, HL< -3SD, bilateral femur curvature, angulation, clinical suspicion of fetal bone dysplasia, ARSA, echogenic intracardiac focus | | *COL1A2* (NM_000089.4) c.2845G > A p.G949S Missense | Dn | P(PS2_VeryStrong, PS4_Moderate, PM1, PM2_Supporting, PP3) | AD | OI, type II/III/IV | TOP |
| 31§ | Fetal short long bone, cleft palate in the current pregnancy. | History of adverse pregnancy with clinical suspicion of PRS (after surgical treatment, the development of the son is basically normal at 1.5 years old). The father of the fetus presented retinal detachment and high myopia. | *COL2A1* (NM_001844) c.2484delG p.G831Dfs*50 Frameshift | Pat | LP (PVS1, PM2_Supporting) | AD | Stickler syndrome I | TB Glossoptosis, feeding difficulties after birth, suspective of PRS |
| 32§ | Fetal short long bones. | The pregnant woman presents short stature, clinical suspicion of pituitary dysplasia | *COL2A1* (NM_001844) c.1808G > A p.G603D Missense | Mat | LP (PS4_P + PM1 + PM2 + PP3) | AD | Spondylometepiphyseal dysplasia congenita, Strudwick type, SEMD | TOP |
| VOUS variants related to fetal findings | | | | | | | | |
| Continued | | | | | | | | |

| Case ID | Fetal skeletal anomaly with or without other UAs | Other indications for PD | Gene/RefSeq Mutation site/type | Origin | ACMG Classification | Inheritance pattern | Associated Disorder | Pregnancy Outcome |
|---|---|---|---|---|---|---|---|---|
| 33 | Fetal BPD − 2.1SD, HC -2.9SD, AC -3.1SD, FL -2.0SD, HL -2.5SD, oligohydramnios, intra-cardiac echogenic foci. | Fetal SNP array: arr[GRCh37] 6p25.3p23(203,878 − 13,411,320) x2 hmz, 6p21.1p11.1(41,305,454 − 58,726,706) x2 hmz, 6q11.1q14.1(61,972,918 − 75,972,465) x2 hmz, 6q22.31q25.1(123,041,062–149,830,858) x2 hmz VOUS | UPD(6)mat | Mat | VOUS | | | Preterm birth at 34 weeks. Normal development at 1.2 years old |
| 34 | Fetal polydactyly/toe deformity, multiple cysts in the fetal abdominal cavity, no amniotic fluid. | | *DYNC2H1* (NM_001377) c.2225T > G p.M742R Missense c.4918T > C p.C1640R Missense | Mat Pat | VOUS (PM2, PM3, PP5) VOUS (PM2) | AR | Short-rib thoracic dysplasia 3 with or without polydactyly | TOP |
| 35 | Feral slender long bones, macrocephaly, polyhydramnios | | *OFD1* (NM_003611) c.1621G > T p.D541Y | Hem | VOUS (PM2, PP3) | XLR | Simpson-Golabi-Behmel syndrome, type 2 | TOP |
| 36 | Fetal short fetal FL and HL | | *NPR2* (NM_003995) c.664 C > T p.R222C c.2720 C > T p.T907M | Pat Mat | VOUS (PM1, PM2, PP3) VOUS (PM1, PM2, PP3) | AR | Acromesomelic dysplasia, Maroteaux type | TOP |
| 37 | Fetal short long bones | | *TRPV4*(NM_021625) c.695G > A p.R232H | Mat | VOUS (PM1, PM2_Supporting, PP3) | AD | Spondylometaphyseal dysplasia, Kozlowski type | TB The infant presented congenital laryngeal chondromalacia, laryngeal obstruction of 2 degrees, causing bronchopneumonia, growth retardation, malnutrition, right pneumothorax, spinal muscular atrophy, single transverse palmar crease, speech retardation, and a different smile from normal children |
| 38 | Fetal talipes equinovarus of both feet, distal arthrogryposis in both hands. | The father has the same abnormal phenotypes. Fetal SNP array: arr[GRCh37] 2p12(78,657,153 − 79,851,089)×4 pat (VOUS) | *TPM2* (NM_003289) c.298G > T p.D100Y Missense | Pat | VOUS (PM1, PM2, PP3) | AD | Arthrogryposis, distal, type 1 A | TOP |

Continued

| Case ID | Fetal skeletal anomaly with or without other UAs | Other indications for PD | Gene/RefSeq Mutation site/type | Origin | ACMG Classification | Inheritance pattern | Associated Disorder | Pregnancy Outcome |
|---|---|---|---|---|---|---|---|---|
| 39 | Fetal small FL/BPD and FL/HC ratios, and short humerus and femur, suspected noncompaction cardiomyopathy, the left ventricular wall is asymmetrical thickening, and mild tricuspid regurgitation | | *ACTN2*(NM_001103.4) c.2428dupA p.T810Nfs*11 Frameshift | Mat | VOUS (PVS1_Strong, PM2_Supporting)) | AD | Myopathy, congenital with structured cores and Z-line abnormalities; Cardiomyopathy, dilated, 1AA, with or without LVNC; Cardiomyopathy, hypertrophic, 23, with or without LVNC; Myopathy, distal, 6, adult onset | TOP |
| 40 | Fetal limb undergrowth, increased NT (7.8 mm), hydrops fetalis, omphalocele, mild tricuspid regurgitation | | *COL2A1* (NM_001844) c.3203G > A p.G1068E Missense | Dn | VOUS | AD | Achondrogenesis, type II | TOP |
| 41 | Fetal short long bones and curved at angles | The pregnant women have osteoporosis, easy fracture, blue sclera, clinical suspicion of incomplete dentinogenesis, OI; short stature (120 cm), prone to fracture in the femur near the waist | *COL1A1* (NM_000088) c.1436G > C p.G479A Missense | - | VOUS (PM2_Supporting, PP3, PP4) | AD | OI, type I | TOP |

**Table 2**. Clinically significant variants detected by WES in 41 fetuses with skeletal anomalies. AD, autosomal dominance, AR: autosomal recessive; ARSA, aberrant right subclavian artery; BCNS, basal cell nevus syndrome; BPD, biparietal diameter; CNV-seq, copy Number variation sequencing; Dn, de novo; FGR, fetal growth restriction; FL, femur length; GDD, global developmental delay; HL, humerus length; hmz, homozygosity; L, lumbar vertebra; Mat, maternal; MICPCH, Mental retardation and microcephaly with pontine and cerebellar hypoplasia; OI, osteogenesis imperfecta; NEDRIHF, Neurodevelopmental disorder with neonatal respiratory insufficiency, hypotonia, and feeding difficulties; NEM, nemaline myopathy; NT, nuchal translucency; Pat, paternal; PCD, Primary Ciliary dyskinesia; PD, prenatal diagnosis; PRS, Pierre-Robin sequence; T, thoracic vertebra; S, sacral vertebrae; SCDO5, spondylocostal dysostosis 5; SD, standard deviation; SEMD, spondylometepiphyseal dysplasia congenita, Strudwick type; SSFSC, Short stature, facial dysmorphism, and skeletal anomalies with or without cardiac anomalies; SHFM4, split-hand/foot malformation 4; SNP array; single nucleotide polymorphism array; TB, term birth; T-C-A haplotype: rs2289292(C > T) & rs3809624(T > C) & rs3809627(C > A); TOP, termination of pregnancy; UA, ultrasound anomaly; VOUS, variant of uncertain significance; XLR: X-linked recessive. § novel variants.

| Case ID | Indications for prenatal diagnosis | CNV result/Pathogenicity | Inheritance | Pregnancy outcome |
|---|---|---|---|---|
| 42 | Fetal abnormal development of fetal hands (clinical suspicion of spider fingers) | seq[GRCh37]7q36.1(151818701_152007230)x)VOUS | Mat | TOP |
| 43* | Fetal gastric vesicles and bladder were not observed, hydramnios, absence of both kidneys, bipedal varus | seq[GRCh37]16p11.2(29449426_30320316)x1 P | Dn | TOP |
| 44 | Fetal flexion contracture, ventriculomegaly and FGR | seq[GRCh37]16p13.11(14947627_16301303)x3 VOUS 16p13.11 recurrent microduplication (neurocognitive disorder susceptibility locus) | Mat | Preterm birth. Erythema after birth, premature closure of fontanelles at five months after birth, GDD, intellectual disability, seizure, absent speech |
| 45 | Absence of fetal nasal bone | arr[GRCh37]Xq28(154101869_154271165)x2 VOUS | Mat | TB<br>Normal development |

**Table 3**. VOUS or incidental CNV results of four fetuses with negative WES results. Dn, de novo; FGR, fetal growth restriction; GDD, global developmental delay; TOP, termination of pregnancy; VOUS, variant of uncertain significance; Mat, maternal.

refused. According to the genotype-phenotype correlation analysis, eight genes involved in short limbs with or without other UAs, and five genes causing the bent BD were identified (Table 2).

There were two cases (Cases 1 and 31) with a history of adverse pregnancy outcomes. One fetus presented with a cleft palate, micrognathia, femur length and humerus length that were less than the expected length, pyelectasis, and hydramnios in the current pregnancy (Table 2, Case 1). An LP variant (c.79delG p.E27Sfs*24) in *BMP2* was identified in this fetus. The same pathogenic variant segregating with the phenotype was found in the mother and an elder brother (neonatal death), both of whom had a cleft palate, short limb, and micrognathia. The second fetus presented with short long bones, and a cleft palate in the ongoing pregnancy (Table 2, Case 31). Almost the same anatomical anomalies had been present in a previous pregnancy of the same parents, and the father had additional retinal detachment and high myopia. An LP variant (c.2484delG p.G831Dfs*50) in *COL2A1* was identified in the ongoing pregnancy. The same variant segregating with the observed manifestations was identified in the father and an elder brother.

In the remaining nine cases with VOUS, in addition to two compound heterogeneous variants in *DYNC2H1* and *NPR2*, we also identified variants in *TRPV4*, *ACTN2*, *TPM2*, *COL2A1*, *COL1A1*, and *OFD1*, and the correlations between the phenotypes and genotypes were consistent or partially consistent. Of note, maternal uniparental disomy (UPD) of chromosome 6 was also detected in Case 33. Due to the lack of evidence for functional verification, these variants were classified as VOUS.

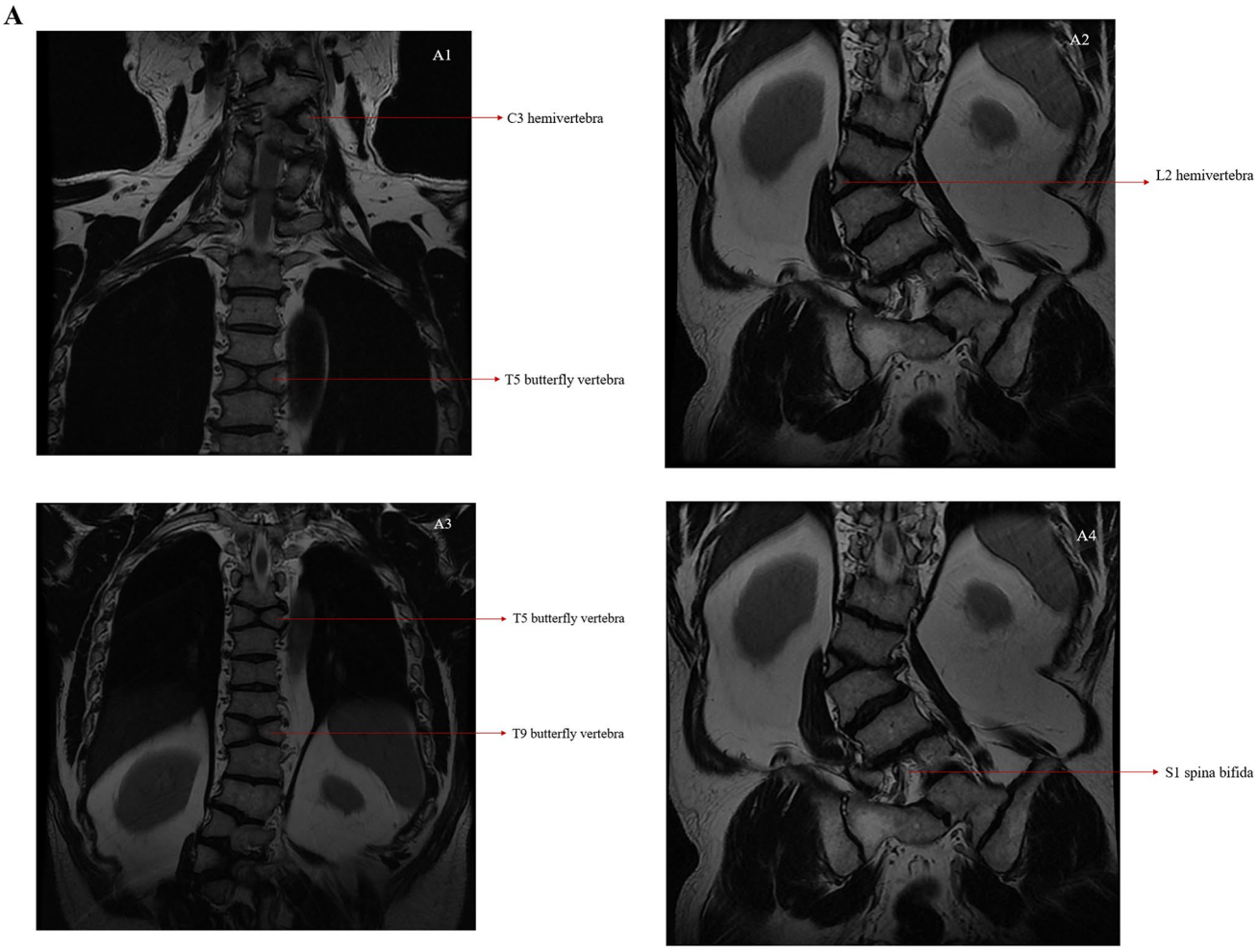
## Discussion

We performed prenatal WES on consecutive fetuses with SKAs that underwent routine genetic testing and increased the diagnostic yield by approximately 41.0% (32/78) following routine genetic testing. This rate is much higher than the previously reported rates of 15% (10/65) and 24% (8/34)[12,18], but lower than the previous result of 64% (35/55)[8] and significantly lower than the results reported in previous studies with small sample sizes ($n < 30$)[19–23]. The difference in the diagnostic rate via WES was closely related to the sample size and specimen selection employed. The size of our cohort was the second largest among the reported studies of fetal SKAs analyzed via WES, and covered a greater number of and more specific phenotypes and genes associated with BD. Thus, compared with other small studies, our diagnostic rate via WES for fetuses with SKAs was relatively objective and reliable. Detailed ultrasound examination is vital for an extended genetic diagnostic strategy. In our study, the most common sonographic features were short long bones ($n = 39$, 50%), followed by abnormal limb morphology ($n = 14$, 17.9%), with genetic diagnosis rates of these two phenotypes being 33.3% (13/39) and 28.6% (4/14), respectively. These were higher than the genetic diagnosis rates of other SKAs. Because the risk of BD was high in our cohort, in cases of fetal short limbs and abnormal limb morphology, with or without additional UAs, molecular genetic testing via WES is considered.

The accurate interpretation of genetic variants is crucial. De novo diagnostic variants were identified in 40.6% (13/32) of our cohort, suggesting that it is especially essential for further prenatal molecular genetic testing after excluding routine genetic abnormalities by karyotyping and microarray analysis when fetal SKAs are examined, Moreover, it should be highlighted that the possibility of parental gonadal mosaicism cannot be ruled out and if present, there may be a recurrence risk of 1.3–9.4%[24,25]; thus, prenatal molecular genetic diagnosis is still advised in future pregnancies.

Nine fetuses with genetically diagnosed results (AD-inherited) inherited the disorder from one of the parents, and the parents of six of these fetuses had similar phenotypes to the fetus, while the other parents (Cases 15) did not have obvious symptoms. This may be related to the incomplete penetrance and high heterogeneity of genetic skeletal disorders.

A history of an undiagnosed fetus (or proband) affected by multiple or recurrent similar structural anomalies yielding a normal karyotype or microarray results in the current pregnancy was highly suggestive of a genetic cause, thus, in cases of a positive family history and/or adverse pregnancy history, WES should be further recommended after routine genetic testing. In Case 1, the pregnant woman had previously experienced adverse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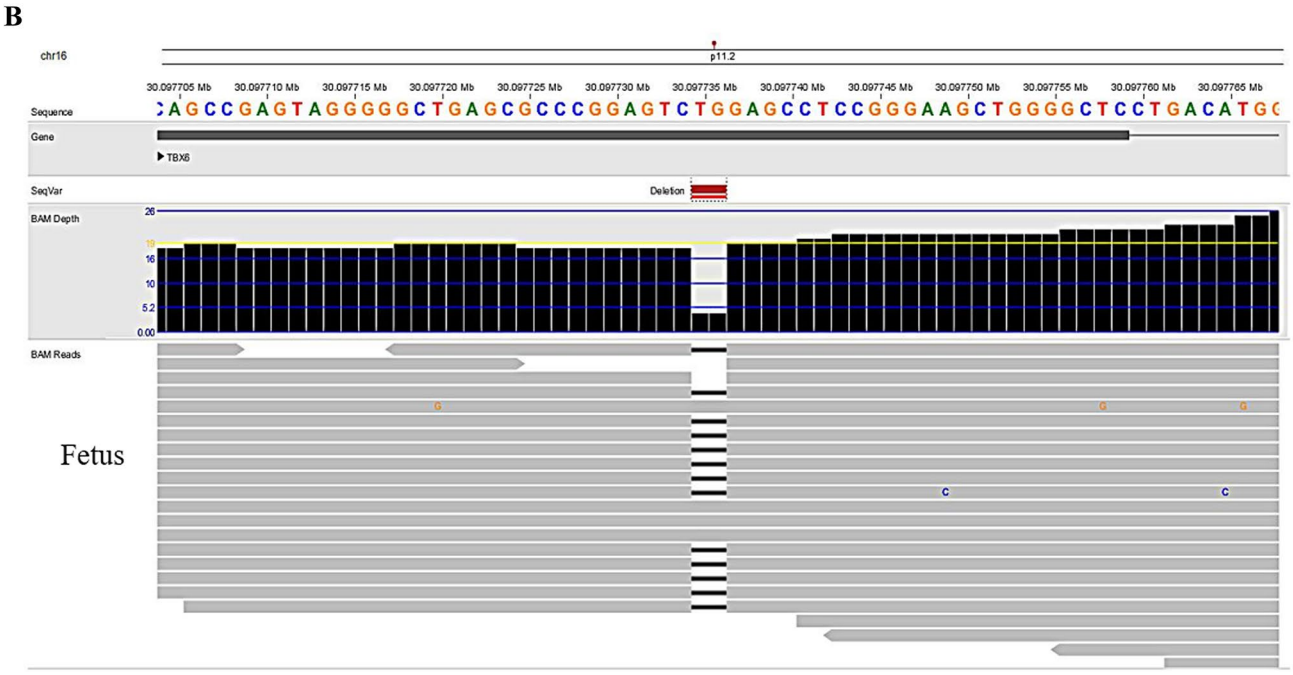
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Sequence
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Deletion
BAM Depth
BAM Reads
Fetus

◀**Fig. 1**. The spine MRI image of the fetus's father and fetus-only WES and familial Sanger sequencing results for Case 13. (**A**). The spine MRI image of the fetus's father; (**B**). Fetus-only WES results for case 13; (**C**). Familial Sanger sequencing revealed that the fetus harbored an inherited paternally frameshift variant, NM_004608: c.1121_1122delCA, in *TBX6*; (**D**). Familial Sanger sequencing results on T-C-A haplotype of（rs2289292(C/T), rs3809624(T/C), and rs3809627(C/A) in *TBX6*. Abbreviation: C: cervical vertebra; L: lumbar vertebra; T: thoracic vertebra; S: sacral vertebrae. (**A**) The spine MRI image of the fetus's father. A1. C3 hemivertebra and T5 butterfly vertebra. A2. L2 hemivertebra. A3. T5 butterfly vertebra and T9 butterfly vertebra. A4. S1 spina bifida. (**B**). Fetus-only WES results for case 13. (**C**). Familial Sanger sequencing revealed that the fetus harbored an inherited paternally frameshift variant, NM_004608: c.1121_1122delCA, in *TBX6*. C1: The fetus; C2 The father; C3: The mother. (**D**). Familial Sanger sequencing results on T-C-A haplotype of (rs2289292(C/T), rs3809624(T/C), and rs3809627(C/A) in *TBX6*. D1: The feus harbored a heterozygous haplotype. D2: The father harbored a heterozygous haplotype. D3: The mother harbored a homozygous haplotype.

outcomes in her first pregnancy, with a fetal cleft palate, micrognathia, and clinical suspicion of Pierre Robin sequence (PRS) in the first pregnancy. Amniocentesis was performed, resulting in unremarkable results for fetal karyotyping and CNV, and the male baby died 2 weeks after birth due to pneumonia and breathing difficulties. An autopsy and further genetic testing were declined. In the current (second) pregnancy, ultrasonographic findings at 19 weeks of gestation revealed fetal micrognathism, a cleft palate, hydramnios, and a dilated left renal pelvis, again indicating suspicion of PRS. Ultrasound findings at $24^{+4}$ weeks of gestation revealed additional UAs (fetal femur length and humerus length were less than expected, pyelectasis, and hydramnios) in addition to the previous observed anomalies. Micrognathia and glossoptosis were confirmed using fetal MRI. Trio-WES revealed a frameshift variant, c.79delG (p. E27Sfs*24) in the *BMP2* gene in the fetus. Sanger sequencing confirmed that the c.79delG gene in *BMP2* was of maternal origin. The pregnant woman exhibited mild symptoms, including a short stature (147 cm), craniofacial anomalies (short nose, anteverted nares, long philtrum, thin upper lip, high palatal arch, and dental crowding), phalangeal abnormalities (only two knuckles in the 5th finger of the right hand), and conductive hearing impairment, but with normal cognition, no cardiac murmur, and a normal electrocardiogram. Based on these findings, a definitive diagnosis of short stature, facial dysmorphism, and skeletal anomalies, with or without cardiac anomaly syndrome (SSFSC, OMIM 235200)[26] was made, and fetal labor was induced.

In Case 13, the fetus presented with a congenital spinal deformity, suspected scoliosis, and an atrial septal defect. The father exhibited short stature (163 cm), and the spine MRI of the fetus's father revealed cervical vertebra 3 (C3) and lumbar vertebra 2 (L2) hemivertebra; thoracic vertebra 5 (T5) and T9 butterfly vertebra; sacral vertebrae 1 (S1)- level spina bifida with different degrees of scoliosis; and C4-5, C6-7 disc herniation. Fetus-only WES revealed an LP frameshift variant, c.1121_1122del p. P374Rfs*112, in *TBX6* associated with spondylocostal dysostosis 5 (SCDO5, OMIM 122600), which is caused by biallelic variation at one locus, that is, a rare loss-of-function variant plus a common suballele in the trans position, similar to the AR mode of inheritance[27]. Because of the low coverage of the untranslated regions and introns of *TBX6* with WES, only a few reads were detected at rs2289292 (C/T:9/2) and rs3809624 (T/C:1/1), and no reads were detected at rs3809627 (Fig. 1.2). Sanger sequencing was performed to determine the origin, revealing that the fetus carried an inherited paternal frameshift variant, c.1121_1122del p. P374Rfs*112, in *TBX6*, as well as an inherited parentally common hypomorphic allele (T-C-A haplotype: rs2289292(C/T), rs3809624(T/C), and rs3809627(C/A)) in *TBX6* (Fig. 1), causing SCDO5[28].

Maternal UPD on chromosome 6 presents a great challenge for genetic counselling prenatally[29]. In Case 33, the fetus exhibited biparietal diameter of -2.1 standard deviation (SD), a head circumference of -2.9SD, an abdominal circumference of -3.1SD, a femur length of -2.0SD, a humerus length of -2.5SD, oligohydramnios, intra-cardiac echogenic foci. Fetal SNP array analysis revealed multiple homozygous segments of a region on chromosome 6. Further parental microarray analysis preliminary revealed maternal segmental iso-UPD of chromosome 6 [UPD (6) mat], and subsequently trio-WES confirmed UPD (6) mat, also known as mixed UPD, including iso-UPD and hetero-UPD (Fig. 2). UPD (6) mat is a rare finding, and its clinical pathogenetic significance is not clear. The clinical manifestations of UPD (6) mat were not obvious[30], but placental dysfunction due to trisomy 6 mosaicism has been reported to cause fetal growth restriction (FGR) as well as preterm birth[30–32]. There has been no reported imprinting effect associated with this segment, and this was confirmed in Case 33.

For fetal SKAs, efficient and accurate prenatal molecular genetic diagnosis is recommended, which may contribute to appropriate genetic counselling, comprehensive pregnancy management, and an understanding of the risk of recurrence in future pregnancies. However, there were still 59.0% (46/78) of cases in our cohort for whom definite diagnostic results were not obtained, including nine fetuses carrying VOUS. For one of these fetuses (Case 41), verification of the parental origin of the VOUS was rejected. Highly suspicious variants were identified in the nine fetuses, but the significance of these genetic variants is uncertain and requires more cases and further functional experiments for verification, which is worthy of attention and provides a certain reference value for similar situations.

Because the correlations between SNVs and indels identified via WES and postnatal phenotypes are very limited, interpreting prenatal WES results, especially VOUS, can be challenging, as the detection of prenatal VOUS may cause considerable stress and anxiety for the affected pregnant couple, which may even lead certain women choosing to terminate the pregnancies. A more detailed and complete fetal clinical evaluation and continuous follow-up would allow for a better assessment of the causal relationship of the variation. With the continuous updating of databases and the rapid development of sequencing technology, pathogenic variations

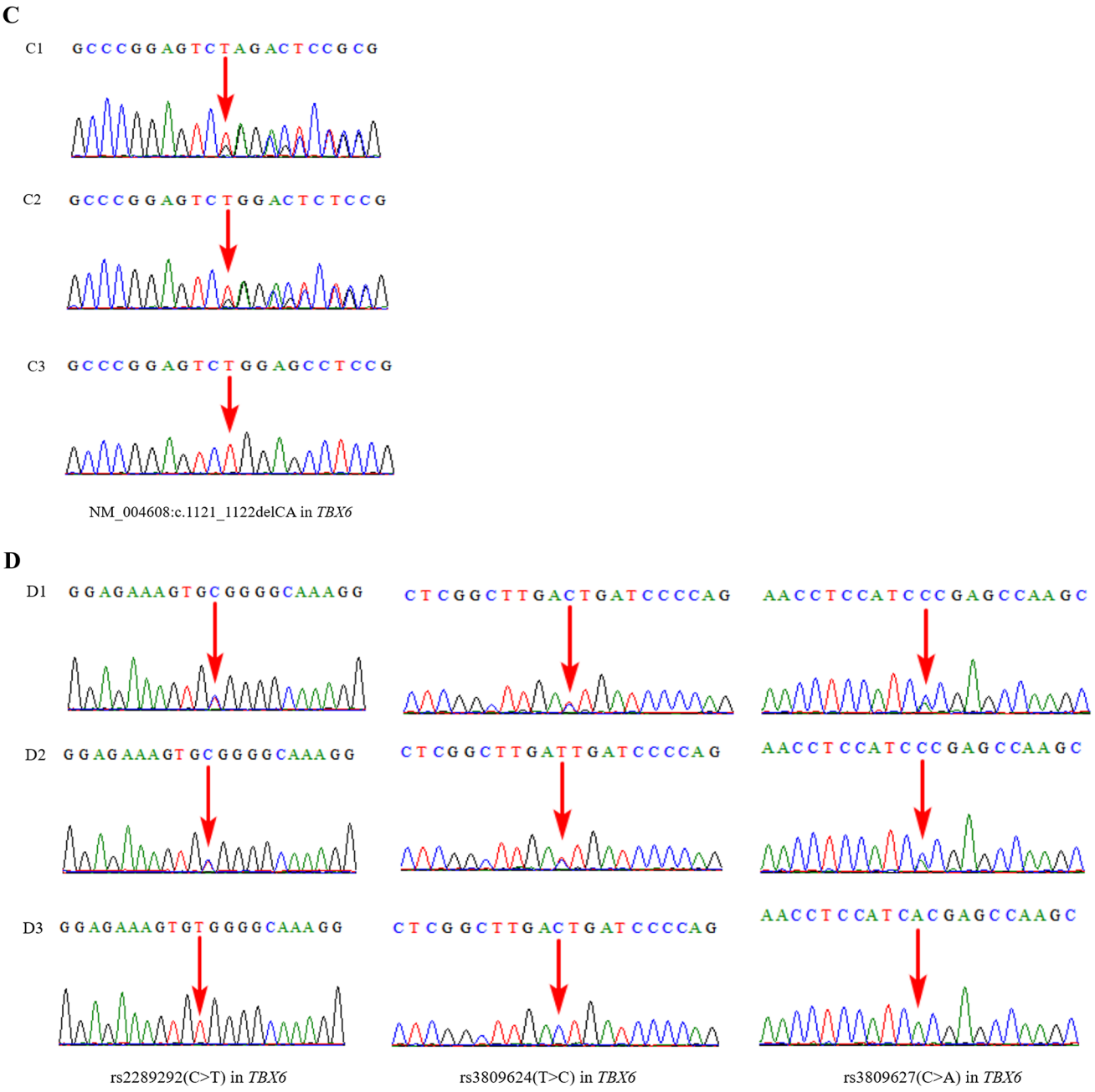


**Figure 1.** (continued)

or new disease-causing genes may be identified via reanalysis of WES, whole genome sequencing, or RNA sequencing data or even via epigenetic studies, which can be a powerful complementary technique when WES results are negative[33]. Further research is essential to explore the etiology of the disorders in these families with negative or VOUS results.

Diagnostic sequence variants were identified in 28 different genes, corresponding to 29 different disorders across 41 fetuses. In 21 cases, the mode of inheritance was AD and in 10 it was AR, and in 1 case it was X-linked recessive. Overall, the detection rate in our study was 41.0% (32/78) and 41 different variants were identified, of which nine were novel variants. Our study further broadened the mutation spectrum of fetal SKAs, and will help improve the management of future pregnancies and genetic counselling of the affected parents. However, more cases of WES for fetuses with SKAs examined via ultrasound should be investigated using WES, to extend the genotype-phenotype correlations and explore comprehensive genetic causes of SKAs. This will provide more reference information and further reveal the clinical efficacy of prenatal WES testing.

Our study has some limitations. First, although it was a prospective single-center study, the sample size was not sufficiently large. Thus, larger population studies are needed, and the follow-up period was not long

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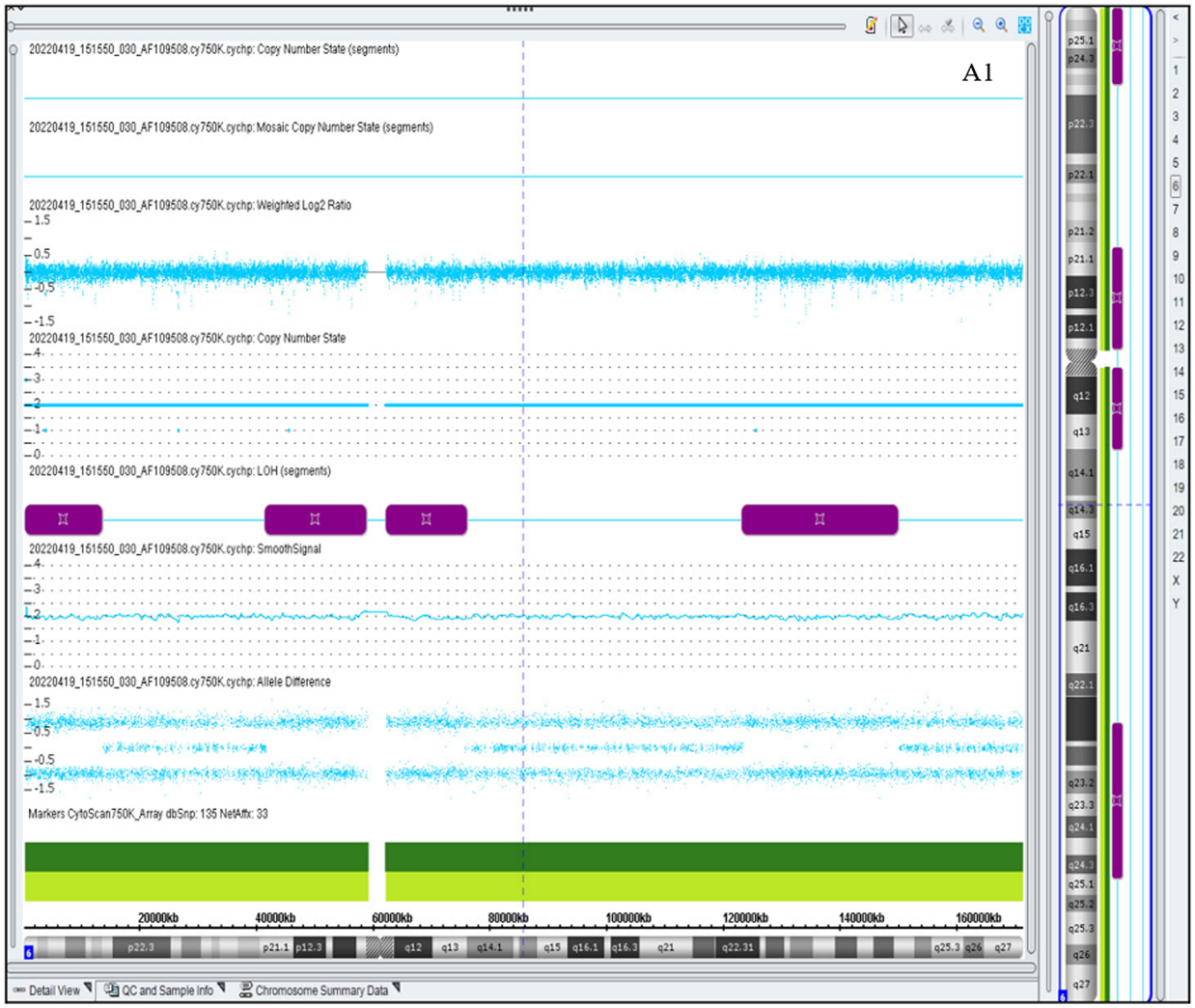
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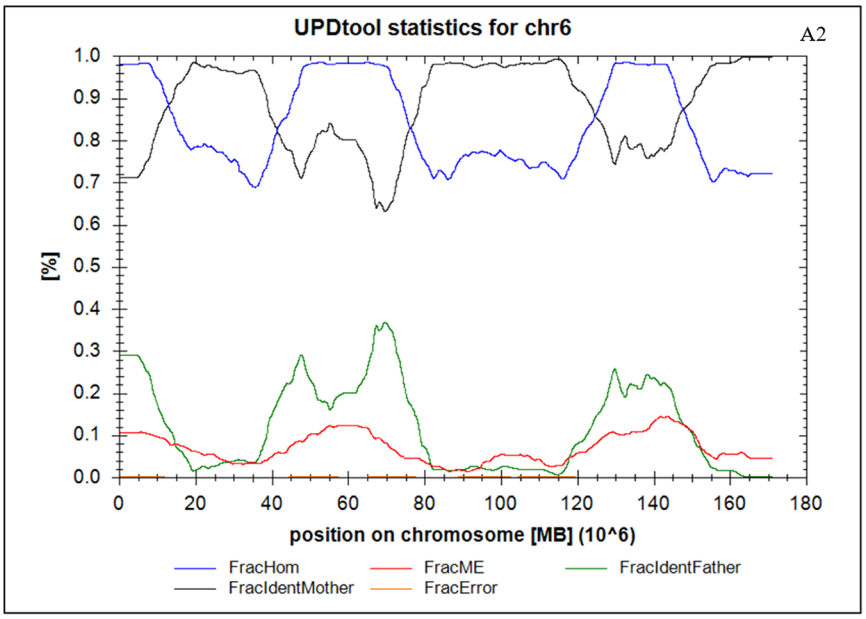
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◀**Fig. 2**. Case 33 was firstly detected with regions of homozygosity (ROHs) and further confirmed as maternal UPD (6) by parental SNP array verification and trio-WES. (**A**). Case 33 was firstly detected with ROHs and further confirmed as maternal UPD (6) by parental SNP array verification; (**B**). Trio-WES result confirmed as maternal UPD 6 (UPD (6) mat). (A). Case 33 was firstly detected with ROHs and further confirmed as maternal UPD (6) by parental SNP array verification. (A1). Detection of a 71.2M segmental ROHs on chromosome 6 using a SNP array. SNP array result: a 71.2 Mb region of homozygosity on chromosome 6 (arr[hg19]6p25.3p23(203,878-13,411,320) x2 hmz,6p21.1p11.1(41,305,454-58,726,706) x2 hmz, 6q11.1q14.1(61,972,918-75,972,465) x2 hmz, 6q22.3 1q25.1(123,041,062-149,830,858) x2 hmz) was detected by SNP array; (A2). Confirmation of maternal UPD (6) using the UPD tool. A1. Detection of a 71.2 Mb segmental ROHs on chromosome 6 using a SNP array. SNP array result: a 71.2 Mb region of homozygosity on chromosome 6 (arr[hg19]6p25.3p23(203,878-13,411,320) x2 hmz,6p21.1p11.1(41,305,454-58,726,706) x2 hmz, 6q11.1q14.1(61,972,918-75,972,465) x2 hmz, 6q22.3 1q25.1(123,041,062-149,830,858) x2 hmz) was detected by SNP array. A2. Confirmation of maternal UPD (6) using the UPD tool. FracHom (blue line): fraction of genotypes that is homozygous; FracME (red line): fraction of Mendelian errors; FracFather (green line): fraction of genotypes identical to the fathers; FracMother (black line): fraction of genotypes identical to the mother; FracError (orange line): fraction of errors (= ME that cannot be explained by UPD). (B). Trio-WES result confirmed as maternal UPD 6 (UPD (6) mat). B1: The fetus; B2: The father; B3: The mother.

**B**

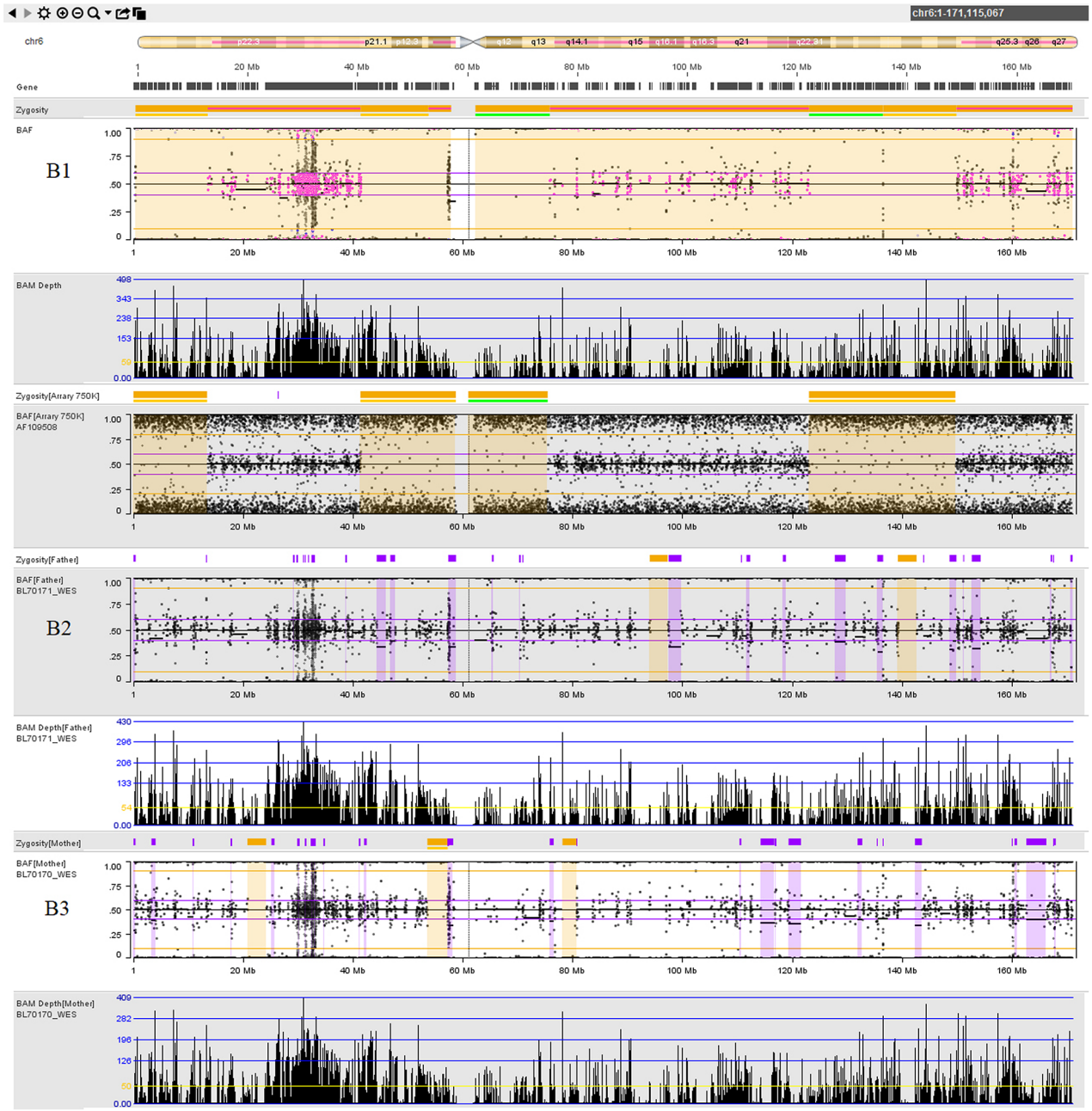


**Figure 2.** (continued)

enough, and therefore, some postnatal clinical phenotypes may have been missed. Second, we did not confirm the pathogenicity of these novel splice-site mutations using a splicing assay by constructing a minigene in vitro.

In conclusion, WES increased the diagnostic yield in fetuses with SKAs, identifying an additional 41% of cases with a genetic cause. Our study further showed that prenatal WES is recommended for fetuses with SKAs following routine testing, to facilitate comprehensive genetic counselling and the assessment of the risk of recurrence in future pregnancies.

## Data availability

Sequence data that support the findings of this study are not publicly available in order to comply with hospital and IRB policy. According to the consent form, sequencing data cannot be accessed without patient's permission. But they are available from the corresponding author on reasonable request.

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## Author contributions

H.X., L.Z., C.J. and A.Y., H.X., L.Z., C.J. and A.Y. prepared the main manuscript and Z.T., L.X., W.L., W.Z., Q.G., R.F., and H.H. prepared prepared figures and tables. All authors have read and approved the final article. Z.T., L.X., W.L., W.Z., Q.G., R.F., and H.H. prepared the experiment. All authors have read and approved the final article.

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## Declarations

## Competing interests

The authors declare no competing interests.

## Additional information

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