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ORIGINAL RESEARCH

The Clinical Utility of Whole-Exome Sequencing in the Prenatal Diagnosis of Fetal Skeletal Dysplasia

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Objective: To investigate the clinical application value of exome sequencing technology in fetuses with sonographically detected skeletal dysplasia.

Methods: A total of 80 pregnant women whose fetuses were diagnosed with sonographically detected skeletal dysplasia at 11^{+0} to 38^{+0} weeks of gestation in our hospital from March 2021 to January 2022 were enrolled in this study. G-banded chromosomal karyotype analysis (KA), chromosomal microarray analysis (CMA) and whole exome sequencing (WES) were simultaneously performed for all cases. Clinical data including maternal age, gestational week, prenatal ultrasound findings, results of KA, CMA and WES, and pregnancy outcomes were collected and subjected to statistical analysis.

Results: Limb shortening was the most common phenotypic manifestation of fetal skeletal dysplasia. The diagnostic yield of KA combined with CMA for fetal skeletal dysplasia was only 7.5% (6/80), while the additional combination with WES identified 43 positive cases, with an overall diagnostic yield of 53.75% (43/80). There was a statistically significant difference in the diagnostic yield between the two detection protocols (χ^2=50.19, P<0.001), with an absolute increase of 46.25% in the diagnostic yield after the integration of WES. The most common clinical disorders caused by pathogenic genes included osteogenesis imperfecta, achondroplasia and thanatophoric dysplasia. The majority of pathogenic variants were de novo mutations, mainly involving the *COL1A1, FGFR2* and *FGFR3* genes. Among the 43 WES-positive cases, 34 pregnancies opted for termination of pregnancy.

Conclusion: Whole-exome sequencing technology holds important application value in the prenatal diagnosis of fetal skeletal system diseases, and provides a more accurate evidence base for genetic counseling and clinical decision-making.

Keywords: whole exome sequencing, technology, fetal skeletal dysplasia, prenatal diagnosis, clinical value

Introduction

Fetal skeletal dysplasia represents a group of phenotypically heterogeneous disorders characterized by abnormalities in the morphology, size and density of the skeletal system. Clinically, it manifests as malformations involving the extremities, thoracic cage, skull and other anatomical sites, with a global incidence of approximately 0.2%.[1] The clinical severity varies drastically across affected cases: severe forms can result in intrauterine or perinatal death, while survivors may suffer from permanent disability due to skeletal malformations. Genetic etiologies include chromosomal aneuploidy, microdeletions/microduplications and monogenic disorders; to date, 771 distinct hereditary bone diseases have been identified, which are associated with 552 genes.[2] Prenatal diagnosis of fetal skeletal dysplasia remains extremely challenging due to the large number of disease subtypes, variable and overlapping phenotypic presentations.[3] Emerging evidence has indicated that more than 50% of fetuses with skeletal dysplasia cannot obtain a definitive

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etiological diagnosis via conventional genetic testing methods (eg, prenatal ultrasonography, karyotype analysis, chromosomal microarray analysis).[4] Whole-exome sequencing (WES), a novel high-throughput and high-resolution next-generation sequencing technology, is capable of detecting single nucleotide variants (SNVs), small insertions and deletions (Indels) as well as de novo mutations. It is particularly suitable for the diagnosis of genetically heterogeneous disorders and exhibits excellent genetic diagnostic performance in fetuses with sonographically detected skeletal dysplasia, although the diagnostic yield varies considerably across different studies with distinct disease spectra and sample sizes.[5] This study aimed to investigate the clinical application value of WES in fetuses with sonographically identified skeletal dysplasia, and to provide a robust evidence base for genetic counseling, pregnancy management and recurrence risk assessment.

Materials and Methods

Participants

A total of 80 pregnant women who underwent prenatal care at our hospital from March 2021 to January 2022, and were found to have fetal skeletal dysplasia on prenatal ultrasound at 11^{+0} to 38^{+0} weeks of gestation, were enrolled in this study. All these patients received G-banded chromosomal karyotype analysis (KA), chromosomal microarray analysis (CMA), and whole-exome sequencing (WES) simultaneously.

Inclusion Criteria

① Fetal skeletal dysplasia was identified on prenatal ultrasound at 11^{+0} to 38^{+0} weeks of gestation, including limb shortening, radial hypoplasia or aplasia, tibial curvature, narrow thoracic cage, strawberry-shaped skull, macrocephaly, microcephaly, nasal bone aplasia, polydactyly, syndactyly, overlapping digits, polydactyly of the feet, and talipes equinovarus/valgus; ② Written informed consent was signed by the pregnant women.

Exclusion Criteria

① The fetus had non-skeletal malformations indicating induced termination of pregnancy or perinatal death caused by other etiologies; ② Incomplete clinical data or loss of follow-up.

This study was approved by the Medical Ethics Committee of Liuzhou Maternal and Child Health Hospital (approval number: ERR-2022-025).

Methods

Sample Collection

Chorionic villi (10–30 mg) or fetal amniotic fluid (30 mL) or umbilical cord blood (2.5 mL) was collected via amniocentesis or cordocentesis, and peripheral venous blood samples (1–3 mL each) were obtained from the fetal parents for reserve use simultaneously. All specimens were stored at 2–8°C under refrigeration until the initiation of laboratory processing.

Fetal Ultrasound Examination

All pregnant women underwent prenatal ultrasound assessments of fetal development at 11^{+0} to 38^{+0} weeks of gestation in accordance with the routine ultrasonic examination protocols specified in the *Guidelines for Prenatal Ultrasonography* and *Standards for Obstetric Ultrasonography.* All fetal growth parameters were measured during the assessments. For the screening of fetal extremity skeletal development, the sequential tracking principle was adopted: ultrasound scanning was performed from the proximal to the distal segments of the extremities, the lengths of fetal long bones were measured, and the length, number, morphology, structure and motor function of the extremities and long bones were systematically observed. Fetal skeletal dysplasia was defined as the presence of the following malformations: limb shortening, radial hypoplasia or aplasia, tibial curvature, narrow thoracic cage, strawberry-shaped skull, macrocephaly, microcephaly, nasal bone aplasia, polydactyly, syndactyly, overlapping digits, polydactyly of the feet, and talipes equinovarus/valgus.

Karyotype Analysis

Fetal chorionic villus, amniotic fluid and umbilical cord blood samples were collected for chromosomal karyotype analysis, which was performed independently through two parallel experimental lines involving routine cell culture, slide preparation and G-banding. Chromosome slides were scanned using an automated chromosome image analysis system manufactured by Zeiss (Germany). A total of 20 metaphase spreads were manually counted across the two lines, with at least 3 karyotypes analyzed per line. In cases of chimerism or abnormal cell lines, the number of counted metaphase spreads was increased to 100. Chromosomal karyotypes were described in accordance with the professional criteria and specifications of the International System for Human Cytogenomic Nomenclature (ISCN 2020).

CMA Detection

Genomic DNA was extracted from samples using the TIANamp Micro DNA Kit. Genome-wide variant detection of fetal DNA was performed with the Affymetrix CytoScan 750K microarray, followed by data analysis using ChAS 3.2 software. Copy number variations (CNVs) were categorized into five pathogenicity grades. The interpretation of CNVs was conducted in accordance with the American College of Medical Genetics and Genomics (ACMG) Standards and Guidelines for the Interpretation of Sequence Variants, classifying CNVs as pathogenic, likely pathogenic, variants of uncertain significance (VUS), benign, or likely benign.

WES Detection

Genomic DNA (150–300 ng) was extracted from fetal samples, and sheared into 100–300 bp fragments using a focused ultrasonicator, followed by the construction of DNA libraries. Sequencing was performed on the Illumina HiSeq 2500, HiSeq X Ten and NovaSeq platforms in accordance with the standard operating protocols. The detected variant data were subjected to bioinformatic analysis: variants with a minor allele frequency (MAF) > 0.05 in the 1000 Genomes Project (1000G) and Exome Aggregation Consortium (ExAC) databases were filtered out. Variants were then annotated, and predictions of their effects on protein function and splice site pathogenicity were conducted in accordance with the guidelines of the American College of Medical Genetics and Genomics (ACMG).[6] Finally, the identified variant loci were classified into five categories: pathogenic, likely pathogenic, uncertain significance, likely benign, and benign.

Genetic Counseling and Pregnancy Outcome Follow-Up

Pregnant women with abnormal whole exome sequencing (WES) results received genetic counseling after obtaining their test reports. For those who chose to continue their pregnancies, outcomes were monitored via telephone follow-up at six months postpartum.

Statistical Methods

Descriptive statistical analysis was performed for all data. Normally distributed continuous variables were expressed as mean ± standard deviation ($\bar{x} \pm s$), and categorical variables were presented as percentages (n/%). The chi-square test was used for intergroup comparisons to analyze differences between categorical variables. A two-tailed P value < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS 26.0 software (IBM Corp., Armonk, NY, USA).

Results

General Materials and Fetal Ultrasound Findings

A total of 80 pregnant women were enrolled in this study. The maternal age was 34.7±6.8 years (range, 19–42 years), and the gestational age was 25.6±12.4 weeks (mean ± standard deviation). Fetal ultrasound findings revealed various anomalies in all cases, including 40 cases with proximal bone shortening (ranging from −1.8 SD to −8 SD), 4 cases with midshaft bone anomalies (including radial aplasia, hypoplasia, brachymorphia and tibial curvature), and 12 cases with distal skeletal anomalies involving abnormal limb posturing, syndactyly, polydactyly, overlapping digits, syndactylous toes, polydactylous toes, and talipes varus/valgus. Moreover, 38 cases presented with cranial, facial, thoracic and

truncal anomalies, among which 16 cases had overlapping skeletal anomalies. Of the 43 cases with positive whole-exome sequencing (WES) results, 34 underwent pregnancy termination, the main reasons being as follows: a definitive diagnosis of fatal, disabling or severe dysfunctional disorders in the fetus; confirmation of severe syndromes with a dominant inheritance pattern by genetic testing; and the presence of severe fetal growth restriction (FGR) complicated by potential developmental anomalies of other systems and intrauterine fetal hypoxia (Table 1).

Karyotype

Chromosomal abnormalities were identified in 6.25% (5/80) of the fetuses, including 2 cases of trisomy 21, 1 case of trisomy 18, 1 case of chromosome 18 chimeric variation, and 1 case of chromosome 9 inversion. Paternal uniparental disomy of chromosome 2 was not detected by karyotype analysis (KA), resulting in a false negative rate of 20%.

CMA

The detection rate of copy number variations (CNVs) was 6.25% (5/80). Notably, chromosomal microarray analysis (CMA) identified an additional case of paternal uniparental disomy (UPD) of chromosome 2 that karyotype analysis had overlooked. The fetus with paternal UPD of chromosome 2 exhibited only mild ultrasound findings, including femoral and humeral shortening (2–4 SD below the mean) and placental thickening. Additionally, a segmental duplication of chromosome 4 was co-detected in a case of mosaicism involving chromosome 18 (Figure 1A–E). Furthermore, CMA failed to detect the inversion of chromosome 9 identified by karyotype analysis, resulting in a false-negative rate of 20%.

WES

Genetic Variants and Pathogenic Findings Detected by Whole-Exome Sequencing

Of the 80 cases, 43 were positive for pathogenic variants by WES (53.75%). Mellis R et al reported a 53% diagnostic yield of WES for fetal skeletal dysplasia,[7] which was consistent with the results of this study. Among the 43 positive cases,7 cases had variants of uncertain significance (VUS), 33 cases were identified with pathogenic or likely pathogenic variants, resulting in a pathogenic detection rate of 33/80 (41.25%), and 2 cases had variants with controversial or uncertain pathogenicity. One case had a family history of ichthyosis, while the detection result was the normal wild-type of FLG c.3321 (Table 1). The diagnostic rate of karyotyping analysis (KA) combined with chromosomal microarray analysis (CMA) for fetal skeletal dysplasia was only 7.5% (6/80). In contrast, 43 positive cases were detected after the combined application of WES, with a detection rate of 53.75% (43/80). The difference in diagnostic rates between the two protocols was statistically significant (χ^2=50.19, P<0.001), with an absolute increase of 46.25% in the diagnostic rate. The most common clinical disorders caused by pathogenic gene variants were osteogenesis imperfecta, achondroplasia and thanatophoric dysplasia, which was consistent with the findings of a previous study by Jin Han.[8] In this study, one patient with an FGFR3 gene variant presented with lethal skeletal dysplasia complicated by a strawberry-shaped skull and a narrow thoracic cage (Figure 2A–C).

Gene Distribution and Mendelian Inheritance Patterns

All mutations identified in this study were validated by Sanger sequencing, with the majority being de novo mutations. Among these, 19 cases were confirmed as autosomal dominant genetic disorders, involving the *COL1A1* (1 case), *FGFR2* (1 case), *FGFR3* (9 cases), *TRPV4* (1 case), *ACAN* (1 case), *PTPN11* (1 case), *NRIP1* (2 cases), *CHD3* (1 case), *IGF1R* (1 case), *CHRND* (1 case), *SMARCD1* (1 case), *BRCA2* (1 case), *PUF60* (1 case), *SCN4A* (1 case), *ZNF462* (1 case) and *TGIF1* (1 case) genes. Three cases were confirmed as autosomal recessive genetic disorders, involving the *RAPSN* (1 case), *WDR35* (1 case) and *LAMA1* (1 case) genes. Four fetuses had X-linked inheritance, including 1 case with exons 49–51 deletion of the *DMD* gene and 3 cases with c.1388G>A and c.1024C>T locus mutations in the *G6PD* gene. See Figure 3.

Types of Genetic Variants

Missense variants were the most frequent, accounting for 30 cases. In addition, there were 2 cases of frameshift variants and 2 cases of in-frame deletions. Copy number variants (CNVs), including chimeric duplications and chromosomal

Table 1 43 Cases of WES-Positive or Wild-Type Fetuses and Pregnancy Outcome

Case	Age (Year)	Gestation (Weeks)	Skeletal Abnormal Phenotype				Non-Skeletal Abnormalities	FGR	KA	CMA	WES	Mutation Type	Clinical Diagnosis	Pregnancy Outcome
			Limb Abnormalities			Trunk, Head, Chest								
			Proximal	Middle Segment	Distal									
1	31	23 weeks and 4 days	HL short, FL short								TRPV4 (NM_021625.5): c.1412_1414del, het	P	Metatropic dysplasia	Induced termination of pregnancy
2	28	13 weeksand 6 days		Right radius hypoplasia (radius shortened by 3.7 mm, right forearm postural abnormality, palm adduction)							MAP1B c.7141C>T (p.Arg2381Trp), het	VUS	Periventricular Nodular Heterotopia Type 9	Term delivery
3	34	29 weeks and 0 days	HL and FL -*4 SD								FGFR3 (NM_000142.4): c.1138G>A (p.Gly380Arg), het	P	Achondroplasia (AD), Lethal Dysplasia Type I (AD), Chondrodysplasia (AD), Lethal Dysplasia Type II (AD), Severe achondroplasia with developmental delay and acanthosis	Induced termination of pregnancy
4	33	22 weeks and 1 day	Lethal Skeletal Dysplasia			Thoracic hypoplasia, strawberry skull					FGFR3 (NM_000142.4): c.742C>T (p.Arg248Cys), het	P	Achondroplasia (AD), Lethal Dysplasia Type I (AD), Chondrodysplasia (AD), Lethal Dysplasia Type II (AD), Severe achondroplasia with developmental delay and acanthosis nigricans (AD)	Induced termination of pregnancy
5	33	38 weeks and 0 days	HL and FL −2 SD			Thoracic hypoplasia					FGFR3 (NM_000142.4): c.1138G>A (p.Gly380Arg), het	P	Lethal Dysplasia Type I (AD), Chondrodysplasia (AD), Achondroplasia (AD), (AD), Lethal Dysplasia Type II (AD), Severe achondroplasia with developmental delay and acanthosis nigricans (AD)	Induced termination of pregnancy

(*Continued*)

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Table 1 (Continued).

Case	Age (Year)	Gestation (Weeks)	Skeletal Abnormal Phenotype				Non-Skeletal Abnormalities	FGR	KA	CMA	WES	Mutation Type	Clinical Diagnosis	Pregnancy Outcome
			Limb Abnormalities			Trunk, Head, Chest								
			Proximal	Middle Segment	Distal									
6	34	27 weeks and 1 day	FL −3.3 SD, HL −2.6 SD				Placental thickening			Presence of paternal uniparental disomy in a region of chromosome 2	A heterozygous variant in the ACAN gene at chr15:89385083, designated as NM_001369268.1: c.742G>A (p.Ala248Thr), in Exon 5|19	P	Multiple Epiphyseal Dysplasia (AD)	Induced termination of pregnancy
7	33	24 weeks and 0 days	FL and HL-2SD				Fetal bilateral lens dysplasia; congenital cataract to be ruled out; fetal hydrops syndrome (generalized skin edema, pleural/abdominal effusion); persistent left superior vena cava (bilateral superior vena cava)				FLG c.3321,WT	NP		Induced termination of pregnancy
8	33	24 weeks and 0 days	Lethal Skeletal Dysplasia (possible lethal dwarfism)								FGFR3 c.742C>T, het	P	Achondroplasia (AD), Lethal Dysplasia (AD)	Induced termination of pregnancy
9	24	28 weeks and 4 days				Absent nasal bone, microcephaly	FGR		46, XN, inv(9) (p12q13)		FGFR3 c.2421A>G, het	P	Lethal Dysplasia (AD), Achondroplasia (AD)	Induced termination of pregnancy
10	27	13 weeks and 1 day	Possible Lethal Skeletal Dysplasia				NT 9.6mm; reversal of alpha wave in fetal ductus venosus blood flow spectrum; fetal cystic hygroma				COL1A1 c.2015_2016delinsT, het	P	Caffey Disease (AD); Combined Osteogenesis Imperfecta and Ehlers-Danlos Syndrome Type I (AD); Osteogenesis Imperfecta Type I (AD); Osteogenesis Imperfecta Type II (AD); Osteogenesis Imperfecta Type III (AD); Osteogenesis Imperfecta Type IV (AD)	Induced termination of pregnancy
11	35	22 weeks and 2 days				Nasal bone hypoplasia	Tetralogy of Fallot				TGIF1 c.268C>T, het	P	Holoprosencephaly Type 4 (AD), incomplete penetrance of TGIF1	Induced termination of pregnancy
12	19	31 weeks and 4 days	FL-1.91SD, HL-2SD								NRIP1 c.3313A>G, het	VUS	Congenital Renal and Urinary Tract Anomalies, Type 3 (AD)	Term delivery

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13	26	26 weeks and 1 day			Bilateral Foot Varus		Persistent left superior vena cava		47, XN, +21	Seq[hg19]dup(21)(q11.2.q22.3) chr21: g.14300000_48129895dup	21q11.2–21q22.3 (chr21:?14,982,509-48084306?)X3 (Exact breakpoints unknown)	P	Down's Syndrome	Induced termination of pregnancy
14	33	24 weeks and 4 days				Possible C6, T5, and T7 hemivertebrae of the spine	Single Umbilical Artery				Deletion of exons 49–51 in the DMD gene	P	Becker Muscular Dystrophy (XLR), Dilated Cardiomyopathy, Type 3B (XL), Duchenne Muscular Dystrophy (XLR)	Preterm Birth, Esophageal Atresia Type III, accompanied by cervical and thoracic vertebral malformations, 13 pairs of ribs (polycostaly), and abnormal hand posture; Patent Ductus Arteriosus and Patent Foramen Ovale
15	23	35 weeks and 0 days	HL-2SD				Polyhydramnios, bilateral pleural effusion				FGFR3 NM_000142.5: c.1138G>A, het	P		Induced termination of pregnancy
16	33	26 weeks and 6 days	FL-3.72SD, HL-2SD				Pericardial effusion, pulmonary artery crossing				LZTR1 (NM_006767.4): c.851G>A, het	LP	Noonan Syndrome Type 2 (AR); Schwannomatosis Type 2 Predisposition (AD)	Induced termination of pregnancy
17	36	16 weeks and 6 days			Bilateral hand syndactyly, bilateral foot syndactyly	Caudal dysgenesis	NT thickening 3.9mm; dextroversion of the heart; ventricular septal defect (VSD); aberrant right subclavian artery; sacrococcygeal tissue protrusion				WDR35 (NM_001006657.2): c.440A>G, het	VUS	Cranial Ectodermal Dysplasia Type 2 (AR); Short Rib-Thoracic Dysplasia Type 7 with or without Polydactyly (AR)	Term delivery
18	36	26 weeks and 1 day	FL-2SD				Polyhydramnios		47, XN, +21	Seq[hg19]dup(21)(q11.2.q22.3) chr21: g.14300000_48129895dup	Duplication of a 40.55 Mb region at p12-q22.3 on chromosome 21	P	Down's Syndrome	Induced termination of pregnancy
19	28	23 weeks and 0 days	FL-4.12SD, Lethal Skeletal Dysplasia		Bilateral foot polydactyly, Absence of the right radius and left radial dysplasia, left hand polydactyly		Bilateral renal pelvis separation		46, XN, add(18)(p11.3)[27]/46, XN[73]	Seq[hg19]dup(4)(q31.3q35.2) chr4: g.151940000–190940000dupseq [hg19]del(18)(p11.32p11.32) chr18:g.12000–2780000delseq [hg19]del(18)(p11.32p11.31) chr18:g.2780000–5800000del	Mosaic duplication of a 38.95 Mb region at q31.3-q35.2 on chromosome 4	P	Chromosome 4q32.1-q32.2 triplication syndrome [OMIM:613603]Syndrome	Induced termination of pregnancy

(*Continued*)

Table 1 (Continued).

Case	Age (Year)	Gestation (Weeks)	Skeletal Abnormal Phenotype				Non-Skeletal Abnormalities	FGR	KA	CMA	WES	Mutation Type	Clinical Diagnosis	Pregnancy Outcome
			Limb Abnormalities			Trunk, Head, Chest								
			Proximal	Middle Segment	Distal									
20	29	17 weeks and 4 days			Bilateral lower extremities postural abnormality, bilateral hands postural abnormality		Double outlet right ventricle; ventricular septal defect (VSD); aberrant right subclavian artery; bilateral ear buds not clearly visualized				G6PD (NM_001042351.3): c.1388G>A, hemi	P	G6PD Deficiency Hemolytic Anemia (Favism) (XL)	Term delivery
21	36	33 weeks and 5 days	FL-1.81SD, HC/FL=5.70								ZNF462 (NM_021224.6): c.344G>A, het	VUS	Weiss-Kruszka Syndrome (AD)	Induced termination of pregnancy
22	27	23 weeks and 3 days	FL-5.10SD, HL-2.0SD			Nasal bone hypoplasia, BPD-3.79SD, HC-4.04SD, AC-4.27SD	Small gastric bubble; increased echogenicity of intra-abdominal bowel; placental thickening	Weight −8.84 standard deviations (−8.84 SD); intermittent disappearance of diastolic flow is observed in the fetal umbilical artery (Type II Fetal Growth Restriction (FGR)).			G6PD (NM_001042351.3): c.1024C>T, het	LP	G6PD Deficiency Hemolytic Anemia (Favism) (XL)	Induced termination of pregnancy
23	31	26 weeks and 5 days				BPD+3.07SD, HC +4.08SD, TCD= +2SD	Right-sided aortic arch of the heart, aberrant left subclavian artery				Duplication of a 40.55 Mb region at p12-q22.3 on chromosome 21	P	Down's Syndrome	Induced termination of pregnancy
24	31	11 weeks and 1 day				Nasal bone hypoplasia	NT3.3mm				G6PD:c.1388G>A, het	P	G6PD Deficiency Hemolytic Anemia (Favism) (XL)	Term delivery

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25	25	29 weeks and 0 days	FL-8.56SD			BPD-5.95SD, HC-7.21SD, AC-3.86SD	Increased echogenicity of fetal intra-abdominal bowel. Low fetal heart rate	FGR			FGFR2 (NM_000141.5): c.755C>G, het	P	Antley-Bixler Syndrome without genital anomalies or steroidogenesis disorder (OMIM:207410), Apert Syndrome (OMIM:101200), Beare-Stevenson Cutis Gyrata Syndrome (OMIM:123790), campomelic dysplasia Syndrome (OMIM:614592), Craniofacial-Skeletal-Cutaneous Dysplasia (OMIM:101600), Crouzon Syndrome (OMIM:123500), Jackson-Weiss Syndrome (OMIM:123150), LADD Syndrome Type I (OMIM:149730), Pfeiffer Syndrome (OMIM:101600), Saethre-Chotzen Syndrome (OMIM:101400) (AD)	Induced termination of pregnancy
26		32 weeks and 5 days	FL-2.8SD			BPD-2SD, HC-2.8SD	Polyhydramnios, fetal bilateral pleural effusion, fetal scalp thickening and edema, increased blood flow resistance of fetal umbilical artery				FGFR3 (NM_000142.5): c.1138G>A, het	P	Achondroplasia (OMIM:100800), Costal Dysplasia (OMIM:146000), Severe Achondroplasia with Developmental Delay and Acanthosis Nigricans (OMIM:616482), Lethal Osteodysplasia Type I (OMIM:187600), Lethal Osteodysplasia Type II (OMIM:187601)	Induced termination of pregnancy
27	28	18 weeks and 4 days			Bilateral foot varus, bilateral upper extremities in a crossed position in front of the chest, and bilateral hands with overlapping fingers	Possible micrognathia					PIK3CA (NM_006218.4): c.1090G>A, het	LP	Cowden Syndrome Type 5 (OMIM:615108), Megalencephaly-Capillary Malformation-Polymicrogyria Syndrome, Somatic Mutation (OMIM:602501)	Induced termination of pregnancy

(*Continued*)

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Table 1 (Continued).

Case	Age (Year)	Gestation (Weeks)	Skeletal Abnormal Phenotype				Non-Skeletal Abnormalities	FGR	KA	CMA	WES	Mutation Type	Clinical Diagnosis	Pregnancy Outcome
			Limb Abnormalities			Trunk, Head, Chest								
			Proximal	Middle Segment	Distal									
28	41	13 weeks and 4 days				Nasal bone hypoplasia	NT 6.4mm; fetal cystic hygroma; possible absence of aortic valve; severe tricuspid valve regurgitation; pericardial effusion; single umbilical artery; reversal of alpha wave in fetal ductus venosus spectrum; reversal of diastolic blood flow in umbilical artery				MSH6 (NM_000179.3): c.2577_2580del, het; FLNC (NM_001458.5) c.5277del, het	LP/LP	Hereditary Nonpolyposis Colorectal Cancer (HNPCC), AD; Limb-Girdle Muscular Dystrophy (LGMD), Dilated Cardiomyopathy (DCM), Isolated Myopathy, AD	Induced termination of pregnancy
29	25	30 weeks and 5 days			Fetal bilateral foot varus, postural stiffness, and bilateral hands postural abnormality		Small fetal gastric bubble; Polyhydramnios (suggesting possible presence of esophageal atresia)				SCN4A (NM_000334.4): c.4737C>A, het; c.4420G>A, het	VUS/VUS	Classic Congenital Myopathy Type 22A (AR); Severe Fetal Congenital Myopathy Type 22B (AR); Hyperkalemic Periodic Paralysis (AD); Hypokalemic Periodic Paralysis Type 2 (AD); Congenital Myasthenic Syndrome Type 16 (AR); Acetazolamide-Responsive Atypical Congenital Myotonia (AD); Congenital Paramyotonia (AD)	Induced termination of pregnancy
30	34	32 weeks and 5 days	FL-2.7SD				Small fetal gastric bubble; Polyhydramnios (suggesting possible presence of esophageal atresia)				PUF60 (NM_078480.3): c.389G>A, het	P	Verheij Syndrome (AD)	Preterm Birth
31	32	33 weeks and 6 days				Ultrasonic measurement of fetal biparietal diameter is at −4.03 standard deviations (−4.03 SD), and ultrasonic measurement of fetal head circumference is at −2.34 standard deviations (−2.34 SD).					BRCA2 (NM_000059.4): c.5722_5723del, het	P	Hereditary Breast and/or Ovarian Cancer (AD)	Induced termination of pregnancy

32	29	28 weeks and 2 days						FGR			LAMA1 (NM_005559.4): c.2953del, het; c.3745G>A, het	LP/VUS	Poretti-Boltshauser Syndrome (AR)	Induced termination of pregnancy
33	30	25 weeks and 1 day						FGR			SMARCD1 (NM_003076.5): c.1429C>T, het	VUS	Coffin-Siris Syndrome Type 11 (AD)	Term delivery
34	35	18 weeks and 4 days			Bilateral foot varus, Possible postural abnormality of fetal bilateral upper extremities and hands	Possible micrognathia	NT thickening				CHRND (NM_000751.3): c.89G>C, het; c.59G>A, het	VUS/LP	Slow-Channel Congenital Myasthenic Syndrome Type 3A (AD); Acetylcholine Receptor Deficiency-Associated Congenital Myasthenic Syndrome Type 3C (AR); Lethal Multiple Pterygium Syndrome (AR); Fast-Channel Congenital Myasthenic Syndrome Type 3B (AR)	Induced termination of pregnancy
35	31	13 weeks and 4 days				Absence of nasal bone	NT: 6.4mm; fetal cystic hygroma; possible absence of aortic valve and pulmonary valve; severe tricuspid valve regurgitation; pericardial effusion/single umbilical artery; reversal of alpha wave in fetal ductus venosus spectrum; reversal of diastolic blood flow in umbilical artery		47,XN, +18	Seq[hg19]dup(18)(p11.32.q23) chr18:g.1–78077248dup	Duplication of an 80.2 Mb region at p11.32-q23 on chromosome 18, and Trisomy 18	P	Edwards Syndrome	Induced termination of pregnancy
36	32	26 weeks and 1 day	FL-2.53SD				Polyhydramnios				FGFR3 (NM_000142.5): c.1138G>A, het	P	Achondroplasia (OMIM:100800), Costal Dysplasia (OMIM:146000), Severe Achondroplasia with Developmental Delay and Acanthosis Nigricans (OMIM:616482), Lethal Osteodysplasia Type I (OMIM:187600), Lethal Osteodysplasia Type II (OMIM:187601)	Induced termination of pregnancy

(*Continued*)

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Table 1 (Continued).

Case	Age (Year)	Gestation (Weeks)	Skeletal Abnormal Phenotype				Non-Skeletal Abnormalities	FGR	KA	CMA	WES	Mutation Type	Clinical Diagnosis	Pregnancy Outcome
			Limb Abnormalities			Trunk, Head, Chest								
			Proximal	Middle Segment	Distal									
37	21	28 weeks and 4 days			Overlapping fingers, bilateral lower extremities postural abnormality	Possible micrognathia	Possible diaphragmatic eventration; bilateral pulmonary hypoplasia; leftward displacement of the heart; bilateral pulmonary arteries being relatively slender; Abdominal Circumference (AC) −2 standard deviations (−2SD); polyhydramnios; gastric bubble is relatively small				RAPSN (NM_005055.5): c.484G>A, hom	LP	Fetal akinesia deformation sequence 2 (AR);Myasthenic syndrome, congenital, 11, associated with acetylcholine receptor deficiency (AR)	Induced termination of pregnancy
38	38	23 weeks and 1 day			Bilateral hands postural abnormality (overlapping fingers)		NF6.8mm				ACTA1 c.49G>A, het	P	Myopathy, scapulohumeroperoneal (AD) (OMIM:616852),Myopathy, actin, congenital, with cores (AD/AR) (OMIM:161800), Myopathy, actin, congenital, with excess of thin myofilaments (AD/AR) (OMIM:161800); Myopathy, congenital, with fiber-type disproportion 1 (AD/AR) (OMIM:255310); Nemaline myopathy 3, autosomal dominant or recessive (AD/AR) (OMIM:161800)	Induced termination of pregnancy
39	26	30 weeks and 5 days				Head circumference is smaller than normal	Ventricular Septal Defect (VSD)				IGF1R c.3473G>A, het	P	Insulin-like Growth Factor 1 Resistance (AD/AR)	Induced termination of pregnancy

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40	27	30 weeks and 5 days	Short Limbs			Thoracic hypoplasia					FGFR3 c.742C>T(p. Arg248Cys), het	P	Bladder Cancer (-); Colorectal Cancer, Somatic (-); Achondroplasia (AD); Seminoma (-); Muenke Syndrome (AD); Lethal Dysplasia Type II (AD); Cervical Cancer (-); Severe Achondroplasia with Developmental Delay and Acanthosis Nigricans (AD); Stiff Little Finger, Tall Stature, and Deafness Syndrome (AR/AD); Achondroplasia (AD); Lethal Dysplasia Type I (AD); Crouzon-Cutaneous-Skeletal Syndrome (AD); Lacrimal Duct-Ear-Tooth-Finger Syndrome (AD); Epidermal Nevus (-)	Induced termination of pregnancy
41	27	29 weeks and 0 days				Biparietal diameter and head circumference are larger than normal					CHD3 (NM_001005271.3): c.5456G>A, het	P	Snijders Blok-Campeau Syndrome (AD)	Induced termination of pregnancy
42	36	31 weeks and 2 days				BPD+1.58SD, HC +1.5SD	Polyhydramnios, hyperechoic material filling the cavum septi pellucidi				PTPN11 (NM_002834.5): c.179G>C, het	P	LEOPARD Syndrome Type 1 (OMIM:151100), Noonan Syndrome Type 1 (OMIM:163950) (AD)	Preterm Birth
43	19	25 weeks and 4 days		Fetal right tibial curvature			Fetal cavum Vergae (i.e., presence of the sixth ventricle) is present; fetal right kidney enlargement, cortical thickening, unclear corticomedullary junction, and increased echogenicity				NRIP1 c.3313A>G, het	VUS	Congenital Renal and Urinary Tract Anomaly, Type 3 (AD)	Induced termination of pregnancy

Notes: "-" indicates that the value of the fetal biometric parameter is below the mean value of the reference range for the corresponding gestational age, and the number following it represents the multiple of standard deviation (SD) from the mean.

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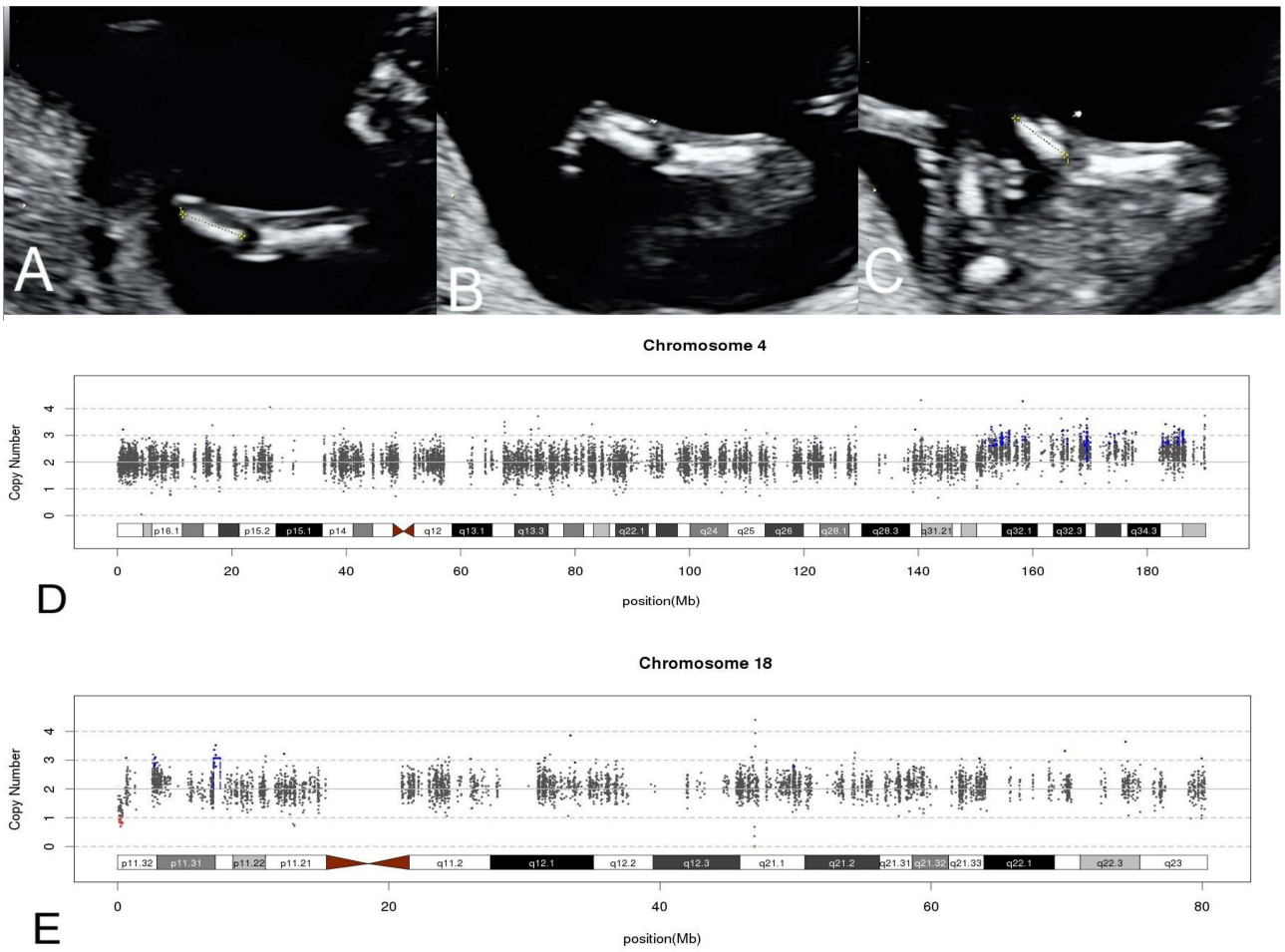

Figure 1 Ultrasound and CMA results for Case 19 are as follows: (**A**) Absence of the right radius, The yellow dashed line marks the ulna, with absent radius. (**B**) Preaxial polydactyly of the left foot. (**C**) Left radial dysplasia, The yellow dashed line marks the ulna, with radial hypoplasia. (**D**) seq[hg19]dup(4)(q31.3q35.2)chr4:g.151940000–-190940000dup. (**E**) seq[hg19]del(18)(p11.32p11.32)chr18:g.12000–2780000del; seq[hg19]del(18)(p11.32p11.31)chr18:g.2780000–5800000del.

segment triploidies, involved chromosomes 4, 18 and 21 with fragment sizes ranging from 38.95 Mb to 80.2 Mb. One case had a large fragment deletion of gene exons (exons 49–51 deletion of the *DMD* gene). Special zygotic variants included hemizygote of *G6PD* c.1388G>A and homozygote of *RAPSN* c.484G>A, while the majority of the remaining variants were heterozygotes.

Discussion

The human genome comprises approximately 180,000 exons, which represent about 1% of the total genome.[9] It is estimated that 85% of pathogenic variants arise from protein-coding regions and splice sites.[10] Whole exome sequencing (WES) can identify over 4,000 monogenic disorders, accounting for more than 85% of fetal pathogenic mutations. However, skeletal developmental abnormalities present in various forms and are linked to a broad spectrum of chromosomal abnormalities and gene variants.

In our study, we identified two cases of trisomy 21; one presented with bilateral talipes varus, while the other exhibited femoral shortening. One case of trisomy 18 showed ultrasound findings of absent nasal bone, emphasizing that chromosomal abnormality screening is a critical factor in the evaluation of skeletal dysplasia. Notably, one fetus with a karyotypic inversion (46,XN,inv(9)(p12q13)) displayed absent nasal bone and microcephaly, which was not detected by chromosomal microarray analysis (CMA). Additionally, a case of paternal uniparental disomy (UPD) of chromosome 2, characterized by femoral and humeral shortening, was overlooked by karyotype analysis (KA). Furthermore, one fetus with an 18q microdeletion combined with mosaic duplication of 4q31.3-q35.2 presented with femoral shortening, lethal

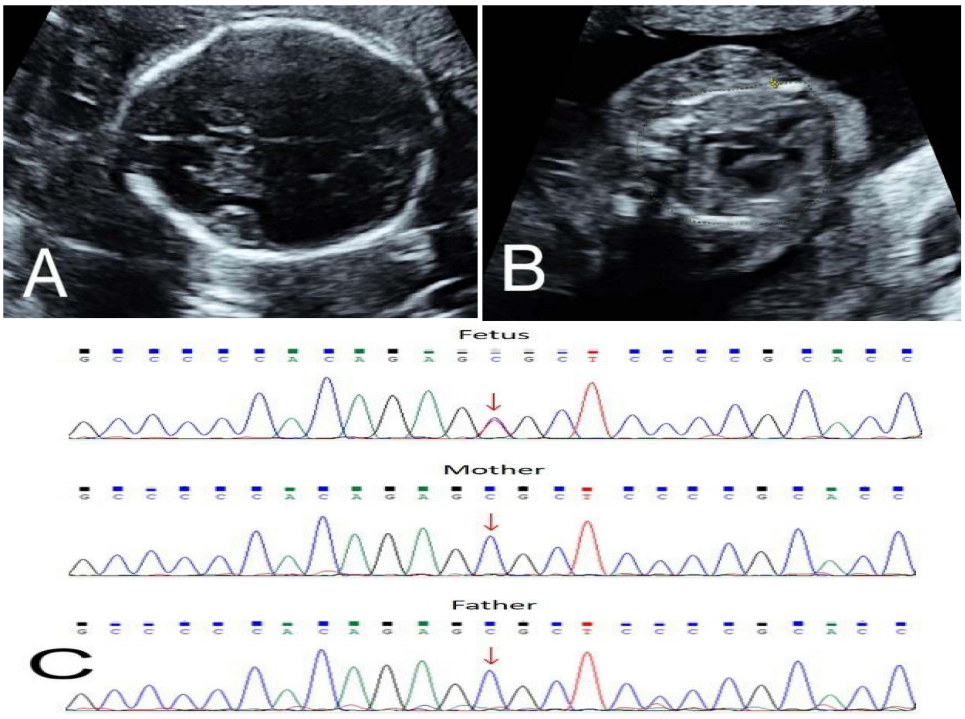

Figure 2 Ultrasound and WES results for Case 4 are as follows: (**A**) Strawtberny skull. (**B**) Thoracic hypoplasia, the yellow dashed area indicates a narrow thorax. (**C**) FGFR3(NM 000142.4):c.742C>T(p.Arg248cys), het. Sanger sequencing validation of the de novo C>T variant in Case 4. The red arrow indicates the C>T nucleotide substitution in the fetal sample, which was not detected in either the maternal or paternal samples, confirming its de novo origin.

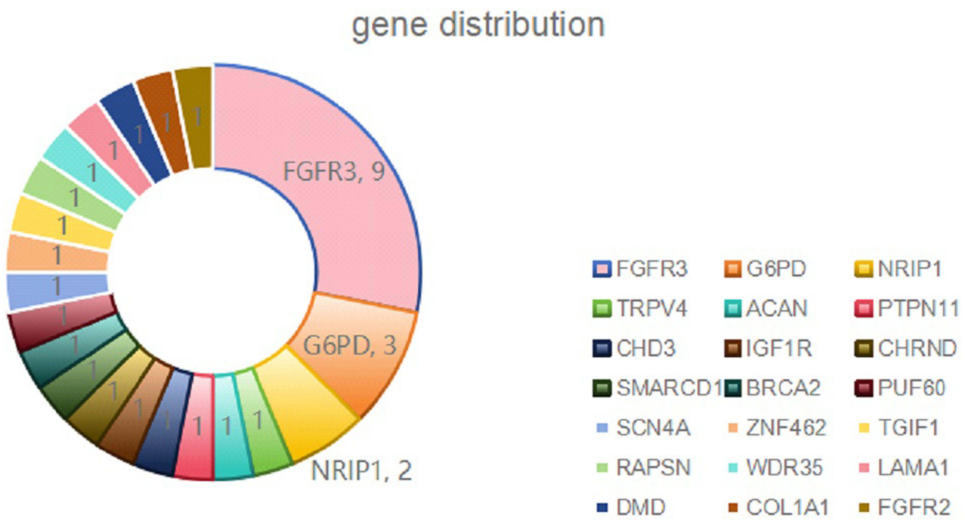

Figure 3 Distribution of genetic variant types in 43 cases of fetal skeletal dysplasia confirmed by WES. Data are presented as percentages (n=43). Numbers represent the number of cases.

skeletal dysplasia, polydactyly, and bilateral renal pelvic dilatation. Although chromosome 18 anomalies typically result in localized skeletal defects, this case involved severe lethal skeletal dysplasia, suggesting that the 4q31.3-q35.2 region may also play a role in skeletal development. These findings underscore the phenotypic complexity arising from multiple variant combinations, necessitating careful consideration in the analysis of genotype-phenotype correlations. Importantly, the combined diagnostic yield of KA and CMA for fetal skeletal dysplasia in our study was only 7.5% (6/80), indicating that a significant proportion of cases may have been missed by these conventional methods.

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WES results revealed 43 positive cases in this study, with a diagnostic yield of 53.75%, representing a 46.25% increase compared with karyotype analysis (KA) combined with chromosomal microarray analysis (CMA). These findings demonstrate that WES has excellent clinical utility in the prenatal diagnosis of fetal skeletal dysplasia. Osteogenesis imperfecta, achondroplasia and thanatophoric dysplasia were the most common clinical disorders associated with pathogenic genes. In addition to well-recognized genotypes such as *COL1A1, FGFR2* and *FGFR3*, several other genes implicated in fetal skeletal dysplasia were identified in this study, which furnishes a vital evidence base for clinical genetic counseling.

Among the common pathogenic genotypes, the *COL1A1* gene encodes the α1 chain of type I collagen, a key component for maintaining skeletal strength and structural integrity. Pathogenic variants in this gene can cause abnormal collagen synthesis and lead to osteogenesis imperfecta (OI), which is clinically characterized by skeletal malformations, increased bone fragility, short stature and a high predisposition to long bone fractures. Osteogenesis imperfecta is an autosomal dominant disorder, and heterozygous pathogenic variants are sufficient to cause the disease. Studies by Kurosaki T and Supek F indicated that milder phenotypes are mostly associated with abnormal synthesis of type I collagen, whereas severe phenotypes are linked to structural defects of collagen.[11,12] In this study, a c.2015_2016delinsT frameshift variant in the *COL1A1* gene was identified in the key structural domain, which results in a truncated protein; the corresponding prenatal ultrasound showed lethal skeletal dysplasia, consistent with the conclusions of the aforementioned studies. The *FGFR2* and *FGFR3* genes belong to the fibroblast growth factor receptor (*FGFR*) family and play critical regulatory roles in skeletal development. A total of 10 cases with variants in these two genes were identified in this study, with the majority being *FGFR3* gene variants. Pathogenic variants in *FGFR2* can cause acrocephaly, syndactyly, and dysplasia of the cranial and facial bones. In one case of this study, a c.755C>G variant in the *FGFR2* gene was detected, and prenatal ultrasound revealed significant femoral shortening and microcephaly. *FGFR3* gene variants represent the most common genetic cause of skeletal dysplasia,[13] which are mainly associated with achondroplasia (ACH) and lethal skeletal dysplasia. Achondroplasia is the most common hereditary dwarfism, an autosomal dominant disorder with complete penetrance; 85% of cases are sporadic de novo dominant variants, clinically manifested as disproportionate short stature, proximal long bone shortening, and potential complications including strawberry-shaped skull, narrow thoracic cage and fetal hydrops syndrome. In addition to the common c.1138G>A and c.742C>T variants, two novel heterozygous missense variants (c.2421A>G and c.755C>G) were identified in this study, and prenatal ultrasound showed long bone shortening of 2 to 4 SD in all corresponding cases.

In addition, several other genotypes associated with fetal skeletal dysplasia were identified in this study. The *TRPV4* gene encodes the transient receptor potential cation channel, which is involved in the regulation of intracellular calcium ion homeostasis. Heterozygous missense variants in this gene can cause multiple skeletal disorders including hand-foot malformations and Kozlowski-type spondylometaphyseal dysplasia.[14–16] The *ACAN* gene encodes aggrecan, a key component of the chondrocyte extracellular matrix, and its variants can lead to chondrodysplasia and hereditary short stature syndromes associated with accelerated bone maturation.[17–19] Aggrecan contains two epidermal growth factor-like motifs, one C-type lectin domain (CLD), and a complement regulatory protein-like motif in the G3 domain.[20,21] Studies have shown that variants in different structural domains exhibit distinct sensitivities to growth hormone and GnRHa therapy, among which patients with *ACAN* variants in the G3 domain have a poor response to postnatal growth hormone treatment.[22] In this study, a heterozygous missense variant of *ACAN* (001369268.1: c.742G>A, p. Ala248Thr) was identified, which was accompanied by paternal uniparental disomy of chromosome 2. This finding suggests that prenatal diagnosis requires the combination of multiple detection technologies to comprehensively assess disease risk and recurrence risk, as well as to perform prenatal monitoring and postnatal evaluation. Variants in the *PTPN11* gene are the most common pathogenic cause of Noonan syndrome (NS), accounting for 50% of pathogenic variants in NS. NS is an autosomal dominant disorder clinically characterized by short stature, craniofacial malformations, short neck, and cardiac anomalies, which is associated with variants in genes regulating the RAS/MAPK signaling pathway. Heterozygous loss-of-function variants in *NRIP1* are the monogenic cause of autosomal dominant congenital anomalies of the kidney and urinary tract (CAKUT) in humans.[23] In one case of this study, a c.3313A>G missense variant in the *NRIP1* gene was detected; prenatal ultrasound indicated right renal enlargement, cortical thickening, unclear corticomedullary differentiation, accompanied by right tibial curvature. This finding suggested that abnormal calcium and

phosphorus metabolism induced by CAKUT impairs normal skeletal mineralization and growth. Variants in the *LZTR1* gene with different genetic transmission patterns can also cause NS.[24] Snijders-Blok-Campou syndrome (SNIBCPS) caused by *CHD3* gene variants is a rare genetic disorder that often affects the nervous and musculoskeletal systems, manifesting as macrocephaly, leg length discrepancy, hypotonia, and may also present with skeletal dysplasia including microcephaly and scoliosis.[25] Variants in the *IGF1R* gene can lead to intrauterine and postnatal growth retardation, which may be accompanied by microcephaly and intellectual disability.[26] *WDR35* is involved in intraflagellar transport and regulates signaling pathways associated with skeletal development. Abnormalities in the Hedgehog (Hh) signaling pathway of skeletal development lead to short rib-polydactyly syndrome (SRPS), with polydactyly being a common accompanying phenotype.[27]

In summary, conventional detection methods including prenatal ultrasound, karyotype analysis (KA) and chromosomal microarray analysis (CMA) play important roles in the diagnosis of fetal skeletal dysplasia but have obvious limitations. A large number of genes are currently known to be associated with fetal skeletal dysplasia; in this study, 16 genes including *COL1A1, FGFR2* and *FGFR3* were linked to autosomal dominant inheritance, while 3 genes including RAPSN and WDR35 were associated with autosomal recessive inheritance. Whole-exome sequencing (WES) has significantly proved the diagnostic rate of fetal skeletal dysplasia and expanded the mutation spectrum for prenatal diagnosis, thereby providing a more precise evidence base for clinical management and genetic counseling. For fetuses carrying pathogenic gene variants, comprehensive genetic counseling should be provided to pregnant women and their families based on the variant type and disease severity; prenatal interventions including pregnancy termination, prenatal monitoring and early postnatal intervention can be implemented as appropriate. Multidisciplinary collaboration involving geneticists, obstetricians, pediatricians and other specialists is crucial for clinical diagnosis and management. In addition, it should be noted that the limited detection range of WES and the incomplete manifestation of fetal phenotypes may result in false negative results, which cannot completely rule out the possibility of genetic diseases. Pregnant women should be informed of the importance of regular reanalysis of sequencing data, and whole-genome sequencing (WGS) should be performed when necessary. For cases with variants of uncertain significance (VUS), the interpretation of results should focus on three key aspects: first, phenotypic correlation analysis, which involves evaluating the matching degree between VUS and fetal phenotypes by integrating clinical manifestations, family history and authoritative databases of the proband; second, parental validation testing, which is used to clarify the genetic origin of VUS, assess the probability of pathogenicity and exclude false positives; third, postnatal follow-up, which requires formulating individualized follow-up protocols, dynamically observing phenotypic development and adjusting the interpretive conclusions of VUS as appropriate. At the same time, ethical considerations should be emphasized: in cases of non-lethal fetal malformations or uncertain VUS results, it is essential to balance the disclosure of genetic information with the anxiety of family members, avoid excessive medical interventions, respect the right to autonomous decision-making, protect patient privacy, and integrate objectivity with humanistic care in clinical practice.

This study has several limitations. First, the sample size was relatively small; future studies should expand the sample size to include more cases with different types of fetal skeletal dysplasia. Second, a definitive diagnosis could not be made for some fetuses even after multiple genetic tests. Combined use of karyotype analysis, chromosomal microarray analysis (CMA) and whole-exome sequencing (WES) still suffers from incomplete genomic coverage, and whole-genome sequencing (WGS) was not performed for cases with negative WES results. Third, some skeletal system anomalies do not constitute severe birth defects, which gives rise to ethical dilemmas during genetic counseling, and the specific implications of such cases warrant further investigation and discussion.

Conclusion

Whole-exome sequencing technology holds important application value in the prenatal diagnosis of fetal skeletal system diseases, and provides a more accurate evidence base for genetic counseling and clinical decision-making.

Ethics Approval and Consent to Participate

This study was approved by the Institutional Review Board (IRB) of Liuzhou Maternal and Child Care Service Centre (Approval Number: IRB-2022-025; Approval Date: July 25, 2022) and conducted in strict accordance with the

principles of the Declaration of Helsinki (2013 revision) and relevant international ethical guidelines for human research. Prior to study enrollment, all participants (or their legal guardians, for minors under 18 years old) were provided with a detailed written explanation of the study objectives, procedures, potential risks (e.g., minimal discomfort from blood sampling), anticipated benefits, and data usage policies. All participants voluntarily signed a written informed consent form after confirming they fully understood the study details and had no remaining questions. Participants retained the right to withdraw from the study at any time without penalty or impact on their subsequent medical care. All personal and research data were de-identified (e.g., replaced with unique study IDs) and stored in password-protected servers to ensure participant privacy and data security.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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The authors declare no competing interests in this work.

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