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Clinical utility of trio whole exome sequencing in fetuses with ultrasound anomalies

Ziye Zeng[1,2†], Lan Zhang[1,2†], Yuqin Zhou[1,2], Xue Zhang[1,2], Hong Yi[1,2], He Li[1,2], Yuqi Liu[1,2], Jian Li[1,2], Qian Chen[1,2], Yulin Chen[1,2], Guiming Yu[1,2], Jing Yi[3], Yana Zhang[4], Hua Zhang[1,2*] and Yanling Dong[1,2*]

Abstract

Introduction Ultrasound scanning anomalies in fetuses are a cause for concern and often necessitate further diagnostic procedures. This retrospective study evaluated the utility of trio whole exome sequencing (trio-WES) in the diagnosis of fetuses with ultrasound anomalies.

Methods We included fetuses diagnosed with fetal ultrasound anomalies referred to the First Affiliated Hospital of Chongqing Medical University between November 2018 and July 2023. Fetal anomalies were classified into structural anomalies, dynamic anomalies, and soft markers. Karyotype analysis, chromosomal microarray analysis (CMA) or copy number variation sequencing (CNV-seq) and trio-WES were performed for the eligible cases. Perinatal outcomes were recorded and evaluated at postnatal follow-up.

Results A total of 316 fetuses were included for the analysis, including 199 (63.0%) cases with structural abnormalities, 63 (19.9%) cases with dynamic abnormalities, and 54 (17.1%) fetuses with ultrasonic soft markers. The diagnostic yield of karyotyping and CMA/CNV-seq was 4.1% (13/316), and Trio-WES achieved an additional diagnosis rate of 15.8% (50/316). Pathogenic or likely pathogenic alleles (P/LP) variants of 132 genes were identified in 125 (39.6%, 125/316) cases, and variant of uncertain significance (VUS) was detected in 81 samples (25.6%, 81/316). Ten cases (3.2%, 10/316,) were found to have pathogenic karyotype or CNVs in supplementary analysis of WES. Fetuses presenting musculoskeletal anomalies and multiple anomalies demonstrated highest diagnostic rates at 36.4% (8/22) and 36.1% (13/36), respectively. The diagnostic rate of fetuses with short femur was 20% (8/40), significantly higher than other ultrasonic soft markers. The modes of inheritance observed in patients with molecular diagnoses were autosomal dominant (AD) in 66.0% cases (33/50), autosomal recessive (AR) in 26.0% cases (13/50), and X-linked (XL) in 8.0% cases (4/50).

Conclusion The integration of CMA/CNV-seq with trio-WES, alongside prenatal ultrasound scanning, holds the promise of significantly enriching our ability to decipher fetal phenotypes. This tripartite approach stands to revolutionize the diagnostic process, offering a more comprehensive and nuanced understanding of the underlying genetic architecture that underpins prenatal anomalies.

†Ziye Zeng and Lan Zhang contributed equally to this work.

*Correspondence:
Hua Zhang
zh2844@gmail.com
Yanling Dong
yanlingdong@hospital.cqmu.edu.cn

Full list of author information is available at the end of the article

Keywords Trio whole exome sequencing, Structural anomalies, Dynamic anomalies, Soft markers, Prenatal diagnosis

Introduction

Fetal abnormalities, affecting 3% of all pregnancies and contributing to around 20% of all perinatal deaths, encompass diverse chromosomal and genetic abnormalities, including aneuploidy, copy number variations (CNVs) and gene-specific pathogenic variants [1–3]. Prenatal ultrasound scanning (USS) of the fetus plays a crucial role in identifying anomalies, ranging from minor defect to severe multisystem disorder, with the detection efficiency from 15 to 85% [4, 5].

Structural anomalies involve non-transient anatomic alterations of organs or limbs development. Nonspecific and often transient minor USS findings, termed "soft markers", include increased NT, short femur, echogenic intracardiac focus, mild ventriculomegaly, enlarged cisterna magna, choroid plexus cysts, echogenic bowel, mild hydronephrosis, absent/hypoplastic nasal bone and single umbilical artery [6, 7]. Anomalies categorized as "dynamic" may regress or worsen during pregnancies including changes in amniotic fluid (anhydramnios, oligohydramnios and polyhydramnios), fetal growth restriction (FGR), effusions (hydrops, pleural or pericardial effusions, ascites, hydroderma) and cystic hygroma [8].

For many years, the standard approach to prenatal diagnosis has involved the use of fetal ultrasound to identify pathogenic abnormalities, followed by conventional karyotyping and/or chromosomal microarray analysis (CMA) using samples from amniotic fluid, chorionic villi, or cord blood DNA. The integration of CMA into prenatal testing has markedly increased the detection rate of chromosomal abnormalities by approximately 13% [9–11]. Array-based molecular cytogenetic analyses, especially CMA, are capable of detecting smaller chromosomal imbalances, such as CNVs, with a resolution down to 50 Kb [12]. In more recent times, the development of whole-exome sequencing (WES) and whole-genome sequencing (WGS) has significantly enhanced diagnostic yields [13–15]. Trio-WES, which includes sequencing the proband and both parents, is recognized for its effectiveness and has contributed to a higher detection rate in prenatal diagnosis [16]. Numerous studies have underscored the value of large-scale sequencing within proband-parent trios as an effective first-line diagnostic tool, influencing clinical decision-making, especially in neonatal and pediatric populations [17, 18]. Consequently, WES is significantly bolstering the arsenal of prenatal diagnostic tools, thereby elevating the standard of care in routine obstetric practice.

However, certain loci bear the dubious distinction of being classified as Likely Pathogenic (LP) or variants of uncertain significance (VUS), presenting interpreters and counselors with a formidable challenge. When ultrasound findings hint at a genetic predisposition to abnormality, the diagnostic matrix is augmented, facilitating a more nuanced understanding. This retrospective inquiry was concerned with the clinical trajectory of 316 fetuses whose ultrasound scans revealed discrepancies, ultimately submitting to trio-WES for a comprehensive molecular genetic appraisal. Such procedures underscore the indispensable role of molecular genetic testing in the armamentarium of prenatal medicine.

Materials and methods

Subjects recruitment

This retrospective cohort study encompassed fetuses exhibiting ultrasound scanning anomalies, who were enrolled at the First Affiliated Hospital of Chongqing Medical University between November 2018 and July 2023. The fetuses with ultrasound abnormalities, subjected to conventional karyotyping, CNV-seq, or CMA, concurrently underwent trio-WES. DNA from the fetuses was extracted from amniotic fluid, cord blood, or cardiac blood, while peripheral blood samples from the parents were analyzed via WES. The study conformed to the principles of the Declaration of Helsinki, obtaining approval from the Institutional Review Board and Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (K2023-580).

Karyotyping analysis

G-banded using trypsin-Giemsa staining for karyotyping following a series of standard protocols. Chromosome karyotype map scanning and acquisition were done using an automatic metaphase chromosome analysis system (GSL-120: Leica Microsystems, Deerfield, IL, USA). Karyotypes were defined according to the international system of Human Cytogenetic Nomenclature (ISCN 2020).

CNV sequencing (CNV-Seq)

Genomic DNA of amniotic fluid cells was extracted using MagPure Buffy Coat DNA Midi KF Kit (Magen, Guangzhou, China) following the manufacturer's instructions. Fifty ng of amniocyte DNA was fragmented with an average size of 150 to 200 bp and DNA libraries were constructed by end repair, ligated with sequencing adaptors, and the modified fragments were amplified by polymerase chain reaction (PCR). The library was enriched by array hybridization using the MGIEasy Exome Capture V4 Probe Set (MGI, Shenzhen, China), followed by elution and postcapture amplification. The products were then analyzed on an Agilent 2100 Bioanalyzer to

estimate the magnitude of enrichment. DNA libraries were sequenced on the BGISEQ-500 (BGI, Shenzhen, China). Sequencing results from each sample were mapped to the human genome reference hg19 and updated to the human genome reference GRCh38 (hg38) with DECIPHER database according to the ISCN 2020. The identified and mapped CNVs were interpreted according to publicly available databases, including the Database of Genomic Variants (DGV); Online Mendelian Inheritance in Man (OMIM); DECIPHER, University of California, Santa Cruz (UCSC); and PubMed. Their pathogenicity was assessed according to the guidelines outlined by the American College of Medical Genetics (ACMG) for the interpretation of copy number variants [19]. CNVs were interpreted and divided into five categories: pathogenic, likely pathogenic, VUS, likely benign, and benign. Following the analysis of trio whole exome sequencing data, all identified variants were curated and submitted to the China National GeneBank Database (CNGBdb; http://www.cngb.org/). This step was taken to contribute to the global genetic variation database and to allow for the potential replication of our findings by other researchers.

Chromosome microarray analysis (CMA)

Genomic DNA was extracted from peripheral blood or amniotic fluid cells of the pregnant woman using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The Infinium Global Screening Array (Illumina, San Diego, CA), containing ~ 700 000 markers, genome-wide marker SNPs and markers targeting all regions of known cytogenetic significance, was used for whole genome scanning. The array was scanned using the iScan microarray scanning system (Illumina, San Diego, CA). Molecular karyotype analysis was performed using KaryoStudio 1.4.3.0 Build 37 software (Illumina, San Diego, CA). Raw data were uploaded to KaryoStudio 1.4.3.0 Build 37 software (Illumina, San Diego, CA) and log R ratios and BAFs were calculated by to a reference 'cluster' generated from a set of 2000 ~ 3000 clinical samples. For each sample, primary analysis consisted of visual inspection of individual chromosome log R ratios and BAF profiles. Automated detection of copy number alterations was performed using KaryoStudio 1.4.3.0 Build 37 software. All identified abnormalities were further characterized by visual inspection of the chromosome log R and BAF plots. CNV was classified according to the ACMG/ClinGen guidelines as: (i) pathogenic (P), (ii) Likely Pathogenic (LP), (iii) Variants of Uncertain Significance (VUS), (iv)Likely Benign (LB), (v) Benign. Following the identification of all variants, including P, LP, and VUS, a comprehensive post-test genetic counseling session was conducted with the families. During these sessions, all variants were reported, and their implications were discussed in depth. Genetic counselors employed clear and empathetic communication to assist families in understanding the findings and to explore their options for further management and reproductive planning.

Bioinformatics

We evaluated the chromosomal region using information from the Online Mendelian Inheritance in Man database (OMIM, http://omim.org/), the DECIPHER da tabase (http://decipher.sanger.ac.uk), the UCSC database (http://genome.ucsc.edu), the DGV database (http://dgv.tcag.ca/dgv/app/home), and the ClinGen database (http://dosage.clinicalgenome.org/).

Whole-exome sequencing (WES)

Five milliliters of venous blood were taken from each of the participants, and genomic DNA was extracted according to the manufacturer's standard procedure for the MagPure Buffy Coat DNA Midi KF Kit (Magen, Guangzhou, China). Genomic DNA was fragmented using Segmentase (BGI, Shenzhen, China) to generate small DNA fragments (e.g., 100–500 bp), which were passed over magnetic beads to enrich the fragments that were 280–320 bp long. The ends were filled in, and then an "A" base was added to the 3' end to allow the DNA fragment to be ligated to an adapter bearing a "T" base at the 3' end. The resulting DNA fragments were amplified by ligation-mediated polymerase chain reaction, purified, and then used to create a library. The library was enriched by array hybridization using the MGIEasy Exome Capture V4 Probe Set (MGI, Shenzhen, China), followed by elution and postcapture amplification. The products were then analyzed on an Agilent 2100 Bioanalyzer to estimate the magnitude of enrichment. DNA libraries were sequenced on the MGIseq-2000 platform (BGI, Shenzhen, China) with an average depth of ~120X (PE100). For the original sequencing data, we removed unqualified sequences from the primary data using a local dynamic programming algorithm to generate "clean reads"0.31 This included low-quality reads, adapter sequences, and reads containing more than 10% Ns, 50% reads with a quality value of less than 5, and an average quality of less than 10. The "clean reads" were then aligned to the human genome reference (hg19) using Burrows Wheeler Aligner (BWA) software, which was followed by the removal of PCR duplicates with the Picard tool, local indel realignment, base quality score recalibration, and joint variant calling with GATK Haplotype Caller. We applied the Variant Quality Score Recalibration (VQSR) method to filter out potential low-quality variants with the default datasets and used the parameters recommended by the GATK toolkit. SNPs and indels occurring in exons and canonical splice sites were further analyzed. Following the guidelines of the

American College of Medical Genetics (ACMG), pathogenic genetic variants (P), likely pathogenic genetic variants (LP), and variants of unknown significance (VUS) were recorded and documented. The positive results of our study included pathogenic genetic variants and likely pathogenic genetic variants. Sanger sequencing was performed to confirm all diagnostic genetic variants. In addition, supplementary CNV analysis based on WES data was performed to detect the aneuploidy, triploidy, chromosome copy number variation (≥ 1 Mb), and loss of heterozygosity (≥ 5 Mb).

Clinical indication categories

Phenotypes observed by ultrasound were standardized using Human Phenotype Ontology (HPO) terms. Abnormalities of different systems were identified. Fetuses were categorized as having a single systematic abnormality or multiple systematic abnormalities. Specific districts for structural anomalies were: central nervous system (CNS), musculoskeletal, genitourinary system, digestive system, cardiovascular system, thorax/respiratory/chest, and craniofacial.

Subsoft markers were increased NT (NT > 3.5 mm), short femur, echogenic intracardiac focus, ventricular dilatation, enlarged cisterna magna, choroid plexus cysts, echogenic bowel, pyelectasis, mild hydronephrosis, absent/hypoplastic nasal bone and single umbilical artery.

Subcategories for dynamic anomalies were amniotic anomalies (polyhydramnios and oligohydramnios), fetal growth restriction (FGR), effusions and hydrops fetalis.

Clinical follow-up

Pregnancy outcomes were classified as live birth, postnatal anomalies, postnatal death, termination of pregnancy (TOP), selective reduction of affected twin, and not available (refusal of follow-up, loss of follow-up and unknown). We perform a clinical follow-up at evaluation six months following birth via electronic medical records or telephone calls as well as routine follow-up assessments annually. If abnormalities were found at follow-up examinations, we would recommend further evaluation by a pediatrician.

Statistical analysis

Statistical analysis was performed using the Pearson chi-square test or Fisher's exact test with SPSS software, version 25.0 (IBM). A p-value < 0.05 was considered statistically significant.

Results

Sample characteristics

A total of 346 fetuses with ultrasound scanning anomalies were referred for an invasive prenatal diagnostic test. Twenty-two cases with insufficient clinical data were excluded, 3 families who refused WES testing and 5 families who did not undergo prenatal WES testing were excluded. Ultimately, 316 families were enrolled and underwent karyotype analysis, CMA/CNV-seq, and trio-WES simultaneously (Fig. 1). Demographic characteristics and prenatal phenotypes were provided in Table 1. The median maternal and paternal age of all pregnancies was 29 (IQR, 27–33) and 31(IQR, 28–34) years respectively, and the median week of gestation was 25.1(IQR, 23.4–29.3) weeks. Among the sources of fetal DNA, 298 (94.3%) samples were from amniotic fluid, 10 (3.2%) samples were from umbilical cord blood, and 8 samples (2.5%) were from cardiac blood. With regard to prenatal phenotype at enrollment, 199 (63.0%) cases had structural anomalies, 63 (19.9%) cases had dynamic anomalies, and 54 (17.1%) fetuses had soft markers. The most frequent phenotype was the FGR (52/316, 16.5%), followed by the genitourinary system anomalies (50/316, 15.8%), short femur (40/316, 12.7%), and multiple systems anomalies (36/316, 11.4%), altogether comprising more than half of all cases.

Diagnostic yields

Of the 316 cases in which karyotyping and CMA/CNV-seq were performed, abnormal karyotypes were detected in 5 cases and pathogenic CNVs were detected in 8 cases. The total diagnostic yield of karyotyping and CMA/CNV-seq was 4.1% (13/316). The case results were shown in supplementary Table 1.

In trio-WES analysis, 158 P/LP variants of 132 genes were identified in 125 cases, representing an 39.6% (125/316) detection rate. Eighty-one cases (25.6%, 81/316) were identified with VUS, involving 96 genes and 142 variants (Supplementary Table 2). Ten cases (10/316, 3.2%) were found to have pathogenic karyotype or CNVs in supplementary analysis of WES. Within the detection range of the WES, 100 (31.6%) cases had negative results and no relevant variants were detected.

Sixty variants, including 18 *de novo* variants, meeting the molecular diagnostic criteria were detected in 50 of the 316 samples. Thirty-seven of these variants have not been reported previously. The overall additional diagnostic rate of WES was 15.8% (50/316, Table 2). There were 42 genes involved in the positive cases, including *FGFR3*, *COL2A1*, *DMD*, *TSC2*, *TBX5*, *COL9A2*, *CREBBP*, *RBM10*, *PEX1*, *FLNB*, *TREX1*and others (Fig. 2, Fig. 4).

Particularly, the c.1138G > A variant was identified within the *FGFR3* gene (MIM: 134934) in three instances, whereas one case harbored the NM_000142.4:c.1123G > T variant. Alterations in the *FGFR3* gene have the potential to engender Achondroplasia (OMIM:100800), Thanatophoric dysplasia, type I (OMIM:187600) or Thanatophoric dysplasia, type II

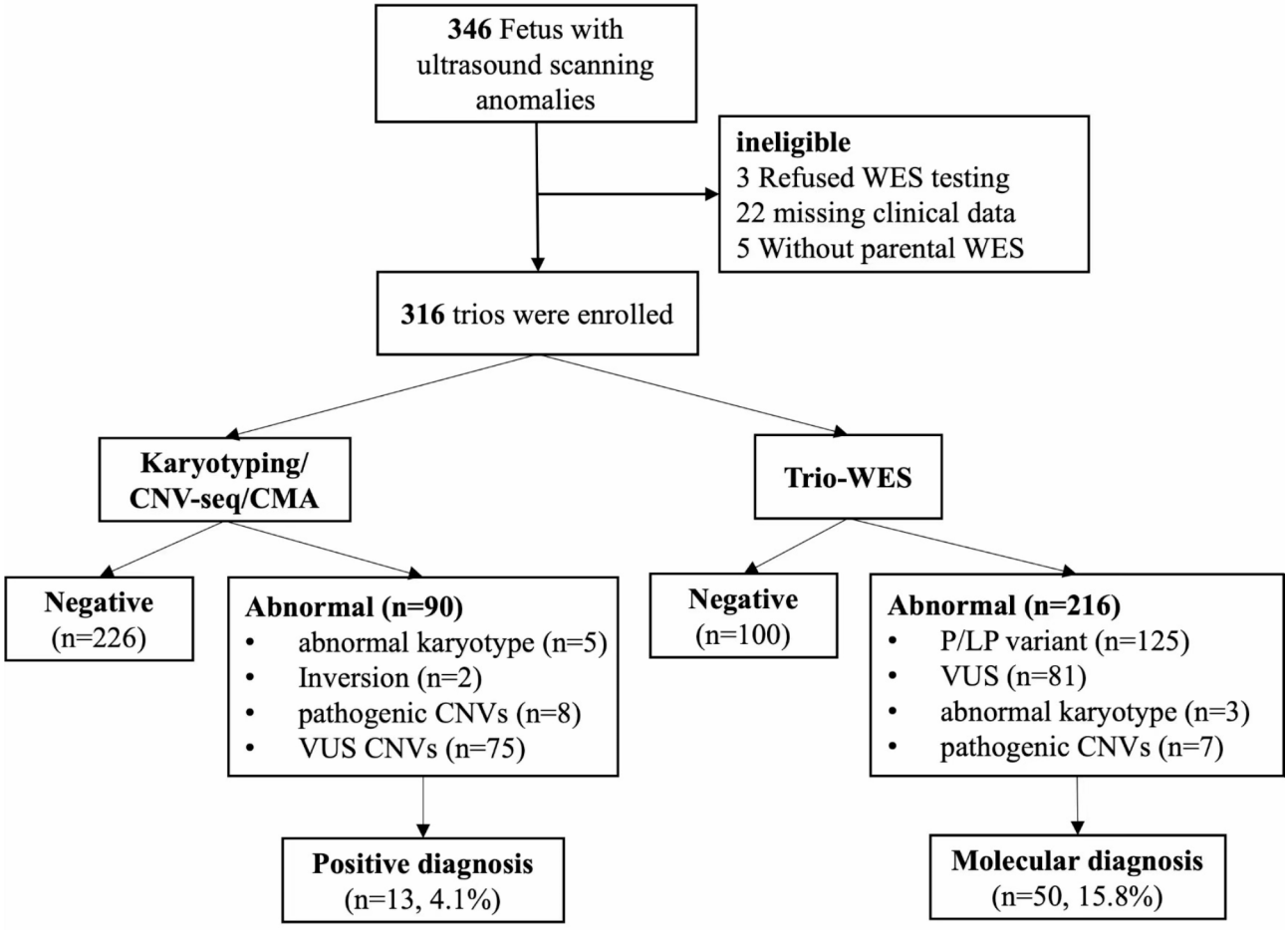

Fig. 1 Flowchart of genetic analysis progression

(OMIM:187601). Achondroplasia is the most prevalent form of chondrodysplasia. Those afflicted exhibit rhizomelic limb shortening, macrocephaly, and distinctive facial characteristics, including frontal bossing and midface retrusion [20, 21]. The fetus under investigation manifested with a short femur, a condition that may have arisen due to the detected variant in *FGFR3*. In another case, the fetus carried a paternal variant in the *COL2A1* gene (NM_001844.4: c.1249G > A), with Achondrogenesis type 2 being caused by heterozygous variants in *COL2A1*, an autosomal dominant (AD) disorder. The father of this fetus indeed exhibited symptoms of short stature. Consequently, the findings from WES were in concordance with the clinical diagnosis and the established inheritance pattern.

Clinical indication categories

Clinical features were converted into standard HPO terms for all cases to facilitate the genotype phenotype correlation analysis. A disease gene was considered potentially relevant to the fetal anomalies if its associated clinical phenotypes meet one of the following criteria: (1) match HPO entry of the fetal phenotype; (2) match the superclass based on HPO or clinical synopsis in OMIM database; (3) be reported in previous cases manifesting the same or similar phenotypes of the fetuses.

The prevalence of genetic diagnoses exhibited variability, contingent upon the anatomical systems affected. Notably, fetuses presenting musculoskeletal anomalies and multiple anomalies demonstrated highest diagnostic rates at 36.4% (8/22) and 36.1% (13/36), respectively, which were significantly higher than those in the other groups ($p<0.05$). A total of 29 fetuses harbored cardiovascular abnormalities, yielding a molecular diagnostic rate of 24.1% (7/29). The diagnostic rate for fetuses presenting with craniofacial abnormalities was 13.0% (3/23). Of the 28 fetuses demonstrating CNS abnormalities, a diagnosis was rendered in 3 instances (10.7%, 3/28). The digestive system presented a diagnostic rate of 11.1% (1/9), while the genitourinary system exhibited a rate of 4.0% (2/50). Fetuses with fetal growth restriction (FGR) demonstrated a rate of 9.6% (5/52). In instances where fetuses exhibited a short femur, the molecular diagnostic rate for this condition was 20.0% (8/40), as illustrated in Fig. 3.

Table 1 Demographic characteristics and phenotypic features

Characteristics		Value
Median maternal age (IQR), years		29 (27–33)
Median paternal age (IQR), years		31 (28–34)
Median gestational age (IQR), weeks		25.1 (23.4–29.3)
Sample types, *n* (%)		
	amniotic fluid	298 (94.3%)
	umbilical cord blood	10 (3.2%)
	cardiac blood	8 (2.5%)
Prenatal phenotype, *n* (%)		
structural anomalies		199 (62.97%)
	genitourinary system	50 (15.8%)
	Multiple systems	36 (11.4%)
	Cardiovascular system	29 (9.2%)
	CNS	28 (8.9%)
	Craniofacial	23 (7.3%)
	Musculoskeletal system	22 (7.0%)
	Digestive system	9 (2.8%)
	Respiratory system	2 (0.6%)
soft markers		54 (17.1%)
	short femur	40 (12.7%)
	Increased NT	7 (2.2%)
	choroid plexus cysts	2 (0.6%)
	enlarged cisterna magna	1 (0.3%)
	pyelectasis	1 (0.3%)
	single umbilical artery	1 (0.3%)
	Multiple soft markers	2 (0.6%)
dynamic anomalies		63 (19.9%)
	FGR	52 (16.5%)
	effusions	6 (1.9%)
	polyhydramnios	3 (0.9%)
	hydrops	2 (0.6%)

IQR denotes interquartile range; CNS central nervous system; NT nuchal translucency; FGR fetal growth restriction

We were unsuccessful in conducting molecular diagnoses for fetuses experiencing 2 instances of respiratory system anomalies, 2 cases of hydrops fetalis, six cases of effusions, 3 cases of polyhydramnios and 14 cases of other soft markers. No additional diagnostic rates were achieved within these cohorts.

Mendelian inheritance

The modes of inheritance observed in patients with molecular diagnoses were autosomal dominant (AD) in 66.0% cases (33/50), autosomal recessive (AR) in 26.0% cases (13/50), X-linked (XL) in 8.0% cases (4/50) (Table 3). More than half of the autosomal dominant disorders were attributed to de novo variants (51.5%, 17/33). Eleven fetuses with autosomal recessive disorders harbored compound-heterozygous variants (84.6%, 11/13), whereas two fetuses (15.4%, 2/13) exhibited homozygous variants inherited from their parents, respectively. Three fetuses with XL disorders inherited the variant from their mothers. We discovered a variant, NM_000276.3:c.665-669delTCATC, in the *OCRL* gene (MIM: 300535). The fetus in case 122 exhibited congenital cataract, aligning with the clinical phenotypes of Lowe syndrome (OMIM: 309000) [22] Additionally, a de novo variant, NM_001039590.2:c.1104T > G, was identified in the *USP9X* gene, which belongs to the peptidase C19 family and encodes a protein analogous to ubiquitin-specific proteases. The *USP9X* gene has been implicated in Mental retardation, X-linked 99, syndromic, female-restricted (OMIM:300968). The fetus of case 286 displayed cataract and an Atrial septal defect on ultrasound.

Clinical follow-up

We successfully conducted follow-up on 284 (89.9%) of the 316 fetuses. Pregnancy outcomes were shown in Table 4. In case 9, the fetus was diagnosed with a de novo variant in the *FGFR3* gene, which is associated with achondroplasia, and subsequent termination was elected due to embryonic damage. In case 28, the fetus was diagnosed with a variant in the *COL2A1* gene, which could potentially lead to chondrodysplasia; however, the parents chose to continue the pregnancy. Postpartum,

Table 2 Fetal molecular diagnosis of WES

ID	Ultrasound Finding	Gene	RefSeq identifier	Variants	ACMG classification criteria	Reported previously	Type	Origin	Zygosity	Disease Association (OMIM)/inheritance pattern	Outcome
4	short femur	*FGFR3*	NM_000142.4	c.1138G > A	PS1 + PS2 + PS3 + PS4 + PM1 + PM2 + PP1 + PP2 + PP4	Yes	P	de novo	het	Achondroplasia (100800)/AD; Thanatophoric dysplasia, type I (187600)/AD; Thanatophoric dysplasia, type II (187601)/AD;	NA
8	cardiovascular	*TBX5*	NM_000192.3	c.1025delA	PVS1_Strong + PM2	unreported	LP	M (had a child with CHD who died)	het	Holt-Oramsyndrome (142900)/AD	NA
9	musculoskeletal	*FGFR3*	NM_000142.4	c.1138G > A	PM1 + PM2 + PP2 + PS2 + PS1 + PS4 + PS3 + PP1	Yes	P	de novo	het	Thanatophoric dysplasia, type I (187600)/AD; Thanatophoric dysplasia, type II (187601)/AD; Achondroplasia (100800)/AD	TOP
11	CNS	*CSPP1*	NM_024790.6	c.2527-2528delAT	PVS1 + PM2 + PM3	Yes	P	M	Com-het	Joubert Syndrome 21 (615636)/AR	ND
				c.2729-2731delinsTC	PVS1 + PM2	unreported	LP	F			
19	short femur	*FGFR3*	NM_000142.4	c.1138G > A	PS1 + PS2 + PS3 + PS4 + PM1 + PM2 + PP1 + PP2	Yes	P	de novo	het	Thanatophoric dysplasia, type I (187600)/AD; Thanatophoric dysplasia, type II (187601)/AD; Achondroplasia (100800)/AD;	TOP
27	short femur	*COL9A2*	NM_001852.3	c.406 C > T	PVS1 + PM2	unreported	LP	M	het	multiple Epiphyseal dysplasia 2 (600204)/AD	LB
28	short femur	*COL2A1*	NM_001844.4	c.1249G > A	PM1 + PM2 + PP2 + PP3	unreported	LP	F (affected)	het	Achondrogenesis type 2 (20061)/AD; Kniest dysplasia (156550)/AD; Spondyloepimetaphyseal dysplasia, Strudwick type (184250)/AD	LB
29	CNS	*CREBBP*	NM_004380.2	c.5837_5838insC	PVS1 + PS2 + PS4_Supporting	Yes	P	de novo	het	Menke-Hennekam syndrome 1 (618332)/AD; Rubinstein-Taybi syndrome 1 (180849)/AD	TOP
30	multiple systems	*ALG3*	NM_005787.5	c.490delG	PVS1 + PM2	unreported	LP	M	Com-het	Congenital disorder of glycosylation, type Id (601110)/AR	TOP
				c.932 + 2T > G	PVS1 + PM2	unreported	LP	F			
33	genitourinary	*TFAP2A*	NM_003220.2	c.154delG	PVS1 + PS2_Supporting + PM2	unreported	P	de novo	het	Branchio-oculo-facial syndrome (113620)/AD	TOP

Table 2 (continued)

ID	Ultrasound Finding	Gene	RefSeq identifier	Variants	ACMG classification criteria	Reported previously	Type	Origin	Zygosity	Disease Association (OMIM)/inheritance pattern	Outcome
37	multiple systems	*PBX1*	NM_002585.3	c.863G > A	PS2_Moderate + PM2 + PP2 + PP3	unreported	LP	de novo	het	Congenital renal and urinary tract anomalies, potentially associated with hearing loss, ear abnormalities, or developmental delay(617641)/AD	TOP
42	multiple systems	*PKHD1*	NM_138694.3	c.2854G > A	PM1 + PM2 + PM3_Strong + PP3 + PP4	Yes	P	F	Com-het	Polycystic kidney disease 4 with or without polycystic liver disease (263200)/AR	TOP
				c.4274T > G	PM2 + PM3_Strong + PP4	Yes	LP	M			
44	cardiovascular	*TSC1*	NM_000368.4	c.1639dupA	PVS1 + PS2 + PM2	unreported	P	de novo	het	Tuberous sclerosis-1 (191100)/AD	TOP
47	craniofacial	*RBM10*	NM_005676.4	c.1318C > T	PVS1 + PM2	unreported	LP	M	hemi	TARP syndrome (311900)/XL	TOP
68	multiple systems	*TREX1*	NM_033629.3	c.294-295insA	PVS1_Strong + PM2 + PM3 + PP1 + PP4	unreported	P	F	com-het	Aicardi-Goutieres syndrome 1 (225750)/AD, AR	TOP
				c.536T > C	PM2 + PM3_Strong + PP4	unreported	VUS	M			
70	musculoskeletal	*FLNB*	NM_001457.3	c.6840dupC	PVS1 + PM2	Yes	LP	F	com-het	Boomerang dysplasia (112310)/AD; Atelosteogenesis, type I (108720)/AD; Atelosteogenesis, type III (108721)/AD;	lost
				c.5134G > T	PM2	Yes	VUS	M			
72	digestive	*SBDS*	NM_016038.2	c.258 + 2T > C	PVS1 + PM3_Very Strong + PP	Yes	P	F	com-het	Shwachman-Diamond syndrome (260400)/AR	TOP
			NM_016038.2	c.637delG	PM2 + PVS1_Strong	unreported	LP	M			
90	CNS	*PEX1*	NM_000466.2	c.2080-2082delGAT	PM1 + PM2 + PM4	unreported	LP	M	com-het	Heimler syndrome 1 (234580)/AR; Peroxisome biogenesis disorder 1 A (214100)/AR; Peroxisome biogenesis disorder 1B (601539)/AR	TOP
			NM_000466.2	c.2927–2A > G	PVS1_Moderate + PM2 + PM3	unreported	LP	F			
92	cardiovascular	*DSC2*	NM_024422.3	c.2398-2399insG	PVS1_Strong + PM2	unreported	LP	F (The couple had a fetus with CHD)	het	Arrhythmogenic right ventricular dysplasia, familial, 11 (610476)/AD, AR	TOP
95	genitourinary	*TFAP2A*	NM_003220.2	EX1-EX7E Del	PVS1 + PM2 + PP1	Yes	P	M	het	Branchi-ooculo-facial syndrome (113620)/AD	LB
98	multiple systems	*DMD*	NM_004006.2	EX45-EX50DUP	PVS1_Strong + PS4 + PM2	Yes	P	M	hemi	Duchenne muscular dystrophy (310200)/XL	NA
101	FGR	*ODC1*	NM_002539.1	EX4-EX6 Dup	PVS1_Strong + PM2	unreported	LP	F	het	Neurodevelopmental disorder with alopecia and brain abnormalities (619075)/AD	TOP

Table 2 (continued)

ID	Ultrasound Finding	Gene	RefSeq identifier	Variants	ACMG classification criteria	Reported previously	Type	Origin	Zygosity	Disease Association (OMIM)/inheritance pattern	Outcome
103	musculoskeletal	*COL1A1*	NM_000088.3	c.869G > A	PS2 + PM2 + PP2 + PP3	unreported	LP	de novo	het	Osteogenesis imperfecta, type II (166210)/AD; Osteogenesis imperfecta, type I (166200)/AD; Osteogenesis imperfecta, type III (259420)/AD;	TOP
105	cardiovascular	*KMT2D*	NM_003482.3	c.1967delT	PVS1 + PM2	Yes	LP	de novo	het	Kabuki Syndrome 1 (147920)/AD	TOP
107	multiple systems	*PKD2*	NM_000297.3	c.2051-2054delACTC	PVS1 + PM2 + PP4	Yes	P	M	het	Polycystic kidney disease 2 (613095)/AD	TOP
112	multiple systems	*ABCA12*	NM_173076.2	c.6116delT	PVS1 + PM2	unreported	LP	M	Com-het	Ichthyosis, congenital, autosomal recessive 4 A (601277)/AR	NA
				c.2563-2570delinsGGCAATT	PVS1 + PM2	unreported	LP	F			
118	multiple systems	*DHCR7*	NM_001360.3	c.1210C > T	PM1 + PM2 + PM3_Very Strong + PP2 + PP3 + PP4	Yes	P	F&M	hom	Smith-Lemli-Opitz syndrome (270400)/AR	TOP
122	craniofacial	*OCRL*	NM_000276.3	c.665-669delTCATC	PVS1 + PM2	unreported	LP	M	hemi	Lowe syndrome (309000)/XL	TOP
141	cardiovascular	*FBXW11*	NM_000505.3	c.1340G > A	PS2 + PM2 + PP2 + PP3	Yes	LP	de novo	het	Neurodevelopmental, jaw, eye, and digital syndrome (618914)/AD	TOP
142	musculoskeletal	*COL2A1*	NM_001844.4	c.2131G > A	PM2 + PM5 + PP2 + PP3	unreported	LP	F	het	Achondrogenesis type 2 (200610)/AD; Kniest dysplasia (156550)/AD; Spondyloepimetaphyseal dysplasia, Strudwick type (184250)/AD	LB
145	FGR	*LZTR1*	NM_006767.3	c.1149 + 1G > T	PVS1_Moderate + PM2 + PM3 + PP4	Yes	LP	M	het	Schwannomatosis 2 (615670)/AD (Typified by incomplete penetrance); Noonan syndrome 10 (616564)/AD; Noonan syndrome 2 (605275)/AD, AR;	LB
146	FGR	*LZTR1*	NM_006767.3	c.1555delC	PVS1 + PM2	unreported	LP	F	het	Schwannomatosis 2 (615670)/AD (Typified by incomplete penetrance); Noonan syndrome 10 (616564)/AD; Noonan syndrome 2 (605275)/AD, AR;	NA
151	cardiovascular	*TBX1*	NM_080647.1	EX1-EX3Dup	PVS1_Strong + PM2	unreported	LP	de novo	het	Tetralogy of Fallot (187500)/AD; Digeorge syndrome (188400)/AD; Velocardiofacial syndrome (192430)/AD	TOP

Table 2 (continued)

ID	Ultrasound Finding	Gene	RefSeq identifier	Variants	ACMG classification criteria	Reported previously	Type	Origin	Zygosity	Disease Association (OMIM)/inheritance pattern	Outcome
152	cardiovascular	*TSC2*	NM_000548.3	c.1432C > T	PVS1 + PS2 + PM2 + PP4	Yes	P	de novo	het	Tuberous sclerosis-2 (613254)/AD	TOP
156	FGR	*COL1A2*	NM_000089.3	c.1089 + 1G > A	PVS1 + PM2	unreported	LP	M	het	Osteogenesis imperfecta, type II (166210)/AD; Osteogenesis imperfecta, type IV (166220)/AD; Osteogenesis imperfecta, type III (259420)/AD;	LB
181	multiple systems	*SUMF1*	NM_182760.3	c.8 C > T	PM2 + PM3 + PP2 + PP	unreported	LP	F	Com-het	Multiple sulfatase deficiency (272200)/AR	TOP
				c.270G > C	PM1 + PM2 + PM3 + PP2 + PP3	unreported	LP	F			
				EX8-EX9EDel	PVS1_Strong + PM2 + PM3_Supporting + PP4	Yes	LP	M			
195	FGR	*COMP*	NM_000095.2	c.1042-1043dupTG	PVS1 + PM2	unreported	LP	M (history of adverse pregnancy)	het	Pseudoachondroplasia (177170)/AD; Carpal tunnel syndrome 2 (619161)/AD; Epiphyseal dysplasia, multiple, 1 (132400)/AD	TOP
197	musculoskeletal	*COL1A2*	NM_000089.3	c.1819G > A	PVS1 + PS2_Supporting + PM2	Yes	P	de novo	het	Osteogenesis imperfecta, type II (166210)/AD; Osteogenesis imperfecta, type IV (166220)/AD; Osteogenesis imperfecta, type III (259420)/AD;	TOP
198	multiple systems	*ZMIZ1*	NM_020338.3	c.2182G > T	PVS1 + PS2_Supporting + PM2	unreported	P	de novo	het	Neurodevelopmental disorder with dysmorphic facies and distal skeletal anomalies (618659)/AD	TOP
238	short femur	*FGFR3*	NM_000142.4	c.1123G > T	PS2_Moderate + PS3 + PS4_Supporting + PM2 + PP4	Yes	P	de novo	het	Achondroplasia (100800)/AD; Thanatophoric dysplasia, type I (187600)/AD; Thanatophoric dysplasia, type II (187601)/AD;	TOP
240	short femur	*NALCN*	NM_052867.2	c.1660G > T	PVS1 + PM2	unreported	LP	F	com-het	Hypotonia, infantile, with psychomotor retardation and characteristic facies 1 (615419)/AR; Congenital contractures of the limbs and face, hypotonia, and developmental delay (616266)/AD	LB
				c.620G > A	PM2 + PM3_Supporting + PP2 + PP3	unreported	VUS	M			

Table 2 (continued)

ID	Ultrasound Finding	Gene	RefSeq identifier	Variants	ACMG classification criteria	Reported previously	Type	Origin	Zygosity	Disease Association (OMIM)/inheritance pattern	Outcome
248	craniofacial	*CTNND1*	NM_001085458.1	c.1902_1903insA	PVS1 + PM2	unreported	LP	F (affected)	het	Blepharocheilodontic syndrome 2 (617681)/AD	TOP
254	musculoskeletal	*LBR*	NM_002296.4	c.1757G > A	PM2 + PM3_Strong + PP3	Yes	LP	F&M	hom	Greenberg skeletal dysplasia (215140)/AR; Rhizomelic skeletal dysplasia with Pelger-Huet anomaly (618019)/AR; Reynolds syndrome (613471)/AD; Pelger-Huet anomaly(169400)/AD	TOP
275	musculoskeletal	*SALL4*	NM_020436.3	c.375delC	PVS1 + PM2	unreported	LP	M (affected)	het	Duane-Radial ray syndrome (607323)/AD; IVIC syndrome (147750)/AD;	TOP
280	short femur	*NPR2*	NM_003995.3	c.844 C > T	PVS1 + PM2 + PM3	Yes	P	F	het	Short stature with nonspecific skeletal abnormalities (616255)/AD; Epiphyseal chondrodysplasia, Miura type (615923)/AD	LB
284	musculoskeletal	*EVC2*	NM_147127.4	c.450 + 2T > C	PVS1 + PM2	unreported	LP	M (History of adverse pregnancy)	het	Weyers acrofacial dysostosis (193530)/AD	NA
286	multiple systems	*USP9X*	NM_001039590.2	c.1104T > G	PVS1 + PS2_Supporting + PM2	unreported	P	de novo	het	Mental retardation, X-linked 99, syndromic, female-restricted (300968)/XL	TOP
288	multiple systems	*FOXC2*	NM_005251.2	c.223dupT	PVS1_Strong + PM2	unreported	LP	de novo	het	Lymphedema-Distichiasis syndrome (153400)/AD	TOP
301	short femur	*COL2A1*	NM_001844.4	c.1142G > T	PS2_Moderate + PM1 + PM2 + PP	unreported	LP	de novo	het	Achondrogenesis type 2 (20061)/AD; Kniest dysplasia (156550)/AD; Spondyloepimetaphyseal dysplasia, Strudwick type (184250)/AD	TOP

Table 2

305	multiple systems	*ABCC6*	NM_001171.5	c.4279G > A c.4404-1G > A	PVS1 + PM2 + PM3 PM2 + PM3_Strong + PP3	Yes Yes	LP LP	M F	Com-het	Arterial calcification, generalized, of infancy, 2 (614473)/AR; Pseudoxanthoma elasticum, forme fruste (177850)/AD	NA

NA: Not available; LB: Live birth; ND: neonatal death; AD, autosomal dominant; AR, autosomal recessive; XL, X-linked; F: inherited from father; M: inherited from mother; Hemi, hemizygous; Het, heterozygous; Hom, homozygous; LP, likely pathogenic; P, pathogenic; TOP: termination of pregnancy; CHD: congenital heart disease

the fetus experienced hypoxia and subsequently developed achondroplasia and an atrial septal defect. In case 85, a variant in the *PKD1* gene was detected in the fetus, which is associated with polycystic nephropathy. Due to the presence of renal anomalies detected during fetal development, the pregnancy was concluded via induced labor at the 34th week, a decision made voluntarily by the parents.

Discussion

This retrospective study, employing trio-WES, scrutinized fetuses that presented with structural anomalies, soft markers, or dynamic anomalies detected via ultrasound during ongoing pregnancies. The overall additional diagnostic rate achieved was 15.8% (50/316), a figure that resonates with the rates reported in several analogous studies, which have documented rates ranging from 10 to 32% [11, 23, 24]. A diverse array of molecular diagnoses was rendered across various anatomical systems, with a predilection for musculoskeletal system (36.4%, 8/22), the multisystem anomalies (36.1%, 13/36) and cardiovascular system (24.1%, 7/29). The lowest yields were for fetuses with genitourinary anomalies (4.0%) and respiratory anomalies (0.0%). Our results demonstrated that the diagnostic yield varied significantly among different phenotypic groups and generally aligned with the range documented in the literature [8]. In most cases, WES was not typically the top choice for isolated soft markers of ultrasound abnormalities. Significantly, the study found that the diagnostic rate for isolated short femur, at 20.0%, was notably higher than other isolated soft markers. It might suggested a need to categorize anomalies of short femur separately from traditional soft markers or to incorporate them into the classification of skeletal system abnormalities during the prenatal diagnosis. Moreover, supplementary analysis of WES unearthed 3 instances of abnormal karyotype and identified 7 cases of pathogenic CNVs. Germline genomic variants encompass a range of types such as chromosome aneuploidies, micro-deletion/duplication, intragenic CNVs, and SNV/InDels. Several studies have indicated that pathogenic or likely pathogenic CNVs and SNV/InDels can be identified concurrently in a subject [25]. In this study, two fetuses (case 70 and case 98) that diagnosed with pathogenic CNVs by chromosome analysis were also found to carry inherited pathogenic gene variants in subsequent WES testing. Failing to consider genetic abnormalities alongside chromosomal abnormalities could potentially increase the risk of fetal abnormalities in future pregnancies. The purpose of prenatal diagnosis is to offer appropriate counseling to prospective parents in order to make informed choices about the ongoing pregnancy, which may also relate to future postpartum management and future fertility decisions. Genetic diseases are diagnosed primarily according to

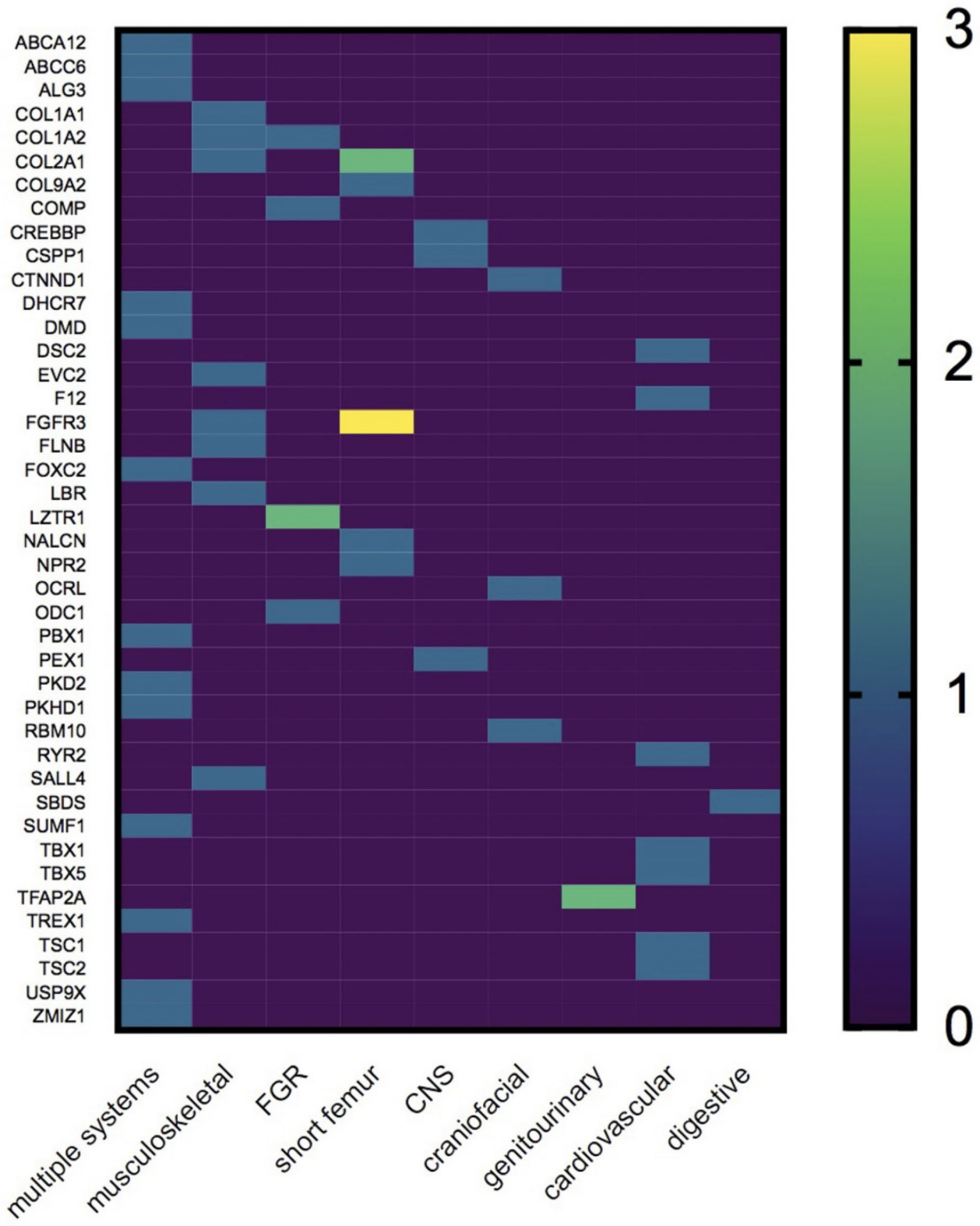

Fig. 2 Molecular diagnostic rates in different type of abnormalities

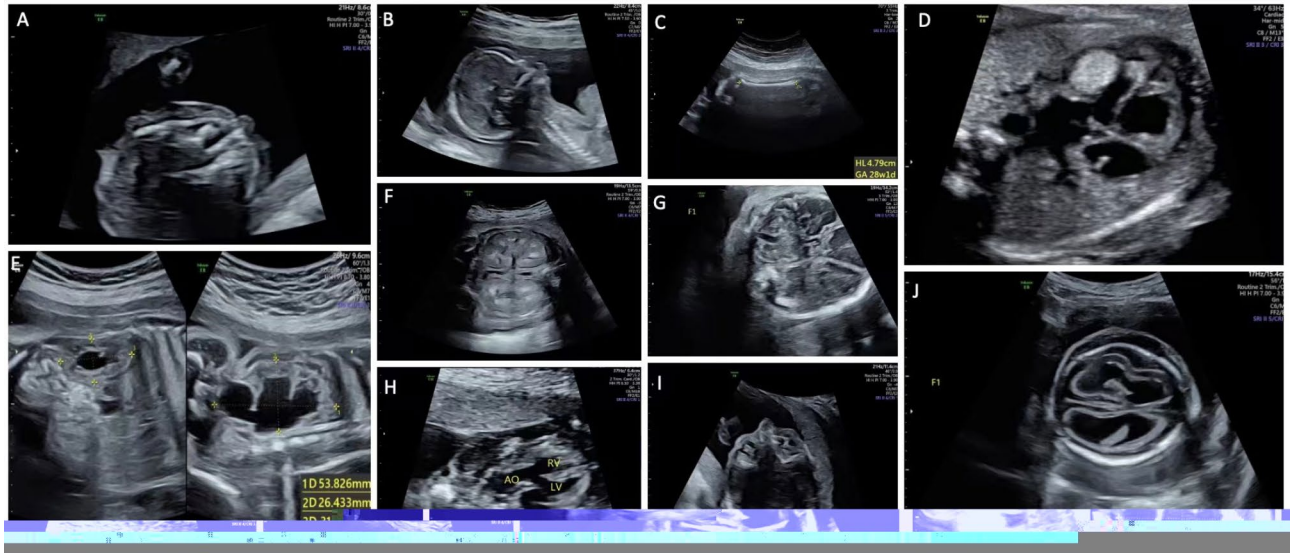

Fig. 3 Ultrasound images of foetal abnormalities. (**A**) 17w cleft lip and palate; (**B**) 18w small mandibular deformity; (**C**) $36w^{4d}$ long bones short for 8 weeks; (**D**) $27w^{5d}$ rhabdomyosarcoma (multiple); (**E**) 26w unilateral hydronephrosis; (**F**) 35w rhabdomyosarcoma; (**G**) 32-35w Blake's cyst (this image shows a normal sized cerebellar earthworm); (**H**) 22w Preeclamptic heart disease (Act IV) with generalised oedema (thickening of the skin, pericardial effusion), intravenous catheter, A-wave reversal, this case is a recipient in TAPS; (**I**) 28-30w Congenital cataract with permanent vitreous hyperplasia, this image shows intra-lens cataract and permanent vitreous hyperplasia; (**J**) $24w^{+3d}$ Pleural and abdominal effusion Skin oedema Cerebral oedema Cerebral sulcus gyrus shallowing Cerebral sulcus gyrus widening Fissure widening Enlarged heart (in a recipient of TTTS with heart failure Total body oedema including cerebral oedema)

Table 3 Inheritance pattern of genes and variants of molecular diagnoses

Mode of Inheritance	Number of Diagnoses	Percent of Diagnoses (%)
AD		66.0%
De novo	17	
Inherited/Mother	9	
Inherited/Father	7	
AR		26.0%
Compound heterozygous	11	
Homozygous	2	
XL		8.0%
De novo	1	
Inherited/Mother	3	

AD, autosomal dominant; AR, autosomal recessive; XL, X-linked

Table 4 Pregnancy outcomes

Pregnancy outcome, *n* (%)	All pregnancies (*n* = 316)	Chromosomal abnormalities (*n* = 13)	molecular diagnoses (*n* = 50)	VUS (*n* = 81)
Live birth	120 (38.0%)	0	6 (12.0%)	35 (43.2%)
Postnatal abnormalities	46 (14.6%)	0	2 (4.0%)	13 (16.0%)
Postnatal death	5 (1.6%)	0	1 (2.0%)	0 (0.0%)
Selective reduction of affected twin	4 (1.3%)	1 (7.7%)	1 (2.0%)	1 (1.2%)
TOP	109 (34.5%)	12 (92.3%)	32 (64.0%)	26 (32.1%)
Refuse follow-up	15 (4.7%)	0	6 (12.0%)	3 (3.7%)
unknown	10 (3.2%)	0	1 (2.0%)	1 (1.2%)
Lost follow-up	7 (2.2%)	0	1 (2.0%)	2 (2.5%)

VUS: Variant of Uncertain Significance; TOP: termination of pregnancy

the patient's clinical phenotype, while determining the fetal phenotype largely relies on obstetric ultrasonography [26]. Additionally, factors leading to variable prenatal clinical presentation include the skill level of medical staff, imaging techniques, and the stage of gestation as observed through prenatal ultrasonography. Therefore, it is essential to provide a comprehensive analysis of genetic variants for fetuses with ultrasonic abnormalities due to limited or uncertain fetal phenotypes. In the present study, the fetal ultrasound examination in case 44 revealed an anomalous ventricular septal echo, which was initially suspected to be rhabdomyoma; however, subsequent karyotype and CMA results were negative. Subsequent WES testing pointed to a de novo variant in *TSC1* (NM_000368.4:c.1639dupA) as a potential causative agent. Conducting WES could aid in offering further diagnosis and identifying new genetic variations.

Variant of Uncertain Significance pose particular dilemmas within the prenatal context and are inadequate to be categorically designated as either pathogenic or benign [27]. The ambiguity surrounding the VUS detected in fetuses can engender considerable distress and complicate decision-making processes for expectant parents. In this study, we identified VUS in 81 instances (25.6%, 81/316), of which 13 fetuses developed abnormalities after birth. After obtaining new clinical phenotypes at follow-up or postpartum, five cases of fetal abnormalities were identified with VUS that likely explaining the phenotype (case 10, 22, 56, 278, 279). In case 56, We discovered a hemi variant in *GPC3* gene (NM_004484.3:c.349 A > G) in a fetus that displayed ultrasonic abnormality of liver and polyhydramnios and diagnosed with hepatic hemangioma after birth. The *GPC3* gene was associated with Simpson-Golabi-Behmel syndrome type 1 (SGBS1) as well as elevated susceptibility to embryonal tumors. It suggests that the importance of VUS should be considered if an infant develops a phenotype associated with the variation during growth and development, thus contributing to early diagnosis and intervention of the disease. We anticipated that with the widespread use of WES in prenatal diagnosis, more VUS will be identified and reclassified, allowing a more precise diagnosis will be possible with integrated genotype–phenotype information.

Determining a precise genetic variant can enhance reproductive counseling for parents and optimize the management of subsequent pregnancies through the implementation of prenatal or preimplantation genetic testing. Of the families ascertained to possess a diagnostic genetic variant, nearly two-thirds (32 out of 50 families) were at risk for recurrence in future pregnancies. A half of these diagnostic variants were autosomal dominant, inheriting from either the father or the mother with a 50% risk of recurrence. Thirteen cases were diagnosed with autosomal recessive diseases, with nearly all associated with a 25% risk of recurrence in future pregnancies. These parents were confirmed as carriers of genetic diseases, highlighting the criticality of genetic carrier screening for congenital defects. In this study, the parental phenotype was predominantly based on self-reporting or observation. A thorough clinical examination of anomalies was not consistently performed. However, in clinical practice, the absence of parental phenotype, family history, and particularly the clinical data of siblings, can engender diagnostic uncertainty. Consequently, a routine clinical examination of parents is imperative. Consequently, family history investigation and Trio-WES testing are particularly important for fetuses with ultrasound scanning anomalies. Furthermore, de novo variants were detected in 42 fetuses (13.3%), involving 39 genes and 44 variants. Recent studies have indicated a correlation between the incidence of de novo point variants in offspring and advanced paternal age [28]. However, it was noteworthy that the fathers of fetuses carrying de novo variants did not demonstrate significantly advanced age in this study. Given the complexity of interpreting NGS results, particularly in the context of VUS and inconclusive diagnoses, we have adhered to current clinical guidelines and recommendations. Our approach to variant interpretation includes a rigorous classification system that aligns with the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) guidelines. We have also provided comprehensive information on the management of VUS, including the potential for ongoing surveillance and the role of expert consultation. Recognizing the profound impact of such findings on families, we have emphasized patient education and psychological support throughout the process.

By depositing our variants in the CNGBdb, we aim to enhance the availability of genetic data for the scientific community and to support future studies in the field of genetic medicine.Previous research has demonstrated that the incomplete manifestation of prenatal phenotypes, coupled with the unavailability of corresponding genetic variant-clinical phenotype correlations, has partially accounted for certain challenges in etiological elucidation [13, 29]. This absence challenges the elucidation of the clinical etiology underlying certain rare variants, underscoring the necessity for the establishment of a comprehensive prenatal fetal ultrasound abnormality and genotype database for the purposes of clinical genetic counseling [30]. Additionally, WES revealed pathogenic variants that were incongruent with the observed anomalies, such as a de novo variant in *ZMIZ1* (NM_020338.3: c.2182G > T); NM_000477.5:

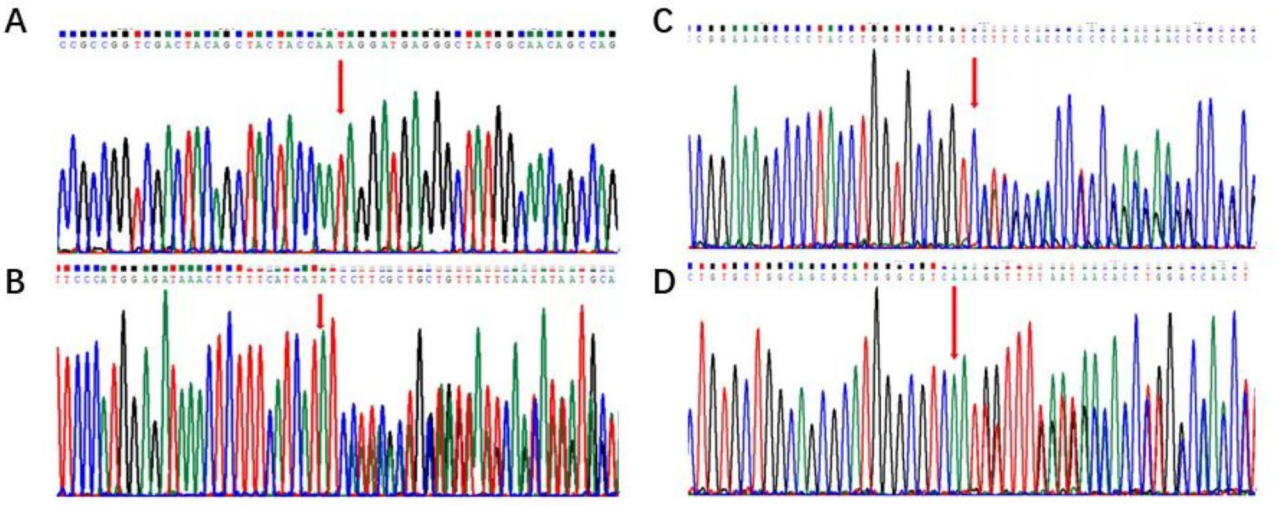

Fig. 4 Sanger sequencing was used to verify the detected variants in WES. (**A**) *RBM10*, NM_005676.4: c.1318 C > T(p.Gin440) in case 47; (**B**) *PEX1*, NM_000466.2:c.2080_2082delGAT (p. Asp694del) in case 90. (**C**) *FLNB*, NM_001457.3:c.6840dupC (p. lle2281Hisfs*23) in case 70; (**D**) *TREX1*, NM_033629.3: c.293 A[2 > 3] (std: c.294dupA alt: c.294_295insA), p. Cys99Metfs*3 in case 68

c.1328 C > A variant in *ALB*; a homozygous variant in *FLG* (NM_002016.1: c.12064 A > T); a de novo variant in *GJA1* (NM_000165.3:c.221_222delAT); a homozygous variant in *DUOX2 (NM_014080.4:c.0482G > T)*; NM_015386.2:c.202-203delTT variant in *COG4*; and various other instances. These findings underscore the intricacies involved in the interpretation of WES results. The inherent challenges of limited fetal ultrasound phenotypes and the absence of a fetal phenotype database were brought to the fore. Existing databases, including ClinVar [31], HGMD [31], and ExAC [32], were fragmented and ill-suited for the interpretation of fetal phenotypes, thereby impeding the precision required for accurate variant ranking in fetal prenatal WES. The postnatal phenotype of the fetus warrants meticulous attention and follow-up, as it provides invaluable context for the clinical implications of the genetic findings. Furthermore, during the analysis of Trio-WES results, we recognized the potential for inherited variants and the possibility of incomplete penetrance. We have carefully considered these factors and have included only those variants that are well-supported by clinical evidence and literature. In cases of genetic heterogeneity and when a precise clinical diagnosis is not available, we have provided a comprehensive discussion of all possible diagnoses, ensuring that the ultrasound findings are fully considered.

In summation, WES has demonstrated an extraordinary capability within the realm of prenatal medicine, albeit with a concurrent array of challenges that encompass practicality, genetic counseling, and economic considerations within clinical practice. The purpose of prenatal diagnosis is to furnish prospective parents with meticulous counseling, thereby equipping them to make an enlightened decision regarding their ongoing pregnancy. The judicious combination of CMA or CNV-seq sequencing with WES may yield substantial value within the scope of prenatal diagnosis. Our investigation has laid the groundwork for subsequent scholarly inquiries, particularly with respect to the integration of WES into conventional prenatal screening protocols. Future endeavors should delve into the cost-effectiveness of this integrated approach and its psychological repercussions on parents, thereby enriching our comprehension of its clinical utility.

Supplementary information

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Supplementary Material 1

Supplementary Material 2

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Author contributions
H.Z. and Y.D. carried out study design. Z.Z. and L.Z. wrote the main manuscript text. Y.Z., G.Y.and Y.Z. prepared Figs. 1, 2, 3 and 4. J.Y., Y.L., J.L., Q.C. and Y.Z. performed the experiments. All authors reviewed the manuscript. X.Z., H.Y. and H.L. prepared Tables 1, 2, 3 and 4. H.Z. and Y.D. carried out study design. All authors read and approved the final manuscript.

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Data availability
The data reported in this study are available in the CNGB Nucleotide Sequence Archive (CNSA: https://db.cngb.org/, accession number: CNP0006094).

Declarations

Ethical approval
Informed consent was obtained from all subjects and/or their legal guardian(s). Ethical approval was obtained for this study from the First Affiliated Hospital of Chongqing Medical University (No. K2023-580).

Consent for publication
Not applicable.

Conflict of interest disclosure
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author details
[1]Department of Obstetrics and Chongqing Fetal Medical Centre, The First Affiliated Hospital of Chongqing Medical University, 1 Youyi Road, Yuzhong, Chongqing 400016, People's Republic of China
[2]Chongqing Fetal Medical Centre, The First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, People's Republic of China
[3]Clin Lab, BGI Genomics, Guizhou 550000, China
[4]Clin Lab, BGI Genomics, Chongqing 400000, China

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