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ARTICLE

Application of Whole-Exome Sequencing in the Genetic Diagnosis of Prenatal Ultrasound Abnormalities

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Abstract

Aims/Background Prenatal diagnosis is a crucial tool in reducing birth defects. Research indicates that whole-exome sequencing (WES) is particularly effective for detecting abnormalities associated with structural ultrasound findings. This study aimed to evaluate the utility of WES in the genetic diagnosis of prenatal ultrasound abnormalities.

Methods A total of 50 pregnant women with prenatal ultrasound abnormalities, diagnosed at Rizhao People's Hospital between January 2023 and May 2024, were enrolled. Amniocytes, abortion tissues, and peripheral blood samples from the couples were collected for family-based WES.

Results WES revealed genetic abnormalities in 20 out of 50 cases, resulting in a detection rate of 40%. The detection rates for specific abnormalities were as follows: skeletal abnormalities (41.7%), cardiovascular abnormalities (54.5%), central nervous system abnormalities (30%), urinary system abnormalities (50%), nuchal translucency thickening/hygroma colli (20%), and facial anomalies/cleft lip and palate (25%). The genetic detection rates for monosystemic and multisystemic abnormalities were 34.2% and 50%, respectively.

Conclusion WES is crucial in the genetic diagnosis of prenatal ultrasound abnormalities, enhancing the accuracy of prenatal diagnostics and facilitating informed genetic counseling.

Key words: prenatal ultrasound abnormalities; whole-exome sequencing (WES); prenatal diagnosis; genetic counseling

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Introduction

Prenatal diagnosis is a crucial tool in reducing birth defects, with approximately 3% of pregnancies presenting with prenatal ultrasound abnormalities (Lord et al, 2019), which contribute to around 20% of perinatal deaths (Osterman et al, 2015). Genetic testing is crucial in evaluating these abnormalities and guiding decisions about pregnancy outcomes and reproductive planning. Cytogenetic and chromosome microarray analyses are commonly used in the genetic diagnosis of prenatal ultrasound abnormalities, with diagnostic yields of approximately 32% and an additional detection rate of 3–5% for specific abnormalities (Hillman et al, 2011; Robson et al, 2017; Yang et al, 2013). However, these methods have limitations. Whole-exome sequencing (WES) has emerged as a valuable tool, enhancing the diagnostic yield by 8–10% for prenatal ultrasound abnormalities (Monaghan et al, 2020).

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Research indicates that WES is particularly effective for detecting abnormalities associated with structural ultrasound findings, such as multiple malformations, fetal bone development abnormalities, cardiovascular defects, central nervous system development abnormalities, genitourinary system malformations, and facial deformities. However, the genetic diagnosis for these conditions varies widely across different systems. In this study, we conducted familial WES on fetuses with prenatal ultrasound abnormalities to evaluate the application value of WES in the genetic diagnosis of fetal structural abnormalities.

Methods

Basic Information

We enrolled 50 pregnant women who presented with fetal ultrasound anomalies between January 2023 and May 2024. The inclusion criteria were as follows: (1) maternal age ranging from 22 to 41 years; (2) gestational age ranging from 11 weeks +5 days to 37 weeks; (3) participants having no severe complications or comorbidities. All participants provided informed consent before surgery, and there were no contraindications for amniocentesis.

Amniotic fluid samples were obtained through ultrasound-guided amniocentesis (n = 41), and tissue samples were collected post-abortion (n = 9). Peripheral blood samples were also collected from the pregnant women and the biological fathers of the fetuses. This study was approved by the Ethics Review Committee of Rizhao People's Hospital (Approval No. 2023-Lunli-13-01).

Experimental and Statistical Methods

DNA Extraction

Genomic DNA was extracted from amniotic fluid cells using the Qiamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). For tissue and cell genomic DNA, the Tissue DNA Kit D3396 (Omega Bio-Tek, Norcross, GA, USA) was employed. Peripheral blood genomic DNA was extracted using the Blood DNA Kit D3396 (Omega Bio-Tek, Norcross, GA, USA). All procedures were performed according to the manufacturer's instructions.

Whole-Exome Sequencing (WES)

Extracted DNA samples were subjected to ultrasonic fragmentation, followed by end repair and adaptor ligation. High-throughput sequencing was performed on the Illumina Novaseq 6000 platform, targeting the whole-exome regions of 20,000 genes using the capture probes from the xGen® Exome Research Panel v1.0 (Integrated DNA Technologies (IDT)). Disease-associated genes were screened based on clinical presentations and analyzed using databases such as Online Mendelian Inheritance in Man (OMIM) (https://www.omim.org/) and MedGen (https://www.ncbi.nlm.nih.gov/medgen). The pathogenicity of gene variants was classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines, aiding in clinical diagnosis, treatment decisions, and genetic counseling.

Bioinformatics Analysis

Raw sequencing data were processed by removing adaptors, and low-quality reads to generate clean reads. The clean reads were aligned to the hg38 reference genome using Burrows-Wheeler-Alignment Tool (BWA) software to generate Binary Alignment/Map (BAM) files. Sequence Alignment/Map tools (SAMtools) (https://doi.org/10.1093/gigascience/giab008) was used to analyze the BAM files, followed by sorting and duplicate removal. Variant sites were identified using Genome Analysis Toolkit (GATK) (https://doi.org/10.1101/201178) and Varscan (https://doi.org/10.1101/gr.129684.111), while Annovar (https://doi.org/10.1093/nar/gkq603) software was employed to annotate variants. The annotation included information on basic mutations, gene-disease associations, mutation frequencies in the general population, inclusion in disease databases, and software-based pathogenicity predictions.

Quality Control

The sequencing data for each sample exceeded 10 Gb, with an average target region depth $>100\times$. Coverage of the target region was 99.5%, with over 99% of the target region sequences at a depth of $>10\times$, 98% at $>20\times$, and 95% at $>30\times$. The average proportion of Q20 was $>90\%$.

Statistical Analysis

Data were analyzed using SPSS version 26.0 (https://www.ibm.com/cn-zh/spss, IBM Corp., Armonk, NY, USA). Categorical variables were expressed as n (%), and comparisons were made using the chi-squared (χ^2) test. A p-value < 0.05 was considered statistically significant.

Results

Age and Gestational Age Distribution

The enrolled gravidas were aged between 22 and 41 years, with a median age of 31. Fetal developmental abnormalities were diagnosed via ultrasound at gestational ages ranging from 11^{+5} to 37 weeks, with a median of 22^{+5} weeks (Fig. 1).

Distribution of Ultrasound Abnormalities by System

Ultrasound-detected structural abnormalities in the 50 cases were classified into the skeletal, cardiovascular, central nervous (CNS), urinary systems, and nuchal translucency (NT) thickening/hygroma colli. The distribution of abnormalities and the number of positive cases within each system are shown in Figs. 2,3.

WES Findings

Among the 12 cases with skeletal system abnormalities identified through prenatal ultrasound, 5 had positive WES results, yielding a detection rate of 41.7%. Of these, 2 cases were associated with short rib thoracic dysplasia Type 3 and polydactyly, linked to mutations in the dynein cytoplasmic 2 heavy chain 1 (*DYNC2H1*) gene. One variant exhibited a probable pathogenicity (LP) mutation, while four variants of uncertain significance (VOUS) were also identified. Additionally, one

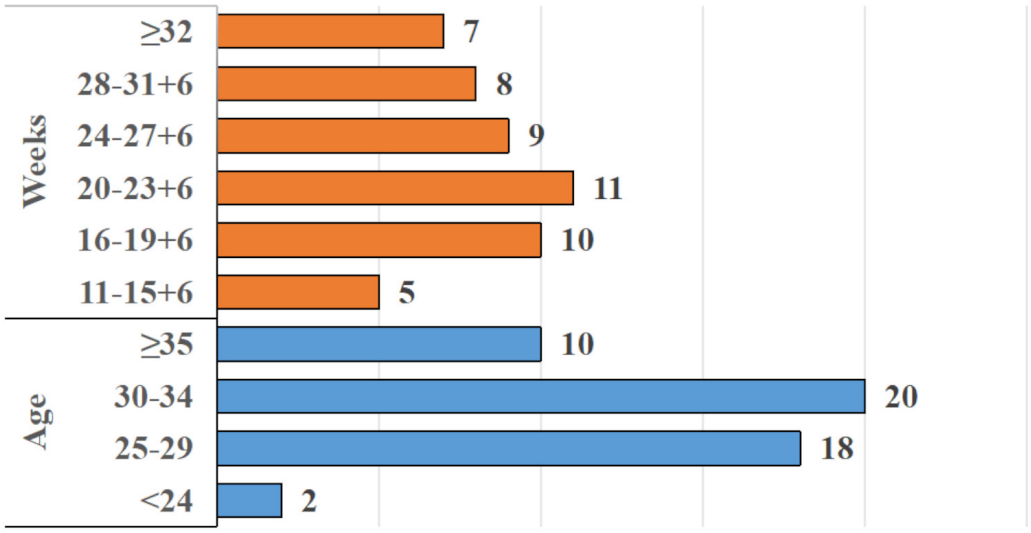

Fig. 1. Distribution of gestational and maternal age at the expected delivery date.

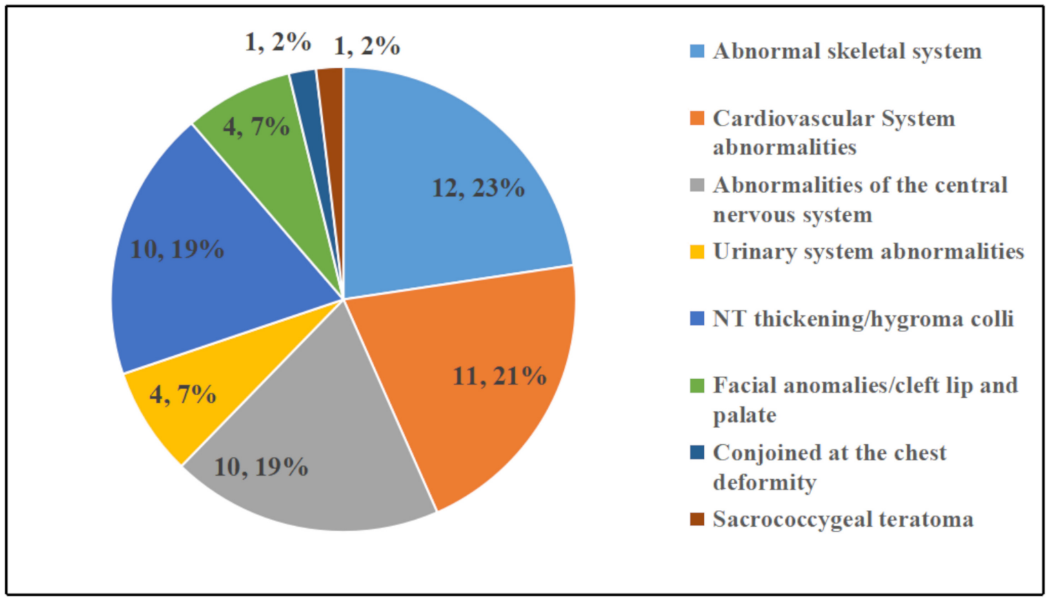

Fig. 2. Number and proportion of cases by system. NT, nuchal translucency.

case was related to achondroplasia, where a pathogenic mutation in the fibroblast growth factor receptor 3 (*FGFR3*) gene was detected. Another case involved mutations in the *FGFR2* gene, associated with Apert Syndrome and Crouzon Syndrome, and exhibited a pathogenic mutation. The final case in this group had a mutation in the myosin II regulatory light chain (*MYLII*) gene, related to distal joint contracture Type 1C, with an LP mutation.

Among the 11 cases with cardiovascular abnormalities, 6 had positive WES results, corresponding to a detection rate of 54.5%. One case was associated with Tetralogy of Fallot and involved an LP and a VOUS mutation in the vacuolar protein sorting 13 homolog B (*VPS13B*) gene. Another case involving complex heart disease was found to have a deletion of exon 1–28 in the Duchenne muscular dystrophy (*DMD)* gene (ChrX: 32441180–33211213), which was classified as

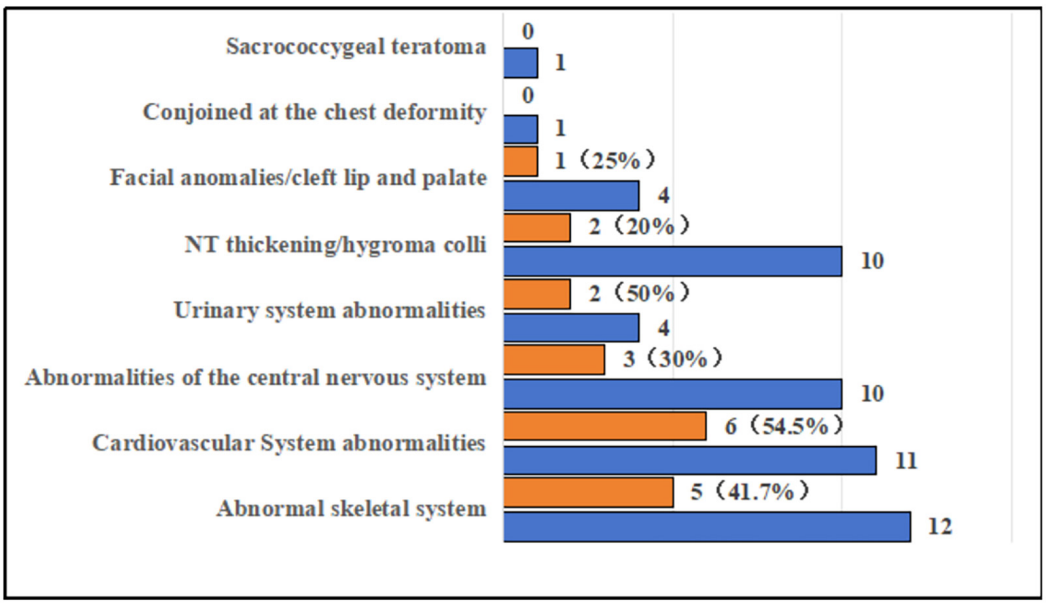

Fig. 3. Disease system and number of positive cases with ultrasound abnormalities (red represents the number of positive cases, blue represents the total number of cases).

pathogenic. A further presented a VOUS mutation in the filamin C (*FLNC*) gene, associated with cardiomyopathy. One rhabdomyoma case was linked to a likely pathogenic mutation in the tuberous sclerosis complex 2 (*TSC2*) gene. Additionally, one case with a ventricular septal defect had a VOUS mutation in the nuclear receptor binding SET (Su (var) 3–9, Enhancer-of-zeste and Trithorax) domain protein 1 (*NSD1*) gene, which is related to Sotos Syndrome 1. Another case with a ventricular septal defect was associated with an LP mutation in the Neurofibroma-Noonan Syndrome-related gene neurofibromin 1 (*NF1*) gene, which is implicated in Neurofibroma-Noonan Syndrome. Lastly, one case of congenital heart disease (CHD) was diagnosed as trisomy 21.

Of the 10 cases with CNS abnormalities, 3 had positive WES results, accounting for 30%. One case involved a VOUS mutation in the L1 cell adhesion molecule (*L1CAM*) gene, linked to Masa Syndrome. Another case exhibited two VOUS mutations in the anthrax toxin receptor 1 (*ANTXR1*) gene, associated with Growth retardation, Alopecia, Pseudoanodontia, and Optic atrophy (GAPO) Syndrome. The final case in this group involved a heterozygous deletion of 301.75 kb on chromosome 6 (6q27, chr6:170282827–170584581), inherited from the mother, which is associated with 6q27 microdeletion syndrome and classified as pathogenic.

Among the 4 fetuses with urinary abnormalities, 2 had positive WES results, corresponding to a detection rate of 50%. Both cases involved mutations in the polycystin 1 (*PKD1*) gene, linked to polycystic kidney disease Type 1. The pathogenic mutation was inherited from the father in one case, while the other presented a newly diagnosed VOUS mutation.

Among the 10 cases presenting with nuchal translucency (NT) thickening/hygroma colli, 2 yielded positive WES results, representing a detection rate of 20%. One of these cases involved a complication of fetal bradycardia, with a VOUS mutation in the guanine nucleotide-binding protein subunit beta 2 (*GNB2*) gene, which is asso-

ciated with Sick Sinus Syndrome Type 4. The other case was diagnosed as trisomy 18. Additionally, one case presenting with cleft lip and palate was found to have a VOUS mutation in the protein phosphatase 3 catalytic subunit alpha (*PPP3CA*) gene, which is implicated in cleft palate development (Table 1).

Overall, WES results revealed that 20 of the 50 cases had positive findings based on abnormal prenatal ultrasound findings, with a positivity rate of 40%. The detection rate for cases with single-system anomalies was 34.2, while the rate for cases with multiple-system anomalies was 50%. However, no statistically significant difference was observed between these two groups ($p = 0.326$, Table 2), likely due to sample selection and relatively small sample size. Among the 50 participants, 26 pregnancies were terminated following comprehensive counseling and full informed consent.

Discussion

Whole-exome sequencing (WES), a method developed through sequence-capture (or targeting) technology with high-throughput sequencing, allows for the detection of coding region abnormalities while also identifying structural variations in coding and non-coding regions, copy number variations (CNVs), site mutations, and mitochondrial variations (Bris et al, 2018). According to the ACMG guidelines, WES is recommended as a genetic diagnostic tool where fetal structural abnormalities are observed, but chromosomal microarray (CMA) testing or karyotyping returns typical results (Monaghan et al, 2020). The application of WES in cases of prenatal ultrasound abnormalities is gradually gaining recognition (Laboratories of Beijing Center et al, 2020; Manickam et al, 2021; Petrovski et al, 2019). In this study, WES identified positive findings in 20 out of 50 subjects, with a positivity rate of 40%. The majority of these cases involved abnormalities in the skeletal system, cardiovascular system, CNS, urinary system, and NT thickening/hygroma colli.

Application of WES in Skeletal Abnormalities

Fetal skeletal dysplasia (SD) is a heterogeneous disorder characterized by abnormal growth and development of the fetal skeleton, with an incidence of about 3.2 per 10,000 live births (Stembalska et al, 2021). The International Association of Osteodysplasia has identified 461 distinct hereditary bone diseases involving 437 genes (Mortier et al, 2019). In the current study, the genes *DYNC2H1*, *FGFR3*, *FGFR2*, and *MYLII* were related to SD. Compound heterozygous variants in the *DYNC2H1* were identified as pathogenic for short rib thoracic dysplasia 3 (SRTD3) (Fang et al, 2023; Guan et al, 2023). Stembalska et al (2022) performed WES on a fetus diagnosed prenatally with asphyxiating thoracic dystrophy (Jeune Syndrome) and identified missense variants in *DYNC2H1*, inherited from the mother (c.7289T>C; p.I2430T) and father (c.12716T>G; p.L4239R). In addition, Chen et al (2024) reported that a Y340C mutation in the *FGFR2* gene could lead to cloverleaf skull formation fetuses, as observed during prenatal ultrasound examination. Research by Khan et al (2024) identified *FGFR3* as a viable drug target for treating chondrodysplasia (disproportionate dwarfism) in cases of related mutations. Furthermore, Yang et al (2022) demonstrated that a significant increase in lubricin

Table 1. WES (whole-exome sequencing) results of 50 fetuses with ultrasound abnormalities.

Number	Ultrasound guidance	Gene	Nucleotide change/mutation	Heterozygosity	Clinical classification	Genetic source	Genetic pattern	Clinical diagnosis	Gestation outcome	Note
1	Short limbs, small chest (thanatophoric dwarfism)	*DYNC2H1* (dynein cytoplasmic 2 heavy chain 1)	c.1912A>G p.M638V c.2225T>G p.M742R c.10953T>A p.D3651E	Het Het Het	VUS VUS VUS	Pat Mat Mat	AR AD AR	Short rib thoracic dysplasia Type 3 with/without polydactyly	Labor induction	
2	Head circumference and femur length discrepancy of 6 weeks, with short femur	*FGFR3* (fibroblast growth factor receptor 3)	c.1138G>A p.G380R	Het	P	De novo	AD	Achondroplasia	Labor induction	
3	Short femur, small chest, underdeveloped thymus	*DYNC2H1* (dynein cytoplasmic 2 heavy chain 1)	c.11035C>T p.Q3679X c.11483T>G p.13828R	Het Het	LP VUS	Pat Mat	AR	Short rib thoracic dysplasia Type 3 with/without polydactyly	Labor induction	Roche translocation on chromosomes 13 and 14
4	Abnormal spinal development, enlarged bilateral ventricles	*FGFR2* (fibroblast growth factor receptor 2)	c.707C>G p.S236C	Het	P	De novo	AD AD	Apert Syndrome Crouzon Syndrome	Labor induction	
5	Bilateral strephenopodia, absence of left tibia, and short right tibia	No abnormality							Labor induction	
6	Femur shorter by 4 weeks, single umbilical artery, head circumference smaller by 2 weeks	No abnormality							Labor induction	

Table 1. Continued.

Number	Ultrasound guidance	Gene	Nucleotide change/mutation	Heterozy-gosity	Clinical classifi-cation	Genetic source	Genetic pattern	Clinical diagnosis	Gestation outcome	Note
7	Hemivertebra	No abnormality							Continuing gestation	
8	Absent nasal bone	No abnormality							Continuing gestation	
9	Fused vertebrae	No abnormality							Continuing gestation	
10	NT2.8, abnormal cervical vertebrae arrangement, spinal cord cone anomaly, absent right kidney	No abnormality							Labor induction	
11	Bilateral strephenopodiac	No abnormality							Continuing gestation	
12	Polyhydramnios, abnormal morphology of hands and feet	*MYLII* (Myosin II regulatory light chain)	c.98C>T p. A33V	Het	LP	De novo	AD/AR	Distal joint contracture Type 1C	Labor induction	
13	Tetralogy of Fallot	*VPS13B* (vacuolar protein sorting 13 homolog B)	c.1121C>T p.T374I c.4683_4684del p.E1562Rfs*7	Het Het	VUS LP	Mat Pat	AR	Tetralogy of Fallot	Labor induction	

Table 1. Continued.

Number	Ultrasound guidance	Gene	Nucleotide change/mutation	Heterozygosity	Clinical classification	Genetic source	Genetic pattern	Clinical diagnosis	Gestation outcome	Note
14	Complex cardiac anomalies (single atrium, single ventricle, persistent truncus arteriosus Type 3, inferior vena cava disconnection, persistent right umbilical vein)	*DMD* (Duchenne muscular dystrophy)	ChrX:32441180–33211213 Exon 1–28 deletion	Het	P	De novo	XR XR XL	Duchenne muscular dystrophy, bayesian muscular dystrophy, dilated cardiomyopathy Type 3B	Labor induction	
15	Diffuse left ventricular wall echo enhancement	*FLNC* (filamin C)	c.2686G>A p. G896R	Het	VUS	Pat	AD	Myofibrillar myopathy Type 5, familial hypertrophic cardiomyopathy Type 26/familial restrictive cardiomyopathy Type 5, distant myopathy Type 4	Continuing gestation	
16	Rhabdomyoma	*TSC2* (tuberous sclerosis complex 2)	c.1397T>C p. L466P	Het	LP	Mat	AD	Rhabdomyoma	Labor induction	Mother has epilepsy
17	Dextrocardia, ventricular septal defect	No abnormality							Continuing gestation	

Table 1. Continued.

Number	Ultrasound guidance	Gene	Nucleotide change/mutation	Heterozygosity	Clinical classification	Genetic source	Genetic pattern	Clinical diagnosis	Gestation outcome	Note
18	Congenital heart disease (complete atrioventricular septal defect, vagus right subclavian artery)	*Trisomy 21* (Down Syndrome)							Labor induction	
19	Ventricular septal defect	*NSD1* (nuclear receptor binding SET (Su (var) 3–9, Enhancer-of-zeste and Trithorax) domain protein 1)	c.3295A>T p.S1099C	Het	VUS	Mat	AD	Sotos Syndrome 1 (ventricular septal defect)	Continuing gestation	
20	Ventricular septal defect, femur shorter than 2 weeks of gestation	*NF1* (neurofibromin 1)	c.731-2A>G	Het	LP	De novo	AD	Neurofibroma - Noonan Syndrome	Labor induction	
21	Short femur (less than 3 weeks), ventricular septal defect	No abnormality							Continuing gestation	

Table 1. Continued.

Number	Ultrasound guidance	Gene	Nucleotide change/mutation	Heterozygosity	Clinical classification	Genetic source	Genetic pattern	Clinical diagnosis	Gestation outcome	Note
22	Right ventricle enlarged by 1.1 mm, abnormal corpus callosum development, cerebellar hypoplasia	*L1CAM* (L1 cell adhesion molecule)	c.3562G>A p.G1188R	Het	VUS	Mat	XR	Masa Syndrome: hydrocephalus, underdeveloped corpus callosum, (female carriers may have mild symptoms)	Labor induction	Maternal chromosome abnormalities 46, XX, inv(1) (p13q21), pericentric inversion, adverse pregnancy and childbirth history (one heterozygous loss, one embryo damage, one ventricular widening with abnormal development of the corpus callosum and cerebellar hypoplasia)
23	Bilateral ventricular dilation, cerebellar vermis missing, third ventricle widened (Dandy walk)	No abnormality							Labor induction	First child diagnosed with CAH (congenital adrenal hyperplasia, without genetic detection)
24	Bilateral ventricular dilation, polyhydramnios		There was a 301.75 kb heterozygous deletion on chromosome 6 6q27 (chr6:170282827–170584581) of CMA, with pathogenicity		P	Mat		6q27 microdeletion syndrome	Labor induction	Mother had an intellectual disability

Table 1. Continued.

Number	Ultrasound guidance	Gene	Nucleotide change/mutation	Heterozygosity	Clinical classification	Genetic source	Genetic pattern	Clinical diagnosis	Gestation outcome	Note
25	Lateral ventricle enlarged	No abnormality							Continuing gestation	
26	Lateral ventricle widened, renal cyst	No abnormality							Continuing gestation	
27	Absence of corpus callosum, arachnoid cyst	No abnormality							Continuing gestation	
28	Lateral ventricle widened	No abnormality							Continuing gestation	
29	Lateral ventricle widened, enhanced echogenic bowel	*ANTXR1* (anthrax toxin receptor 1)	c.1307A>G p.V436S c.943A>G p.T315A	Het Het	VUS VUS	Pat Mat	AR	GAPO (Growth retardation, Alopecia, Pseudoanodontia, and Optic atrophy) Syndrome (ventricular enlargement)	Continuing gestation	
30	Dilation of posterior cranial fossa pool (14.9 mm)	No abnormality							Continuing gestation	
31	Dilation of bilateral ventricles and third ventricle, ventricular septal defect	No abnormality							Labor induction	
32	Polycystic kidney	*PKD1* (polycystin 1)	c.9761_9768del GCCACGCT p.Q3254Lfs*3	Het	P	Pat	AD	Polycystic kidney disease Type 1	Labor induction	Husband had polycystic kidneys

Table 1. Continued.

Number	Ultrasound guidance	Gene	Nucleotide change/mutation	Heterozygosity	Clinical classification	Genetic source	Genetic pattern	Clinical diagnosis	Gestation outcome	Note
33	Right renal hydronephrosis	No abnormality							Continuing gestation	
34	Multicystic dysplastic kidney	*PKD1* (polycystin 1)	c.11333C>A p.T3778N	Het	VUS	Mat	AD	Polycystic kidney disease Type 1 (adult polycystic kidney disease)	Labor induction	
35	Left kidney deficiency	No abnormality							Continuing gestation	
36	NT 3.4 mm	No abnormality							Continuing gestation	
37	NT 2.9 mm	No abnormality							Continuing gestation	
38	NT 2.7 mm	No abnormality							Continuing gestation	
39	Cervical lymphatic cyst, bradycardia	*GNB2* (guanine nucleotide-binding protein subunit beta 2)	c.964G>A p.D322N	Het	VUS	Mat	AD	Sinus Syndrome Type 4	Labor induction	
40	Cervical lymphatic cyst	No abnormality							Labor induction	
41	Cervical lymphangiocystic tumor, systemic edema	*Trisomy 18* (Edwards Syndrome)							Labor induction	

Table 1. Continued.

Number	Ultrasound guidance	Gene	Nucleotide change/mutation	Heterozygosity	Clinical classification	Genetic source	Genetic pattern	Clinical diagnosis	Gestation outcome	Note
42	NT 4.0 mm	No abnormality							Continuing gestation	
43	NT 4.5 mm	No abnormality							Continuing gestation	
44	NT 3.5 mm	No abnormality							Continuing gestation	
45	Cleft lip and palate	No abnormality							Labor induction	
46	Cleft lip and palate	*PPP3CA* (protein phosphatase 3 catalytic subunit alpha)	c.788C>T p.P263L	Het	VUS	Mat	AD	Cleft palate	Labor induction	
47	Situs inversus	No abnormality							Continuing gestation	
48	Right ear malformation	No abnormality							Labor induction	
49	Thoracoabdominal conjoined malformation	No abnormality							Labor induction	
50	Sacrococcygeal teratoma	No abnormality							Continuing gestation	

*: Nonsense variation. Het, heterozygous mutation; P, pathogenic; LP, probable pathogenic; VUS, variants of uncertain significance; De novo, de novo mutation; Mat, maternal inheritance; Pat, paternal inheritance; AD, autosomal dominant inheritance; AR, autosomal recessive inheritance; XR, X-linked recessive inheritance; XL, X-linked inheritance; CMA, chromosomal microarray.

Table 2. Detection rates for single and multiple ultrasound abnormalities based on WES results (Note: $\chi^2 = 0.965$; $p = 0.326$).

Ultrasound system classification	n	Positive (cases)	Positivity rate (%)
Single system anomalies	38	13	34.2
Multiple system anomalies	12	6	50

(Proteoglycan 4, PRG4) could result in a curved finger joint disease, while common mutations such as those in collagen Type I alpha (*COL1A*) were also observed (Jelin et al, 2022).

In the present study, the genetic positivity rate for skeletal system abnormalities was 41.7% (5/12), which is consistent with previous studies that reported detection rates of 15.4% (10/65) (Lord et al, 2019), 24% (8/34) (Petrovski et al, 2019), 39% (28/72) (Normand et al, 2018), and 29.8% (37/124) (Fu et al, 2021).

Application of WES in Cardiovascular Abnormalities

Congenital heart defects (CHD) are the most common structural fetal abnormalities, with approximately 3–12% associated with genetic mutations (Hopkins et al, 2019; Russell et al, 2018). In this study, gene mutations associated with cardiac abnormalities included *VPS13B*, *FLNC*, *TSC2*, *NSD1*, and *NF1*. Previous research by Assimopoulos et al (2022) identified the association between *VPS13B* and CHD, while Lee et al (2023) demonstrated that *FLNC* mutations were strongly associated with CHD in a family with patrilineal sex chromosome chimerism. Tuberous sclerosis (TSC) is a multisystem genetic disorder often presented as rhabdomyoma in fetal development, and *TSC2* mutations have been frequently identified using WES (Pavlicek et al, 2021; Yang and Li, 2022; Young et al, 2021). Saacks et al (2022) detected *NSD1* mutations in patients with CHD in South Africa, while Yi et al (2019) uncovered *NF1* mutations in fetuses with CHD in their exploration of the genetic mechanisms behind ectopic heart syndrome (Htx).

Our study found a positivity rate of 54.5% (6/11) for cardiovascular abnormalities. Other studies have similarly demonstrated the potential of WES to enhance the detection of fetal CHD, reporting positivity rates of 11.1% (9/81) (Lord et al, 2019), 5% (4/77) (Petrovski et al, 2019), and 11.7% (24/205) (Fu et al, 2021).

Application of WES in CNS Abnormalities

Currently, the mechanism underlying over 80% of fetal CNS abnormalities, as identified through chromosomal karyotyping and CNV analysis, remains largely undetermined. However, many are suspected to be related to monogenic diseases (Yaron et al, 2022). Our findings associate fetal CNS abnormalities with mutations in the *L1CAM* and *ANTXR1* genes. *L1CAM* plays a central role in neurogenesis and axonal guidance (Gasparotto et al, 2023), while *ANTRX1* mutations are implicated in GAPO syndrome, a rare autosomal recessive disorder. Additionally, Gomes and Witwer (2022) observed that *L1CAM* expression is limited to neurons and upregulated during cancer progression. Damagatla et al (2024) reported a novel *ANTXR1* variation leading to premature protein truncation, underscoring the diag-

nostic utility of WES in such conditions. Further, Przyklenk et al (2024) explored the underlying mechanisms of *ANTXR1* deficiency, revealing its contribution to the aging phenotype in human fibroblasts through defects in nuclear structure and actin dynamics.

Additionally, mutations in other genes, such as glutaminase (*GLS*), have been linked to CNS abnormalities, including developmental epileptic encephalopathy (DEE) (Bazgir et al, 2024). Moreover, Alsehli et al (2024) identified single-gene mutations associated with posterior fossa abnormalities (PFA) involving genes such as *MPL* (Myeloproliferative Leukemia Virus Oncogene), *C5orf42* (Chromosome 5 Open Reading Frame 42), *ISPD* (Inositol-3-Phosphate Synthase Domain Containing), *PDHA1* (Pyruvate Dehydrogenase E1 Alpha Subunit), *PNPLA8* (Patatin-Like Phospholipase Domain Containing 8), *JAM3* (Junctional Adhesion Molecule 3), *UMOD* (Uromodulin), *NEK8* (NIMA-Related Kinase 8), *HNF1B* (Hepatocyte Nuclear Factor 1 Homeobox B), and *BBS2* (Bardet-Biedl Syndrome 2), further emphasizing the genetic diversity underlying CNS disorders. Our study also identified incidental mutations in *HSPD1* (Heat Shock Protein Family D Member 1) and *GRIN2B* (Glutamate Ionotropic Receptor NMDA Type Subunit 2B) in 2 cases (7% of the cases). WES has demonstrated its value in diagnosing agenesis of the corpus callosum (ACC), with reported diagnostic rates as high as 47% in this study, while Blayney et al (2024) reported a detection rate of 32% for CNS abnormalities by WES.

Application of WES in Urinary Abnormalities

Congenital anomalies of the kidney and urinary tract (CAKUT) account for 20–30% of congenital structural abnormalities (Uy and Reidy, 2016). In our study, we detected *PKD1* mutations associated with polycystic kidney disease. Heterozygous loss-of-function mutations in *PKD1* typically lead to autosome dominant polycystic kidney disease (ADPKD) in adulthood, while Zheng et al (2024) reported that bisallelic low-level *PKD1* mutations can result in the prenatal onset of polycystic kidney disease. Additionally, Lei et al (2017) identified pathogenic variants in *UMOD*, *NEK8*, *HNF1B*, and *BBS2* in CAKUT fetuses with/without other structural abnormalities during genetic etiology testing using WES, and two incidental variants in *HSPD1* and *GRIN2B*.

Our findings revealed a detection rate of 50% (2/4) for urinary abnormalities. This is consistent with a previous study that reported detection rates of 31% for multisystem urinary tract malformations (UTMs) and 16% for isolated cases (Sonner et al, 2024). Additionally, Jelliffe-Pawlowski et al (2015) reported a detection rate of 15.7% (3/19) for bilateral renal abnormalities.

Application of WES in NT Thickening/Hygroma Colli

Increased NT thickening is associated with genetic diseases (Jelliffe-Pawlowski et al, 2015). Dai et al (2023) used various diagnostic techniques to assess NT thickening in fetuses and identified heterozygous variants in genes including *SOS1* (Son of Sevenless Homolog 1), *COL2A1* (Collagen Type II Alpha 1 Chain), *LZTR1* (Leucine Zipper Like Transcription Regulator 1), and *BRAF* (B-Raf Proto-Oncogene,

Serine/Threonine Kinase), all of which were classified as VOUS. Furthermore, Ji et al (2023) demonstrated that comprehensive prenatal genetic testing, including chromosomal microarray analysis (CMA) and WES, improved outcomes for fetuses with NT thickening between 3.0–3.49 mm.

In our study, a *GNB2* mutation was identified in a fetus with NT thickening and bradycardia as well as one case of trisomy 18. Detection rates reported in the literature vary. For instance, the detection rate in fetuses with NT ≥4.0 mm was 3.2% (3/93) (Lord et al, 2019), while for those with NT ≥3.5 mm, it was 12% (6/51) (Petrovski et al, 2019).

Application of WES in Multiple Fetal Malformations

A multicenter prospective cohort study in the UK reported a 15.4% (22/143) detection rate for multiple fetal system structural abnormalities using WES after excluding cases of aneuploidy and CNVs. This involved the identification of 20 pathogenic or potentially pathogenic genetic variants (Lord et al, 2019). Numerous studies have shown an increasing detection rate for genetic factors and multiple fetal system structural abnormalities. For example, detection rates were reported as 37.5% (39/104) by Normand et al (2018), 33.7% (30/89) by Vora et al (2020), 29.2% by Lai et al (2022), and 38.9% by Lei et al (2021). Comparison between multiple and single malformations have shown significant differences in detection rates: 19% vs. 6% (Jelin et al, 2022), and 19.9% (30/151) vs. 12.2% (122/996) (Fu et al, 2021). These findings highlight WES as a valuable tool for identifying genetic factors in fetuses with multiple system structural abnormalities.

Interpretation of WES Results

When discussing WES results, the following points should be addressed during consultations: (1) For individuals receiving negative reports, it is essential to emphasize that a comprehensive evaluation is required to determine the prognosis, as negative results do not guarantee the absence of abnormalities; (2) For pathogenic or likely pathogenic variants, a thorough evaluation should be conducted, and individuals should be informed about the associated genetic conditions, recurrence risks, and other relevant implications; (3) For variants of uncertain significance (VUS), it is important to communicate that the clinical significance is indeterminate at this time, and that the interpretation for genetic variants may evolve with future research. Objective, scientifically grounded counseling is necessary to guide reproductive decision-making and assess potential risks.

Conclusion

In summary, prenatal WES plays a pivotal role in the genetic diagnosis of fetal ultrasound abnormalities, enabling more accurate predictions of fetal prognosis and recurrence risk in future pregnancies. However, there is a need to standardize clinical indications, technical procedures, data interpretation, reporting protocols, and pre- and post-test consultations to optimize the application of WES in prenatal diagnostics.

Key Points

- Prenatal chromosome karyotyping, family-based WES, and chromosomal microarray analysis (CMA) should be used together to improve the diagnostic rate of fetal ultrasound-detected structural abnormalities.
- Indications for WES in families with ultrasound-detected fetal abnormalities should be further standardized to enhance clinical practice.
- Based on the findings of WES, better counseling and guidance can be provided to repregnant couples.
- The findings of this study offer valuable guidance for reproductive decision-making.

Availability of Data and Materials

The data and materials related to this study will be available upon reasonable request from the corresponding authors.

Author Contributions

MS, HC and LQ designed the research. MS was in charge of quality control. HC and LQ carried out the implementation. DL and YX collected the data and YX performed the statistical analysis. DL wrote the manuscript. XW took part in analysing data, writing the manuscript, literature review, English translation and editing. All authors contributed to important editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Review Committee of Rizhao People's Hospital (No. 2023-Lunli-13-01). All participants provided informed consent.

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Conflict of Interest

The authors declare no conflict of interest.

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