Research article

# Simultaneous CNV-seq and WES: An effective strategy for molecular diagnosis of unexplained fetal structural anomalies

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## ARTICLE INFO

## ABSTRACT

Background: Fetal structural anomalies are detected by ultrasound in approximately 3 % of pregnancies. Numerous genetic diagnostic strategies have been widely applied to identify the genetic causes of prenatal abnormalities. We aimed to assess the value of simultaneous copy number variation sequencing (CNV-seq) and whole exome sequencing (WES) in diagnosing fetuses with structural anomalies.

Methods: Fetuses with structural anomalies detected by ultrasound were included for eligibility. After genetic counseling, WES and CNV-seq were performed on DNA samples of fetuses and their parents. All detected variants were evaluated for pathogenicity according to ACMG criteria, with the final diagnosis was determined based on ultrasound results and relevant family history.

Results: The diagnostic rate of 174 fetuses with prenatal ultrasound abnormalities was 26.44 %, higher than that achieved through either CNV or WES analysis alone. Furthermore, the highest diagnostic rate was observed in fetuses with multiple system anomalies, accounting for 50 % of the total diagnostic yield, followed by skeletal system anomalies at 45.45 %. Three cases with multiple system abnormalities were found to have a dual diagnosis of pathogenic CNVs and SNV variants, representing 1.72 % of the total cohort. 38 pregnant women in their third trimester of pregnancy (27 weeks+) participated in this study, and 23.68 % received a confirmed genetic diagnosis. Finally, 31 women (67.39 %) voluntarily terminated their pregnancy following the testing and extensive genetic counseling.

Conclusions: Our study demonstrated that the simultaneous CNV-seq and WES analyses are beneficial for the molecular diagnosis of underlying unexplained structural anomalies in fetuses.

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This strategy is more efficient in elucidating prenatal abnormalities with compound problems, such as dual diagnoses. Furthermore, the simultaneous strategy has a shorter turnaround time and is particularly suitable for families with structural anomalies found in the third trimester of pregnancy.

## 1. Introduction

Fetal structural anomalies, ranging from a single minor defect to fatal multisystem anomalies, are detected by ultrasound in approximately 3 % of pregnancies [1]. Previous studies have shown that chromosomal aneuploidy, uniparental disomy, copy number variations, single nucleotide variations (SNVs), and short insertion-deletions (Indels) are the most common genetic causes [2–5]. In general, conventional karyotype testing has a diagnostic rate of 8 %–10 % [6]. Chromosomal microarray analysis (CMA) or low-pass genome sequencing (CNV-seq) can detect up to an additional 10 % of short indels and duplications [7]. Recently, two significant cohort studies indicate that whole-exome sequencing (WES) can provide an additional 8.5%–10 % of diagnostic rate for structural abnormalities, even when karyotype and CMA results are negative [4,5].

The strategy of sequential karyotype, CMA or CNV-seq, and whole exome sequencing has been extensively used in the clinical setting of prenatal abnormalities. Although it can improve the diagnosis rate, there are still some limitations: First, in the cases carrying pathogenic CNVs and SNVs simultaneously [8], the sequential strategy is more likely to cause missed diagnosis or insufficient follow-up health care. Second, this strategy requires a long turnaround time (up to 50 days), which is unsuitable for pregnancies where ultrasound anomalies are detected late, especially those in the third trimester [9,10]. Simultaneous CNV-seq and WES can overcome these challenges and have met the needs for prenatal diagnosis in some research [11]. However, evidence supporting the clinical feasibility of simultaneous CNV-seq and WES for detecting the genetic causes of fetal structural malformations remains limited. Here, we report a single-center experience of simultaneous CNV-seq and WES in 174 fetal structural abnormalities, aiming to explore the feasibility and diagnostic rate of this strategy in these pregnancies.

## 2. Material and methods

### 2.1. Patients

The families of 174 fetuses with structural abnormalities identified by ultrasound and indicated for prenatal genetic testing were recruited from Chenzhou First People's Hospital. Written consent forms were obtained from each family, and the study was supported by the ethnic approval ((Research) No. 2020081) of Chenzhou First People's Hospital. Fetal samples were obtained by an invasive diagnostic procedure such as amniocentesis, chorionic villus sampling, or cordocentesis. Parental peripheral blood samples were obtained for trio analysis or validation. Genetic counseling was provided both before and after prenatal genetic testing, and pregnant couples were informed of the testing results. The pregnancy outcome of each family was followed up.

### 2.2. WES

WES was performed by inputting 150–300 ng of genomic DNA from each sample. First, 80–200 ng of genomic DNA from each sample was sheared using the Covaris S220 Focused Ultrasonicator (Covaris, Woburn, MA, USA). The fragmented DNA was further processed using AMPure XP Beads (Life Sciences, Indianapolis, IN, USA) to obtain 100-300 bp fragments. Library construction including end repair, A-tailing, adapter ligation, and 7 cycles of PCR amplification was then performed. The PCR products were then heat denatured to form single-stranded DNA, followed by circularization with DNA ligase, and the remaining linear molecule was digested with exonuclease. After the construction of the DNA nanoballs, exome capture using the MGIEasy Exome Capture V4 probe (MGI) was followed by paired-end read sequencing (2 × 100 bp read length) on the MGISEQ-2000 platform with an average depth of at least 100-fold. Analysis of the exome sequencing data was performed as previously described [12]. Our WES can detect other types of variations related to the phenotype of the subject. These variations can involve large fragments of genomic copy number variation, such as deletion or duplication intervals (≥1 Mb). Additionally, the test can also detect chromosome aneuploidy, triploidy, and loss of heterozygosity (LOH) intervals (≥5 Mb). The accuracy of the prompt content has not been verified and will be displayed in the attached report.

### 2.3. CNV-seq

CNV-seq using whole-genome amplification was performed according to the manufacturer's instructions (BGI, Wuhan, China). Briefly, the DNA was fragmented into 200-300bp fragments, followed by end repair, adaptor ligation, PCR amplification, purification, and quantitation. The eligible cDNA libraries were then sequenced using the BGISEQ-500 platform based on NGS technology (BGI, Wuhan, China). Chromosomal aneuploidy and whole-genome CNVs (resolution 0.1 Mb) and chromosomal mosaicism (>10 %) were detected with the method as previously reported [13].

*2.4. Data interpretation and reporting*

Detected CNVs and SNV variants were interpreted according to the standards of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen) [14]. Among a large number of SNVs/INDELs, We have prioritized potential causative SNVs/INDELs from a vast collection based on the following criteria: (1) SNVs/INDELs that are either absent or have a minor allele frequency of ≤1 % in the Exome Aggregation Consortium (ExAC) and Genome Aggregation Database (gnomAD) databases, indicating a variant evidence classified as PM2; (2) evidence of familial segregation that is consistent with the inheritance pattern of the variants, which can be supported by PS2/PM6/PM3; (3) supporting evidence from published research (such as PS1/PS3/PS4/PM5); (4) identification of null variants (PVS1); (5) assessment of conservation levels and predicted impact on coding and non-coding sequences (PP3); and (6) relevance to the clinical phenotype of the fetus (PP4). All selected variants were assessed for pathogenicity based on the ACMG guidelines and the ClinGen Sequence Variant Interpretation Working Group according to the updated recommendations for the ACMG criteria [15–17].

The positive results to explain the fetal phenotype included P/LP variants that were consistent with the inheritance pattern and associated with the phenotype of related disorders, and the variants of unknown significance (VUS) in the disease-causing genes that were associated with the fetal phenotype. Otherwise, the remaining results were reported as negative or non-diagnostic. The pregnant women and their partners were informed of the ACMG incidental findings [18], and secondary findings were only reported if they agreed in the pre-test informed consent process as per the ACMG document [19].

## 3. Results

### 3.1. Study cohort features

Between April 2019 and June 2022, 174 pregnant women with fetuses diagnosed with structural abnormalities via ultrasound at the First People's Hospital of Chenzhou were enrolled in this study. The median gestational age was 23 weeks, ranging from 11 to 36 weeks. 14 fetuses had a family history of affected siblings or at least one affected parent, but no molecular diagnosis was conducted (Table 1). Various sample types, including 31 cord blood samples, 25 chorionic villus, 8 abortion tissues, and 110 amniotic fluid samples, were used for WES and/or CNV-seq analysis (Table 1). The median turnaround times (TAT) for both WES and CNV-seq was 14 days (range 10–21 days). In this study, we aimed to shorten the TAT of simultaneous CNV-seq and whole-exome sequencing, allowing pregnant women could obtain results faster. The median TAT for simultaneous CNV-seq and whole-exome sequencing were 14–21 days as compared with TAT of up to 50 days for sequential strategy (Table 1).

### 3.2. Diagnostic rate based on prenatal ultrasound

In total, 15 cases (8.62 %) were identified with pathogenic CNVs through CNV-seq and 34 cases (19.54 %) diagnosed with pathogenic SNVs via WES (Table 2). We utilized HPO terms to standardize ultrasound-identified phenotypes and detected fetal abnormalities across 10 different systems, including single-system and multi-system anomalies. Our findings indicate that while the combined diagnostic approach does not enhance the diagnostic rate for some fetuses with clinical abnormalities, the diagnosis rate for fetuses with cardiac, multiple system, nuchal, and skeletal abnormalities is significantly higher when using the combined methods of CNV and WES compared to a single method. Anomalies of the multiple systems and skeletal system exhibited the highest diagnostic

**Table 1**
Clinical details of the study cohort.

| Cohort characteristics | Number |
| --- | --- |
| **Total** | 174 |
| **Gestational weeks (median)** | 23 weeks (range 11–36 weeks) |
| **Maternal age (median)** | 27 (range 18–49) |
| **Sample types** | |
| Amniotic fluid | 110 |
| Cord blood | 31 |
| Chorionic villus | 25 |
| Abortion tissues | 8 |
| **Family history** | 14 |
| **Turnaround time (TAT)** | |
| WES | 14 days (range 10–21 days) |
| CNV-seq | 14 days (range 10–21 days) |
| Sequential strategy | up to 50 days |
| Simultaneously CNV-seq and WES | 14–21 days |
| **Initial DNA** | |
| WES | 150–300 ng |
| CNV-seq | 200 ng |
| Sequential strategy | 350–550 ng |
| Simultaneously CNV-seq and WES | 100–280 ng |
| **Follow-ups** | 87.9 % (153/174) |

**Table 2**

Distribution of diagnosis rates among anatomical systems of fetuses in the present cohort.

| Clinical category | CNV | WES | Combined detection |
|---|---|---|---|
| Brain | 0/12 (0) | 4/12 (33.33) | 4/12 (33.33) |
| Facial | 0/12 (0) | 2/12 (16.67) | 2/12 (16.67) |
| Cardiac | 2/34 (5.88) | 3/34 (8.82) | 5/34 (14.71) |
| Thoracoabdominal | 0/4 (0) | 0/4 (0) | 0/4 (0) |
| Genitourinary | 0/22 (0) | 4/22 (18.18) | 4/22 (18.18) |
| Skeletal | 1/11 (9.09) | 4/11 (36.36) | 5/11 (45.45) |
| Intrauterine growth restriction | 0/10 (0) | 1/10 (10) | 1/10 (10) |
| Nuchal | 4/24 (16.67) | 2/24 (8.33) | 6/24 (25) |
| Multisystem | 8/30 (26.67) | 10/30 (33.33) | 15/30 (50) |
| Others | 0/15 (0) | 4/15 (26.67) | 4/15 (26.67) |

Data are given as n/N (%); Combined detection: fetuses that were diagnosed by CNV or WES.

rates with the combined methods, at 50 % and 45.45 % respectively. Fetuses with the affected brain (33.33 %) exhibited a higher diagnosis rate than the overall diagnosis rate. No successful diagnoses were made for fetuses with thoracoabdominal anomalies in our cohort. Other anatomical anomalies had varied diagnosis rates, all below the overall diagnosis rate.

### 3.3. Genetic sequencing analysis

In our study, the overall diagnostic yield achieved with the simultaneous CNV-seq and WES approach was 26.44 %, whereas the diagnostic yield for CNV testing alone was 6.9 %, and for WES testing alone was 17.82 % (Fig. 1). The combined diagnostic approach outperformed the individual testing methods in diagnosing fetal ultrasound anomalies. Furthermore, three patients (6.52 %) were identified with dual pathologies, underscoring the risk of missed diagnoses with sequential diagnostic approaches.

The diagnostic results of chromosomal variations are shown in Table 2, Fifteen pathogenic or likely pathogenic CNVs were identified in 15 patients, with the highest diagnostic rate in multiple systems anomalies (26.67 %, 8/30) and nuchal anomalies(16.67 %, 4/24), followed by skeletal anomalies (9.09 %, 1/11) and cardiac anomalies (5.88 %, 2/34). The detailed CNV information is included in Table 3. Moreover, a pathogenic CNV, known as trisomy 21 was identified in two cases of nuchal anomalies, one case of multiple anomalies involving both skeletal and cardiac systems, and one case of skeletal anomaly. CNV-seq offers significant advantages over WES alone by providing timely detection of structural variations, including smaller copy number variations. In our study, the deletion regions in case 7 (1.4 Mb deletion at Chr22: 20393660–21796237 (GRCh37/hg19)) and case 43 (1.4 MB deletion at Chr22: 18887652–20307698 (GRCh37/hg19)) encompassed the TBX1 gene, whose mutations largely explain the phenotypes associated with most DiGeorge syndromes. These syndromes related to the identified deletions may account for the fetal cardiac ultrasound abnormalities. For families opting to receive CNV-seq test results, the findings are sent to a prenatal geneticist for consultation, either to proceed with the pregnancy or termination of the pregnancy. Of the 15 fetuses with structural variations detected through CNV-seq, 12 families opted for pregnancy termination, and 3 pregnancies ended in stillbirth (Table 3).

According to the diagnosis of WES, 34 fetuses were detected to have pathogenic sequence variants (SNVs and small InDels) (Table 4). Among all the anomaly groups, skeletal anomalies had the highest diagnostic rate of 36.36 % (4/11), then brain anomalies (33.33 %, 4/12) and multiple systems anomalies (33.33 %, 10/30), then followed by other systems anomalies (26.67 %, 4/15), genitourinary anomalies (18.18 %, 4/22), facial anomalies (16.67 %, 2/12), intrauterine growth restriction (10 %, 1/10), cardiac anomalies (8.82 %, 3/34), and nuchal anomalies (8.83 %, 2/24). Similar to CNV-seq, WES didn't identify any pathogenic variants in thoracoabdominal anomalies. The WES analysis detected 54 causative variants in 35 fetuses, including 25 novel mutation. Among
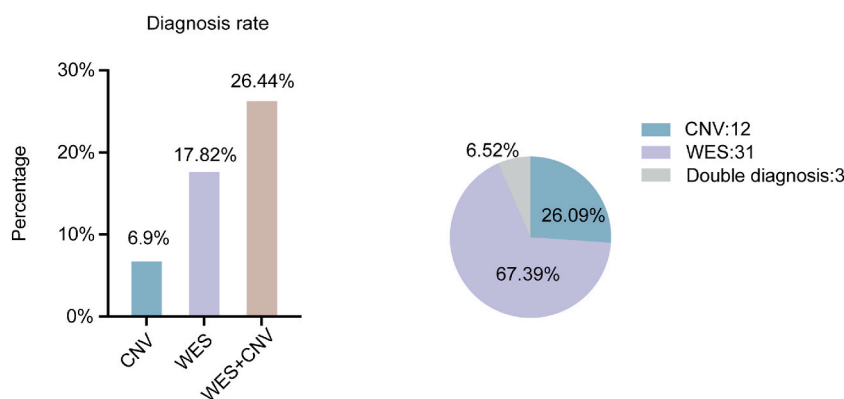


**Fig. 1.** Diagnostic rate of fetal structural anomalies for CNV testing alone, whole-exome sequencing (WES) alone, and combined genetic analysis methods.

**Table 3**

Pathogenic (P) and likely pathogenic (LP) copy-number variants (CNVs) detected by copy number variants sequencing among 174 cases of fetal structural anomalies.

| ID | Clinical findings | Phenotype category | CNVs | CNV classification | Sex | Author(s) and Year | Outcome |
|---|---|---|---|---|---|---|---|
| 7 | Single ventricle; Transposition of the great arteries | Cardiac | seq[GRCh37]22q11.21del (g.20393660-21796237) × 1 | P | Unknown | Liping Zhao et al., 2020 [20] | TOP |
| 17 | Intrauterine growth restriction; Renal hypoplasia | Multisystem | seq[GRCh37]4p16.3p16.1del (g.10004-7675299) × 1 | P | Unknown | Chih-Ping Chen et al., 2020 [21] | TOP |
| 43 | Arrhythmogenic right ventricular dysplasia; Coronary artery aneurysm; Pericardial effusion | Cardiac | seq[GRCh37]22q11.21q11.21del (g.18887652-20307698) × 1 | P | Unknown | Jawad, A F et al., 2001 [22] | TOP |
| 57 | Ventricular septal defect; Short long bone; Intrauterine growth restriction; | Multisystem | 47,XX,+21 | P | Female | Chih-Ping Chen et al., 2021 [23] | Stillbirth |
| 58 | Cystic hygroma | Large NT/ Nuchal | seq[GRCh37]14q11.2q21.3dup (g.20484708-g.50777838) × 3 | P | Unknown | Luo, Huayu et al., 2018 [24] | TOP |
| 69 | Ventricular septal defect; Coarctation of aorta; Choroid plexus cyst | Multisystem | 47,XX,+18 | P | Female | M A Lizárraga et al., 2021 [25] | Stillbirth |
| 83[a] | Ventricular septal defect; Nasal bone loss | Multisystem | seq[GRCh37]21p13q22.3dup(g.1-48129895) × 1 | P | Unknown | Chih-Ping Chen et al., 2021 [23] | TOP |
| 87[a] | Short long bone; Intrauterine growth restriction; Oligohydramnios | Multisystem | seq[GRCh37]2p25.3q37.3dup(g.1-243199373) × 1 | P | Unknown | This study | TOP |
| 116 | Cystic hygroma | Large NT/ Nuchal | seq[GRCh37]21p13q22.3dup(g.1-48129895) × 1 | P | Unknown | Chih-Ping Chen et al., 2021 [23] | TOP |
| 131 | Nuchal translucency of 3.5 mm | Large NT/ Nuchal | seq[GRCh37]21p13q22.3dup(g.1-48129895) × 1 | P | Unknown | Chih-Ping Chen et al., 2021 [23] | TOP |
| 132 | Absent fetal nasal bone; Short humerus and femur; Persistent left superior vena cava | Skeletal | seq[GRCh37]21p13q22.3dup(g.1-48129895) × 1 | P | Unknown | Chih-Ping Chen et al., 2021 [23] | TOP |
| 146 | Stillbirth | Multisystem | 47,XX,+22 | P | Female | Shuang Hu et al., 2023 [26] | Stillbirth |
| 159 | Nuchal translucency of 5.4 mm | Large NT/ Nuchal | seq[GRCh37]Xp22.33p11.22del (g.1_50242846) × 1 | P | Male | Dai, H-L et al., 2023 [27] | TOP |
| | | | seq[GRCh37]Xp11.22q28del (g.50242846_154926263) × 1 | P | | This study | |
| 172[a] | Exencephaly; Prominent umbilicus; Talipes calcaneovarus; Cardiac anomalies; Endocardial cushion defect | Multisystem | seq[GRCh37]2q35q35del (g.215500643-215843259) × 1 | P | Unknown | This study | TOP |
| 192 | Abnormal thoracic and lumbar vertebrae; Butterfly vertebrae; Hemivertebrae; Vertebral fusion | Multisystem | seq[GRCh37]16p11.2p11.2del (g.29443654_30283046) × 1 | P | Unknown | Andrée Delahaye et al., 2012 [28] | TOP |

Large NT/Nuchal, large nuchal translucency; P, pathogenic; LP, likely pathogenic; TOP, termination of pregnancy.

[a] Indicates patients have a dual diagnosis of pathogenic CNVs and SNV variants.

pathogenic variant detected by whole-exome sequencing in fetuses with prenatal ultrasound abnormalities, missense mutations were the most common (Fig. 2). Following those were nonsense mutations. Brain abnormalities were mainly characterized by deletion mutations. KEGG enrichment analysis of these pathogenic variant genes showed significant enrichment primarily in metabolic pathways (Fig. 3). Additionally, six pathogenic/likely pathogenic variants in TSC2, FGFR3, and G6PD were found to be causative in at least two fetuses, with half of these fetuses also carrying other variants. Of the 34 families with sequence variants detected through WES, after receiving genetic counseling and reproductive guidance from prenatal genetics physician, 25 opted for pregnancy termination, and 9 resulted in live births (Table 3).

**Table 4**

Pathogenic (P) and likely pathogenic (LP) variants detected by whole-exome sequencing among 174 cases of fetal structural anomalies.

| ID | Clinical findings | Phenotype category | Gene | Zygosity | Disease (OMIM ID) | Inheritance pattern | Mutation origin | Variant(s) | Author(s) and Year | Outcome |
|---|---|---|---|---|---|---|---|---|---|---|
| 5 | Polyhydramnios | Other | RAPSN | Comp het | Fetal akinesia deformation sequence 2 (618388) | AR | mat | NM_005055.4:c.484G > A(p. Glu162Lys) | Lore Winters et al., 2017 [29] | TOP |
| | | | | | | | pat | NM_005055.4:c.280G > A(p. Glu94Lys) | Natera-de Benito, D et al., 2017 [30] | |
| 8 | Cystic hygroma | Large NT/ Nuchal | RIT1 | Het | Noonan syndrome 8 (615355) | AD | de novo | NM_006912.5; c.268A > G(p. M90V) | Zilong Qiu et al., 2020 [31] | TOP |
| 10 | Abnormal renal pelvis morphology; Hydrops fetalis | Genitourinary | PKLR | Comp het | Pyruvate kinase deficiency (266200) | AR | pat | NM_000298.5:c.1462C > T(p. Arg488*) | Baronciani, L et al., 1998 [32] | Livebirth |
| | | | | | | | mat | NM_000298.5:c.330_331delCG (p.Gly111Aspfs*18) | This study | |
| | | | G6PD | Hemi | Hemolytic anemia, G6PD deficient (favism) (300908) | XL | mat | NM_001042351.1:c.1024C > T (p.Leu342Phe) | Qi Jiang et al., 2020 [33] | |
| 11 | Pelvic dysplasia; Abnormal renal pelvis morphology | Multisystem | P3H1 | Comp het | Osteogenesis imperfecta, type VIII (610915) | AR | pat | NM_001243246.2:c.1914+1G > A | Liliane Todeschini de Souza et al., 2021 [34] | TOP |
| | | | | | | | mat | NM_001243246.2:c.652G > T (p.Glu218*) | Cabral, Wayne A et al., 2007 [35] | |
| 21 | Short long bone | Skeletal | GPX4 | Comp het | Spondylometaphyseal dysplasia, Sedaghatian type (250220) | AR | pat | NM_001039848.1: c.547_548dupTG(p. Trp183Cysfs*3) | This study | TOP |
| | | | | | | | mat | NM_001039848.1: c.549_552delGATG(p.Trp183*) | This study | |
| 22 | Ectopic kidney; Renal dysplasia; Genitourinary | Genitourinary | DHCR7 | Comp het | Smith-Lemli-Opitz syndrome (270400) | AR | pat | NM_001360.2:c.278C > T(p. Thr93Met) | Fitzky et al., 1998 [36] | TOP |
| | | | | | | | mat | NM_001360.2:c.862G > A(p. Glu288Lys) | M Wisch-Baumgartner et al., 2001 [37] | |
| 29 | Arterial calcification; Pulmonary artery atresia; Edema; Polyhydramnios | Multisystem | ENPP1 | Hom | Hypophosphatemic rickets, autosomal recessive, 2 (613312) | AR | pat | NM_006208.2:c.1742C > T(p. Pro581Leu) | This study | Livebirth |
| 45 | Cardiac rhabdomyoma | Cardiac | TSC2 | Het | Tuberous sclerosis-2 (613254) | AD | pat | NM_001318829.2:c.910T > C (p.Trp304Arg) | Clinvar database | TOP |
| 54 | Ventriculomegaly; Diacele enlargement; Dilated third ventricle | Brain | PDHA1 | Het | Pyruvate dehydrogenase E1-alpha deficiency (312170) | XL | de novo | NM_000284.3: c.934_940delAGTAAGA(p. Ser312Valfs*12) | Dahl et al., 1990 [38] | TOP |
| | | | BUB1B | Het | Premature chromatid separation trait (176430); Mosaic variegated aneuploidy syndrome 1 (257300) | AD,AR | mat | NM_001211.5: c.2405_2406insCC(p. Trp803Hisfs*10) | This study | |
| 66 | Absent fetal nasal bone; Increased distance between eyes | Facial | ANKRD11 | Het | KBG syndrome (148050) | AD | mat | NM_013275.5: c.2848_2849insG(p. Asp950Glyfs*68) | This study | TOP |

**Table 4** (*continued*)

| ID | Clinical findings | Phenotype category | Gene | Zygosity | Disease (OMIM ID) | Inheritance pattern | Mutation origin | Variant(s) | Author(s) and Year | Outcome |
|---|---|---|---|---|---|---|---|---|---|---|
| 74 | Talipes calcaneovarus | Skeletal | RAB11B | Het | Neurodevelopmental disorder with ataxic gait, absent speech, and decreased cortical white matter (617807) | AD | de novo | NM_004218.3:c.368G > A(p. Gly123Asp) | This study | TOP |
| 75 | Left ventricular hypertrophy; Left ventricular outflow tract stenosis | Cardiac | PLD1 | Comp het | Cardiac valvular dysplasia 1 (212093) | AR | pat | NM_002662.4:c.2083C > T(p. Arg695Cys) | Priya Ranganath et al., 2023 [39] | TOP |
| | | | | | | | mat | NM_002662.4:c.2024G > A(p. Arg675Gln) | Lahrouchi, Najim et al., 2021 [40] | |
| 76 | Cardiac rhabdomyoma; Ventriculomegaly | Multisystem | TSC2 | Het | Tuberous sclerosis-2 (613254) | AD | pat | NM_000548.3:c.1372C > T(p. Arg458*) | Park JH et al., 2018 [41] | Livebirth |
| 83[a] | Ventricular septal defect; Nasal bone loss | Multisystem | RPL11 | Het | Diamond-Blackfan anemia 7 (612562) | AD | pat | NM_000975.3:c.126G > C(p. Gln42His) | This study | TOP |
| 84 | Absent right kidney | Genitourinary | FRAS1 | Comp het | Fraser syndrome 1 (219000) | AR | pat | NM_025074.6:c.2407G > A(p. Val803Met) | Clinvar database | TOP |
| | | | | | | | mat | NM_025074.6:c.10606G > A(p. Gly3536Ser) | This study | |
| 87[a] | Short long bone; Developmental retardation; Oligohydramnios | Multisystem | LBR | Het | Pelger-Huet anomaly (169400); Rhizomelic skeletal dysplasia with or without Pelger-Huet anomaly (618019) | AD, AR | mat | NM_002296.3:c.43C > T(p. Arg15*) | Hoffmann, Katrin et al., 2002 [42] | TOP |
| 106 | Ectopic kidney; Renal Dysplasia | Genitourinary | PBX1 | Het | Congenital anomalies of kidney and urinary tract syndrome with or without hearing loss, abnormal ears, or developmental delay (617641) | AD | mat | NM_002585.3:c.780C > G(p. Tyr260*) | This study | Livebirth |
| 110 | Cataract | Other | OCRL | Hemi | Lowe syndrome (309000) | XL | mat | NM_000276.3:c.940-9_943delAACTCATAGGTTC | Keita Nakanishi et al., 2023 [43] | Livebirth |
| 111 | Absence of cerebellar vermis; Abnormal skull morphology; Encephalocele | Brain | CSPP1 | Hom | Joubert syndrome 21 (615636) | AR | mat | NM_024790.6: c.2244_2245delAA(p. Glu750Glyfs*30) | This study | TOP |
| 119 | Absence of cerebellar vermis | Brain | TMEM67 | Comp het | COACH syndrome 1 (216360) | AR | pat | NM_153704.5:c.2204T > C(p. Val735Ala) | This study | TOP |
| | | | | | | | mat | NM_153704.5:c.1175C > G(p. Pro392Arg) | Clinvar database | |
| 125 | Nuchal cystic hygroma; Abnormal renal pelvis morphology | Multisystem | PTPN11 | Het | LEOPARD syndrome 1 (151100) | AD | de novo | NM_002834.3:c.214G > C(p. Ala72Pro) | Kosaki, Kenjiro et al., 2002 [44] | TOP |
| 135 | Cleft lip with cleft palate | Facial | CDH1 | Het | Blepharocheilodontic syndrome 1 (119580) | AD | mat | NM_004360.3:c.454C > T(p. Gln152*) | This study | TOP |
| 137 | Nuchal translucency of 3.7 mm | Large NT/ Nuchal | CYP21A2 | Comp het | Hyperandrogenism, nonclassic type, due to 21-hydroxylase deficiency (201910) | AR | pat | NM_000500:c.549+1G > C | Leandro Simonetti et al., 2018 [45] | Livebirth |
| | | | | | | | mat | NM_000500:EX3 DEL | This study | |
| 147 | Polyhydramnios | Other | PLEC | Comp het | Epidermolysis bullosa simplex 5A, Ogna type (131950); | AD,AR | pat | NM_000445.3:c.10526G > A(p. Arg3509His) | Clinvar database | Livebirth |

**Table 4** (*continued*)

| ID | Clinical findings | Phenotype category | Gene | Zygosity | Disease (OMIM ID) | Inheritance pattern | Mutation origin | Variant(s) | Author(s) and Year | Outcome |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Muscular dystrophy, limb-girdle, autosomal recessive 17 (613723) | | mat | NM_201384.1:c.12C > T(p. (His4 = )) | This study | |
| | | | | | | | mat | NM_000445.3:c.9751C > G(p. Leu3251Val) | This study | |
| | | | MYH11 | Comp het | Aortic aneurysm, familial thoracic 4 (132900); Megacystis-microcolon-intestinal hypoperistalsis syndrome 2 (619351) | AD,AR | pat | NM_001040113.1:c.1523G > A (p.Arg508His) | This study | |
| | | | | | | | mat | NM_001040113.1: c.5819_5820insCA(p. Gln1941Thrfs*20) | Alhopuro, Pia et al., 2008 [46] | |
| 148 | Lethal bone dysplasia | Skeletal | FGFR3 | Het | Achondroplasia (100800) | AD | mat | NM_000142.4:c.1118A > G(p. Tyr373Cys) | Otsuka M et al., 2011 [47] | TOP |
| 150 | Increased head circumference; Short humerus; Short femur; Situs inversus visceralis; Dextrocardia | Multisystem | DYNC2H1 | Comp het | Short-rib thoracic dysplasia 3 with or without polydactyly (613091) | AR | mat | NM_001080463.1:c.11747delG (p.Gly3916Valfs*23) | This study | TOP |
| | | | | | | | pat | NM_001080463.1:c.427G > C (p.Ala143Pro) | This study | |
| 161 | Polyhydramnios | Other | CLCN5 | Hemi | Dent disease 1 (300009) | XL | mat | NM_001127899.1:c.941C > T (p.Ser314Leu) | Qiaoping Chen et al., 2021 [48] | Livebirth |
| 172[a] | Exencephaly; Prominent umbilicus; Talipes calcaneovarus; Cardiac anomalies; Endocardial cushion defect | Multisystem | VANGL2 | Het | Neural tube defects (182940) | AD | pat | NM_020335.2:c.937+1G > T | This study | TOP |
| | | | G6PD | Hemi | | XL | mat | NM_001042351.1:c.95A > G(p. His32Arg) | This study | |
| 177 | Nuchal cystic hygroma; Short long bone | Multisystem | FGFR3 | Het | Achondroplasia (100800) | AD,AR | de novo | NM_000142.4:c.1948A > G(p. Lys650Glu) | Chih-Ping Chen et al., 2020 [49] | TOP |
| | | | COL1A2 | Het | Combined osteogenesis imperfecta and Ehlers-Danlos syndrome 2 (619120) | AD,AR | mat | NM_000089.3:c.2506G > A(p. Ala836Thr) | Clinvar database | |
| 178 | Ventricular septal defect; Common arterial trunk; Bilateral cleft lip; Hypoplasia of Proximal radius; Triangular shaped phalanges of the hand; Talipes calcaneovarus; Single umbilical artery | Multisystem | CHD7 | Het | CHARGE syndrome (214800) | AD | de novo | NM_017780.3:c.4908delG(p. Glu1636Aspfs*4) | This study | TOP |

(*continued on next page*)

**Table 4** (*continued*)

| ID | Clinical findings | Phenotype category | Gene | Zygosity | Disease (OMIM ID) | Inheritance pattern | Mutation origin | Variant(s) | Author(s) and Year | Outcome |
|---|---|---|---|---|---|---|---|---|---|---|
| 188 | Short long bone; Small rib cage; Lethal bone dysplasia | Skeletal | COL2A1 | Het | Achondrogenesis, type II or hypochondrogenesis (200610) | AD | mat | NM_001844.4:c.2473G > A(p. Gly825Arg) | This study | TOP |
| 191 | Intrauterine growth restriction | Growth | DYRK1A | Het | Intellectual developmental disorder, autosomal dominant 7 (614104) | AD | de novo | NM_101395.2:c.-76-2A > G | This study | Livebirth |
| 196 | Single atrium and ventricle | Cardiac | FANCA | Comp het | Fanconi anemia, complementation group A (227650) | AR | mat | NM_000135.2:c.70G > T(p. Glu24*) | Moghrabi, Nabil N et al., 2009 [50] | TOP |
|  |  |  |  |  |  |  | pat | NM_000135.2:c.4225C > T(p. Arg1409Trp) | Richards, Sue et al., 2015 [51] |  |
| 204 | Meningoencephalocele | Brain | CC2D2A | Comp het | Joubert syndrome 9 (612285) | AR | pat | NM_001080522.2: c.3070_3071delAGinsC(p. Arg1025Glufs*7) | This study | TOP |
|  |  |  |  |  |  |  | mat | NM_001080522.2:c.2848C > T (p.Arg950*) | Daimin Xiao et al., 2017 [52] |  |

Het, heterozygosity; Hom, homozygosity; Com het, compound heterozygosity; Hemi, hemizygote; P, pathogenic; LP, likely pathogenic; AR, autosomal recessive inheritance; AD, autosomal dominant inheritance; XL, X-linked inheritance; De novo, neither of the parents had the same variants; Pat, Father had the same variants; Mat, Mother had the same variants.

[a] Indicates patients have a dual diagnosis of pathogenic CNVs and SNV variants.
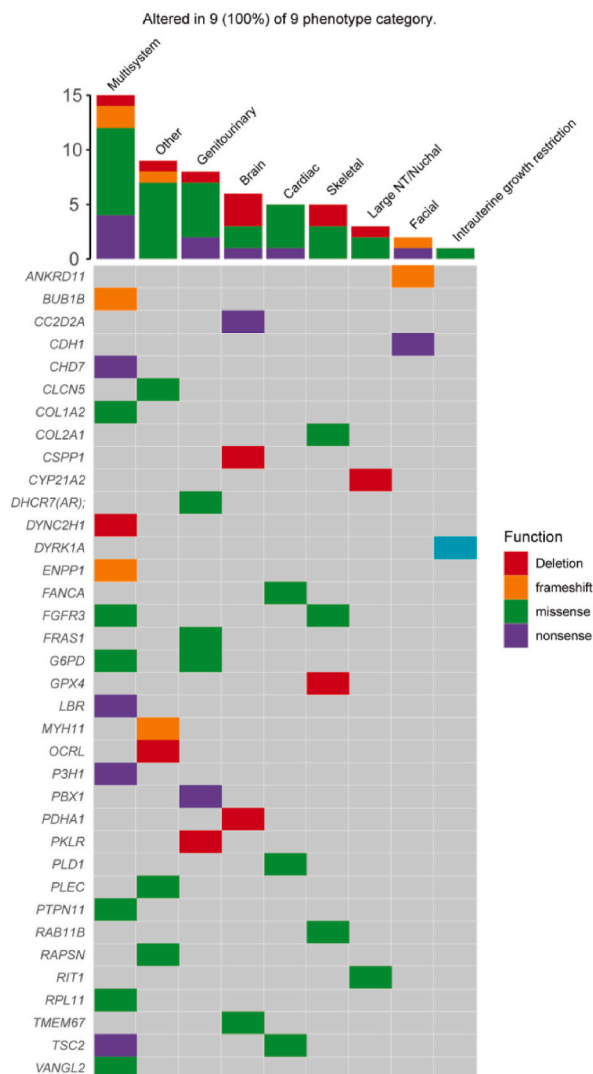
**Fig. 2.** Pathogenic (P) and likely pathogenic (LP) variants type among phenotype category detected by whole-exome sequencing.

### 3.4. Patients with dual diagnoses

In addition to these results, we identified three cases with both pathogenic CNVs and SNV variants (Table 3, Table 4), representing 1.72 % of all involved patients. All three fetuses with dual diagnoses were diagnosed with multisystem anomalies. In case 83, ultrasound findings including a fetal ventricular septal defect and absence of the nasal bone. A new variant, *RPL11*:c.126G > C(p. Gln42His), identified by WES, was determined to be likely pathogenic. The pathogenic SNV on the *RPL11* gene was reported to be associated with diamond-blackfan anemia 7 (OMIM#612562), an autosomal dominant disorder that can cause ventricular septal defect and ostium secundum atrial septal defect. The fetus was simultaneously detected for trisomy 21 syndrome (OMIM#190685) by CNV-seq, which was known to contribute to heart malformations and characteristic facial features. Case 87 showed intrauterine growth restriction (IUGR) and small kidneys through ultrasound. CNV-seq revealed a 7.6-Mb deletion at 4p16.3-p16.1, previously linked to Wolf-Hirschhorn syndrome (OMIM#194190). The affected fetuses exhibit intrauterine growth restriction, renal hypoplasia, and prenatal growth deficiency. It is important to note that the pathogenic SNV on the *LBR* gene in the autosomal dominant form was not found to be associated with the skeletal phenotype (OMIM#169400), which confirmed that the CNV was solely causal of the skeletal phenotype in this particular case. Case 172 showed exencephaly, a prominent umbilicus, cardiac anomalies, and other multisystem diseases. A pathogenic CNV in 2q35 (342.61 kb deletion) altered the *FN1* gene, which has been associated with genu varum and leg length discrepancy. The pathogenic SNV on the *G6PD* gene has been shown to cause neonatal jaundice and hemolytic anemia, providing additional information. The dual diagnosis provides compelling evidence for the genetic pathogenicity of fetuses, particularly those with symptoms in multiple anatomical systems.
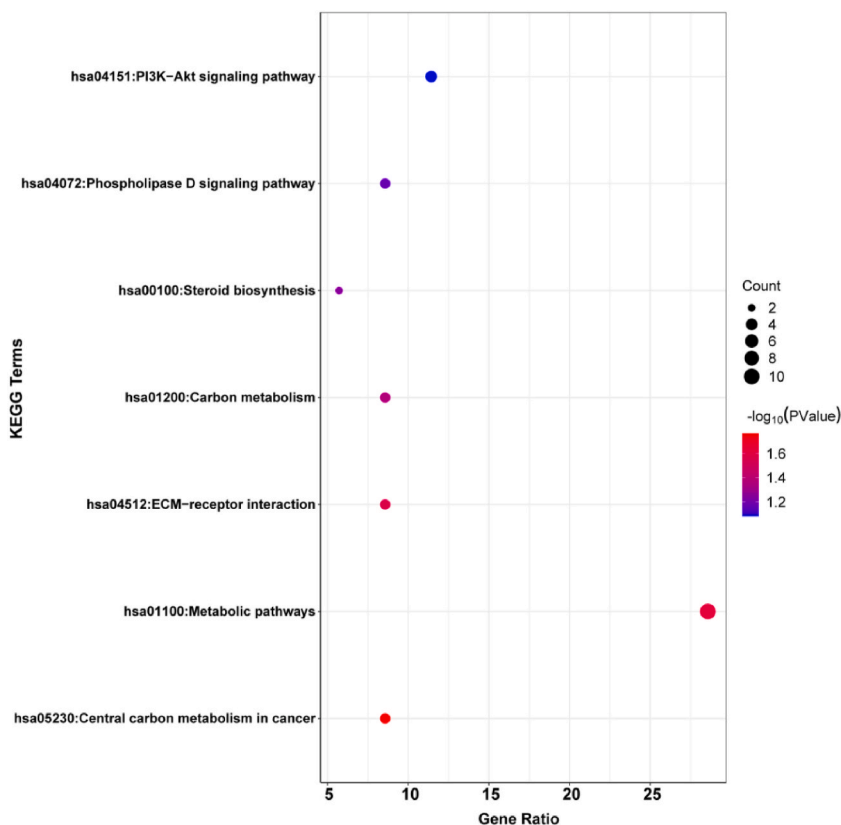
**Fig. 3.** KEGG enrichment analysis of pathogenic (P) and likely pathogenic (LP) variants detected by whole-exome sequencing.

## 4. Discussion

This study was one of the studies to simultaneously perform CNV-seq and WES analyses in fetuses with structural anomalies on ultrasound scans [8,11,53], yielding a diagnostic rate of 26.44 %, demonstrating a comprehensive evaluation of the genetic causes of these affected patients. CNV-seq and WES analyses interpreted 6.90 % and 17.82 % of fetal malformations, respectively. In addition, 1.72 % of enrolled patients had dual genetic diagnoses, with both pathogenic CNVs and SNV variants. These findings are comparable to the study by Chen et al. [8] in Hubei Province, China, which reported a diagnostic rate was 23.67 %, supporting the increased diagnostic rate and feasibility of using a combined strategy for fetal structural abnormalities.

Previous studies have shown that CNV-seq provides an additional 6%–10 % of genetic diagnoses [2,6,8,53–55], which is similar to our study. All these studies included more than 100 patients, suggesting that the actual incidence of pathogenic CNVs is likely on this scale. However, certain patient subtypes may exhibit much higher levels of diagnostic CNVs. For example, a study from Changzhou, China, focusing on congenital heart defects, found 24.5 % chromosomal abnormalities using CMA, including 11.5 % aneuploidies and 13.0 % clinical CNVs [56]. Another study focusing on congenital heart disease reported a similar rate of 14.3 % causative CNVs [26]. Studies involving more than 100 patients and using WES analysis reported the diagnostic yields ranged from 8.5 % to 49.6 %, depending on whether the focus was on all affected fetuses or some affected patients or different ethnic groups [4,5,8,53,54,57–62]. In our cohort, WES analyses yielded an 19.54 % causative diagnosis rate, which was comparable to cohorts with a similar number of unselected patients, higher than the larger cohorts, but lower than some specific types of affected fetuses. These differences may result from regional variations in disease occurrenceor focus on specific disease types within selected cohorts.

The highest diagnostic rate performed by CNV-seq and WES simultaneously in fetuses were multiple system or skeletal anomalies, 50 %, and 45.45 % respectively, suggesting that these clinical signs detected by prenatal ultrasound screening may serve as specific clues for genetic diagnoses, which aligns with previous studies [8]. Patients with central nervous system involvement also had a high genetic diagnostic rate (33.33 %), similar to a previous study on fetuses [6], indicating that genetic diagnosis can effectively aid in identifying the causes of this disease cohort. These three systems also had a high diagnostic yield in the WES analyses, with diagnosis rates exceeding 30 %, demonstrating that the clinical benefit of simultaneous CNV-seq and WES analysis was far greater than individual diagnostic strategy. In other words, fetuses with congenital structural abnormalities in these systems are recommended to undergo simultaneous WES and CNV-seq analysis to obtain the best diagnosis. Notably, fetuses with amniotic fluid abnormalities and brain anomalies were diagnosed solely through WES, with the genetic variants identified in over 30 % of cases. Based on these findings, it might be reasonable to prioritize WES testing over CNV-seq for such patients.

TAT was an critical concern for pregnant couples, especially for pregnant women in the third trimester. Previous studies have shown that the average TAT for a sequential diagnostic approach involving karyotype, CMA, and WES exceeds 4 weeks [63], which is not ideal for couples in the late-stage pregnancies. For congenital structural anomalies detected during the third-trimester pregnancies, the TAT was unacceptable and required urgent reduction. Simultaneous CNV-seq and WES could reduce the TAT to 21 days, making it a more feasible option for prenatal patients, especially in the third trimester. In this study, a total of 38 third-trimester patients (27 weeks+) were included, of which 23.68 % received a confirmed genetic diagnosis, representing 21.84 % (38/174) of the total cohort (Table 1). These findings indicate that at least in the Chenzhou region of Hunan Province, one-fifth of fetuses with congenital structural anomalies were diagnosed during the third trimester.

Economic efficiency is also a key consideration in the selection of prenatal diagnostic testing methods. While simultaneous CNV-seq and WES strategy can rapidly aid in diagnosing the causes for certain patients. For most pregnant women, clinical phenotypes remain essential for guiding diagnostic decisions. If ultrasound diagnosis has already confirmed prenatal symptoms suggestive of chromosomal disorders in the fetus, rapid diagnostic methods like karyotyping and CMA should be prioritized to exclude these conditions. Moreover, the simultaneous CNV-seq and WES testing is more suitable for pregnant women nearing delivery. Thus, it aids in quickly diagnosing the causes for these pregnant women, facilitating rapid clinical management and prognostic measures.

In our study, three patients (case 83, case 87, and case 172) were diagnosed with a dual diagnosis of pathogenic CNVs and SNV variants. The dual-diagnostic rate in our study was 1.72 %, which is comparable to the rate reported in the previous study (1.04 %) [8]. The current standard sequential karyotype-CMA-WES strategy in these cases could lead to the loss of important genetic variant information in such cases. If a pathogenic CNV is identified in cases following this sequential strategy, the workflow would be stopped and exome sequencing (ES) would be omitted. The available data on dual diagnoses in patients with prenatal anomalies are still limited, as few studies have utilized a simultaneous diagnosis strategy. Future research is needed to clarify the real incidence of dual-diagnosis pathogenic variants and to establish accurate precise counseling and medical guidelines for these patients.

Moreover, it is recommended to provide timely management and consultation for these pregnancies in the early stages of pregnancy, in order to assess potential risks for the couple in the future. In the subgroup of families with a positive diagnosis, 43 families received a definitive diagnosis, and 34 couples opted to terminate the pregnancy after counseling about the likely phenotype of the postnatal fetus. The results from these studies demonstrated the benefits of the simultaneous strategy in detecting prenatal structural anomalies, particularly during the third trimester of pregnancy. These findings strongly support recommending the simultaneous strategy as a first-line approach for prenatal structural anomalies.s.

## 5. Conclusions

In this study, simultaneous CNV-seq and WES analysis during pregnancy were employed for clinical diagnosis of CNVs and SNVs in fetuses with ultrasound abnormalities. Despite the ongoing issue of high costs, our results suggest that this diagnostic method is more suitable for prenatal diagnosis of the genetic etiology in fetuses with non-severe abnormalities, providing crucial reference for parents considering pregnancy termination. This diagnostic method also offers a more accurate reference in confirming the genetic etiology of malformed fetuses, especially in cases where clinical phenotypes of SNV and CNV pathogenic variations may be easily confused. Finally, pre-test genetic counseling is essential for the implementation of this combined strategy, facilitating the selection of appropriate populations for simultaneous CNV-seq and WES analysis in clinical diagnosis. Future research should focus on identify the population characteristics that are suitable for specific diagnostic strategies.

## CRediT authorship contribution statement

**Haoqing Zhang:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Xinglan He:** Writing – original draft, Data curation, Conceptualization. **Yuankun Wang:** Writing – original draft, Supervision, Methodology, Formal analysis, Conceptualization. **Caiyun Li:** Visualization, Methodology, Investigation, Data curation. **Hongguo Jiang:** Supervision, Data curation. **Shuai Hou:** Resources, Investigation, Data curation. **Dongqun Huang:** Investigation, Formal analysis, Data curation. **Wenqian Zhang:** Supervision, Data curation. **Jufang Tan:** Supervision, Investigation. **Xiaoyun Du:** Visualization, Supervision, Investigation. **Yinli Cao:** Methodology, Data curation. **Danjing Chen:** Resources, Investigation, Data curation. **Haiying Yan:** Resources, Methodology. **Lingling Peng:** Methodology, Data curation. **Dongzhu Lei:** Writing – review & editing, Resources, Project administration, Investigation, Data curation, Conceptualization.

## Consent to publish

Written informed consent was obtained from all subjects involved in the study.

## Additional information

No additional information is available for this paper.

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**Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Data availability**

The data are available upon request.

**References**

[1] M. Persson, S. Cnattingius, E. Villamor, J. Soderling, B. Pasternak, O. Stephansson, M. Neovius, Risk of major congenital malformations in relation to maternal overweight and obesity severity: cohort study of 1.2 million singletons, Bmj-Brit Med J 357 (2017) 2563, https://doi.org/10.1136/bmj.j2563.
[2] R.J. Wapner, C.L. Martin, B. Levy, B.C. Ballif, C.M. Eng, J.M. Zachary, M. Savage, L.D. Platt, D. Saltzman, W.A. Grobman, et al., Chromosomal microarray versus karyotyping for prenatal diagnosis, New Engl J Med 367 (2012) 2175–2184, https://doi.org/10.1056/NEJMoa1203382.
[3] S.C. Hillman, S. Pretlove, A. Coomarasamy, D.J. McMullan, E.V. Davison, E.R. Maher, M.D. Kilby, Additional information from array comparative genomic hybridization technology over conventional karyotyping in prenatal diagnosis: a systematic review and meta-analysis, Ultrasound Obstet. Gynecol. 37 (2011) 6–14, https://doi.org/10.1002/uog.7754.
[4] J. Lord, D.J. McMullan, R.Y. Eberhardt, G. Rinck, S.J. Hamilton, E. Quinlan-Jones, E. Prigmore, R. Keelagher, S.K. Best, G.K. Carey, et al., Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study, Lancet 393 (2019) 747–757, https://doi.org/10.1016/S0140-6736(18)31940-8.
[5] S. Petrovski, V. Aggarwal, J.L. Giordano, M. Stosic, K. Wou, L. Bier, E. Spiegel, K. Brennan, N. Stong, V. Jobanputra, et al., Whole-exome sequencing in the evaluation of fetal structural anomalies: a prospective cohort study, Lancet 393 (2019) 758–767, https://doi.org/10.1016/S0140-6736(18)32042-7.
[6] Y. Yaron, G.V. Ofen, A. Mory, H.N. Zunz, A. Kurolap, S.A. Bar, G.D. Brabbing, D. Marom, S.L. Ben, F.H. Baris, et al., Exome sequencing as first-tier test for fetuses with severe central nervous system structural anomalies, Ultrasound Obstet. Gynecol. 60 (2022) 59–67, https://doi.org/10.1002/uog.24885.
[7] F. Fu, R. Li, Y. Li, Z.Q. Nie, T. Lei, D. Wang, X. Yang, J. Han, M. Pan, L. Zhen, et al., Whole exome sequencing as a diagnostic adjunct to clinical testing in fetuses with structural abnormalities, Ultrasound Obstet. Gynecol. 51 (2018) 493–502, https://doi.org/10.1002/uog.18915.
[8] X. Chen, Y. Jiang, R. Chen, Q. Qi, X. Zhang, S. Zhao, C. Liu, W. Wang, Y. Li, G. Sun, et al., Clinical efficiency of simultaneous CNV-seq and whole-exome sequencing for testing fetal structural anomalies, J. Transl. Med. 20 (2022) 10, https://doi.org/10.1186/s12967-021-03202-9.
[9] N.A. Miller, E.G. Farrow, M. Gibson, L.K. Willig, G. Twist, B. Yoo, T. Marrs, S. Corder, L. Krivohlavek, A. Walter, et al., A 26-hour system of highly sensitive whole genome sequencing for emergency management of genetic diseases, Genome Med. 7 (2015) 100, https://doi.org/10.1186/s13073-015-0221-8.
[10] C.J. Saunders, N.A. Miller, S.E. Soden, C.J. Dinwiddie, A. Noll, N.A. Alnadi, N. Andraws, M.L. Patterson, L.A. Krivohlavek, J. Fellis, et al., Rapid whole-genome sequencing for genetic disease diagnosis in neonatal intensive care units, Sci. Transl. Med. 4 (2012) 135–154, https://doi.org/10.1126/scitranslmed.3004041.
[11] Q. Qi, Y. Jiang, X. Zhou, H. Meng, N. Hao, J. Chang, J. Bai, C. Wang, M. Wang, J. Guo, et al., Simultaneous detection of CNVs and SNVs improves the diagnostic yield of fetuses with ultrasound anomalies and normal karyotypes, Genes-Basel (2020) 11, https://doi.org/10.3390/genes11121397.
[12] G. Bergant, A. Maver, L. Lovrecic, G. Cuturilo, A. Hodzic, B. Peterlin, Comprehensive use of extended exome analysis improves diagnostic yield in rare disease: a retrospective survey in 1,059 cases, Genet. Med. 20 (2018) 303–312, https://doi.org/10.1038/gim.2017.142.
[13] Z. Dong, J. Zhang, P. Hu, H. Chen, J. Xu, Q. Tian, L. Meng, Y. Ye, J. Wang, M. Zhang, et al., Low-pass whole-genome sequencing in clinical cytogenetics: a validated approach, Genet. Med. 18 (2016) 940–948, https://doi.org/10.1038/gim.2015.199.
[14] E.R. Riggs, E.F. Andersen, A.M. Cherry, S. Kantarci, H. Kearney, A. Patel, G. Raca, D.I. Ritter, S.T. South, E.C. Thorland, et al., Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen), Genet. Med. 22 (2020) 245–257, https://doi.org/10.1038/s41436-019-0686-8.
[15] S. Richards, N. Aziz, S. Bale, D. Bick, S. Das, J. Gastier-Foster, W.W. Grody, M. Hegde, E. Lyon, E. Spector, et al., Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of medical genetics and genomics and the association for molecular pathology, Genet. Med. 17 (2015) 405–424, https://doi.org/10.1038/gim.2015.30.
[16] D.B. Zastrow, H. Baudet, W. Shen, A. Thomas, Y. Si, M.A. Weaver, A.M. Lager, J. Liu, R. Mangels, S.S. Dwight, et al., Unique aspects of sequence variant interpretation for inborn errors of metabolism (IEM): the ClinGen IEM Working Group and the Phenylalanine Hydroxylase Gene, Hum. Mutat. 39 (2018) 1569–1580, https://doi.org/10.1002/humu.23649.
[17] T. Brandt, L.M. Sack, D. Arjona, D. Tan, H. Mei, H. Cui, H. Gao, L. Bean, A. Ankala, G.D. Del, et al., Adapting ACMG/AMP sequence variant classification guidelines for single-gene copy number variants, Genet. Med. 22 (2020) 336–344, https://doi.org/10.1038/s41436-019-0655-2.
[18] S.S. Kalia, K. Adelman, S.J. Bale, W.K. Chung, C. Eng, J.P. Evans, G.E. Herman, S.B. Hufnagel, T.E. Klein, B.R. Korf, et al., Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics, Genet. Med. 19 (2017) 249–255, https://doi.org/10.1038/gim.2016.190.
[19] K.G. Monaghan, N.T. Leach, D. Pekarek, P. Prasad, N.C. Rose, The use of fetal exome sequencing in prenatal diagnosis: a points to consider document of the American College of Medical Genetics and Genomics (ACMG), Genet. Med. 22 (2020) 675–680, https://doi.org/10.1038/s41436-019-0731-7.
[20] L. Zhao, H. Luo, G. Luo, X. Qiu, Y. Liao, [Prenatal diagnosis and genetic analysis of a 46,XN,del(11)(q14q22) fetus], Zhonghua Yi Xue Yi Chuan Xue Za Zhi 37 (2020) 879–882, https://doi.org/10.3760/cma.j.issn.1003-9406.2020.08.018.
[21] C.P. Chen, L.K. Wang, S.R. Chern, P.S. Wu, S.W. Chen, F.T. Wu, L.F. Chen, Y.Y. Chen, W. Wang, Wolf-Hirschhorn syndrome: Prenatal diagnosis and molecular cytogenetic characterization of a de novo distal deletion of 4p (4p16.1–> pter) in a fetus with facial cleft and preaxial polydactyly, Taiwan. J. Obstet. Gynecol. 59 (2020) 425–431, https://doi.org/10.1016/j.tjog.2020.03.016.
[22] A.F. Jawad, D.M. McDonald-Mcginn, E. Zackai, K.E. Sullivan, Immunologic features of chromosome 22q11.2 deletion syndrome (DiGeorge syndrome/velocardiofacial syndrome), J. Pediatr. Urol. 139 (2001) 715–723, https://doi.org/10.1067/mpd.2001.118534.
[23] C.P. Chen, C.H. Chan, S.R. Chern, P.S. Wu, S.W. Chen, F.T. Wu, D.D. Town, M.S. Lee, W. Wang, Prenatal diagnosis and molecular cytogenetic characterization of a small supernumerary marker chromosome derived from chromosome 15 in a pregnancy associated with recurrent Down syndrome, Taiwan. J. Obstet. Gynecol. 60 (2021) 152–156, https://doi.org/10.1016/j.tjog.2020.11.023.

[24] H. Luo, Q. Xiao, W. Su, S. Chen, M. Jiang, G. Xiao, [Genetic analysis of a fetus with partial 18p tetraploidy syndrome], Zhonghua Yi Xue Yi Chuan Xue Za Zhi 35 (2018) 719–722, https://doi.org/10.3760/cma.j.issn.1003-9406.2018.05.023.

[25] M.A. Lizarraga, S. Mintegui, E.J. Sanchez, J.M. Galdeano, E. Pastor, A. Cabrera, [Heart malformations in trisomy 13 and trisomy 18], Rev. Esp. Cardiol. 44 (1991) 605–610.

[26] S. Hu, X. Kong, Molecular delineation of de novo small supernumerary marker chromosomes in prenatal diagnosis, a retrospective study, Taiwan. J. Obstet. Gynecol. 62 (2023) 94–100, https://doi.org/10.1016/j.tjog.2022.06.018.

[27] H.L. Dai, X. Zhou, X.F. Guang, Turner syndrome, Qjm-Int J Med 116 (2023) 136–137, https://doi.org/10.1093/qjmed/hcac224.

[28] A. Delahaye, P. Bitoun, S. Drunat, M. Gerard-Blanluet, N. Chassaing, A. Toutain, A. Verloes, F. Gatelais, M. Legendre, L. Faivre, et al., Genomic imbalances detected by array-CGH in patients with syndromal ocular developmental anomalies, Eur. J. Hum. Genet. 20 (2012) 527–533, https://doi.org/10.1038/ejhg.2011.233.

[29] L. Winters, E. Van Hoof, L. De Catte, K. Van Den Bogaert, T. de Ravel, L. De Waele, A. Corveleyn, J. Breckpot, Massive parallel sequencing identifies RAPSN and PDHA1 mutations causing fetal akinesia deformation sequence, Eur. J. Paediatr. Neurol. 21 (2017) 745–753, https://doi.org/10.1016/j.ejpn.2017.04.641.

[30] B.D. Natera-de, A. Topf, J.J. Vilchez, L. Gonzalez-Quereda, J. Dominguez-Carral, J. Diaz-Manera, C. Ortez, M. Bestue, P. Gallano, M. Dusl, et al., Molecular characterization of congenital myasthenic syndromes in Spain, Neuromuscul. Disord. 27 (2017) 1087–1098, https://doi.org/10.1016/j.nmd.2017.08.003.

[31] Z. Qiu, W.T. Chang, Y.C. Chou, K.C. Wen, Y. Ziying, K. Yuen, X. Cai, T.Y. Chang, H.C. Lai, P.L. Sung, Prenatal case of RIT1 mutation associated Noonan syndrome by whole exome sequencing (WES) and review of the literature, Taiwan. J. Obstet. Gynecol. 61 (2022) 535–538, https://doi.org/10.1016/j.tjog.2022.03.025.

[32] L. Baronciani, P. Bianchi, A. Zanella, Hematologically important mutations: red cell pyruvate kinase (2nd update), Blood Cell Mol. Dis. 24 (1998) 273–279, https://doi.org/10.1006/bcmd.1998.0193.

[33] Q. Jiang, Z.H. Deng, H.Y. Chen, H. Yang, W.J. Liu, [Gene mutants and their clinical characteristics of G6PD deficiency among children in luzhou area], Zhongguo Shi Yan Xue Ye Xue Za Zhi 28 (2020) 996–1000, https://doi.org/10.19746/j.cnki.issn.1009-2137.2020.03.046.

[34] L.T. de Souza, R.R. Nunes, M.O. de Azevedo, F.T. Maria, A new case of osteogenesis imperfecta type VIII and retinal detachment, Am. J. Med. Genet. 185 (2021) 238–241, https://doi.org/10.1002/ajmg.a.61934.

[35] W.A. Cabral, W. Chang, A.M. Barnes, M. Weis, M.A. Scott, S. Leikin, E. Makareeva, N.V. Kuznetsova, K.N. Rosenbaum, C.J. Tifft, et al., Prolyl 3-hydroxylase 1 deficiency causes a recessive metabolic bone disorder resembling lethal/severe osteogenesis imperfecta, Nat. Genet. 39 (2007) 359–365, https://doi.org/10.1038/ng1968.

[36] B.U. Fitzky, M. Witsch-Baumgartner, M. Erdel, J.N. Lee, Y.K. Paik, H. Glossmann, G. Utermann, F.F. Moebius, Mutations in the Delta7-sterol reductase gene in patients with the Smith-Lemli-Opitz syndrome, P Natl Acad Sci Usa 95 (1998) 8181–8186, https://doi.org/10.1073/pnas.95.14.8181.

[37] M. Witsch-Baumgartner, J. Loffler, G. Utermann, Mutations in the human DHCR7 gene, Hum. Mutat. 17 (2001) 172–182, https://doi.org/10.1002/humu.2.

[38] H.H. Dahl, C. Maragos, R.M. Brown, L.L. Hansen, G.K. Brown, Pyruvate dehydrogenase deficiency caused by deletion of a 7-bp repeat sequence in the E1 alpha gene, Am. J. Hum. Genet. 47 (1990) 286–293.

[39] P. Ranganath, V. Vs, I. Rungsung, A. Dalal, S. Aggarwal, Next generation sequencing in a case of early onset hydrops: closing the loop on the diagnostic odyssey!, Fetal Pediatr. Pathol. 42 (2023) 103–109, https://doi.org/10.1080/15513815.2022.2058660.

[40] N. Lahrouchi, A.V. Postma, C.M. Salazar, D.M. De Laughter, F. Tjong, L. Piherova, F.Z. Bowling, D. Zimmerman, E.M. Lodder, A. Ta-Shma, et al., Biallelic loss-of-function variants in PLD1 cause congenital right-sided cardiac valve defects and neonatal cardiomyopathy, J. Clin. Invest. (2021) 131, https://doi.org/10.1172/JCI142148.

[41] J.H. Park, C. Lee, M.S. Chang, K. Kim, S. Choi, H. Lee, H.S. Lee, K.C. Moon, Molecular characterization and putative pathogenic pathways of tuberous sclerosis complex-associated renal cell carcinoma, Transl Oncol 11 (2018) 962–970, https://doi.org/10.1016/j.tranon.2018.05.010.

[42] K. Hoffmann, C.K. Dreger, A.L. Olins, D.E. Olins, L.D. Shultz, B. Lucke, H. Karl, R. Kaps, D. Muller, A. Vaya, et al., Mutations in the gene encoding the lamin B receptor produce an altered nuclear morphology in granulocytes (Pelger-Huet anomaly), Nat. Genet. 31 (2002) 410–414, https://doi.org/10.1038/ng925.

[43] K. Nakanishi, K. Nozu, R. Hiramoto, S. Minamikawa, T. Yamamura, J. Fujimura, T. Horinouchi, T. Ninchoji, H. Kaito, N. Morisada, et al., A comparison of splicing assays to detect an intronic variant of the OCRL gene in Lowe syndrome, Eur. J. Med. Genet. 60 (2017) 631–634, https://doi.org/10.1016/j.ejmg.2017.08.001.

[44] K. Kosaki, T. Suzuki, K. Muroya, T. Hasegawa, S. Sato, N. Matsuo, R. Kosaki, T. Nagai, Y. Hasegawa, T. Ogata, PTPN11 (protein-tyrosine phosphatase, nonreceptor-type 11) mutations in seven Japanese patients with Noonan syndrome, J. Clin. Endocrinol. Metab. 87 (2002) 3529–3533, https://doi.org/10.1210/jcem.87.8.8694.

[45] L. Simonetti, C.D. Bruque, C.S. Fernandez, B. Benavides-Mori, M. Delea, J.E. Kolomenski, L.D. Espeche, N.D. Buzzalino, A.D. Nadra, L. Dain, CYP21A2 mutation update: comprehensive analysis of databases and published genetic variants, Hum. Mutat. 39 (2018) 5–22, https://doi.org/10.1002/humu.23351.

[46] P. Alhopuro, D. Phichith, S. Tuupanen, H. Sammalkorpi, M. Nybondas, J. Saharinen, J.P. Robinson, Z. Yang, L.Q. Chen, T. Orntoft, et al., Unregulated smooth-muscle myosin in human intestinal neoplasia, P Natl Acad Sci Usa 105 (2008) 5513–5518, https://doi.org/10.1073/pnas.0801213105.

[47] M. Otsuka, M. Mizuki, J. Fujita, S. Kang, Y. Kanakura, Constitutively active FGFR3 with Lys650Glu mutation enhances bortezomib sensitivity in plasma cell malignancy, Anticancer Res. 31 (2011) 113–122.

[48] Q. Chen, Y. Cao, L. Xu, Q. Liu, X. Wu, Bartter-like syndrome as the initial presentation of dent disease 1: a case report, Front Pediatr 9 (2021) 725251, https://doi.org/10.3389/fped.2021.725251.

[49] C.P. Chen, T.Y. Chang, T.W. Lin, S.R. Chern, S.W. Chen, S.T. Lai, T.Y. Chuang, W. Wang, Prenatal diagnosis of hydrancephaly and enlarged cerebellum and cisterna magna in a fetus with thanatophoric dysplasia type II and a review of prenatal diagnosis of brain anomalies associated with thanatophoric dysplasia, Taiwan. J. Obstet. Gynecol. 57 (2018) 119–122, https://doi.org/10.1016/j.tjog.2017.12.020.

[50] N.N. Moghrabi, M.A. Johnson, M.J. Yoshitomi, X. Zhu, M.J. Al-Dhalimy, S.B. Olson, M. Grompe, C.S. Richards, Validation of Fanconi anemia complementation Group A assignment using molecular analysis, Genet. Med. 11 (2009) 183–192, https://doi.org/10.1097/GIM.0b013e318193ba67.

[51] S. Richards, N. Aziz, S. Bale, D. Bick, S. Das, J. Gastier-Foster, W.W. Grody, M. Hegde, E. Lyon, E. Spector, et al., Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of medical genetics and genomics and the association for molecular pathology, Genet. Med. 17 (2015) 405–424, https://doi.org/10.1038/gim.2015.30.

[52] D. Xiao, C. Lv, Z. Zhang, M. Wu, X. Zheng, L. Yang, X. Li, G. Wu, J. Chen, Novel CC2D2A compound heterozygous mutations cause Joubert syndrome, Mol. Med. Rep. 15 (2017) 305–308, https://doi.org/10.3892/mmr.2016.6007.

[53] J. Zhou, Z. Yang, J. Sun, L. Liu, X. Zhou, F. Liu, Y. Xing, S. Cui, S. Xiong, X. Liu, et al., Whole genome sequencing in the evaluation of fetal structural anomalies: a parallel test with chromosomal microarray plus whole exome sequencing, Genes-Basel (2021) 12, https://doi.org/10.3390/genes12030376.

[54] L. Lei, L. Zhou, J.J. Xiong, Whole-exome sequencing increases the diagnostic rate for prenatal fetal structural anomalies, Eur. J. Med. Genet. 64 (2021) 104288, https://doi.org/10.1016/j.ejmg.2021.104288.

[55] F. Lu, P. Xue, B. Zhang, J. Wang, B. Yu, J. Liu, Estimating the frequency of causal genetic variants in foetuses with congenital heart defects: a Chinese cohort study, Orphanet J. Rare Dis. 17 (2022) 2, https://doi.org/10.1186/s13023-021-02167-8.

[56] Y. Xing, Y. Zhang, J. Chen, F. Wu, M. Yuan, G. Zou, Y. Yang, F. Zhou, J. Zhou, L. Sun, Prenatal diagnosis for fetuses with isolated and non-isolated congenital heart defects using chromosomal microarray and exome sequencing, Prenat. Diagn. 42 (2022) 873–880, https://doi.org/10.1002/pd.6168.

[57] A. Kucinska-Chahwan, M. Geremek, T. Roszkowski, J. Bijok, D. Massalska, M. Ciebiera, H. Correia, I. Pereira-Caetano, A. Barreta, E. Obersztyn, et al., Implementation of exome sequencing in prenatal diagnosis and impact on genetic counseling: the polish experience, Genes-Basel (2022) 13, https://doi.org/10.3390/genes13050724.

[58] N.L. Vora, K. Gilmore, A. Brandt, C. Gustafson, N. Strande, L. Ramkissoon, E. Hardisty, A. Foreman, K. Wilhelmsen, P. Owen, et al., An approach to integrating exome sequencing for fetal structural anomalies into clinical practice, Genet. Med. 22 (2020) 954–961, https://doi.org/10.1038/s41436-020-0750-4.

[59] K. Diderich, K. Romijn, M. Joosten, L. Govaerts, M. Polak, H.T. Bruggenwirth, M. Wilke, M.A. van Slegtenhorst, Y. van Bever, A.S. Brooks, et al., The potential diagnostic yield of whole exome sequencing in pregnancies complicated by fetal ultrasound anomalies, Acta Obstet. Gynecol. Scand. 100 (2021) 1106–1115, https://doi.org/10.1111/aogs.14053.

[60] R. Li, F. Fu, Q. Yu, D. Wang, X. Jing, Y. Zhang, F. Li, F. Li, J. Han, M. Pan, et al., Prenatal exome sequencing in fetuses with congenital heart defects, Clin. Genet. 98 (2020) 215–230, https://doi.org/10.1111/cge.13774.

[61] T.Y. Lei, F. Fu, R. Li, Q.X. Yu, K. Du, W.W. Zhang, Q. Deng, L.S. Li, D. Wang, X. Yang, et al., Whole-exome sequencing in the evaluation of fetal congenital anomalies of the kidney and urinary tract detected by ultrasonography, Prenat. Diagn. 40 (2020) 1290–1299, https://doi.org/10.1002/pd.5737.

[62] M. Chen, J. Chen, C. Wang, F. Chen, Y. Xie, Y. Li, N. Li, J. Wang, V.W. Zhang, D. Chen, Clinical application of medical exome sequencing for prenatal diagnosis of fetal structural anomalies, Eur. J. Obstet. Gynecol. Reprod. Biol. 251 (2020) 119–124, https://doi.org/10.1016/j.ejogrb.2020.04.033.

[63] M. Pratt, C. Garritty, M. Thuku, L. Esmaeilisaraji, C. Hamel, T. Hartley, K. Millar, B. Skidmore, S. Dougan, C.M. Armour, Application of exome sequencing for prenatal diagnosis: a rapid scoping review, Genet. Med. 22 (2020) 1925–1934, https://doi.org/10.1038/s41436-020-0918-y.