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Diagnostic performance of chromosomal microarray and whole exome sequencing in fetal structural anomalies: a single-center retrospective study

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Abstract

Background Fetal structural anomalies detected by prenatal ultrasound were reported in 3–5% of pregnancies, and they range from a single, minor anomaly to multiple systemic anomalies. Tests designed to identify the genetic factors (chromosomal or monogenic) responsible for the vast majority of fetal structural anomalies play a crucial role in prenatal diagnosis. A retrospective analysis was performed to assess the diagnostic yield and clinical efficiency of prenatal chromosomal microarray analysis (CMA) and whole-exome sequencing (WES) in diagnosis of fetuses with structural anomalies, and to explore genotype–phenotype correlations.

Methods We retrospectively analyzed 391 fetuses referred for genetic testing due to sonographically detected structural anomalies. CMA was performed in 310 cases, and WES was conducted in 81 cases with prior negative CMA results. Detected copy number variants (CNVs) and sequence variants were classified according to ACMG guidelines. Cases were stratified into nine phenotypic categories based on ultrasonographic findings.

Results CMA identified pathogenic CNVs in 13/310 cases (4.2%) and variants of uncertain significance (VUS) in 13/310 (4.2%). WES revealed pathogenic or likely pathogenic variants in 18/81 cases (22.2%) and VUS in 9/81 cases (11.1%). The highest diagnostic yield for CMA was observed in fetuses with cardiovascular anomalies (12%), and for WES in those with central nervous system anomalies (46.1%) and skeletal system anomalies (38.8%). Notably, eight novel likely pathogenic variants were identified. Incidental findings were detected in 2 WES cases.

Conclusions CMA and WES provide complementary value in the genetic evaluation of fetal structural anomalies. While CMA remains a robust first-tier test, WES significantly enhances diagnostic yield, especially in cases with negative CMA and specific phenotypic features. Accurate ultrasonographic phenotyping and appropriate test selection are crucial for maximizing diagnostic outcomes in prenatal settings.

Keywords Fetal diagnosis, Genotype–phenotype correlation, Whole-exome sequencing, Fetal anomalies, Chromosome abnormalities

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Introduction

Fetal malformations detected by ultrasonographic examination have been reported in approximately 3% of fetuses [1–3]. Heart defects, skeletal dysplasia, congenital anomalies, genitourinary system anomalies, craniofacial anomalies, and nervous system anomalies are the most common fetal structural anomalies [1]. Major fetal structural malformations occur in 2–3% of pregnancies and are responsible for approximately 25% of perinatal deaths [2, 4–6]. Aneuploidies, chromosomal structural rearrangements, and monogenic diseases are genetic causes that are associated with fetal structural anomalies. Conventional cytogenetic analysis is used as the first step in routine prenatal diagnosis, and the diagnostic value has been reported to be approximately 30% in prenatal samples with fetal ultrasonographic anomalies. The diagnostic value of conventional cytogenetic analysis used to detect chromosomal anomalies in cases with fetal structural abnormalities has been reported to be approximately 30% [2, 7, 8]. In the remaining cases, CMA and/or WES tests are used in the next step to detect the etiology of fetal anomalies. Chromosomal microarray, which detects submicroscopic changes not detected by standard karyotyping, contributes 10% to the diagnostic value in structurally malformed fetuses. The diagnostic value of prenatal WES varies between 8.5 and 39.4% in different studies [4, 9].

The genetic etiology of fetal structural anomalies remains unknown in the majority of cases (more than 60%) [9]. The data obtained through comprehensive genetic evaluations using tests such as chromosome microarray and whole-exome sequencing will contribute to the establishment of the phenotype-genotype relationship. They will be beneficial for elucidating the pathogenesis and genetic causes of fetal structural anomalies. The diagnostic yield of prenatal WES is primarily determined by accurate indications [9–11]. With the use of comprehensive studies such as WES and CMA, the number of variants of unknown significance (VUS) is increasing, and it is important to interpret these variants correctly. The pathogenic effects of these variants can be clarified

by collecting data from new patients, which will benefit the establishment of phenotype-genotype correlation.

It was aimed to evaluate the diagnostic contribution of CMA and WES in a cohort of fetuses with structural anomalies and to assess the genotype–phenotype correlations across major phenotypic categories. We also present novel variants and discuss their relevance to prenatal genetic counseling and decision-making.

Material & method

Study design and cohort

This study was designed as a retrospective study aiming to analyze the genetic data (CMA or WES) of 391 fetuses with structural anomalies detected by prenatal ultrasonography. All cases were referred to a single tertiary center for genetic evaluation between April 2020 and April 2024. CMA was performed in 310 cases, and WES was applied to 81 cases with negative CMA results. Fetuses referred solely due to abnormal serum screening, high-risk non-invasive prenatal testing (NIPT), maternal anxiety, or familial known variants were excluded. Cases were categorized into nine major phenotypic groups based on ultrasonographic findings: Multisystem anomalies, Cardiovascular anomalies, Skeletal system anomalies, Central nervous system/cranial anomalies, Hydrops fetalis, Increased nuchal translucency (NT), Cystic hygroma, Urogenital anomalies/oligohydramnios, Digestive system anomalies/polyhydramnios (Table 1).

Sample collection and DNA extraction

Genomic DNA was isolated from chorionic villus sampling (CVS), amniotic fluid, fetal tissue, or cord blood using the QIAamp DNA Blood or Tissue Kits (QIAGEN, Germany).

Chromosomal microarray analysis

Microarray analysis of fetal genomic DNA samples from 310 fetuses was performed using the Affymetrix Cytoscan Optima kit by the Affymetrix CytoScan test (Life Technologies, USA) and the Illumina HumanCytoSNP-12 kit (Life Technologies, USA) by the Illumina

Table 1 Diagnostic yields of CMA and WES by phenotypic group

Phenotypic Group	CMA Diagnostic Yield (%) n:310	WES Diagnostic Yield (%) n:81
Central Nervous System/Cranial Anomalies	5.5%	46.1%
Skeletal System Anomalies	8.8%	38.8%
Hydrops Fetalis	8.0%	33.3%
Cardiovascular System Anomalies	12.0%	33.3%
Multisystem Anomalies	9.5%	28.5%
Urogenital Anomalies/Oligohydramnios	5.5%	28.5%
Digestive System Anomalies/Polyhydramnios	4.1%	33.3%
Increased Nuchal Translucency (NT)	8.5%	0%
Cystic Hygroma	6.6%	0%

Abbreviations: LP likely pathogenic, P pathogenic, VUS variant of unknown significance

Iscan platform. Analysis of the array data was performed using Chromosome Analysis Suite software and BlueFuse software. Analysis of the obtained data and evaluation of pathogenicity were performed using DECIPHER, Database of Genomic Variants (DGV), ClinVar, and Franklin Genoox programs.

Whole-exome sequencing (WES)

Whole-exome sequencing was conducted using both the MGI DNBSEQ-G400 (MGI Tech, China) and Illumina NovaSeq 6000 (Illumina, San Diego, USA) platforms. Library preparation was performed using the Twist Exome 2.0 kit. The generated libraries were sequenced aiming to achieve a minimum of 20x read depth for more than 98% of the targeted bases. Sequencing data were aligned to the human reference genome GRCh38 using Franklin Genoox software (<https://franklin.genoox.com/clinical-db/home>). Bioinformatic analysis of Whole-exome sequencing data was performed using the Franklin by Genoox Analysis program. Variant annotation and filtering were performed using a comprehensive set of public databases (ClinVar, the Human Gene Mutation Database (HGMD), dbSNP), as well as population frequency datasets (Genome Aggregation Database (gnomAD), Exome Aggregation Consortium (ExAC), 1000 Genomes Project). Functional predictions were assessed using in silico tools, including PolyPhen-2, SIFT, MutationAssessor, and SpliceAI.

Variant classification

Variant classification was performed based on ACMG recommendations using a combination of standardized evidence criteria established to assess the pathogenic impact of the variant. These criteria include PVS1 (variants in genes where the disease mechanism is loss-of-function), PM2 (extremely rare variants not found in population databases or with low population frequency), and PP3 (variants suggested by many prediction tools to have a deleterious effect). Considering the data from functional studies, variant location and segregation analysis results, additional criteria such as PS1, PM1, and PP1 were also applied. Each variant's classification as pathogenic, likely pathogenic, or variant of uncertain significance (VUS) was determined by integrating these evidence codes following ACMG recommendations [12]. The detected variants were classified as 'Pathogenic,' 'Likely pathogenic,' 'Variant of unknown clinical significance (VUS),' 'Likely Benign,' or 'Benign' according to specific ACMG/AMP guidelines [12]. Only pathogenic, likely pathogenic, and clinically relevant VUS variants were reported. Variants unrelated to the referral phenotype (e.g., neurodevelopmental disorders not detectable prenatally) were categorized as incidental findings. Pre-test counselling was conducted to ensure that the fetal

incidental findings, which are defined in the ACMG guideline, will be reported. The reporting criteria for fetal incidental findings were done according to ACMG guidelines [13].

Statistical analysis

Statistical analysis was performed using Fisher's exact test. The significance of differences in the diagnostic yield of chromosomal microarray analysis (CMA) and whole exome sequencing (WES) tests among phenotypic groups was assessed statistically. Statistical comparisons were made both at the overall level and within each of the nine defined phenotypic categories. A p -value of <0.05 was considered statistically significant.

Results

Study cohort

A total of 391 fetuses with sonographically identified structural anomalies were included. CMA was performed in 310 cases, and WES was applied in 81 fetuses with previously negative CMA results. The most common referral indications for CMA were multisystem anomalies (23.5%), cardiovascular anomalies (16%), and increased nuchal translucency (11.2%), respectively. The most common referral indications for WES were multisystem anomalies (25.9%), skeletal system anomalies (22.2%), and hydrops fetalis (16%), respectively.

Diagnostic yield of chromosomal microarray analysis (CMA)

Of the 310 fetuses who underwent CMA, CNVs were detected in 26 cases (8.3%). Pathogenic or likely pathogenic CNVs were found in 13 cases (4.2%), and variants of uncertain significance (VUS) in another 13 cases (4.2%) (Table 1). The highest diagnostic yield was observed in cardiovascular anomalies (12%), multisystem anomalies (9.5%), skeletal anomalies (8.8%), and increased NT (8.5%). The lowest yield was found in fetuses with digestive system anomalies (4.1%, 1/24) and cystic hygroma (6.6%, 1/15). The diagnostic rates of the detected variants according to phenotypic categories were represented in Table 1. Three cases were diagnosed with established CNV syndromes, including 9q34.3 deletion (Kleefstra syndrome), 15q13.3 microdeletion syndrome, and 22q11.21 microduplication syndrome (Table 2). Deletions/duplications were detected in 26 of 310 patients: 13 deletions (6 pathogenic/likely pathogenic, 7 VUS) and 13 duplications (7 pathogenic/likely pathogenic, 6 VUS). In one case, the patient had two different copy number variants. The smallest chromosomal abnormality was a 195 kb heterozygous deletion of the 16q24.1 region, and the largest abnormality was a 34.5 MB heterozygous duplication of the 12p13.33p11.1 region. The maximum size of pathogenic/likely pathogenic deletions was

Table 2 Genotypic and phenotypic characteristics of fetuses with CNV variants

Case	Indication	Chromosome location	Variant type	Size	Zygosity	ACMG Classification	Associated CNV Syndrome
1	omphalocele, nazal bone hypoplasia, choroid plexus cyst, cardiomegaly	7q32.2q34(130724131–142269186)	deletion	11,5 MB	Heterozygous	Pathogenic	NA
2	sacral agenesis	18p11.32p11.21(736718–14850105)	deletion	14.1 MB	Heterozygous	Pathogenic	NA
3	situs inversus, VSD, clubfoot	15q11.1q11.2(20221245–22274367)	deletion	2.1 MB	Heterozygous	VUS	NA
4	right aortic arc anomaly	15q13.2q13.3(3078,466–32622039)	deletion	1.8 MB	Heterozygous	Pathogenic	15q13.3 microdeletion syndrome
5	increased NT, cystic hygroma	16p11.2(28503803–29331350)	duplication	827 kb	Heterozygous	VUS	NA
6	congenital heart defect, heterotaxy	17q25.3(80583397–81044553)	deletion	461 kb	Heterozygous	VUS	NA
7	increased NT, thoracic hemivertebra, frontal bossing, single umbilical artery, bilateral pelviectasis, craniosynostosis	17q21.31(44222477–44744848)	deletion	522 kb	Heterozygous	VUS	NA
8	megacystitis, oligohydramnios	Xp22.33(63303–881031)/Yp11.32(13303–831031)	duplication	0.8 MB	Heterozygous	VUS	NA
9	Multiple fetal anomalies	18p11.32p11.21(64847–15024061)	duplication	15 MB	Heterozygous	Pathogenic	NA
10	polyhydramnios, tracheo-esophageal fistula	17q21.31(40940906–41563104)	deletion	622 kb	Heterozygous	VUS	NA
11	Shortness of long bones, increase in cardiothoracic ratio, nasal bone hypoplasia	13q12.12(22980144_24300788)	duplication	1.3 MB	Heterozygous	VUS	NA
12	polydactyly	7p11.2q11.21(56899324–63437954)	duplication	6.5 MB	Heterozygous	likely pathogenic	NA
13	abdominal wall defect, ectopia cordis, Cantrell's pentalogy	Xp22.31(6499633–8118384)	duplication	1.6 MB	Heterozygous	VUS	NA
14	Increased NT, Fallot tetralogy	16q11.2q12.1(46470077–52453763)	duplication	5.6 MB	Heterozygous	VUS	NA
15	Hydrops fetalis	Xp22.31(6568449–8188133)	deletion	1.6 MB	Heterozygous	Pathogenic	NA
16	ARSA	2q32.1(187120595–187890972)	deletion	770 kb	Heterozygous	VUS	NA
17	Spina bifida, choroid plexus cyst	2q13(110116258–110630891)	deletion	514 kb	Heterozygous	VUS	NA
18	Micrognathia, nasal bone hypoplasia	22q11.21(18143467–21560807)	duplication	3.4 MB	Heterozygous	Pathogenic	22q11 duplication syndrome
19	Pes equinovarus	9q34.3(137507241–138125937)	deletion	618 kb	Heterozygous	Pathogenic	9q34.3 deletion syndrome (Kleefstra syndrome)
20	Increased NT, cleft lip/palate	13q14.1 2q21.1(44807390–56562962)	deletion	11.8 MB	Heterozygous	Pathogenic	NA
21	Increased NT, hydrops fetalis	21q11.2q22.3(13634137–46677460) 7q36.1(151487899–152710542)	duplication duplication	21 MB 1.2 MB	Heterozygous Heterozygous	Pathogenic VUS	NA NA
22	diaphragmatic hernia, micro-melia, cystic hygroma	12p13.33p11.1(64621–34629700)	duplication	34.5 MB	Heterozygous	Pathogenic	NA
23	Cardiac anomaly	8p22(17430503–18458211)	Duplication	1 MB	Heterozygous	VUS	NA
24	AVSD, right aortic arch(U sign+), ARSA, oligohydramnios	19p13.3(267039–6126787)	duplication	5.9 MB	Heterozygous	Pathogenic	NA
25	increased NT	16q24.1(86545831–86741626)	deletion	195 kb	Heterozygous	VUS	NA
26	Multisystem anomalies	13q33.1q34(104331905–115092569) 11q24.1q25(122131711–134934196)	deletion duplication	10 MB 12 MB	Heterozygous Heterozygous	Pathogenic Pathogenic	NA NA

14.1 MB. The smallest size of pathogenic/likely pathogenic deletions was 618 kb. The maximum size of pathogenic/likely pathogenic duplications was 34.5 MB, and the smallest size of pathogenic/likely pathogenic duplications was found to be 514 kb.

Diagnostic yield of whole exome sequencing (WES)

Among the 81 WES cases, pathogenic or likely pathogenic variants were detected in 18 fetuses (22.2%). An additional nine fetuses (11.1%) carried VUS. The overall yield of reportable variants (pathogenic + VUS) was 33.3%. The highest diagnostic yield was observed in central nervous system anomalies (46.1%), skeletal anomalies (38.8%), hydrops fetalis (33.3%), and multi-system anomalies (28.5%). No pathogenic variants were detected in fetuses with isolated increased NT or cystic hygroma. The diagnostic rates of the detected variants according to phenotypic categories were represented in Table 1. A total of 34 different variants in 30 genes were detected in 27 of 81 cases: three frameshift, four stop gain, five splicing variants, twenty-one missense variants, and 1 Non-frameshift insertion/deletion variant. A compound heterozygous variant was detected in 1 case. Autosomal dominant (AD) conditions were detected in 11 of 20 cases, autosomal recessive (AR) conditions in 18 cases, X-linked recessive (XLR) conditions in 1 case, and X-linked dominant (XLD) conditions in 1 case (Table 3). Details of individual gene variants, associated phenotypes, and ACMG classifications are presented in Table 3. Incidental pathogenic or likely pathogenic variants unrelated to the fetal phenotype were identified in 2 WES cases (2.4%). These included variants in PKD1 and CFTR, not associated with any prenatal ultrasound findings in the respective fetuses.

Statistical comparison of diagnostic yields

Statistical analysis revealed that there were significant differences in diagnostic yields between CMA and WES in all phenotypic groups ($p < 0.05$ for each comparison). The overall diagnostic yield for pathogenic/likely pathogenic variants was significantly higher in the WES cohort (22.2%) compared to the CMA cohort (4.2%) ($p < 0.001$). WES demonstrated markedly higher yields in fetuses with central nervous system anomalies (46.1% vs. 5.5%, $p < 0.001$), skeletal anomalies (38.8% vs. 8.8%, $p < 0.001$), and hydrops fetalis (33.3% vs. 8.0%, $p < 0.001$). In contrast, for fetuses with isolated increased nuchal translucency or cystic hygroma, WES identified no pathogenic variants, at the same time CMA contributed limited findings (8.5% and 6.6%, respectively), with both differences remaining statistically significant ($p = 0.004$ and $p = 0.019$).

Discussion

This study retrospectively analyzed the clinical and genotypic findings of 391 prenatal cases with fetal structural anomalies. CMA yielded pathogenic or likely pathogenic variants in 4.2% of cases, and this result aligns with prior studies, which have reported CMA diagnostic yields between 4% and 10% in fetuses with sonographic anomalies [14, 15]. The highest yield was observed in fetuses with cardiovascular anomalies (12%), consistent with prior reports supporting CMA as a first-tier test for congenital heart defects [6, 16, 17]. Interestingly, the overall VUS rate in our CMA cohort (4.2%) was slightly higher than the 1.6–3.4% reported in earlier studies, potentially reflecting higher resolution platforms and broader database integration [6, 14–16]. Lowest diagnostic rates of CNVs were observed for gastrointestinal system anomalies (4.1%, 1/24). Notably, 11.5% of CNV-positive cases were consistent with known CNV syndromes, including Kleeftstra syndrome, 15q13.3 microdeletion, and 22q11.2 duplication syndrome, emphasizing the clinical relevance of submicroscopic chromosomal imbalances. 15q13.3 Microdeletion syndrome was detected in a case with the right aortic arch anomaly. 15q13.3 Microdeletion syndrome is characterized by a broad spectrum of neurodevelopmental disorders with no or mild dysmorphological features. We detected a 9q34 microdeletion in a case with pes equinovarus. 9q34 microdeletion causes Kleeftstra syndrome, which is characterized by intellectual disability, hypotonia, speech delay, autism spectrum disorder, dysmorphic facial features, and varying additional clinical features. 22q11.2 duplication syndrome was detected in cases with micrognathia and nasal hypoplasia. 22q11.2 duplication syndrome is characterized by a highly variable phenotype, and one of the phenotypic features is micrognathia, which was presented in our case. Pathogenic/possibly pathogenic incidental copy number variants were detected in 8 cases. Among the cases with incidental findings—variants not related to the fetal ultrasound anomalies—two patients were diagnosed with CNV syndromes.

WES achieved a diagnostic yield of 22.2% for pathogenic or likely pathogenic variants, with the highest rates seen in central nervous system anomalies (46.1%) and skeletal anomalies (38.8%). Negative WES results were observed in cases with increased NT and cystic hygroma because the majority of fetuses presenting with increased nuchal translucency (NT) or cystic hygroma are typically diagnosed through CMA or conventional karyotyping. Consequently, the likelihood of identifying additional monogenic causes via WES in these cases is reduced. The low diagnostic yield observed in the WES group with increased NT or cystic hygroma in our study may primarily be due to the prior exclusion of cases already diagnosed by CMA. This reflects the typical clinical workflow

Table 3 Genotypic and phenotypic characteristics of fetuses with WES variants

Case	Indication	Gene	Variant/dbSNP Number	Zygosity	VARIANT TYPE	ACMG Classification	Associated Disorder/inheritance
1	cardiac anomaly, enlarged heart, polyhydramnios	PKP2 NM_001005242.3	c.511 A>T/p.Arg171*	homozygous	stop gain	Likely pathogenic	Arrhythmogenic Right Ventricular Dysplasia AD/AR
2	ascites, limb abnormality, hydrops fetalis, hydrothorax	CHRNA1 NM_005199.5	c.202 C>T/p.Arg68* rs764266722	homozygous	stop gain	Pathogenic	Multiple Pterygium Syndrome AR
3	skeletal anomalies, syndactyly	FGFR2 NM_000141.5	c.755 C>G/p.Ser252Trp rs79184941	heterozygous	mis-sense	Pathogenic	FGFR2 related disease AD
4	increased nuchal fold, fetal skin edema, cystic hygroma, hydrops fetalis	STAC3 NM_145064.3	c.670+ 2T > A rs112253539	homozygous	Splice donor	Pathogenic	Congenital myopathy AR
5	posterior fossa cyst, hypoplastic cerebellum, corpus callosum agenesis, ventriculomegaly, strawberry shape skull	FKBP NM_024301.5	c.1048del p.A350Pfs*78	homozygous	frame-shift	Likely Pathogenic	Muscular dystrophy-dystroglycanopathy AR
6	narrow thorax, clover leaf cranium, flat nose, short limbs	ROR2 NM_004560.4 FAT4 NM_001291303.3	c.1386 + 1G > A c.9494 C>T/p. Thr3165Met rs770333760	homozygous homozygous	splice donor mis-sense	Likely Pathogenic VUS	Robinow Syndrome AR Van Maldergem Syndrome AR
7	short stature, skeletal abnormalities, intestinal dilation, brain abnormality	ARSL NM_000047.3 NSD1 NM_022455.5	c.410G>C/p.Gly137Ala rs80338711 c.5990 A>G/p. Tyr1997Cys rs797045825	heterozygous heterozygous	mis-sense mis-sense	Pathogenic Pathogenic	Chondrodysplasia punctata XLR Sotos Syndrome AD
8	hydrops fetalis, ascites, pleural effusion	GALNS NM_000512.5	c.239 C>T/p.Ser80Leu rs1209154325 c.1139 + 2T > G	heterozygous heterozygous	mis-sense splice donor	Pathogenic Likely Pathogenic	Mucopolysaccharidosis IVA AR
9	renal agenesis, ventriculomegaly	FGFR2 NM_000141.5	c.1052 C>G/p. Ser351Cys rs121918502	heterozygous	mis-sense	Pathogenic	Apert syndrome AD
10	arthrogryposis, camptodactyly, intrauterine death, hydrops	RYR1 NM_000540.3 CFTR NM_000492.4	c.13013_13032del/ p.Ala4338Glyfs*238 rs193922856 c.3038 C>T/p. Pro1013Leu rs193922516	homozygous homozygous	splice site frame-shift	Likely Pathogenic VUS	Congenital myopathy 1B AR Cystic Fibrosis AR
11	skeletal anomalies, narrow thorax, craniosynostosis, pes equinovarus, tibia bowing, short limbs	FGFR3 NM_000142.5	c.746 C>G/p. Ser249Cys rs121913483	heterozygous	mis-sense	Pathogenic	Achondroplasia AD
12	short long bone, skeletal anomalies, narrow chest, narrow thorax, frontal bossing	INPPL1 NM_001567.4	c.1179dupC/p. Ile394Hisfs*72	homozygous	frame-shift	Likely Pathogenic	Opsismodysplasia AR

Table 3 (continued)

Case	Indication	Gene	Variant/dbSNP Number	Zygosity	VARIANT TYPE	ACMG Classification	Associated Disorder/inheritance
13	ventriculomegaly, hydrocephaly, abnormality of brain morphology	CSF1R NM_001288705.3	c.121 C>T/p.Arg41*	homozygous	Stop gain	Likely Pathogenic	Brain abnormalities, neurodegeneration, and dysosteosclerosis AR
14	polyhydramnios, intestinal dilation, honeycomb appearance of the bowel	SLC26A3 NM_000111.3	c.239G>T/p.Gly80Val	homozygous	mis-sense	Likely Pathogenic	Diarrhea 1, secretory chloride, congenital AR
15	oligohydramnios, abnormal skull, prenatal onset, polydactyly, microcephaly	B9D2 NM_030578.4	c.513 C>G/p.Tyr171* rs143680317	homozygous	Stop gain	Likely Pathogenic	Joubert syndrome 34 AR Meckel Syndrome, Type 10 AR
16	increased NT, choroid plexus cyst, cardiac anomaly	NOTCH1 NM_017617.5	c.3524_3525del7 p.Tyr1175Serfs*7	heterozygous	mis-sense	Likely Pathogenic	Adams-Oliver syndrome 5 AD Aortic valve disease 1 AD
17	cerebellar atrophy, choroid plexus cyst, cerebellar hypoplasia, ventriculomegaly	PKD1 NM_001009944.3	c.2545G>A/p.Val849Met rs749138783	heterozygous	mis-sense	Likely Pathogenic	Polycystic Kidney Disease AD
18	hydronephrosis, brain anomaly	MED12 NM_005120.3	c.2881 C>T/p.Arg961Trp rs80338758	heterozygous	mis-sense	Likely Pathogenic	Hardikar syndrome XLD
19	neural tube defect, hydronephrosis	VANGL2 NM_020335.3	c.936G>A p.Glu312= rs567154551	heterozygous	splice region	VUS	Neural tube defects AD
20	ascites, polyhydramnios, edema, hydrops fetalis	PIEZO1 NM_001142864.4	c.4018 C>T/p.Arg1340Cys rs888911518	heterozygous	mis-sense	VUS	Dehydrated hereditary stomatocytosis with or without pseudohyperkalemia and/or perinatal edema AD
21	polydactyly, cystic kidneys	MKKS NM_170784.3	c.871G>T/p.Val291Phe	heterozygous	mis-sense	VUS	Bardet-Biedl syndrome 6 AR
22	iugr, cortical malformation, contractures, cortical atrophy, cerebellar hypoplasia, ventriculomegaly	THOC6 NM_024339.5 DYNC1H1 NM_001376.5	c.553T>C/p.Ser185Pro rs1335063379 c.784 C>G/p.Leu262Val	homozygous heterozygous	mis-sense mis-sense	VUS VUS	Beaulieu-Boycott-Innes syndrome AR Cortical dysplasia, complex, with other brain malformations 13 AD
23	fetal skin edema, cystic hygroma, limb anomaly, skeletal anomalies, increased nuchal translucency, echogenic bowel	CRB2 NM_173689.7 NEK1 NM_001199397.3	c.676G>C/p.Glu226Gln rs745315528 c.838T>A p.Phe280Ile	homozygous homozygous	mis-sense mis-sense	VUS VUS	Ventriculomegaly with cystic kidney disease AR Short-rib thoracic dysplasia 6 with or without polydactyly AR

Table 3 (continued)

Case	Indication	Gene	Variant/dbSNP Number	Zygosity	VARIANT TYPE	ACMG Classification	Associated Disorder/inheritance
24	corpus callosum agenesis, occipital encephalocele, ventriculomegaly, polydactyly	TCTN2 NM_017780.4	c.1456T>C/p. Arg312Gln rs955811265	homozygous	mis-sense	VUS	Meckel syndrome 8 AR
25	intrauterine death, cardiac anomaly, pes equinovarus	CHD7 NM_017780.4	c.935G>A/p.Arg312Gln rs955811265	heterozygous	mis-sense	VUS	Charge Syndrome AD
26	cardiac anomaly, renal anomaly, skeletal abnormalities	CWC27 NM_005869.4	c.1412G>A p.Arg471Lys rs779259350	homozygous	mis-sense	VUS	Retinitis pigmentosa with or without skeletal anomalies AR
27	anhydramnios, hypoplastic lung, polycystic kidneys	PKD1 NM_001009944.3	c.9467_9469dup p.Gly3156dup	heterozygous	non-frame-shift	VUS	Polycystic Kidney Disease AD
		TRIO NM_007118.4	c.7475 C>A/p. Ser2492Tyr rs774046957	heterozygous	mis-sense	VUS	Deafness 28 AR

Abbreviations: AR autosomal recessive, AD autosomal dominant

where WES is generally considered as a secondary test following negative CMA results. Similarly, Daum et al. (2019) reported a 25% diagnostic yield in fetuses with structural anomalies referred for exome sequencing, with the highest yields observed in those with multiple malformations and brain anomalies [18]. No pathogenic variants were detected in fetuses with isolated increased nuchal translucency, consistent with our findings. These results support the selective use of WES based on specific phenotypic features to maximize diagnostic efficiency. The current study's pathogenic diagnostic rate for prenatal WES analysis was consistent with previous studies' diagnostic rates (ranging from 10 to 30%) [1–3, 19]. These results are aligned with meta-analyses and cohort studies that highlight the superior yield of WES in fetuses with single-system or syndromic malformations, especially when conventional tests are non-diagnostic [1–3, 19]. Several studies give different diagnostic rates, which were affected by several factors such as cohort size variant interpretation, and prenatal indications (Table 4) [4, 20–27]. Consistent with recent literature higher diagnostic rates of WES were observed in cases with musculoskeletal anomalies (38,8%, 7/18) and central nervous system anomalies (46,1%, 6/13) [4, 28–30]. The results reaffirm the role of WES in the evaluation of monogenic disorders in these systems.

VUS and incidental findings in prenatal exome sequencing can create challenges in genetic counseling due to uncertainty in their clinical meaning [13]. This uncertainty may affect parental decision-making

and cause anxiety. To address this, segregation analysis was recommended for all patients with VUS, and the parents were informed about VUS variant reporting by pretest counselling. Additionally, in line with guidelines, only VUS variants related to fetal ultrasound findings or family history were reported [13]. This approach helps to minimize unnecessary concern and supports clearer counseling. Overall, careful communication and follow-up studies are essential to manage the complexities of VUS in prenatal diagnosis. The VUS rate in our WES cohort was 11.1%, within the expected range of 10–20% reported in recent studies [19, 27, 31]. While interpretation challenges remain, especially in prenatal settings with limited phenotype information, integrating databases and cross-referencing with parental data may reduce uncertainty. In our prenatal exome sequencing cohort, incidental findings unrelated to fetal ultrasonographic anomalies were identified in two cases. These variants were not associated with the observed fetal phenotype but were detected as part of the comprehensive exome analysis.

Incidental sequence variants predicted to be unrelated to referral reasons were detected in 2 cases with PKD1 and CFTR gene variants. The case with a likely pathogenic heterozygous PKD1 gene variant had central nervous system anomalies but no polycystic kidneys. The other incidental variant was found in the CFTR gene. Still, the fetus had no prenatal findings of cystic fibrosis, such as echogenic bowel, the absence of the gallbladder, and dilated bowel. Fetal ultrasonographic findings of this

Table 4 Comparison of studies reporting on diagnostic yield of prenatal exome sequencing and prenatal chromosomal microarray

Reference	No cases	Cohort characteristics	Method	Diagnostic yield	Comments
Zhang et al., 2024 [20]	174	retrospective; any anomaly	WES+ CNV-seq	WES;17.8% CNV-seq;6.9%	WES; Higher yield with skeletal anomalies 36.36% CNV-seq; Higher yield with multisystem organ abnormalities (26.67%)
Xiang et al., 2024 [21]	375	retrospective; any anomaly	WES+ CNV-seq	WES;19.7% CNV-seq;5.1%	WES; Higher yield with skeletal anomalies (56.7%) CNV-seq; Higher yield with multisystem organ abnormalities(10%)
Zeng et al., 2025 [22]	316	retrospective; any anomaly	Trio WES+ CNV-seq	WES;15.8% CNV-seq;4.1%	Higher yield with skeletal anomalies (36.1%) And multisystem organ Abnormalities (36.4%)
Diderich et al., 2024 [23]	629	retrospective; any anomaly	WES	WES;14%	Higher yield with multisystem organ abnormalities (21.7%)
Lord et al., 2019 [24]	610	prospective; any anomaly	Trio WES	WES 8.5%	Higher yield with multisystem organ abnormalities (15.4%)
Petrovski et al., 2019 [25]	234	prospective; any anomaly	Trio WES	10%	Higher yield with multisystem organ abnormalities (19%)
Chong et al., 2019 [26]	1129	prospective; Group 1; increased NT Group 2; any anomaly	CMA	Group 1; 5.9% Group 2; 7.2%	Higher yield with Cardiac anomalies (20%)
Fu et al., 2022 [4]	1618	Prospective and retrospective; any anomaly	Trio WES	14.2% %	Higher yield with skeletal anomalies (30.4%)
Vora et al., 2020 [27]	102	Prospective and retrospective; any anomaly	Trio WES	20.6%	Higher yield with multisystem organ abnormalities
Recent study	391	retrospective; any anomaly	WES CMA	WES; 22.2% CMA; 4.2%	Higher yield with central nervous system anomalies (46.1%) Higher yield with cardiac anomalies (12%)

Abbreviations: CNV copy number variants, CNV-seq CNV-sequencing, WES whole exome sequencing

case with incidental CFTR gene variants were arthrogryposis, camptodactyly, and hydrops fetalis. No variant relevant to fetal ultrasonographic findings was detected in this case, but homozygous Class 3 CFTR gene variants were detected. 12 likely pathogenic variants in PKP2, ROR2, FKRP, GALNS, RYR1, INPPL1, CSF1R, SLC26A3, B9D2, NOTCH1, PKD1, MED12 genes were detected in our study. Among the 12 likely pathogenic variants identified, eight variants are novel, as they have not been previously reported in the scientific literature or recorded in any public variant databases. The remaining four variants have been documented solely in public databases but lack representation in published studies.

The discovery of novel variants associated with fetal structural anomalies shows that WES analysis is a powerful tool for establishing the phenotype-genotype relationship in prenatal diagnosis. For example, the NOTCH1

novel variant was found in fetuses with increased NT and choroid plexus cysts at early gestation. NOTCH1 causes autosomal dominant Adams-Oliver syndrome (OMIM:616028) and Aortic valve disease (OMIM:109730). The cardiac anomaly (aortic stenosis) was manifested in the following weeks of late pregnancy. The mother has a ventricular septal defect, and the mother's sister has a bicuspid aortic valve. No genetic study had been done before for these patients, and we also found that they have the same variant. The baby was born with severe cardiac anomalies (severe aortic anomalies), and the phenotype was more severe than that of the mother and maternal aunt. Identifying novel variants and their entry into the databases will help predict how these variants will give ultrasonographic findings in the prenatal period. The identification of incidental variants in genes such as PKD1 and CFTR highlights one of the

major dilemmas of prenatal exome sequencing—how to manage findings unrelated to the referral phenotype. Although such variants may not immediately affect prenatal decision-making, they have potential implications for postnatal follow-up and genetic counseling. Current professional guidelines suggest that such results should be discussed during pretest counseling, and our findings support that recommendation [13]. Although the significant contribution of WES and CMA analysis to prenatal diagnosis cannot be ignored, VUS variants and incidental variants can cause difficulties in counseling and prenatal decision-making.

The significant differences in diagnostic yields between CMA and WES across all phenotypic subgroups underscore the complementary roles of these technologies in prenatal diagnosis. WES offered substantially higher diagnostic returns in cases involving the central nervous system, skeletal system, and hydrops fetalis. These findings statistically validate the phenotypic prioritization of WES as a second-tier test following negative CMA results. Interestingly, the absence of the diagnostic findings in WES for fetuses with isolated increased NT and cystic hygroma supports previous literature suggesting limited monogenic etiology in these isolated findings. The statistical significance of these observations reinforces the clinical utility of phenotype-driven testing strategies.

A significant strength of this study is the relatively large, well-characterized cohort and the standardized classification of ultrasound phenotypes. Furthermore, the combined use of CMA and WES enables a layered diagnostic approach that reflects real-world clinical practice. However, there are several limitations. The study is single-center and retrospective, which may limit generalizability. First, due to its retrospective design, not all patients underwent both chromosomal microarray (CMA) and whole-exome sequencing (WES). WES was performed only in a subset of cases based on DNA availability, clinical prioritization, and parental consent. Consequently, a significant portion of CMA-negative cases were not further evaluated by WES, which may have limited the assessment of the actual added value of exome sequencing. Fu et al. (2018) conducted a study with a design similar to ours, where not all CMA-negative cases underwent WES [31].

Among 3949 fetuses, 18.2% had abnormal karyotypes. In those with normal karyotypes, CMA identified pathogenic CNVs in 8.2% (138/1680). WES was performed in a selected subgroup of 196 fetuses with normal karyotype and CMA results, revealing likely diagnostic variants in 24% of cases. These results support the added value of WES following normal CMA, even when applied to a selected group. Second, although the combined use of CMA and WES is emphasized as a layered diagnostic approach, in this study, the two methods were applied to different patient subgroups. This may have introduced

a degree of non-uniformity in phenotype distribution across diagnostic categories, potentially affecting the generalizability of some conclusions. Our study categorized cases into phenotypic groups comprising both isolated and non-isolated structural anomalies. Previous literature has shown that diagnostic yields differ significantly between isolated and non-isolated anomalies, with isolated cases typically having lower yields. The predominance of non-isolated cases in our cohort may have therefore influenced the overall diagnostic yield reported for each group. Future studies with larger and more balanced cohorts are needed to perform stratified analyses better to understand the impact of anomaly complexity on diagnostic outcomes. Additionally, the accuracy of genotype-phenotype correlations is dependent on the quality and timing of prenatal imaging. Some cases had limited sonographic data, particularly in early gestation, which may affect variant interpretation.

Despite these limitations, our findings support the routine integration of CMA and WES into the prenatal diagnostic workflow for fetuses with structural anomalies. The identification of both known and novel pathogenic variants enhances our understanding of fetal phenotypes, facilitates informed reproductive decision-making, and contributes to the expansion of prenatal variant databases.

Conclusion

This study demonstrates that the combined application of chromosomal microarray analysis and whole-exome sequencing provides substantial diagnostic value in pregnancies complicated by fetal structural anomalies. CMA remains a valuable first-tier test, particularly in cases with cardiovascular and multisystem anomalies. WES significantly enhances the diagnostic yield, especially in fetuses with central nervous system and skeletal malformations, and contributes to novel gene discovery and genotype-phenotype correlation.

Our findings support the integration of WES into routine prenatal diagnostic protocols, especially when CMA is uninformative and detailed phenotyping is available. As genomic technologies and variant interpretation frameworks evolve, their clinical implementation will further refine prenatal counseling, risk assessment, and pregnancy management. CMA and WES are powerful and complementary tools for prenatal genetic diagnosis. In our cohort, WES significantly increased diagnostic yield in cases with negative CMA results, particularly in central nervous system and skeletal anomalies. The integration of both tests enhances genotype-phenotype correlations and offers critical insights for clinical management. Future studies with prospective designs and larger cohorts are needed to define further optimal diagnostic strategies and the clinical utility of prenatal exome sequencing.

Abbreviations

CMA	chromosomal microarray analysis
WES	whole exome sequencing
CNVs	copy number variants
VUS	Variant of unknown clinical significance
NT	nuchal translucency
AR	autosomal recessive
XLR	X-linked recessive
XLD	X-linked dominant

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Authors' contributions

LO conceived and designed the study, analyzed and interpreted the data, and wrote the manuscript. SA performed the experiments and analyzed the data. EU performed the experiments and analyzed the data. All authors reviewed and approved the final version of the manuscript. All authors accept responsibility for the entire content of the manuscript and have approved its submission.

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Data availability

The datasets generated and/or analysed during the current study are available in the ClinVar repository, <https://www.ncbi.nlm.nih.gov/clinvar/>. Additional anonymized data are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by the Yüsek İhtisas University Medical School Ethical Committee and was conducted in accordance with the Declaration of Helsinki (as revised in 2013). All procedures performed in this study involving human participants were conducted in accordance with the ethical standards of the institutional and/or national research committee. Written informed consent was obtained from all individual participants included in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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