The Diagnostic Utility of Prenatal Microarray in High-Risk Pregnancies: A Single-Center Experience to Enhance Reproductive Care and Risk Stratification

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Article

The Diagnostic Utility of Prenatal Microarray in High-Risk Pregnancies: A Single-Center Experience to Enhance Reproductive Care and Risk Stratification

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Abstract

Background/Objective: Prenatal cytogenetic testing is essential for pregnancies at high risk of chromosomal abnormalities. While conventional karyotyping detects large aneuploidies and structural rearrangements (>5–10 Mb), chromosomal microarray analysis (CMA) identifies smaller copy number variants (CNVs), increasing diagnostic yield by approximately 5%. CMA is now recommended as the first-tier test for evaluating fetal structural anomalies detected by ultrasound. **Method:** From March 2023 to September 2024, 344 prenatal samples were analyzed using conventional karyotyping and SNP-based CMA. Karyotyping was performed via flask culture, and CMA was conducted using the Infinium Global Screening Array Cyto (GSA-Cyto) on the Illumina iScan platform. CNVs were interpreted using NxClinical v6.0 and curated databases including ClinVar, DECIPHER, OMIM, ClinGen, and others. Results were aligned to the GRCh37/hg19 reference genome. **Results:** Chromosomal abnormalities were identified in 57/344 cases (16.5%). Of these, 39 were numerical chromosomal anomalies and 18 were pathogenic or likely pathogenic CNVs. Notably, 11 CNVs (3.2%) were undetectable by conventional karyotyping, emphasizing the added value of CMA. **Conclusion:** CMA enhances prenatal diagnostic accuracy by detecting submicroscopic CNVs that are not visible with conventional methods, supporting its routine use in prenatal genetic evaluation.

Keywords: microarray analysis; prenatal diagnosis; amniocentesis; chorionic villus sampling; fetal blood sampling

1. Introduction

Conventional cytogenetic analyses are typically offered as the first option in prenatal diagnosis to couples at high risk of having a child with a chromosomal abnormality. However, conventional cytogenetic analyses can detect aneuploidy and large chromosomal rearrangements of 5–10 megabases (Mb) in size. Thanks to advances in prenatal cytogenetic diagnosis, in recent years, fluorescence in situ hybridization (FISH) and chromosomal microarray analysis (CMA) have begun to be used in addition to conventional cytogenetic analyses. Recently, chromosomal microarray (CMA), CGH array, or SNP array (depending on the internal technology used in their design and reading) have been added to the group of genomic tools used in genetic diagnosis. Chromosomal microarray analysis (CMA) is a cytogenetic molecular technique that can detect microscopic and submicroscopic chromosomal abnormalities smaller than 5 Mb with high sensitivity in patients. Specifically, SNP CMA can identify genetic changes as small as 50–100 kilobases (kb). This enables CMA to provide approximately 100 times higher resolution compared to conventional karyotyping, depending on the probe spacing and platform used. The ability to examine the genome at this high

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resolution has led to the discovery of widespread copy number variations (CNVs) in the human genome, including polymorphic variations in healthy individuals and novel pathogenic copy number imbalances [1]. This has had a major impact on genetic diagnosis over the past decade [2]. Furthermore, CMA provides additional clinically useful information in approximately 5% (range: 2.3–8.3%) of cases [3,4]. While CMA provides higher resolution information compared to conventional cytogenetic analyses, it cannot detect low-level mosaicism and balanced chromosomal rearrangements. In addition, it can lead to the detection of chromosomal variants of uncertain significance (VOUS) and present challenges in interpreting these findings. With the more frequent use of genome-wide and high-resolution array platforms, the prevalence of VOUS is steadily increasing.

A high-risk pregnancy may be indicated by factors such as risk determination in prenatal maternal serum screening (MSS) tests, advanced maternal age (AMA), intrauterine growth restriction (IUGR), increased nuchal translucency (NT), the detection of a structural anomaly in a prenatal ultrasound, a family history of chromosomal abnormalities, and risk determination in NIPT tests. CMA has become the first-tier technique for genetic follow-up when structural anomalies are detected in prenatal ultrasound [5].

The detection rate of pathogenic copy number variations (CNVs) can vary depending on the indication for prenatal diagnosis. For example, studies have reported pathogenic CNVs at a rate of 0–15.0% in fetuses with increased nuchal translucency (NT; ≥2.5 to 3.5 mm, corresponding to the 95th to 99th percentile in the general population) or cystic hygroma [6,7] whereas this rate has been reported as high as 18–22% in all cases of coronary heart disease (CHD). Among pregnancies with CHD, the most common causes are trisomy 21 and 18, along with 22q11 microdeletion [8,9]. Other frequently affected organ systems associated with pathogenic CMA results are the skeletal system, genitourinary system, and central nervous system [10–12]. There is limited information regarding the incidence of clinically significant CNVs in fetuses with ultrasound soft marker abnormalities such as echogenic intracardiac focus (EICF), mild ventriculomegaly, enlarged cisterna magna, choroid plexus cysts (CPCs), thickened nuchal fold, echogenic bowel, mild hydronephrosis and in pregnant women who undergo invasive prenatal testing due to other indications such as advanced maternal age (AMA), abnormal MSS results, and abnormal Non-Invasive Prenatal Test (NIPT) results. Additionally, no studies from Turkey have been found that include a cohort of this size. In this study, we report a retrospective cohort study of 344 high-risk pregnancies that underwent prenatal diagnosis at our center using G-banding karyotyping along with CMA. These analyses were performed due to the observation of a structural anomaly during prenatal diagnosis or the suspicion of a high-risk pregnancy.

2. Materials and Methods

2.1. Ethics

The research received ethical approval from the Ankara Etlik City Hospital Scientific Research Evaluation and Ethics Committee with the document number: AESH-BADEK-2024-876.

In this study, the chromosomal microarray analysis (CMA) results of high-risk pregnancies that underwent prenatal diagnosis at Ankara Etlik City Hospital between March 2023 and September 2024 were evaluated. As of now, 344 pregnant women have undergone CMA as part of their prenatal diagnosis, and various chromosomal anomalies have been detected in 57 patients. The data used in the research will only include genetic report results, and access will be restricted to researchers. The identities of the patients included in the study will remain confidential.

2.2. Inclusion Criteria:

- Patients with an indication for prenatal CMA due to prenatal maternal serum screening (MSS) tests risk with increased NT, advanced maternal age (AMA), intrauterine growth restriction (IUGR), increased nuchal translucency (NT), the detection of a structural anomaly or a soft

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marker in a prenatal ultrasound, a family history of chromosomal abnormalities, and risk determination in NIPT tests.
- Patients who signed an informed consent form agreeing to undergo prenatal genetic testing.

2.3. Exclusion Criteria:

- Patients with biochemical risks in prenatal screening but without increased nuchal translucency, prenatal USG abnormalities, or parental karyotype anomalies are required for a prenatal CMA indication.
- Patients who did not sign the informed consent form and declined prenatal genetic testing.

2.4. Parameters to be Examined:

- Presence of possible aneuploidy
- Presence of possible microdeletions/microduplications
- Mosaicism
- Uniparental disomy (UPD)

2.5. Karyotype Analysis:

Samples obtained from patients, such as chorionic villus sampling (CVS), amniocentesis (AS), or fetal cordocentesis, depending on gestational age, were subjected to cell culture via the flask method for genetic testing.

2.6. CMA Analysis:

In patients meeting the inclusion criteria, chromosomal microarray analysis (CMA) was performed in addition to conventional cytogenetic analysis. For this purpose, DNA was first isolated from prenatal samples. Maternal DNA was also isolated by obtaining a peripheral blood sample from the mother. The isolated DNAs were compared to exclude maternal contamination, and CMA analysis was then initiated. Chromosomal microarray analysis was performed using Infinium Global Screening Array Cyto (GSA-Cyto) chips on the Illumina iScan platform. Copy number variations were detected and visualized using the NxClinical (v.6.0) analysis software developed by Biodiscovery. The relevant genomic positions were reported based on the Human Genome Build 37 (GRCh37/hg19) reference assembly. The obtained DATA were evaluated using current databases, including PubMed, OMIM, DGV, ClinVar, DECIPHER, and ClinGen.

2.7. Statistical Analysis:

Statistical analysis of the DATA in the study was performed using SPSS 25 for Windows and the R programming language. Quantitative variables (discrete or continuous numerical variables) were expressed as mean and standard deviation when they showed normal distribution, otherwise as median and interquartile range (IQR). Qualitative (nominal and ordinal) variables were explained using numbers and percentages. Ordinal variables were arranged in the table according to their hierarchical order.

3. Results

Pregnant women enrolled in the study were aged 17 to 45 years, with an average age of 30.66 years, and they were 9–33 weeks pregnant, with an average of ~19.93 ± 1.63 weeks. A total of 344 prenatal samples were analyzed by chromosomal microarray analysis (CMA). The clinical indications for testing included abnormal ultrasound findings, congenital anomalies, multiple anomalies, increased nuchal translucency (NT), central nervous system (CNS) anomalies, skeletal anomalies, biochemical risk, family history, hydrops fetalis, positive non-invasive prenatal testing (NIPT) results, advanced maternal age (AMA), cystic hygroma, intrauterine growth restriction (IUGR), and

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amniotic fluid abnormalities (anhydramnios/oligohydramnios). The distribution of abnormal findings according to clinical indications is summarized in Table 1.

Table 1. Abnormal results of overall patients.

Sample	Results (Hg19)	Week	USG	Maternal Age	Group
AS	16p13.11(14975292_16295863)x1	23+4	Enlarged ventricle	30	USG findings
AS	16p12.2p11.2(21575087_29319922)x1	26	Enlarged ventricle	35	USG findings
AS	Trisomy 21	21+2	Hepatic calcification, echogenic cardiac focus	28	USG findings
AS	15q11.2(22766739_23226254)x1	22	Ambiguous genitals, hydronephrosis	20	Congenital anomaly
AS	Trisomy 21	23+2	Renal pyelectasis	33	Congenital anomaly
AS	Trisomy 13	24+3	Ventriculomegaly, renal pyelectasis, hypospadias, coarctation of the aorta	38	Multiple findings
AS	Trisomy 18	30	Anal atresia, polyhydramniosis, IUGR, single umblical artery	24	Multiple findings
AS	Trisomy 21	18+3	Renal pelviectasis, AVSD[a]	36	Multiple findings
AS	8p23.3p23.1(170692_12009597)x3, 9p24.3p11.2(10201_44888946)x3, 9q13q22.33(68158106_101087286)x3	16+6	Cleft palate, CHD	35	Multiple findings
AS	Klinefelter Syndrome	22	Pulmonary stenosis, cleft lip and palate, renal pelviectasis, thymus hypoplasia	26	Multiple findings
AS	Trisomy 18	28	Clenched hand, VSD	26	Multiple findings
AS	Trisomy 18	22	IUGR, clench hand, mandibular hypoplasia, VSD, horseshoe kidney	35	Multiple findings
AS	Trisomy 21	17	Duodenal atresia, NT:6mm	39	Multiple findings
AS	Trisomy 13	23	Inferior Vermis Hypoplasia, Polyhydramnios, Mesochardia, TGA[b]	37	Multiple findings
Chord sample	Trisomy 13	24	Cleft Lip/Palate, Hyperecogenic Bowel, Hypoplastic Left Heart, Aortic Coarctation, Holoprosencephaly	24	Multiple findings
AS	22q11.21(18877787_21461607)x1, Di George	28	Truncus Arteriosus, hypoplastic tymus, VSD	35	CHD
AS	Trisomy 21	21+4	Hypoplastic nasal bone, AVSD[a]	33	CHD

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AS	14q32.2q32.33(99718925_107289511)x1	32	Craniosynostosis, hypoplastic left heart, aortic hypoplasia, doubled collecting system of the left kidney	25	CHD
AS	Klinefelter Syndrome	16+3	D-TGA[b]	38	CHD
AS	Turner Syndrome	25	Aort hypoplasia	21	CHD
AS	4p16.3p11(84414_49620838)x3, 13q11q12.11(19020095_21578150)x1	23+2	Pulmonary hypoplasia, VSD, Fallot tetralogy, overriding aorta, clenched hand	23	CHD
CVS	Turner Syndrome	14+1	Hypoplastic left heart	23	CHD
AS	Trisomy 13	21+5	AVSD[a]	23	CHD
AS	Trisomy 21	17+2	VSD, echogenic liver focus	37	CHD
AS	11q23.3q25(119110984_134946504)x1, 11q23.3(118545797_119103406)x3	22+4	Hypoplastic left heart	31	CHD
AS	Xq27.2q28(140856453_155234707)x1, 4q28.3q35.2(134134331_190484505)x3	23	VSD, truncus arteriosus, left-sided gall bladder	33	CHD
AS	Trisomy 18	22	IUGR, Perimembranous VSD	35	CHD
AS	13q21.33q33.2(73157290_105760332)x1	30	Vernian hypoplasia, Pes equinovarus	25	CNS anomaly
AS	16p11.2(29323692_30364805)x3	22+5	Hydrocephaly, lemon sign, cerebellar hypoplasia, left multicyclic dysplastic kidney, Sacral meningomyelocele.	37	CNS anomaly
AS	Trisomy 21	16+5	Alobar holoprosencephaly	37	CNS anomaly
CVS	Trisomy 18	12	NT:7mm	43	Increased NT
CVS	Trisomy 21	12+5	NT:5, Cystic hygroma	32	Increased NT
AS	10p11.1(38784659_39150257)x1 , 10q11.22q11.23(49262918_51832748)x1	16	NT 2.6	36	Increased NT
AS	Trisomy 21	13+4	NT 5, diffuse edema, echogenic cardiac focus	38	Increased NT
C.V.S	4q31.3q35.2(155190509_191044208)x3	13	NT:4mm	39	Increased NT
AS	Trisomy 13	18	Polydactyly of the right foot, hyperecogenic heart	37	Skeletal anomaly
AS	Xp22.31(6453470_8126718)x0	15+4	N	23	Biochemical risk
AS	4q22.2q22.3(94006191_97808388)x1	17	N	34	Biochemical risk
AS	15q11.2(22766739_23226254)x1	22	N	20	Other
AS	47,XYY	20+4	CSP[c]	38	Other
C.V.S	6q14.3q22.31(85761559_120871846)x1	NA	NA	25	Other

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AS	Mosaic UPD of chromosome 3	20	CSP[c]	41	Other
AS	Trisomy 18	17	Megacystit, clenched hand, hydrops, club foot, VSD	40	Hydrops
AS	Trisomy 21	28	Hydrops, polyhydramniosis	34	Hydrops
AS	Yp11.31p11.2(2657176_10057648)x2,Yq11.1q11.221(13133499_19567718)x2,Yq11.222q11.223(20804835_24522333)x0	20	N	24	NIPT risk
AS	Trisomy 21	19+2	Fallot tetralogy	35	NIPT risk
C.V.S	Trisomy 21	13+5	NIPT Tr.21 risk	24	NIPT risk
AS	16q11.2q23.1(46501717_75493481)x3	14	N	24	NIPT risk
AS	Trisomy 21	17	NT:3.4MM	17	NIPT risk

[a] Atrioventricular septal defect, [b] Transposition of Great Arteries, [c] Cavum septi pellucidi

Overall, chromosomal abnormalities were detected in 57 cases, corresponding to a total abnormality detection rate of 16.5% (The screenshots of the abnormal results have been included as supplemental material.). Among these, 18 cases involved pathogenic or likely pathogenic copy number variations (P/LP CNVs) and 39 cases involved numerical chromosomal abnormalities (aneuploidies). A total of 11 cases with CNVs that could not be detected by conventional cytogenetic analysis were identified (Table 2).

Table 2. Abnormal Result ratios of prenatal microarrays, where conventional karyotyping was normal.

Results (Hg19)	**Size**	**Detected by Karyotyping**	**Week**	**USG**
16p13.11(14975292_16295863)x1	**1.32 Mb**	**No**	23+4	Enlarged ventricle
16p12.2p11.2(21575087_29319922)x1	**7.74 Mb**	**Yes**	26	Enlarged ventricle
15q11.2(22766739_23226254)x1*	**460 Kb**	**No**	22	Ambiguous genitals, hydronephrosis
8p23.3p23.1(170692_12009597)x3	**11.8MB**			
9p24.3p11.2(10201_44888946) x3	**44.8Mb**	**Yes**	16+6	Cleft palate, CHD
9q13q22.33(68158106_101087286) x3	**33Mb**			
22q11.21(18877787_21461607)x1	**2.583 kb**	**No**	28	Truncus Arteriosus, hypoplastic tymus, VSD
14q32.2q32.33(99718925_107289511)x1	**7.5 Mb**	**Yes**	32	Craniosynostosis, hypoplastic left heart, aortic hypoplasia, doubled collecting system of the left kidney
4p16.3p11(84414_49620838) x3, 13q11q12.11(19020095_21578150)x1	**49.5 Mb** **2.6Mb**	Yes No	23+2	Pulmonary hypoplasia, VSD, Fallot tetralogy, overriding aorta, clenched hand

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11q23.3q25(119110984_134946504) x1	**15.8Mb**	Yes	22+4	Hypoplastic left heart
11q23.3(118545797_119103406)x3	**558Kb**	No		
Xq27.2q28(140856453_155234707)x1	**14.2Mb**	Yes	23	VSD, truncus arteriosus, left-sided gall bladder
4q28.3q35.2(134134331_190484505)x 3	**56.3 Mb**			
13q21.33q33.2(73157290_105760332)x1	**33 Mb**	**Yes**	30	Vermian hypoplasia, Pes equinovarus
16p11.2(29323692_30364805)x3	**1.04Mb**	No	22+5	Hydrocephaly, lemon sign, cerebellar hypoplasia, left multicyclic dysplastic kidney, Sacral meningomyelocele.
10p11.1(38784659_39150257) x1,	**366Kb**	No	16	NT 2.6
10q11.22q11.23(49262918_51832748)x1	**2.6 Mb**			
4q31.3q35.2(155190509_191044208)x3	**36Mb**	Yes	13	NT:4mm
Xp22.31(6453470_8126718)x0	**1.7Mb**	No	15+4	N
4q22.2q22.3(94006191_97808388)x1	**3.8Mb**	No	17	N
15q11.2(22766739_23226254)x1*	**460 Kb**	No	22	N
6q14.3q22.31(85761559_120871846)x1	**35.1 Mb**	Yes	NA	NA
Mosaic UPD of whole chromosome 3		No	20	CSP
Yp11.31p11.2(2657176_10057648)x2,	**7.4Mb**	Yes	20	N
Yq11.1q11.221(13133499_19567718)x2,	**6.4 Mb**			
Yq11.222q11.223(20804835_24522333)x0	**3.7Mb**			
16q11.2q23.1(46501717_75493481)x3	**29Mb**	Yes	14	N

The distribution of chromosomal abnormalities across different clinical indications is visually summarized in Figure 1. The highest diagnostic yield was observed in the cystic hygroma group, where chromosomal abnormalities were identified in 83% of cases. This was followed by cases with high-risk non-invasive prenatal testing (NIPT) results (58%) and those presenting with multiple sonographic findings (26%). Notably, the group with congenital heart disease (CHD) showed a substantial yield of 37%, whereas increased nuchal translucency (NT) and central nervous system (CNS) anomalies yielded lower detection rates of 15% and 8.8%, respectively (Table 3).

Table 3. Detection Rates of Chromosomal Abnormalities According to Clinical Indications.

Indications	**N**	**Abnormal**	**P/LP CNV**	**Aneuploidi**	**Abnormal Rate (%)**
USG findings	41	3	2	1	0.073
Congenital anomaly	39	2	1	1	0.053
Multiple indications	38	10	1	9	0.26
CHD	35	13	5	8	0.37

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CNS anomaly	34	3	2	1	0.088
Increased NT	33	5	2	3	0.15
Skeletal anomaly	26	1	-	1	0.038
Biochemical risk[1]	19	2	1	1	0.105
Other (Family history)	18	3	2	1	0.17
Hydrops	15	2	-	2	0.14
NIPT risk	12	7	2	5	0.58
AMA	21	1	-	1	0.047
Cystic hygroma	6	5	-	5	0.83
IUGR	5	-	-	-	0
Anhydramnios/oligohydramnios	3	-	-	-	0
Total	**344**	**57**	**18**	**39**	**0.165**

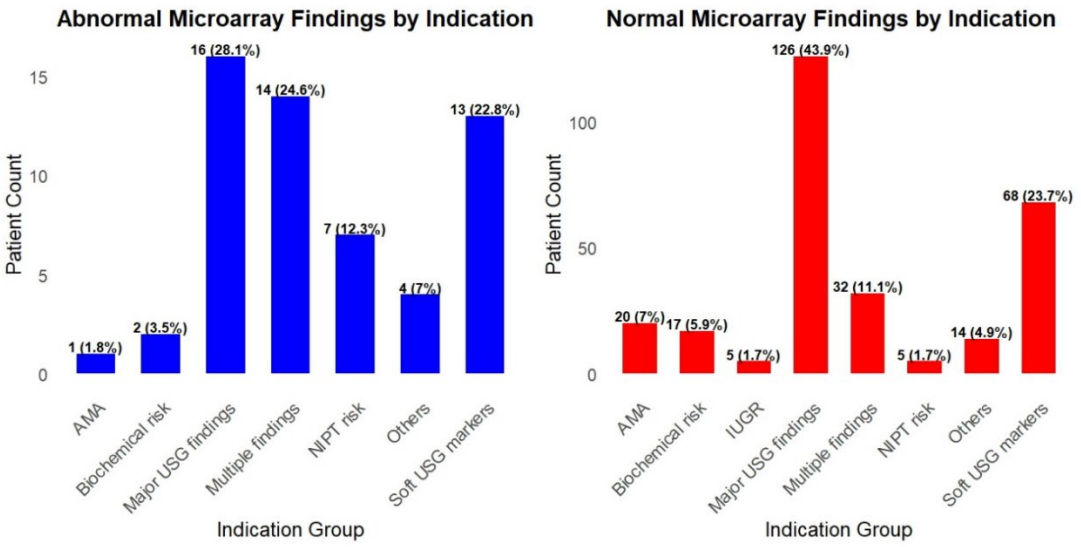

Figure 1. Patient distribution of abnormal and normal result groups in terms of indications.

Among the 41 cases with ultrasound abnormalities, abnormalities were detected in 7.3%, including two pathogenic CNVs and one aneuploidy. Congenital anomalies were identified in 39 cases, with a 5.3% abnormality rate.

Notably, in the group with multiple sonographic findings (n=38), the abnormality rate increased significantly to 26%, highlighting the cumulative risk when multiple structural anomalies are present. Multiple sonographic findings are detailed in Table 4.

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Table 4. Multiple congenital anomaly distribution according to systems.

	CVS*	CNS*	GUS*	GIS*	CFM*	Skeletal	Others
P1			Ilial atresia	Pelviectasis			
P2					Cleft palate		Nasal bone: 6MM
P3	Coarctation of the aorta		Eophageal atresia,			Hypoplastic radius and ulna, left hemihypoplasia	
P4	Ventriculomegaly,			Pelviectasis			
P5	Ventriculomegaly, ARSA						
P6	Ventriculomegaly, Coarctation of the aorta			Renal pyelectasis, hypospadias			
P7	VSD						NT:5.5
P8	Hemivertebra						NT:5.5
P9	Echogenic intracardiac focus, VSD						NT:6 mm
P10	Single umbilical artery		Anal atresia	Polyhydramniosis			IUGR
P11	AVSD			Renal pelviectasis			
P12	Hypoplastic left heart						Hydrops
P13		Occipital cephalocele, Corpus callosum dysgenesis, spina bifida				Hypoplastic thorax	NT:9 mm, IUGR
P14	CHD				Cleft palate		
P15						Club foot, clenched hand	Hydrops fetalis
P16	Pulmonary stenosis			Renal pelviectasis	cleft lip and palate		Thymus hypoplasia
P17	Muscular vsd			Renal pelviectasis,			
P18	Hypoplastic left heart,			Tubular hypoplasia			
P19	Aortic arch anomaly,	Tethered cord, CSP					
P20	pulmonary artery hypoplasia		Omphalocele	Polyhydramnios		Clenched hands	
P21	Coarctation of the aorta, Ebstein anomaly						Hydrops fetalis
P22	VSD					Clenched hand	
P23			Omphalocele				Hydrops fetalis

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P24	VSD			Horseshoe kidney	mandibular hypoplasia	Clenched hand	IUGR
P25				Renal pelviectasis, polyhydramniosis			
P26	Echogenic cardiac focus, VSD			Oligohydroamniosis			
P27	VSD					Clenched hand, rocker bottom feet	
P28	Ventriculomegaly,	Hydrocephalus				Clench Hand, Pes Echinovarus,	Cystic Hygroma, Pleural Effusion
P29	Tetralogy Of Fallot	Encephalocele			Hypertelorism		
P30	Echogenic cardiac focus						NT: 6.2 mm
P31	Tricuspid Atresia, Right Ventricular Hypoplasia,			Polyhydroamniosis			Diaphragmatic Hernia
P32		Subarachnoid hemorrhage, AMA					Edema,Hydrops fetalis,
P33	Unilateral cardiac ventriculomegaly, VSD			Polyhydroamniosis			
P34						Bilateral pes equinovarus, narrow thorax	NT:7.71mm
P35	Ectopia Cordis		Omphalocele				Cystic Hygroma, NT:6.3mm
P36		Encephalocele					NT:6mm
P37		Choroid plexus cyst	Echogenic bowel				, NT:4mm
P38	Ventriculomegaly				cleft lip		
P39						Bilateral pes equinovarus, narrow thorax	NT:7.71mm
P40			Duodenal atresia				NT:6mm
P41	Mesocardia, TGA	Inferior Vermis Hypoplasia		Polyhydramnios			
P42	Hypoplastic Left Heart, Aortic Coarctation,	Holoprosophechaly	Hyperecogenic Bowel		Cleft Lip/Palate		
P43	Truncus Arteriosus, VSD						Hypoplastic tymus

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P44	Pulmonary stenosis, Fallot tetralogy, right aortic arch,					Hypoplasia of the thymus
P45	Hypoplastic left heart, aortic hypoplasia,		Double collecting system of the left kidney	Craniosynostosis		
P46	Pulmonary hypoplasia, VSD, Fallot tetralogy, overriding aorta				Clenched hand	
P47	VSD, truncus arteriosus	Left-sided gall bladder				

*__CVS__: Cardiovascular System, __CNS__: Central Nervous System, __GUS__: Genitourinary System, __CFM__: Craniofacial Morphology

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In the hydrops fetalis group (n=15), chromosomal abnormalities were detected in two cases (14%), both corresponding to aneuploidies. No pathogenic findings were identified in cases with isolated IUGR (n=5) or amniotic fluid abnormalities (n=3).

Among the 19 cases tested due to biochemical risk factors, two chromosomal abnormalities were detected (10.5%). In the "other" category, including cases with positive family history (n=18), the abnormality rate was 17%.

In the AMA group (n=21), only one chromosomal abnormality was detected (4.7%), suggesting a relatively lower diagnostic yield when AMA was the sole indication for testing.

These results underscore the clinical value of chromosomal microarray analysis (CMA) particularly in pregnancies with multiple or specific sonographic anomalies, while also emphasizing the lower likelihood of pathogenic findings in isolated or less specific indications.

4. Discussion

Array-based methods, especially SNP microarrays, are frequently used in prenatal diagnosis. SNP-microarray can detect > 1 kb microdeletions and microduplications with a higher resolution than karyotyping and does not require cell culture. In 2013, the American College of Obstetrics and Gynecology (ACOG) recommended the use of CMA instead of traditional karyotyping for invasive prenatal diagnosis when one or more ultrasound anomalies are detected in the fetüs [13]. In this study, we analyzed the results of 344 prenatal SNP-microarray cases to assess the abnormal findings associated with different prenatal diagnosis indications, and we showed that CMA could detect an additional (11/344, 3.2%) genetic abnormalities compared to karyotype analysis. This rate has been considered consistent with previous studies [4,5]. The overall abnormal rate in our cohort was 16.5%, with pathogenic/likely pathogenic copy number variants (P/LP CNVs) detected in 5.2% of cases and aneuploidy identified in 11.3%. In a study conducted by Wapner et al., more than 4,000 samples from 29 centers were analyzed, and cases that were reported as having normal karyotypes by conventional methods were re-evaluated using CMA [3]. As a result, small deletions and duplications (CNVs) were identified in 6% of the cases. The study concluded that CMA is beneficial in diagnosing aneuploidies and unbalanced rearrangements but may be insufficient for detecting balanced translocations and triploidy. In a review by Callaway et al., CMA was applied to pregnant women who had normal results from conventional karyotyping [14]. The rate of CNV detection ranged between 0.8% and 5.5%, with an average rate of 2.4%. In these pregnant women, the incidence of abnormal fetal ultrasound (US) findings ranged from 6.0% to 11.1%, with an average of 6.5%. The review also included an analysis of pregnant women with abnormal fetal US findings and reported that CNVs were detected in 7% of fetuses with abnormal US. Based on these findings, the authors suggested that CMA could be recommended as a first-tier test. In a study conducted in Turkey involving 320 patients, the CNV detection rate was reported to be 12.3% [15]. The abnormality rates varied significantly depending on clinical indications, highlighting the differential diagnostic yield of prenatal microarray analysis across different risk categories.

In our study, among the highest detection rates, cystic hygroma (83%) and high-risk NIPT results (58%) showed the strongest correlation with chromosomal abnormalities. These findings are consistent with previous studies suggesting that cystic hygroma is frequently associated with aneuploidy, particularly Turner syndrome and trisomy 21, 18, or 13 [16]. Similarly, the high diagnostic yield in cases with abnormal NIPT results underscores the efficacy of NIPT as a screening tool for common chromosomal aneuploidies.

Cases with multiple indications (26%) and those with major structural anomalies such as central nervous system (CNS) abnormalities (8.8%), as well as cases with increased nuchal translucency (15%), showed a higher abnormality rate. This highlights the importance of detailed fetal ultrasound evaluation in guiding prenatal genetic testing. The presence of congenital anomalies as a standalone indication yielded a lower diagnostic rate (5.3%). Given that congenital anomalies are generally known to have multifactorial inheritance, this finding is not surprising (Figure 2).

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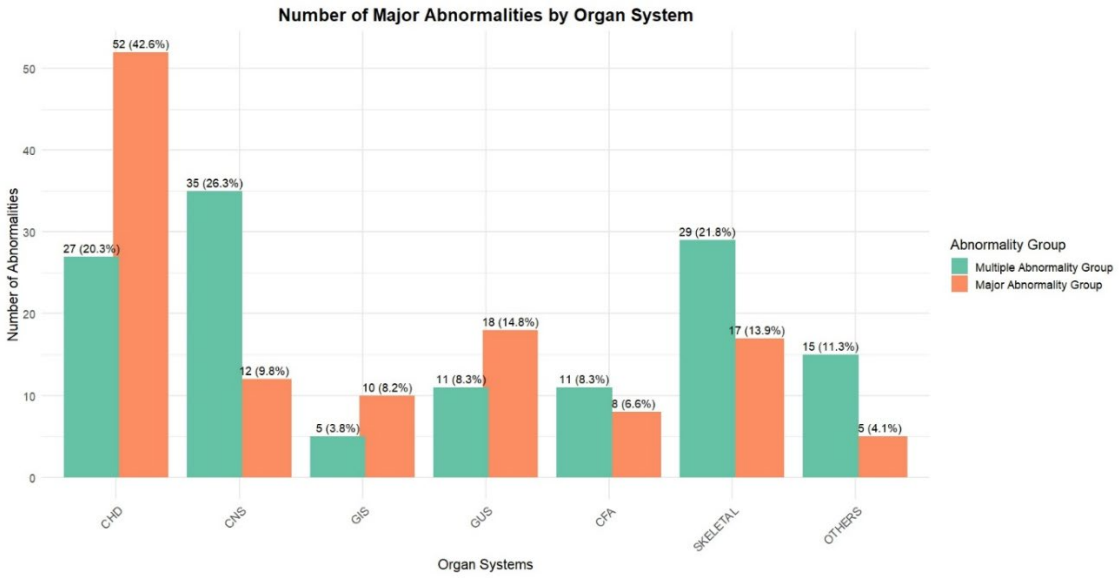

Figure 2. Abnormal Results Rates according to the type of USG abnormalities.

As expected, cases with skeletal anomalies (3.8%) showed a relatively lower rate of abnormal findings. This may reflect the limitations of chromosomal microarray in detecting single-gene disorders or non-structural genetic etiologies associated with these phenotypes. Similarly, intrauterine growth restriction (IUGR) and anhydramnios/oligohydramnios cases did not yield any abnormal microarray results in our cohort. This may be due to the limited sample size of our cohort, which consists of only 344 patients. However, it should also be considered that such conditions may result from multifactorial inheritance or non-genetic etiologies.

Our findings emphasize the importance of selecting appropriate prenatal genetic testing strategies based on clinical indications. In cases with ultrasound (USG) findings in addition to prenatal diagnosis indications such as biochemical risk or advanced maternal age, CMA should be planned simultaneously with prenatal diagnosis. While microarray analysis provides higher-resolution chromosomal anomaly detection and a more precise phenotype expectation, complementary approaches such as whole-exome sequencing (WES) or targeted gene panels may be required in cases with suspected monogenic disorders. Additionally, the identification of P/LP CNVs in certain cases highlights the necessity of accurate genetic counseling to discuss potential implications for fetal prognosis and familial recurrence risks.

As far as we know, our study is the largest and most comprehensive conducted in Türkiye. Future studies with larger cohorts, along with the integration of CMA methods into the prenatal diagnosis process, will be crucial for further refining the prenatal diagnostic approach. Our results contribute to the growing body of evidence supporting the role of prenatal microarray analysis, particularly in high-risk pregnancies with structural anomalies or positive NIPT findings.

5. Conclusion

Our study highlights the diagnostic value of prenatal chromosomal microarray analysis in a cohort of 344 cases with various clinical indications. The overall abnormality detection rate was 16.5%, with significant variations across different prenatal indications. The highest diagnostic yields were observed in cases with cystic hygroma and high-risk NIPT results, confirming the strong association between these findings and chromosomal abnormalities. In contrast, indications such as skeletal anomalies, isolated congenital anomalies, and advanced maternal age showed lower detection rates, suggesting that additional genetic testing approaches, such as whole-exome sequencing (WES) or targeted gene panels, may be necessary in selected cases.

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Our findings reinforce the importance of integrating prenatal ultrasound, biochemical screening, and non-invasive prenatal testing (NIPT) results into the decision-making process for genetic testing. The identification of pathogenic and likely pathogenic CNVs in certain cases highlights the necessity of comprehensive genetic counseling to discuss clinical implications and recurrence risks.

As prenatal genetic testing continues to advance, future research involving larger cohorts and advanced genomic technologies is expected to play a crucial role in refining diagnostic strategies. Expanding the use of genome-wide sequencing approaches may enhance our ability to detect underlying genetic etiologies in fetuses with unexplained structural anomalies. Overall, our results contribute to the growing body of evidence supporting the role of prenatal microarray analysis in high-risk pregnancies and emphasize the need for a personalized, multidisciplinary approach in prenatal diagnosis.

Author Contributions: A.B., H.S., M.T.A., U.C.T., and S.S. have performed patient evaluation and data collection. All authors had full access to all of the DATA in the study, and take responsibility for the accuracy of the DATA analysis. A.B. designed the study and wrote the manuscript. A.B., H.S., M.T.A., and İ.K. contributed to editing/reviewing the final version. All authors checked and arranged the final version of the manuscript and agree to be accountable for all aspects of the work.

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Institutional Review Board Statement: All of the procedures were carried out in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from the participants for molecular genetic analysis and the publication of patient DATA prior to their enrolment in the study. The research received ethical approval from the Ankara Etlik City Hospital Scientific Research Evaluation and Ethics Committee with the document number: AESH-BADEK-2024-876.

Informed Consent Statement: All patients were informed about the study, and verbal and written consent forms from patients or their parents were obtained.

Data Availability Statement: The DATA used and analyzed during this study are available from the corresponding author on reasonable request.

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Abbreviations:

The following abbreviations are used in this manuscript:

ACOG	American College of Obstetrics and Gynecology
AS	Amniocentesis
AVSD	Atrioventricular septal defect
CFM	Craniofacial morphology
CGH	Comparative genomic hybridization
CHD	Coronary heart disease
CMA	Chromosomal microarray analysis
CNS	Central nervous system
CNV	Copy number variants
CPC	Choroid plexus cysts
CSP	Cavum septi pellucidi
CVS	Cardiovascular system
CVS	Chorionic villus sampling
EICF	Echogenic intracardiac focus
GUS	Genitourinary System

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IUGR	Intrauterine growth restriction
LP	Likely pathogenic
MSS	Maternal serum screening
NIPT	Non-Invasive Prenatal Test
NT	Nuchal translucency
P	Pathogenic
SNP	Single nucleotide polymorphism
TGA	Transposition of Great Arteries
UPD	Uniparental disomy
US	Ultrasound
VOUS	Variants of uncertain significance
WES	Whole-exome sequencing

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