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ASSESSMENT OF CHROMOSOMAL MICROARRAY FINDINGS IN THE MOLECULAR DIAGNOSIS OF CONGENITAL HEART DISEASES

KONJENİTAL KALP HASTALIĞININ MOLEKÜLER TANISINDA KROMOZOMAL MİKRODİZİN BULGULARININ DEĞERLENDİRİLMESİ

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Abstract

Objective: Congenital heart disease (CHD) is one of the most frequently encountered congenital anomalies, resulting in significant mortality ratios. Early diagnosis of CHD is critical for effective disease management. Therefore, molecular approaches could provide valuable insights into the differential and early diagnosis of CHD. In the present study, we evaluated the efficiency and outcomes of the chromosomal microarray (CMA) method in patients with clinically diagnosed CHD.

Material and Methods: The study included 113 patients with CHD from a single center in Türkiye. CMA was performed using the Agilent Technologies array comparative genomic hybridisation system. Variants were classified based on the guidelines of the American College of Medical Genetics and Genomics (ACMG).

Results: Participants were classified as isolated (n=25) or syndromic CHD with additional conditions, including developmental delays, intellectual disabilities, growth retardation, other organ abnormalities, or dysmorphic features (n=88). CMA identified pathogenic copy number variants (CNVs) in 12 patients (10.6%). All patients with pathogenic CNVs belonged to the syndromic group (12/88; 13.6%). The most common CNVs were in the 22q11.2 region. Additionally, three CNVs identified in two patients had unique breakpoints that had not previously been reported.

Conclusion: The current study substantiated the findings reported in the literature and demonstrated the diagnostic efficacy of CMA,

Öz

Amaç: Konjenital kalp hastalığı (KKH) yüksek mortalite oranlarıyla en sık karşılaşılan konjenital anomaliler arasındadır. Hastalığın etkin bir şekilde yönetimi için erken tanı kritiktir. Bu noktada, moleküler yaklaşımlar, KKH'nin erken ve ayırıcı tanısı için önem arz etmektedir. Bu çalışmada, klinik olarak KKH tanısı almış hastalarda kromozomal mikrodizin yönteminin sonuçları ve etkinliği değerlendirilmiştir.

Gereç ve Yöntemler: Çalışmaya Türkiye'den 113 KKH'li hasta dahil edilmiştir. Kromozomal mikrodizin, katılımcıların periferik kanından izole edilen genomik DNA ile Agilent Technologies platformu kullanılarak gerçekleştirilmiştir. İşlem sonunda varyantlar, Amerikan Tıbbi Genetik Derneği kriterleri doğrultusunda sınıflandırılmıştır.

Bulgular: Katılımcılar, izole (n=25) ya da gelişme geriliği, bilişsel yetersizlik, büyüme geriliği, diğer organ anomalileri ya da dismorfik bulgular gibi ek özellikleri içeren sendromik KKH (n=88) olarak iki gruba ayrılmıştır. Kromozomal mikrodizin ile 12 hastada (%10,6) patojenik kopya sayısı değişimi (KSD) tespit edilmiştir. Bu hastaların tamamı, sendromik grupta yer almaktadır. En sık KSD, 22q11.2 bölgesinde görülmüştür.

Sonuç: Bu çalışma, literatürde raporlanan sonuçları doğrulamış ve özellikle sendromik KKH vakalarında kromozomal mikrodizin yönteminin verimliliğini kanıtlamıştır. Bu çalışma, ek klinik bulgular ve

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particularly in cases of syndromic CHD. This study is expected to offer new insights into the current literature through additional clinical findings and previously unreported CNVs.

Keywords Congenital heart disease · chromosomal microarray · copy number variation

daha önce bildirilmemiş CNV'lerin raporlanması ile literatüre katkı sağlamaktadır.

Anahtar Kelimeler Konjenital kalp hastalığı · Kromozomal mikrodizin · Kopya sayısı varyasyonu

INTRODUCTION

Congenital heart disease (CHD) is one of the most common congenital disorders and the leading cause of recurrent infant death. CHD can be defined as defects in the structure of the heart that occur before birth, with a high prevalence ratio of 6-13 per 1,000 newborns. However, due to advancements in therapeutic approaches, more than 90% of patients now survive into adulthood (1-3). Therefore, the early and differential diagnosis of CHD is critically important (4, 5).

During the prenatal period, CHD can be diagnosed using ultrasound and foetal echocardiography (6, 7). Nevertheless, if prenatal screening is not performed, newborns with suspected CHD should be immediately diagnosed. In newborns, various methods, including echocardiograms, electrocardiograms, chest X-rays, and pulse oximetry, are generally used to diagnose CHD (2). Nonetheless, clinical examinations may not provide sufficient information for diagnosis, particularly in cases with severe congenital cardiac abnormalities (8). Moreover, the aetiology of CHD can be grouped into syndromic, non-syndromic inherited, or non-syndromic isolated categories, which limits the effectiveness of conventional screening methods for differential diagnosis (9). Here, molecular approaches would be strong candidates for the differential diagnosis of CHD, especially in the era of next-generation sequencing (NGS) (10).

Many genes, particularly those associated with cardiac development, have been identified as the factors contributing to the molecular aetiology of CHD (11). Nonetheless, the genetic background of a remarkable ratio of especially sporadic cases could not be illuminated (12). The known mechanisms result from single-nucleotide variants (SNVs) in critical genes that are inherited in an autosomal dominant or recessive manner. However, in addition to SNVs, aneuploidies and copy number variants (CNVs) have also been shown to contribute to CHD (13-15). Hence, regarding the first-tier screening tests, the chromosomal microarray (CMA) method could be a powerful tool for the molecular diagnosis of CHD (16). Accordingly, CMA has been continuously studied for isolated or syndromic CHD cases to identify novel associated genes and document novel variants that may be pathogenic in a specific population (17-21). Nevertheless, such studies are still necessary to confirm the pathogenicity of the previously reported variants and list new ones that may be linked to the CHD.

In the present study, we evaluated the CMA results of patients with CHD in a single centre in Türkiye. The study highlighted the importance of the CMA approach in the molecular and differential diagnosis of CHD cases.

MATERIALS AND METHODS

Participants

One hundred and thirteen patients with congenital heart anomalies referred to the Department of Medical Genetics at Gazi University Hospital in Ankara, Türkiye, between 2018 and 2024 were included in the study. All patients underwent a complete cardiac evaluation, echocardiogram, medical history, and physical examination. Patients with only CHD and/or minor dysmorphic findings were evaluated in the isolated group, whereas those with one or more additional findings, including developmental delays, intellectual disabilities, growth retardation, other organ anomalies, or major dysmorphic features beyond CHD, were assessed in the syndromic group. Patients with isolated patent ductus arteriosus (PDA) that closed within the first six weeks were excluded from the study. CHD was classified into five types: septal defects, obstructive defects, conotruncal defects, multiple defects (CHDs in two or more groups), and others (such as cardiomegaly, aortic dilatation, dextrocardia, partial pulmonary venous return anomaly, and Ebstein anomaly) according to the National Birth Defects Prevention Study (22).

For genetic analysis, conventional karyotyping, when possible, was routinely performed to detect any aneuploidies. CMA was performed when karyotyping results were normal to identify affected regions in detail or to clarify the origin or specific region of the detected marker chromosome. Fluorescence in situ hybridisation (FISH) analysis was also performed in patients with a derivative chromosome, a marker chromosome, or a large deletion/duplication to confirm or clarify the abnormalities.

Further molecular tests, such as gene panels or exome sequencing, were performed for precise molecular diagnosis if both karyotyping and CMA results were normal. The results of these tests were not included in this study. However, in syndromic cases for which variants were detected by CMA, additional pathogenic variants related to findings other than

CHD were documented. Parental CMA and karyotype analysis was recommended for patients with pathogenic variants or VUS, but it could only be performed in seven families, depending on family acceptance.

Chromosomal microarray

Genomic DNA was isolated from the peripheral blood samples of the participants using the QIAmp Blood Mini Kit (Qiagen, Switzerland). The SurePrint G3 ISCA v2 CGH 8x60K Array or GenetiSure Cyto CGH Microarray Kit, 8 X 60K (Agilent Technologies, Santa Clara, CA, USA) was used for the CMA process according to the manufacturer's routine protocol.

CMA data were primarily analysed using Agilent CytoGenomics software (v3.0.6.6), in which alignments were carried out using the human genome (Chr37). Gains and losses were determined by the threshold of Log2>0.5 and <−0.5. No size threshold was applied during the analysis. Database of Genomic Variants (DGV; www.dgv.tcag.ca), GnomAD (https://gnomad.broadinstitute.org), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar), UCSC Genome Bioinformatics (https://genome.ucsc.edu), Database of Chromosome Imbalance and Phenotype in Humans using Ensembl Resources (Decipher; https://decipher.sanger.ac.uk), PubMed (https://www.ncbi.nlm.nih.gov/pubmed), Online Mendelian Inheritance of Man (OMIM; https://www.ncbi.nlm.nih.gov/omim), and our in-house databases were used for the variant analyses. Using the American College of Medical Genetics and Genomics criteria, the CNVs were categorised as benign, likely benign, of unknown clinical significance, likely pathogenic or pathogenic (23). Those with diagnostic variants found in the targeted panel or exome sequencing tests were excluded from the VUS group. This study was approved by the Gazi University Ethics Committee (Date: 08.07.2025, No: 11).

RESULTS

Clinical findings

Of the 113 patients, 25 (22.1%) had isolated heart abnormalities, whereas 88 (77.9%) were syndromic cases with a range of secondary findings, including developmental delays, intellectual disabilities, growth retardation, other organ anomalies or major dysmorphic features. The patients' ages varied greatly from newborns (13 days old) to 17 years old. Among the syndromic group, 41% had septal defects, 25% had obstructive defects, 4% had conotruncal defects, 11% had multiple defects and 7% had other defects such as cardiomegaly, aortic dilatation, dextrocardia, partial pulmonary venous return anomaly and Ebstein anomaly. In the isolated CHD group, multiple defects were identified in 13%, obstructive defects in 8%, septal and conotruncal defects in 1% and other defects in 2% (Table 1). The classification of findings other than CHD in the syndromic group based on their frequencies is shown in Table 2. The most common non-CHD finding was neurodevelopmental delay or intellectual disability at a ratio of 32%.

Table 1. Types of heart defects in patients

Types of heart defects	Number of patients in the syndromic group	Pathogenic CNV ratio	Number of patients in the isolated group	Pathogenic CNV ratio
Septal defects	41	12%	1	-
Obstructive defects	25	8%	8	-
Conotruncal defects	4	-	1	-
Multiple defects*	11	18%	13	-
Others**	7	42%	2	-

*Patients with cardiac anomalies that belong to two or more groups (e.g., VSD+PS). ** Patients with cardiac anomalies that cannot be specifically classified into the groups mentioned above (such as cardiomegaly, aortic dilatation, dextrocardia, partial pulmonary venous return anomaly, and Ebstein anomaly). CNV: Copy number variant.

Table 2. Other findings accompanying congenital heart disease in the syndromic group

Type of the anomaly	Number of patients*	Frequency in the total group
Renal anomalies	11	12%
Skeletal defects	7	7.9%
Neurodevelopmental delay and intellectual disability	29	32%
Eye anomalies	10	11%
Hearing problems	6	6.8%
Gastrointestinal problems	3	3.4%
Growth retardation	18	20%
Dysmorphic features	20	22%
Others (endocrinologic, immunologic, or metabolic problems)	12	13%

*Patients who have multiple findings are included in more than one group, so the total number exceeds 88.

CMA results

According to the CMA analyses, pathogenic or likely pathogenic (LP) variants were detected in 12 patients (12/113; 10.6%), all of whom were from the syndromic group (13.6%) (Table 3). The total number of pathogenic or LP variants was 13. In the analysis of the CHD groups, pathogenic CNVs were detected in 42% of the "others," 18% of the "multiple defects," 12% of the "septal defects" and 8% of the "obstructive" groups.

Table 3. Pathogenic or likely pathogenic CNVs according to the ACMG 2020 guidelines in patients with CHD

Patient	Age	Gender	Loci	Start/stop points (hg19)	Size (kb)	Del/Dup	P/LP	Inheritance	Diagnosis	Karyotype/FISH	Clinical findings
P-01	1y 2 m	F	7q11.23	72,766,313-74,133,332	1,367	Del	P	N/A	Williams syndrome	46, XX	Supravalvular aortic stenosis, DD, dysmorphic features
P-02	4 m	M	22q11.21	18,894,835-21,407,690	2,513	Del	P	N/A	22q11.2 deletion syndrome	46, XY	Coarctation of the aorta, developmental delay
P-03	13d	F	11q24.3-q25	130,490,570-134,934,196	4,444	Del	P	Maternal translocation: 46,XX,t(11;19) (q24.2;p13.3)	11q distal deletion	46,XX,der(11) (11pter11q24.2::19p13.319pter).	Hypoplasia of the arcus aorta, left pulmonary artery stenosis, IUGR
			19p13.3	281,067-5,510,383	5,229	Dup	P		19p distal duplication	ish der(11) (D11S2071+,D11S1037-, pVYS251A+) mat	
P-04	5 m 10d	F	22q11.1-q11.21	16,197,005-18,651,673	2,455	Dup	P	Paternal (affected)	Cat-eye syndrome	Mosaic marker chromosome mos 47,XX, +mar[23/50]/46,XX[27/50]. ish mar (14/22) (D14Z1/ D22Z1+,wcp22+,wcp14+,D22S75,ARSA)	VSD, anal atresia
P-05	4y 9 m	M	22q11.1-q11.21	16,054,691-18,651,673	2,597	Dup	P	Paternal (affected)	Cat-eye syndrome	Mosaic marker chromosome mos 46,XY[25/46]/47,XY, +mar[21/46].ish der(22)(D14Z1/ D22Z1+,wcp22+)	VSD, anal atresia, vesicoureteral reflux, seizure, hypothyroidism, growth delay
P-06	11y	M	8p23.1	7,169,490-11,805,960	4,636	Del	P	N/A	8p23.1 deletion syndrome	46,XX,del(8) (p23.1p23.1)	Cardiomyopathy, ASD, learning difficulties, hypogonadism
P-07	1 m 25d	M	22q11.21	18,894,835-21,505,417	2,611	Dup	P	Paternal (with no clinical findings)	22q11.2 duplication syndrome	Normal	ASD, PDA, hypotonia, seizure, respiratory failure, central hypothyroidism
P-08	13y 4 m	F	22q11.21	18,807,822-21,440,514	2,633	Del	P	N/A	22q11.2 deletion syndrome	Normal	ASD, VSD, pinealoblastoma, scoliosis
P-09	4 m 29d	F	18q21.31-q23	53,964,914-77,954,165	23,989	Del	P	*de novo*	18q21.31-q23 deletion	46,XX,del(18) (pterq21.31:).ish del(18)(D18Z1+, D18S1390-)	Ebstein anomaly, growth retardation, hearing loss
P-10	11 m	F	18p11.32-p11.21	14,316-15,024,061	15,010	Tetrasomy	P	*de novo*	Supernumerary isochromosome 18p	47,XX,+i(18)(p10). ish i(18p) (D18Z1+,D18S552++, wcp 18+)	VSD, hypotonia, microcephaly, dysmorphic findings
P-11	2y 10 m	M	17q11.2	28,941,066-30,342,666	1,402	Dup	P	Maternal (with no clinical findings)	17q11.2 duplication	N/A	PFO, PDA, BAV, nail dystrophy
P-12	14 y	M	16p11.2	29,238,593-30,332,581	1,094	Dup	LP	N/A	16p11.2 duplication	Normal	Dilatation of the aorta, MVP, albinism*, microcephaly

CNVs: Copy number variants, CHD: Congenital heart disease, Loci: Chromosomal location, Del: Deletion, Dup: Duplication, P: Pathogenic, LP: Likely pathogenic, IUGR: Intrauterine growth retardation, VSD: Ventricular septal defect, ASD: Atrial septal defect, PDA: Patent ductus arteriosus, PFO: Patent foramen ovale, BAV: Bicuspid aortic valve, MVP: Mitral valve prolapse, DD: developmental delay, N/A: Not available, FISH: fluorescent *in situ* hybridisation, F: Female, M: Male, d: Day, m: Month, y: Year, kb: Kilobases *: A pathogenic variant in the *TYR* gene associated with albinism was detected in this patient's exome sequencing analysis.

Table 4. Variants of unknown significance in patients with isolated congenital heart disease

Patient	Age	Gender	Chr Loci	Start/stop points (hg19)	Size (kb)	Del Dup	Genes	Inheritance	Karyotype	Clinical findings
P-13	7y 8 m	F	3p22.2	37,953,551-38,104,718	151	Dup	*VILL*, *CTDSPL*, *PLCD1*, *DLEC1*	N/A	N/A	Aortic root dilatation
P-14	3 m 25d	F	8q24.22	131,664,618-131,852,616	188	Del	*ADCY8* (ex12-18)	N/A	46, XY	Pulmonary stenosis
P-15	2 m	M	7p14.3	34,360,422-34,464,168	104	Del	*NPSRAS1* (last exon)	N/A	46,XY,inv(8) (p21.3q11.23) mat	Coarctation of the aorta, VSD, PDA

VSD: Ventricular septal defect, PDA: Patent ductus arteriosus, N/A: Not available, VSD: Ventricular septal defect, PDA: Patent ductus arteriosus, F: Female, M: Male, d: Day, m: Month, y: Year, kb: Kilobases

Table 5. Variants of unknown significance in the syndromic congenital heart disease patients

Patient	Age	Gender	Chr Loci	Start/stop points (hg19)	Size (kb)	Del/Dup	Genes	Inheritance	Karyotype	Clinical findings
P-16	2y 6 m	F	13q12.12	24,260,840-24,489,283	228	Del	*MIPEP, PCOTH,C1QTNF9B*	N/A	Normal	ASD, elevated TSH, sparse hair
P-17	28d	M	7q31.1	110,228,593-110,520,379	292	Del	*IMMP2L* (last exon)	Maternal (with no clinical findings)	Normal	Coarctation of the aorta, interrupted vena cava inferior, seizures
P-18	1y 2 m	F	4q13.3-q21.1	76,136,017-77,179,493	1,043	Dup	*G3BP2, RCHY1, FAM47E, CXCL11, CXCL9, USO1, SDAD1, ODAPH, CXCL10, PPEF2, CDKL2, NAAA, NUP54, SCARB2, THAP6, ART3*	N/A	Normal	ASD, lung hypoplasia, hypogammaglobulinemia
P-19	7 m 4d	M	8p23.1 - p22	12,586,413-14,797,679	2,211	Del	*SGCZ, LONRF1, C8orf48, DLC1, and TRMT9B*	Maternal (with no clinical findings)	Normal	Coarctation of the aorta, VSD, PDA, bifid thumb
P-20	17y	F	3q26.1	163,292,600-164,569,792	1,277	Del	• *(LINC01324, MIR1263)*	N/A	Normal	Pulmonary stenosis, microcephaly, and dysmorphic findings

Chr Loci: Chromosomal location, Del: Deletion, Dup: Duplication, N/A: Not-available, TSH: Thyroid stimulating hormone, VSD: Ventricular septal defect, ASD: Atrial septal defect, PDA: Patent ductus arteriosus, F: Female, M: Male, d: Day, m: Month, y: Year, kb: Kilobases

No pathogenic CNVs were found in the conotruncal group, which comprised 4% of the syndromic cohort.

The variants included deletions, which accounted for a ratio of 6/13 (46.2%) and duplications, which represented a ratio of 7/13 (53.8%), whereas a tetrasomy was detected in a single case. The sizes of the affected regions varied dramatically, ranging from 1,094 to 15,010 kb. Among the pathogenic variants, the deletion or duplication of the 22q11.21 loci was consistently realised in five patients. Two patients exhibited three CNVs with distinct breakpoints: one patient (P-03) had two CNVs caused by a maternal translocation between chromosomes 11 and 19, while the other (P-09) had a *large de novo deletion* in the 18q region. Parental CMA and karyotype analysis was performed on seven patients with pathogenic or LP variants. Among these, two cases of 22q11.1-q11.21 duplication (P-04 and P-05), associated with Cat-eye syndrome, were inherited from fathers with a history of anal atresia. The 22q11.2 duplication in P-07 was inherited from a father without any clinical findings and the 17q11.2 duplication in P-11 was inherited from a mother with no clinical findings. The 18q21.31-q23 deletion in P-09 and the supernumerary isochromosome 18p in P-10 were determined to be *de novo*.

P-12 showed duplication of 16p11.2, probably related to the CHD findings and microcephaly. Whole-exome sequencing further revealed a homozygous pathogenic variant in the tyrosinase (*TYR*) gene, which was linked to albinism in the patient (24).

Variants of unknown significance (VUS) were identified in eight patients: three presented in isolated cases and five were in the syndromic group (8/113; 7%) (Tables 4, 5). Parental CMA analysis was performed on two syndromic patients with VUS (P-17 and P-19), revealing that their CNVs were inherited from mothers with no clinical findings. Due to potential incomplete penetrance or variable expressivity, these CNVs were ultimately classified as VUS.

DISCUSSION

This study outlined the efficacy of the CMA in the diagnosis of CHD in a Turkish cohort from a single centre. We found the CMA method were informative for 12 patients (12 out of 113; 10.6%). These patients were part of the syndromic group (12 out of 88; 13.6%). When reviewed by the CHD groups, the "others" category, including conditions such as cardiomegaly, aortic dilatation, dextrocardia, partial pulmonary venous return anomaly, Ebstein anomaly and others, had the highest CMA diagnosis rate of 42%. The next most common was the group with multiple defects at 18%, followed by septal defects at 12% and obstructive defects at 8%. The conotruncal anomaly group, with the fewest patients (4.4%), showed no pathogenic CNV. These diagnostic rates have also varied across different studies documented in the literature (17, 21, 25).

CHD is a heterogeneous condition with a multifactorial aetiology involving both genetic and environmental factors. It is one of the most common birth defects, and early diagnosis is essential (26). For newborns, pulse oximetry is an effective tool for the clinical diagnosis of CHD (27, 28). However, additional extra-cardiac symptoms can complicate the overall diagnosis. Furthermore, investigating the cause of CHD could help prevent the condition in future generations (29, 30). Therefore, molecular diagnosis may be a suitable approach for the early and differential diagnosis of CHD (31). At the molecular level, over 400 genes have been linked to CHD. As a result, genetic testing strategies such as exome or whole-genome sequencing could assist in identifying disease-causing variants (3). Significant chromosomal anomalies, including aneuploidies like those seen in Down syndrome and Turner syndrome, as well as microdeletions such as 22q11.2 deletion syndrome and Williams syndrome, are pathogeneses that may feature CHD symptoms (32). Additionally, traditional cytogenetic methods, such as karyotyping and FISH, are crucial for diagnosing CHD within the genetic diagnosis algorithm (33, 34). However, the limitations of these approaches, such as high cost, low accuracy and time consumption, highlight the usefulness of CMA (18, 35). For instance, in patients with a translocation-derived chromosome (P-03) or large deletions (P-06 and P-09) detectable via cytogenetic and FISH studies, CMA helped identify breakpoints at the molecular level. Clarifying these breakpoints and understanding genotype-phenotype relationships are crucial for accurate genetic counselling and effective disease management. Likewise, in cases P-04, P-05 and P-10 involving marker chromosomes, CMA enabled the determination of both the origin and boundaries of the markers.

Numerous studies have documented the diagnostic success of CMA in neonatal and paediatric cases (17, 18, 20, 21). However, further research is necessary to fully assess the potential of CMA in CHD diagnosis, reclassify variants of unknown significance (VUS), and discover new related genes (19). In the current cohort, the diagnostic yield (the percentage of disease-related pathogenic or LP variants) of CMA was 10.6% (12 out of 113 patients) for all patients and 13.6% (12 out of 88) for the syndromic group. These values were low compared with those of previous studies. In one study involving 514 American patients, the diagnostic yield of CMA was 9.3% for isolated cases and 20.6% for syndromic ones, with an overall yield of 18.5% (17). Another study underlined the yield of CMA as 17.9% in isolated and 33.8% in syndromic cases within a cohort of 104 Chinese patients (18). These studies demonstrate that the effectiveness of CMA in CHD diagnosis varies by population and depends on the cohort size. Additionally, as expected, the diagnostic yield was higher in syndromic cases across all studies.

Multiple studies have confirmed the high frequency of CNVs in 22q11 and that alterations in the copy numbers of key genes in this region may influence cardiac transcription factors (36). For example, a study involving foetuses with cardiac anomalies from Türkiye found that the 22q11 deletion was one of the commonly detected variants (37). In the present study, two patients (P-02 and P-08) had 22q11.21 deletion syndrome, one patient (P-07) had 22q11.21 duplication syndrome, and two third-degree relatives (P-04 and P-05) had cat-eye syndrome. Both patients with cat-eye syndrome had anal atresia and ventricular septal defect (VSD). Patient P-05 had additional conditions, including vesicoureteral reflux, seizures, hypothyroidism, and growth delay. Interestingly, neither patient exhibited iris coloboma, a common feature of the syndrome. Research indicates that the spectrum of phenotypic findings can vary depending on the level of tissue mosaicism, which is a recognised aspect of this syndrome (38). The duplication in these cases was inherited from the affected fathers who only had anal atresia.

The 11q distal deletion and 19q distal duplication resulting from a maternal translocation [t(11;19) (q24.2;p13.3)] were identified in case P-03, who exhibited hypoplasia of the aortic arch, left pulmonary artery stenosis, and intrauterine growth restriction (IUGR). Cardiac anomalies have been documented in cases of 19q distal duplication (39). IUGR occurs in both chromosomal abnormalities (40). In the case of P-09, who has an 18q21.31-q23 deletion and Ebstein anomaly, there have been reports of congenital heart defects at a rate of 24%. The Ebstein anomaly has been relatively and rarely shown to be associated with this deletion (41, 42). Chromosome analysis and CMA revealed a supernumerary isochromosome 18p in P-10, who also had VSD, hypotonia, microcephaly, and

dysmorphic features consistent with previously described patients (43, 44).

Patient 12, who had aortic dilatation, exhibited duplication of the 16p11.2 region. Duplications in this region can lead to varying degrees of intellectual disability, autism, microcephaly and behavioural problems (45). No previous reports have linked aortic dilatation with this duplication. However, a case has been reported with a deletion of 16p11.2, aortic arch hypoplasia, and severe aortic coarctation (46). Additionally, a patient with a 0.5 Mb duplication has been described with transposition of the great arteries, pulmonary valve stenosis, ventricular and atrial septal defects, and attention deficit hyperactivity disorder (47). Whole-exome sequencing of P-12 revealed no variants that could explain the cardiac anomaly. Therefore, the aortic dilatation might be related to this duplication. The sequencing also revealed a pathogenic variant in the *TYR* gene, which was responsible for the albinism seen in this patient. Detecting an extra variant associated with albinism emphasises the importance of comprehensive evaluation of test results and family and population studies to achieve more precise clinical insights.

Overall, CMA successfully detected CNVs in a significant number of patients in this cohort. However, the VUSs documented in the study require additional cohort or molecular studies for the reclassification of those variants.

CONCLUSION

The current study evaluated the effectiveness of CMA in the molecular diagnosis of CHD within a Turkish cohort. Our results will enhance the existing literature, especially regarding pathogenic CNVs with unique breakpoints. In addition, we expect that the CNVs of uncertain clinical significance reported here will become more understandable as more clinical data are included in future research.

Ethics Committee Approval	Ethics committee approval was received for this study from the ethics committee of Gazi University Ethics Committee (Date: 08.07.2025, No: 11).
Informed Consent	Written informed consent was obtained from patients who participated in this study.
Peer Review	Externally peer-reviewed.
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