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Chromosomal Microarray Analysis as a Diagnostic Tool in Congenital Heart Diseases

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Keywords
Congenital heart disease · Copy number variations · Microarray · Microdeletion · Microduplication

Abstract
Introduction: Congenital heart diseases are a group of diseases present at birth, including anatomical and physiological abnormalities of the heart. They are the most common birth defects observed in the populations. The etiology is quite diverse. Although they mostly show a multifactorial inheritance pattern, chromosome abnormalities, copy number variations, single gene diseases, and environmental factors are involved in the etiology. Even though the etiology can be detected at a higher rate in syndromic cases, it has not been elucidated in most syndromic and non-syndromic cases. Our study aimed to detect copy number variations in syndromic and non-syndromic cases through chromosomal microarray analysis, to reveal the diagnostic value of the method, and to determine possible new loci. ***Methods:*** Patient files, photographs, and laboratory results of 85 cases (55 syndromic and 30 non-syndromic) who had congenital heart disease and chromosomal microarray analysis were retrospectively evaluated. The differences between the groups were analyzed with Chi-square and Mann-Whitney U tests. ***Results:*** Pathogenic/likely pathogenic copy number variations were detected in 32.7% (18/55) of the syndromic case group and 6.7% (2/30) of the non-syndromic case group. The diagnostic efficacy of chromosomal microarray analysis in the diagnosis and the age at the time of admission were statistically significant between groups. ***Conclusion:*** Our study suggest that the chromosomal microarray analysis is a valuable diagnostic tool to elucidate the etiology of congenital heart diseases.

Introduction

Congenital heart diseases (CHDs) are anatomical or physiological anomalies of the heart and/or great vessels present at birth. CHD are still a common cause of severe morbidity and mortality. The incidence of CHD are 8–12 per 1,000 live births, although there are differences due to

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geography and birth rates [1–3]. When bicuspid aortic valve, physiological peripheral pulmonary stenosis, mitral valve prolapse and patent ductus arteriosus in preterm newborns are excluded, ventricular septal defect is the most common among CHD, followed by secundum atrial septal defect and patent ductus arteriosus [4].

The etiology of the majority of CHD is still unclear but assumed to be multifactorial. It is thought that the multifactorial etiology results from the combination of genetic predisposition and environmental stimulants, which have not yet been identified. Environmental factors include the fetal-placental-maternal environment and could be identified in 2% of CHD [5]. Molecular karyotyping (array, CGH-array, SNP-array) and next-generation sequencing techniques have accelerated the discovery of etiologies.

In the majority of patients with CHD, other organs and systems are not affected. The genetic etiology of non-syndromic CHD is more difficult to establish compared to syndromic CHD. This difficulty arises from genetic heterogeneity, incomplete penetration, polygenic etiology, and non-genetic factors affecting CHD. Genetic etiology can be revealed in 3–17% of non-syndromic cases [6–11]. Single gene disorders are observed in 3–5% of syndromic CHD, gross chromosomal abnormalities/aneuploidies in 8–10%, and pathogenic copy number variations in 3–25% [6, 7].

Genes involved in the etiology of syndromic CHD may also play a role in non-syndromic CHD. For example, *PTPN11* (Noonan syndrome), *TBX5* (Holt-Oram syndrome) genes have also been identified in isolated CHD [12, 13]. Studies have revealed that mosaicism has a small role in the etiology of CHD. Isolated CHD has been detected in patients with mosaic trisomies without involvement of other systems [14]. In addition, cardiac transcription factors including *NKX2-5*, *GATA4*, *TBX5*, *MEF2C*, and *HEY2* have been explored in studies on heart tissues, and increased somatic mutation frequencies have been reported compared to the unaffected heart tissue of the same patient [15]. MicroRNAs and epigenetic rearrangements are other mechanisms that are effective in the genetic etiology of CHD [16]. In the current study, we aimed to detect copy number variations in syndromic and non-syndromic cases, to reveal the diagnostic value of the chromosomal microarray analysis and to determine possible new loci.

Material and Methods

Our study was a cross-sectional and retrospective study from our clinical genetics department over a period of 6 years. The study consisted of cases with CHD and who performed chromosomal microarray. Cases with CHD, either isolated or as part of a group of symptoms, were included in the study. The cases were selected from 2 groups: syndromic and non-syndromic. To determine the groups, the symptoms, findings, anamnesis, growth parameters, physical examinations, and imaging findings of the cases were examined. Syndromic cases encompassed those with CHD exhibiting major and/or multiple minor anomalies, whereas non-syndromic cases presented isolated CHD. Patients with bicuspid aortic valve in adults, physiological peripheral pulmonary stenosis, non-congenital mitral valve prolapse and patent ductus arteriosus of preterm newborns, and non-syndromic gestational diabetes in the antenatal history were not included in the case groups. Syndromes like trisomy 21, trisomy 18, trisomy 13, and monosomy X which can be diagnosed by conventional karyotyping by G-banding were excluded from the study. Families who did not agree to participate in the study were not included in the study. Among the syndromic cases, 50.9% were female and 49.1% were male, while among the non-syndromic cases, 33.3% were female and 66.7% were male. DNA was isolated from peripheral blood using the QiAamp DNA Blood Mini Kit (cat. No. 51106, Qiagen, Hilden, Germany). DNA purity and quality were confirmed by agarose gel electrophoresis. DNA concentration was measured with Qubit (Life Technologies, Singapore). Chromosomal microarray analysis was conducted using Illumina HumanCytoSNP-12v2.1 (299.140 SNP, 6.2 kb median probe range) protocol. Data were analyzed with Bluefuse Multi v4.5 (Illumina). Detected copy number variations were classified according to the recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen) [17]. Differences between groups were analyzed using Chi-square and Mann-Whitney U tests. Informed consent forms were obtained from the cases/parents included in our study.

Results

Chromosomal microarray analysis was performed in 85 children (55.2% [47] male, 44.7% [38] female). There were 50.9% (28) female and 49.1% (27) male cases in the syndromic cases, 33.3% (10) female and 66.7% (20) male in the non-syndromic cases. Ages at admission to our clinic were taken according to the decimal system. The mean age at presentation of the syndromic cases was 2.58 ± 4, while the mean age at presentation of the non-syndromic group was 6.67 ± 5.46. Age at admission was

Table 1. Syndromic cases clinical findings and laboratory results

Case number	Age, years, gender	CHD type	Clinical findings	Chromosome analysis results	CMA results
4	4, male	Ventricular septal defect (VSD), patent foramen ovale (PFO), tetralogy of fallot (ToF)	Underweight (<−2 SDS), short stature, neurodevelopmental delay, strabismus, frequent infections, hypothyroidism, facial dysmorphism	46,XY,del(2)(q12)	arr[GRCh37] 2q12.2q14.1(106423310_117292626)x1
5	9, male	Secundum atrial septal defect (ASD), complete Atrioventricular septal defect (AVSD)	Underweight (<−2 SDS), short stature, neurodevelopmental delay, microcephaly, microphthalmia, cryptorchidism, bilateral persistent pupillary membrane, iridocorneal endothelial syndrome, facial dysmorphism	46,XY,del(3)(p25)	arr[GRCh37] 3p26.3p25.3(93949_10742366)x1
6	5, female	ToF, pulmonary valve stenosis (PVS), pulmonary valve regurgitation (PVR)	Neurodevelopmental delay, hypocalcemia, hypoparathyroidism, facial dysmorphism	46,XX	arr[GRCh37] 22q11.21q11.21(18646514_21462353) x1
7	9, male	Secundum ASD, VSD, interatrial septal aneurysm	Underweight (<−2 SDS), short stature, hypoparathyroidism, strabismus, facial dysmorphism	46,XY	arr[GRCh37] 2q37.3q37.3(242517966_243029573)x1
8	0, male	Secundum ASD, VSD, hypoplastic transverse arch-aortic arch-isthmus-left ventricle	Underweight (<−2 SDS), short stature, hypospadias, club foot, single umbilical artery, facial dysmorphism	46,XY,add(6)(p25::?)	arr[GRCh37] 6q25.3p25.1(206749_6454059)x1 arr[GRCh37] 7q22.3q36.3(106014461_159128556)x3
9	0, female	VSD, interrupted aortic arch	Microcephaly, facial dysmorphism	46,XX	arr[GRCh37] 22q11.21(18877787_21462353)x1
11	1, female	VSD, PFO, PVS, peripheral pulmonary stenosis (PPS)	Microcephaly, neurodevelopmental delay, facial dysmorphism	46,XX	arr[GRCh37] 7q11.23(72766313_74133332)x1
15	14, female	VSD	Neurodevelopmental delay, pituitary adenoma, dysthymia, bilateral sensorineural hearing loss, scoliosis, facial dysmorphism	46,XX	arr[GRCh37] 22q11.21(18894835_21505417)x1
26	15, male	ToF, PVS, right sided arcus aorta, PVR	Short stature, neurodevelopmental delay, hypocalcemia, facial dysmorphism	46,XY	arr[GRCh37] 22q11.21(18919942_21505417)x1

Table 1 (continued)

Case number	Age, years, gender	CHD type	Clinical findings	Chromosome analysis results	CMA results
28	3, male	Secundum ASD, VSD	Epilepsy, neurodevelopmental delay, short stature, polydactyly, astigmatism, lingual frenulum, leukomalacia, facial dysmorphism	46,XY	arr[GRCh37] 1q41(217811927_222887724)x1
33	0, female	Secundum ASD	Underweight (<−2 SDS), microcephaly, neurodevelopmental delay, renal ectasia, radioulnar synostosis, facial dysmorphism	46,XX der(10)t(10;22)(q26;q13)	arr[GRCh37] 10q26.13q26.3(124294520_135427143)x1 arr[GRCh37] 22q13.31q13.33(48324773_51197838)x3
44	4, female	Secundum ASD, PPS, interatrial septal aneurysm, hypoplastic left heart, aortic valve regurgitation (AVR)	Microcephaly, neurodevelopmental delay, facial dysmorphism	46,XX	arr[GRCh37] 7q11.23(72401199_74257046)x1
45	1, male	PVS, PPS	Microcephaly, neurodevelopmental delay, facial dysmorphism	46,XY	arr[GRCh37] 7q11.23(72765457_74335449)x1
46	0, female	PFO, PPS	Neurodevelopmental delay, hypothyroidism, renal stone, facial dysmorphism	46,XX	arr[GRCh37] 7q11.23(72701768_74257046)x1
48	2, male	PFO, PPS	Underweight (<−2 SDS), microcephaly, neurodevelopmental delay, facial dysmorphism	46,XY	arr[GRCh37] 7q11.23(72765457_74257046)x1
50	5, female	VSD	Underweight (<−2 SDS), short stature, microcephaly, neurodevelopmental delay, facial dysmorphism	46,XX	arr[GRCh37] 5p15.33p15.2(38139_12206786)x1 arr[GRCh37] 5q34q35.3(162095410_180705539)x3
53	0, male	Patent ductus arteriosus (PDA)	Microcephaly, neurodevelopmental delay, facial dysmorphism	46,XY	arr[GRCh37] 7q11.23(72722981_74282048)x1 arr[GRCh37] 10q21.3(68382135_68527738)x1
55	0, female	PFO	Underweight (<−2 SDS), short stature, microcephaly, neurodevelopmental delay, facial dysmorphism	46,XX,r(5)	arr[GRCh37] 5p15.33p14.3(50093_20474807)x1 arr[GRCh37] 5q35.3(180063182_180696806)x1

Table 2. Non-syndromic cases clinical findings and laboratory results

Case number	Age, years, gender	CHD type	Chromosome analysis results	Microarray analysis results
N29	6, female	Complete AVSD	46,XX	arr [GRCH38] 17q12 (36110820_37836317)x3
N30	0, male	VSD, right ventricular hypertrophy, left ventricular hypertrophy, interventricular septum hypertrophy, right sided arcus aorta	46,XY	arr [GRCH38] 22q11.21 (18907714_21105931)x3

compared with Mann-Whitney U test, p value was found 0.001 ($p < 0.05$). There was a significant difference between the ages of admission between the two groups.

Pathogenic/likely pathogenic copy number variations were determined by chromosomal microarray analysis method in 32.7% (18/55) of syndromic cases. Additional clinical features frequently observed in these cases were facial dysmorphism (18/18), neurodevelopmental delay (15/18), microcephaly (10/18), short stature (8/18), and underweight (8/18). Other features were ophthalmological findings (strabismus, bilateral persistent pupillary membrane, iridocorneal endothelial syndrome, microphthalmia, strabismus, astigmatism), endocrine disorders (hypothyroidism, hypocalcemia, hypoparathyroidism), urogenital system findings (cryptorchidism, hypospadias, renal ectasia, renal stone), bilateral sensorineural hearing loss, skeletal findings (clubfoot, polydactyly, radioulnar synostosis). Although abnormal results were detected in the karyotype analysis in 5 of these cases, chromosomal microarray analysis study was still needed to determine the precise location of related gene regions. Copy number variations were detected in 6.7% (2/30) of non-syndromic cases. A statistically significant difference was observed between the syndromic and non-syndromic groups (χ^2: 7.327, p: 0.006792). In the syndromic case group, 22q11.2 deletion syndrome was found in 4 cases and Williams syndrome was found in 6 cases. Parental reciprocal translocations were determined in 2 cases.

In the non-syndromic case group, a 1.758 Mb duplication (chr17:36110820_37836317) in the 17q12 chromosome region and a 2.565 Mb duplication (chr22:18907714_21105931) in the 22q11.21 chromosome region were detected (N29, N30). Clinical findings and chromosomal microarray analysis results of syndromic and non-syndromic cases in which copy number variations was detected are provided in Tables 1 and 2.

Discussion

Syndromic cases (especially facial dysmorphisms, skeletal findings) show a higher incidence of genomic variants [18]. Chromosomal microarray analysis is a frequently used method for detecting copy number variations. Considering the limitations of conventional cytogenetic methods, it plays an important role in elucidating the genetic etiopathogenesis of diseases. It has been recommended as a first-line test in cases of developmental delay/cognitive retardation, autism spectrum disorder, and multiple congenital anomalies since 2010 [19]. Although the underlying genetic causes in CHD can be explained at a higher rate in syndromic cases than in non-syndromic cases, it is still not fully elucidated in both groups. It is very important to make a more precise diagnosis for clinical genetics purposes such as revealing the genetic etiology of CHD, determining the risk of recurrence for the parents and family members at risk, evaluation and follow-up of possible non-cardiovascular findings related to the variant, determining the risk of neurodevelopmental delay in newborns and infants.

In our study, pathogenic/likely pathogenic copy number variations were determined by chromosomal microarray analysis method in 32.7% (18/55) of syndromic and in 6.7% (2/30) of non-syndromic cases. There are not many studies in the literature analyzing the significance of chromosomal microarray analysis in identification of CHD etiology. Our results were consistent with the rates reported in the literature, which range from 3 to 25% in syndromic cases and 3–17% in non-syndromic cases [6–11]. The difference of the admission age between the groups was also significant. It was presumed that the severity and mystery of the syndromic conditions affected the behavior of seeking consultation of the families and health professionals at an earlier age.

17q12 duplication syndrome (*OMIM* # 614526) inherited from a healthy asymptomatic mother was detected in a case with non-syndromic complete

atrioventricular septal defect (N29). This situation was thought to be related to the reduced penetrance and variable expressivity of 17q12 microduplication syndrome [20]. In the literature, an isolated heart anomaly (mirror-image dextrocardia, patent ductus arteriosus, double aortic arch, tracheal stenosis) has been reported in a case with 17q12 duplication syndrome [21]. Our case is the first case of isolated atrioventricular septal defect reported in the literature.

22q11.2 duplication syndrome (*OMIM* # 608363), inherited from a healthy asymptomatic mother, was detected in a case with non-syndromic, isolated heart anomalies (ventricular septal defect, right ventricular hypertrophy, left ventricular hypertrophy, interventricular septum hypertrophy, right sided arcus aorta). This situation is associated with the detection of a highly variable phenotype in 22q11.2 duplication syndrome [22].

Limitations of our study include number of patients in the non-syndromic case group, and the chromosomal microarray method used had a relatively lower resolution. These results suggest that the chromosomal microarray analysis method is a crucial diagnostic tool in defining the genetic etiology of CHD, especially in syndromic cases. More studies are needed to understand of the complex genetics of CHD. Copy number variations analyzes can guide the identification of previously unidentified genes and regions and elucidate the etiology of CHD. For this reason, other systems should definitely be evaluated in patients with CHD. We recommend chromosomal microarray analysis as a first-tier diagnostic test, especially in syndromic cases.

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Statement of Ethics

Ethics committee approval for our study was received from İnönü University Scientific Research and Publication Ethics Board in accordance with the Declaration of Helsinki (2019/41, December 17, 2019). Informed consent forms were obtained from the cases/parents included in our study.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Zeynep Esener participated in the design of the study, performed physical examinations of the cases, took anamnesis and family history, performed array study and analysis, and compiled the cases. Kübra Ates performed physical examinations of the cases and took anamnesis and family history. Murat Ozturk performed physical examinations of the cases and took anamnesis and family history. Cemsit Karakurt participated in cardiological evaluation, echocardiography and electrocardiography of cases. Ozlem Elkiran participated in cardiological evaluation, echocardiography, and electrocardiography of cases. Ibrahim Tekedereli participated in the design of the study and performed array analysis.

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article. Chromosomal microarray analysis images and additional data of cases can be shared upon request.

References

1 Hoffman J. The global burden of congenital heart disease. Cardiovasc J Afr. 2013;24(4):141–5. https://doi.org/10.5830/CVJA-2013-028

2 Gaskin K, Kennedy F. Care of infants, children and adults with congenital heart disease. Nurs Stand. 2019;34(8):37–42. https://doi.org/10.7748/ns.2019.e11405

3 van der Linde D, Konings EE, Slager MA, Witsenburg M, Helbing WA, Takkenberg JJ, et al. Birth prevalence of congenital heart disease worldwide: a systematic review and meta-analysis. J Am Coll Cardiol. 2011;58(21):2241–7. https://doi.org/10.1016/j.jacc.2011.08.025

4 Pate N, Jawed S, Nigar N, Junaid F, Wadood AA, Abdullah F. Frequency and pattern of congenital heart defects in a tertiary care cardiac hospital of Karachi. Pak J Med Sci. 2016;32(1):79–84. https://doi.org/10.12669/pjms.321.9029

5 Cowan JR, Ware SM. Genetics and genetic testing in congenital heart disease. Clin Perinatol. 2015;42(2):373–93, ix. https://doi.org/10.1016/j.clp.2015.02.009

6 Nees SN, Chung WK. The genetics of isolated congenital heart disease. Am J Med Genet C Semin Med Genet. 2020;184(1):97–106. https://doi.org/10.1002/ajmg.c.31763

7 Pierpont ME, Brueckner M, Chung WK, Garg V, Lacro RV, McGuire AL, et al. Genetic basis for congenital heart disease: revisited – a scientific statement from the American Heart Association. Circulation. 2018;138(21):e653–711. https://doi.org/10.1161/CIR.0000000000000606

8 Soemedi R, Wilson IJ, Bentham J, Darlay R, Topf A, Zelenika D, et al. Contribution of global rare copy-number variants to the risk of sporadic congenital heart disease. Am J Hum Genet. 2012;91(3):489–501. https://doi.org/10.1016/j.ajhg.2012.08.003

9 Wu XL, Li R, Fu F, Pan M, Han J, Yang X, et al. Chromosome microarray analysis in the investigation of children with congenital heart disease. BMC Pediatr. 2017;17(1):117. https://doi.org/10.1186/s12887-017-0863-3
10 Erdogan F, Larsen LA, Zhang L, Tumer Z, Tommerup N, Chen W, et al. High frequency of submicroscopic genomic aberrations detected by tiling path array comparative genome hybridisation in patients with isolated congenital heart disease. J Med Genet. 2008;45(11):704–9. https://doi.org/10.1136/jmg.2008.058776
11 Mermer S, Sahin DA. Array comparative genomic hybridisation results of non-syndromic children with the conotruncal heart anomaly. Cardiol Young. 2022;32(2):301–6. https://doi.org/10.1017/S104795112100473X
12 Weismann CG, Hager A, Kaemmerer H, Maslen CL, Morris CD, Schranz D, et al. PTPN11 mutations play a minor role in isolated congenital heart disease. Am J Med Genet A. 2005;136(2):146–51. https://doi.org/10.1002/ajmg.a.30789
13 Smemo S, Campos LC, Moskowitz IP, Krieger JE, Pereira AC, Nobrega MA. Regulatory variation in a TBX5 enhancer leads to isolated congenital heart disease. Hum Mol Genet. 2012;21(14):3255–63. https://doi.org/10.1093/hmg/dds165
14 Manheimer KB, Richter F, Edelmann LJ, D'Souza SL, Shi L, Shen Y, et al. Robust identification of mosaic variants in congenital heart disease. Hum Genet. 2018;137(2):183–93. https://doi.org/10.1007/s00439-018-1871-6
15 Reamon-Buettner SM, Spanel-Borowski K, Borlak J. Bridging the gap between anatomy and molecular genetics for an improved understanding of congenital heart disease. Ann Anat. 2006;188(3):213–20. https://doi.org/10.1016/j.aanat.2005.10.007
16 Muntean I, Toganel R, Benedek T. Genetics of congenital heart disease: past and present. Biochem Genet. 2017;55(2):105–23. https://doi.org/10.1007/s10528-016-9780-7
17 Riggs ER, Andersen EF, Cherry AM, Kantarci S, Kearney H, Patel A, et al. Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen). Genet Med. 2020;22(2):245–57. https://doi.org/10.1038/s41436-019-0686-8
18 Wurfbain LF, Cox IL, van Dooren MF, Lachmeijer AMA, Verhoeven VJM, van Hagen JM, et al. Diagnostic gene panel testing in (non)-syndromic patients with cleft lip, alveolus and/or palate in the Netherlands. Mol Syndromol. 2023;14(4):270–82. https://doi.org/10.1159/000530256
19 Manning M, Hudgins L; Professional Practice and Guidelines Committee. Array-based technology and recommendations for utilization in medical genetics practice for detection of chromosomal abnormalities. Genet Med. 2010;12(11):742–5. https://doi.org/10.1097/GIM.0b013e3181f8baad
20 Mefford H. 17q12 recurrent duplication. In: Adam MP, Feldman J, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, et al, editors. GeneReviews®. Seattle (WA); 1993.
21 Liu L, Wang HD, Cui CY, Wu D, Li T, Fan TB, et al. Application of array-comparative genomic hybridization in tetralogy of Fallot. Medicine. 2016;95(49):e5552. https://doi.org/10.1097/MD.0000000000005552
22 Yu A, Turbiville D, Xu F, Ray JW, Britt AD, Lupo PJ, et al. Genotypic and phenotypic variability of 22q11.2 microduplications: an institutional experience. Am J Med Genet A. 2019;179(11):2178–89. https://doi.org/10.1002/ajmg.a.61345