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Evaluation of Genomic Variants in Non-syndromic Congenital Heart Disease in Turkish Pediatric Group

Non-sendromik Konjenital Kalp Hastalığı Tanısı Almış Pediyatrik Türk Grupta Genomik Varyantların Değerlendirilmesi

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ABSTRACT

Objective: Congenital heart disease (CHD) is the most common congenital malformation in the population, and so far, all the known genetic factors could explain only 20-25% of the cases.

Material and Methods: In this study, microarray analysis was performed, and next-generation sequencing of *Myosin Heavy Chain 6 (MYH6)*, *NK2 Homeobox 5 (NKX2-5)*, *GATA Binding Protein 4 (GATA4)*, *Notch Receptor 1 (NOTCH1)*, and *T-Box Transcription Factor 1 (TBX1)* genes, which are known to be involved in the etiology of non-syndromic CHD, was performed in 40 patients with isolated cardiac defects between the ages of 0-18 and 40 age-matched controls.

Results: In microarray analysis, 9 novel copy-number variations (CNVs) that were not reported in population databases, and included *OMIM* genes were detected in 1.5% (6/40) of the patients. Even though the detected CNVs had not been previously associated with CHDs and were classified as Variant of Uncertain Significance (VUS), overall CNV count burden in the patient group was significantly higher than in the control group. Also, there were no pathogenic/likely pathogenic sequence variants in *MYH6, NKX2-5, GATA4, NOTCH1*, and *TBX1* genes. The c.700C>T [p.(Arg234Cys)] and c.5949C>G [p.(Asn1983Lys)] in the *NOTCH1* gene were classified as VUS and have not been detected in the control group.

Conclusion: Although microarray technologies and candidate gene sequencing are useful diagnostic tools, routine genetic testing of sporadic non-syndromic CHD patients is controversial. We believe

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Giriş: Konjenital kalp hastalıkları (KKH) toplumda en sık görülen malformasyonlar olmakla birlikte bilinen genetik faktörler tüm olguların yaklaşık %20-25'lik kısmını açıklayabilmektedir.

Gereç ve Yöntem: Bu çalışmada 0-18 yaş arasında izole KKH tanısı alan 40 olgu ve benzer yaş grubundaki 40 kontrol bireyin periferik kan örneklerinde, KKH etiyolojisinde rol oynadığı bilinen *Miyozin Ağır Zincir 6 (MYH6), NK2 Homeobox 5 (NKX2-5), GATA Bağlayıcı Protein 4 (GATA4), Notch Reseptörü 1 (NOTCH1)* ve *T-Box Transkripsiyon Faktörü 1 (TBX1)* genlerinin yeni nesil dizi analizi yöntemleriyle dizilenmesi ve mikrodizin analizleri çalışılmıştır.

Bulgular: Mikrodizin analizi sonucunda olguların %1,5'unda (6/40) *OMIM* geni içeren ve popülasyon veritabanlarında daha önce bildirilmemiş 9 kopya sayısı değişimi tespit edilmiştir. Tespit edilen kopya sayı varyasyonları daha önce KKH ile ilişkilendirilmemiş ve klinik önemi belirsiz (KÖB) varyant olarak sınıflandırılmış olsa da, hasta grubundaki toplam CNV yükü kontrol grubuna kıyasla anlamlı derecede daha fazla olarak değerlendirilmiştir. İlave olarak olgu grubunda dizi analizi ile değerlendirilen *MYH6, NKX2-5, GATA4, NOTCH1* ve *TBX1* genlerinde patojenik/olası patojenik olarak sınıflandırılan herhangi bir sekans varyantı saptanmamıştır. *NOTCH1* geninde, kontrol grubunda bulunmayan ve KÖB olarak sınıflandırılan c.700C>T p.(Arg234Cys) ve novel c.5949C>G p.(Asn1983Lys) varyantları tespit edilmiştir.

Sonuçlar: Mikrodizin teknolojileri ve aday genlerin dizilenmesi önemli tanısal araçlar olmasına rağmen, sporadik vakalarda geniş kapsamlı

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ABSTRACT

that still remains a challenge to interpret the variants detected in multifactorial CHD with complex etiology, and further studies are needed.

Keywords: Next-generation sequencing, microarray analysis, congenital heart disease

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genetik testlerin rutin tanıda uygulanması tartışmalıdır. Kompleks etiyolojiye sahip multifaktöriyel kalıtım gösteren KKH'larda saptanan varyantların klinik öneminin yorumlanması oldukça güçtür ve ileri çalışmalara ihtiyaç duyulmaktadır.

Anahtar Kelimeler: Yeni nesil dizi analizi, mikrodizin analizi, konjenital kalp hastalıkları

INTRODUCTION

Congenital heart disease (CHD) is the most common congenital malformation in the population with an estimated frequency of 1-2%. Every year approximately 1.35 million new patients are born with the condition, and it still remains one of the most important causes of childhood mortality and morbidity in well-developed countries (1).

Most of the patients, approximately 75-80%, have isolated cardiac defects without any extra-cardiac malformation or global developmental delay, intellectual disability. The rest manifest the cardiac defects as a part of a syndrome such as aneuploidies, monogenic, or microdeletion/duplication syndromes (2).

Large-scale epidemiological studies revealed that a genetic or environmental cause can be identified in approximately 20-30% of CHDs (3). Among the genetic causes, chromosomal aneuploidies and gross structural chromosome aberrations that could be detected by conventional cytogenetic analyses are responsible for 20-25%; submicroscopic chromosomal rearrangements such as 22q11.2 deletion syndrome and Williams syndrome are responsible for 10-12%; and monogenic syndromes such as Kabuki syndrome and Noonan syndrome are responsible for 3-5% (4). It becomes even more compelling in the context of the non-syndromic CHD group. Positive family history increases the risk of recurrence depending on the type of defect (3.4 to 79.1 times) in siblings, and monozygotic twin studies show a higher concordance than the rest of the population (5,6). Also, consanguinity of the parents has been shown to increase the risk (7). Non-syndromic cardiac defects are mostly diagnosed as sporadic multifactorial malformations resulting from complex genetic mechanisms or environmental factors. To further define the genetic contributions to the causes, developmental steps in the embryonic and fetal period are studied in particular, and several genes coding transcription factors, cardiac structural proteins, or molecules responsible for the signal transduction pathways are evaluated (8).

In recent studies, it is estimated that copy-number variations (CNV), which involve the loss and gain of genomic material more than 1 kb, is responsible for approximately 3-10% of the non-syndromic CHD patients. Similarly, next-generation sequencing (NGS) of the candidate genes revealed that sequence variants are responsible for 2% (9). Many genes have so far been identified as responsible for the non-syndromic CHDs, such as *Myosin Heavy Chain 6 (MYH6), NK2 Homeobox 5 (NKX2-5), GATA Binding Protein 4 (GATA4), Notch Receptor 1 (NOTCH1), and T-Box Transcription Factor 1 (TBX1)* (10-12).

Over the past decade, with the development of massive parallel sequencing techniques and microarray technologies, it has become possible to elucidate the monogenic pathogenic variants contributing to specific phenotypes and identify candidate genes. Therefore, this study aimed to perform microarray analysis and NGS on previously implicated genes in Turkish pediatric cases of non-syndromic CHD.

MATERIALS AND METHODS

Study Group

A group of 40 patients was included in the study. All patients were diagnosed by echocardiography at Pediatric Cardiology clinics and then evaluated at Medical Genetics clinics for syndromic traits and pedigree analysis. Inclusion criteria for the patients were not to have extra-cardiac anomaly or global developmental delay or intellectual disability. All 40 age-matched (8±4.6) control samples, consisting of 18 females and 22 males, were examined by echocardiography before being included in the study. The control group was included in the microarray analysis to assess the contrast in size and CNV count burden between two groups. Written informed consent was obtained from legal representatives before collecting blood samples in accordance with the Declaration of Helsinki. The study was approved by the Non-interventional Clinical Research Ethics Committee of Eskişehir Osmangazi University (decision number: 18, date: 25.06.2019).

Microarray Method

DNA was extracted from peripheral blood samples of all cases using the A.B.T. DNA Purification Kit (TM), according to the manufacturer's recommendations. For microarray analysis, the Agilent Comparative Genomic Hybridization + Single Nucleotide Polymorphism Microarray Kit (4x180K) was used. DNA digestion, labeling, purification, and hybridization procedures were performed according to the manufacturer's instructions, and after post-hybridization washes, the scanning process was performed.

Next-Generation Sequencing

Peripheral blood samples were collected from all patients, and by the Magna Pure Compact LC (Roche Applied) extraction kit gDNA was isolated according to the manufacturer's recommendations. Amplification of all exons and exon/intron boundaries of *NKX2-5, TBX1, NOTCH1, MYH6*, and *GATA4* genes in each pool of 51 amplicons was done by TaqDNA polymerase using a Thermal Cycler (Thermo Fisher Scientific Inc.). Library preparation was done with NEXTERA XT Index Kit V2, and sequence analysis on a micro flow cell was performed with the MiSeq instrument (Illumina, Inc.).

Analysis of Sequence Variants

Sequence alignment to the reference genome and the quality filter were performed by MiSeq Reporter v2.3 software. Single nucleotide variants, small insertion variants, and deletion variants were filtered. Coverage depth and quality scores were controlled using

the integrative genomics viewer database. The variant classification according to the American College of Medical Genetics (ACMG) criteria was done using the platforms such as Varsome and Franklin (13). Non-synonymous variants that were not reported in population databases (GnomAD) or had a minor allele frequency of <0.01 were noted as rare variants. The functional impact of missense variants was assessed using *in silico* prediction tools: MutationTaster (http://www.mutationtaster.org/), Prediction of Effects of Human nsSNPs (http://genetics.bwh.harvard.edu/pph2/), Scale-Invariant Feature Transform (http://sift.jcvi.org/), and Human Splicing Finder (http://www.umd.be/HSF/). The dbSNP (https://www.ncbi.nlm.nih.gov/snp/) and ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/) databases were used for literature review.

Analysis of Copy-Number Variations

Feature Extraction 3.0.2.11 and Agilent Cytogenomics 3.0.2.11 software were used for data extraction and analysis, respectively. Conservative log2 ratio thresholds were taken as 0.3 and -0.3, respectively. Genomic regions with at least 5 probes were included for analysis. DECIPHER database v11.12 (https://www.deciphergenomics.org/), the Database of Genomic Variant (http://dgv.tcag.ca/dgv/app/home), and PubMed were used as resources to assess the variants detected. Franklin Genoox database (https://franklin.genoox.com/clinical-db/home), and ClinGen CNV Interpretation Calculator (https://cnvcalc.clinicalgenome.org/cnvcalc/) were used to classify the variants according to ACMG/ClinGen Technical Standards for CNVs (14).

Statistical Analysis

Data analysis was performed with IBM SPSS 21.0. Relationships between means of a continuous variable were evaluated by Independent Samples t-test analysis.

RESULTS

In this study, a total of 40 patients were included: 14 males and 26 females. The mean age of the patients was noted as 6±5.1. According to the diagnostic criteria, 13 septal defects (32.5%), 12 left ventricle outflow defects (30%), 7 conotruncal anomalies (17.5%), 4 complex cardiac anomalies (10%), 2 right ventricle outflow defects (5%), 1 atrioventricular canal defect (2.5%), 1 pulmonary venous return anomaly (2.5%) were detected. The cardiac anomalies of the patients are given in Table 1. The incidence of consanguinity among the parents of the patients was 7.5% (3/40).

Results of Next-generation Sequencing

Patients with non-syndromic congenital heart defects were included in the study; previously defined structural cardiac protein-coding and transcription factor genes were sequenced by NGS methods. Mean coverage was 152x for 98.10% of the targeted amplicons of the *NOTCH1* gene, 134x for 98.40% of the targeted amplicons of the *MYH6* gene, 171x for 99.30% of the targeted amplicons of the *GATA4* gene, 117x for 99.70% of the targeted amplicons of the *NKX2-5* gene and 105x for 99.10% of the targeted amplicons of the *TBX1* gene. There were no pathogenic or likely pathogenic variants detected in this study, but in 5% (2/40) of patients, variants of uncertain significance (VUS) that might be clinically significant were detected. The detected VUS variants and clinical findings of the variant carriers are summarized in Table 2.

The c.700C>T, [p.(Arg234Cys)] variant in the *NOTCH1* gene has been detected in case 34, who was a 9-month-old female patient. She was born to healthy, non-consanguineous parents. There was no prenatal exposure to teratogens during the pregnancy, nor was there a family history of CHD. She was diagnosed with secundum ASD and pulmonary stenosis at 6 months of age. The variant was classified as VUS according to the ACMG criteria. Segregation analysis revealed the variant occurred de novo.

c.5949C>G [p.(Asn1983Lys)] variant in the *NOTCH1* gene has been detected in case 40, who was a 14-year-old male patient. He was born to healthy, non-consanguineous parents. There was no prenatal exposure to teratogens during the pregnancy or family history of CHD. He was diagnosed with bicuspid aortic valve and aortic stenosis after the birth. This variant was a novel change that was not previously reported in databases, and the allele frequency was not available at the GnomAD database. The variant was classified as VUS according to the ACMG criteria. Segregation analysis revealed, the variant occurred *de novo*.

Table 1. Clinical features of the patients

Cardiac anomaly type	n
Septal defects	13
Left ventricle outflow defects	12
Conotruncal anomalies	7
Complex cardiac anomalies	4
Right ventricle outflow defects	2
Atrioventricular canal defects	1
Pulmonary venous return anomaly	1
Total	40

Table 2. Variations detected in uncertain significance in this study

Case No	Gene transcript	cDNA protein	Zygosity Rs Identification	GnomAD allele frequency	Pathogenicity prediction (SIFT, mutationtaster)	Type of congenital heart defect
34	NOTCH1 NM_017617.5	c.700C>T [p.(Arg234Cys)]	Heterozygous rs567890045	0.00000688	Damaging, disease causing	Pulmonary stenosis and secundum atrial septal defect
40	NOTCH1 NM_017617.5	c.5949C>G [p.(Asn1983Lys)]	Heterozygous (-)	(-)	Damaging, disease causing	Bicuspid aorta, aortic stenosis

Results of Microarray Analysis

There were a total of 62 CNVs detected in the patient group and 27 CNVs in the control group. Among the 62 CNVs, five were detected in both control and study groups. Among the patient and control groups, CNV detection rates were 82% (33/40) and 22.5% (9/40), respectively (odds ratio: 16.2381 95% confidence interval: 5.3905 to 48.9145, p<0.0001). The burden of CNV counts (total CNVs/ total individuals) was significantly higher (p<0.001) in the patient group compared to the control group, Among the CNVs detected in patients, 45.1% (28/62) were copy-number losses and 54.8% (34/62) were copy-number gains. 14.51% (38/62) of the CNVs were previously reported in DGV more than once, therefore classified as ''benign''. Also, 12.9% (8/62) of CNVs did not have any genes included in the region. Pathogenic or likely pathogenic variants were not detected in our study, but we have identified VUS CNVs in 15% (6/40) of the patient group. 14.5% (9/62) were are summarized in Table 3.

Case 13 was a 1-year-4-month-old female patient who was diagnosed with perimembranous VSD. She had a 55.749 kb gain at the region 6p25.3 and a 272.22 kb gain at the region 17q25.3. Neither of the aberrations has been previously reported in CHD. Gain of the 6p25.3 region included the *FOXCUT* and *FOXC1* genes. The *FOXC1* gene was reported to be haploinsufficient and was previously associated with Anterior segment dysgenesis 3, multiple types (OMIM #301631) and Axenfeld-Rieger syndrome, type 3 (OMIM #602482). The *FOXCUT* gene encodes a long non-coding RNA, which was suggested to be a regulator of the *FOXC1* transcript and involved in the proliferation and migration processes of tumor cells. Gain of the related region was not reported in the DECIPHER database. The 17q25.3 (79,638,223-79,910,442) region included 17 *OMIM* genes *(CCDC137, HGS, MRPL12, SLC25A10, GCGR, MCRIP1, P4HB, ARHGDIA, ALYREF, ANAPC11, PCYT2, NPB, SIRT7, MAFG, PYCR1, NOTUM, ARL16)*. *GCGR, P4HB, ARHGDIA,* and *PYCR1* were morbid genes in the region, but they have not been previously associated with congenital heart defects. This gain was not reported in the DECIPHER database.

Case 23 was a 7-year-5-month-old male patient who was diagnosed with aortic coarctation and bicuspid aortic valve. He had a 358,299 kb gain at 3q13.31 and an 81.09 kb gain at the 8q24.11 region. The gain at the 3q13.31 region includes the *OMIM* gene, *TUSC7*. In the DECIPHER database, a patient (DECIPHER ID: 384018) who has autism was reported with a gain of a similar region, but it was classified as likely benign and had been maternally inherited. Gain at the 8q24.11 region includes the *EXT1* gene, which encodes the exostosin-1 protein. Loss of the *EXT1* gene causes multiple exostoses type 1, but gain of the gene is not associated with any CHD.

Case 26 was a 4-year, 3-month-old male patient who was a diagnosed with d-TGA. He had a 166,784 kb gain at 4q32.1. The region includes three OMIM genes (*ASIC5, TDO2* and *CTSO*) but they have not been reported as morbid genes. Gain of a similar region was reported in DECIPHER database, previously in a patient with intellectual disability (DECIPHER ID: 345189) but not reported in a patient who was diagnosed with CHD.

Case 28, was a 14-year-3-month-old male patient who has been diagnosed with TOF. He had a 638.515kb gain at Xq21.31. The region included the *PCDH11X OMIM* gene. The *PCDH11X* gene belongs to the protocadherin gene family and has not been associated with any phenotype to date. It was reported to be expressed mainly in brain, and ovary tissues. In addition to that, it is implicated in cell-cell communication and dendritic synaptic plasticity, and is suggested as a candidate gene for dyslexia. Gain of a similar region was reported previously in the DECIPHER database in a patient with intellectual disability and obesity (DECIPHER ID: 258856) who did not have any cardiac findings.

Case 35 was a 17-year-old female patient, who was diagnosed with a bicuspid aortic valve. She had 169.936 kb loss at 19p13.3. The region included three *OMIM* genes (*TLE2, TLE6* and *ZNF77).* The *TLE2* gene has not been previously associated with any phenotype but is known to act as a corepressor in the negative regulation of the canonical Wnt signaling pathway (15).

Table 3. CNVs that were not reported previously in DGV and included genes

Case No	Locus	Size (kb)	Aberration type	Aberration	OMIM genes included	ACMG classification
13	6p25.3	55.749	Gain	arr[GRCh37]6p25.3(1,556,504_1,612,252)x3	*FOXCUT, FOXC1*	VUS
	17q25.3	272.22	Gain	arr[GRCh37]17q25.3(79,638,223_79,910,442) x3	*CCDC137, ARL16, HGS*	VUS
23	3q13.31	358.299	Gain	arr[GRCh37]3q13.3(116,229,141_116,587,439)x3	*TUSC7*	VUS
	8q24.11	81.09	Gain	arr[GRCh37]8q24.1(118,757,607_118,838,696)x3	*EXT1*	VUS
26	4q32.1	166.784	Gain	arr[GRCh37]4q32.1(156,740,213_156,906,996)x3	*ASIC5, TDO2, CTSO*	VUS
28	Xq21.31	638.515	Gain	arr[GRCh37]Xq21.3 (91,632,904_92,271,418) x3	*PCDH11X*	VUS
35	19p13.3	169.936	Loss	arr[GRCh37] 19p13.3 (2,876,148_3,046,083)x1	*ZNF77, TLE2, TLE6*	VUS
36	6q27	98.904	Gain	arr[GRCh37] 6q27 (168,954,929_169,053,833)x3	*SMOC2*	VUS
	13q21.32	95.998	Gain	arr[GRCh3713q21.3 (67,459,885_67,555,883)x3	*PCDH9*	VUS

VUS: Variant of Uncertain Significance, ACMG: American College of Medical Genetics

TLE6 gene is associated with preimplantation embryonic lethality, autosomal recessive (OMIM #612399). It regulates spermatogonia proliferation and cell cycle progression, but is not associated with the pathogenesis of congenital heart defects. *ZNF77* gene has not been previously associated with any phenotype and is predicted to enable DNA-binding transcription repressor activity.

In Case 36, a 10-year-3-month-old male patient diagnosed with a bicuspid aortic valve, a 98.904 kilobases gain at 6q27 and a 95.998 gain at the 13q21.32 regions were detected. gain at the 6q27 region includes *SMOC2* gene. This gene Dentin dysplasia, type I, characterized by microdontia and misshapen teeth, and with autosomal recessive (OMIM #125400), but not with any CHD. The gain at the 13q21.32 region included the *PCDH19* gene. This gene is associated with developmental and epileptic encephalopathy type 9, X-linked (OMIM #300088), but not with any CHD.

DISCUSSION

Congenital heart defects are the most common birth defects all around the world. It remains one of the most important reasons for childhood mortality and morbidity (16). Given the multifactorial nature of the condition, it is challenging to identify the genetic etiology, thereby making it difficult to provide proper genetic counseling to patients. However, thanks to advances in molecular technologies, information on the molecular pathology of the disease has begun to emerge (17).

This study aimed to perform microarray analysis and NGS of *NKX2-5, MYH6, GATA4, NOTCH1* and *TBX1* genes in the Turkish pediatric group with non-syndromic CHD. Microarray analysis is the most important tool for identifying CNVs in routine practice. Among other genetic conditions, it has emerged as a useful tool for the diagnosis of both isolated and syndromic CHDs (18). More recently, in addition to common pathogenic microdeletion syndromes that include extracardiac abnormalities such as 22q11.2 deletion, CNVs that include dosage-sensitive genes important for cardiogenesis, or include regulatory elements, have been identified in isolated CHDs. It is known that CNVs are the underlying mechanism of 3-25% of syndromic CHDs while they account for 3-10% of the non-syndromic CHDs.

In our study, we did not detect any pathogenic or likely pathogenic CNVs, but we have identified CNVs in 15% (6/40) of the patient group. Previous studies described in Table 4 show that pathogenic/likely pathogenic CNVs are detected in the patient cohorts, including both syndromic and non-syndromic CHDs (20-24). Since our patient group included only non-syndromic CHDs and there was no positive family history, we assumed that there were no pathogenic/likely pathogenic CNVs detected.

Besides gene content and pathogenicity classification, researchers also suggest that the total number and size of the CNVs are higher in CHD patients than in control groups. Several studies indicate that the overall CNV count burden is likely higher in CHD patients compared to the control group. Similar to previous studies in the literature, CNV counts were detected significantly higher in the patient group compared to the control group in our study (25,26).

Since the advent of massive parallel sequencing technologies, our understanding of the genetics of CHDs has rapidly expanded (27). Targeted or non-targeted sequencing (whole exome sequencing, whole genome sequencing) technologies have been applied to both syndromic and non-syndromic forms of CHD. Causative variants are mostly identified in familial non-syndromic patients, but due to the multifactorial nature of the CHDs it is not possible to identify a pattern presuming Mendelian inheritance (28,29).

NKX2-5, GATA4, TBX1, NOTCH1, and *MYH6* genes have been identified as strong candidates for non-syndromic CHDs in the past decades (4,30,31). In our study, we have detected a total of 103 variants in the five genes by the NGS technique. There were no pathogenic or likely pathogenic variants detected in this study; however, in 5% (2/40) of patients, VUS were detected. Dong et al. (32) have evaluated 73 CHD probands from consanguineous Turkish families with whole exome sequencing and detected causative genetic alterations in 13.7% of the patients. It was assumed that the detection rate of this study was high because of the consanguinity among the parents and the contribution of recessive variants (9.6%), which is lower in contrast to our study (32). Blue et al. (33) reported pathogenic variants at 57 genes that have been previously associated with both syndromic and non-syndromic CHDs in five

Table 4. Microarray studies in CHDs in the literature

Studies	Patient included	CHD type	Array platform	Pathogenic CNVs detected
Erdogan et al. (20) 2008	105	Non-syndromic	244K Agilent	5/105 (4.7%)
Greenway et al. (21) 2009	114	Non-syndromic syndromic	Affymetrix SNP 6.0	11/114 (9.6%)
Breckpot et al. (22) 2011	46	Non-syndromic syndromic	Affymetrix SNP 6.0	2 /46 (4.3%)
Goldmuntz et al. (23) 2011	58	Syndromic	Affymetrix 100K	12/58 (20.7%)
Tomita-Mitchell et al. (24) 2020	945	Non-syndromic syndromic	Affymetrix SNP 6.0	35/945 (4.3%)
Our study	40	Non-syndromic	Agilent, 180K	-ca

CNV: Copy-number variation, CHD: Congenital heart disease

probands (5/16) with positive family history. It was noted that the high prevalence of the pathogenic variants in the study was due to the inclusion of exceptionally familial cases. Similar to that, Jia et al. (34) evaluated 36 CHD patients from 13 families by targeted NGS analysis of 57 CHD-related genes and detected potentially disease-causing variants in 46% (6/13) of the families. Also, in a study done by Pulignani et al. (35) 68 non-syndromic CHD patients (57 sporadic and 11 familial) were evaluated by next-generation sequencing of 16 candidate genes. They have detected 20 possible disease-causing variants out of 68 patients. We believe that the lower detection rate in our study, compared to previously reported ones, is due to the inclusion of both familial and syndromic cases in other studies. Also, it might differ because of the distinct variant classification criteria that are used and the different number of genes covered in targeted NGS panels.

The *NOTCH1* gene is located at the 9q34 region and consists of 34 exons. It encodes a protein that belongs to an essential intracellular signaling receptor family that has important functions such as cell proliferation, cell death, and cell fate decisions during embryogenesis (36). It was reported that during cardiogenesis, activation of the NOTCH1 receptor function in the epithelial-mesenchymal transition of the endocardial cushion development and the formation of the semilunar valves (37). It is well known that truncating variants of the *NOTCH1* gene are associated with the development of CHDs, especially BAV and left ventricular outflow tract malformations. The c.700C>T [p.(Arg234Cys)] variant was previously reported (rs567890045), but has not been associated with any phenotype. It is located at codon 234 in the extracellular EGF-like domain of the protein. Since the variant is located adjacent to the O-glycosylation site of 232 amino acids, it was predicted to alter posttranslational modification and intracellular interactions of the protein (38). c.5949C>G [p.(Asn1983Lys)] is a novel variant that was reported in the literature. The variant is present in the ankyrin repeat domain of the protein, and there have been several functional studies indicating that such variants might disrupt the proper folding of the protein.

Study Limitations

Although it was designed as a case-control study, and age-matched controls with normal echocardiography have been included, we believe that one of the limitations of the study that might have influenced the results is the limited number of patients who were involved in our study. Second, in the NGS analysis, only five autosomal dominant candidate genes, which have the strongest association, were included; therefore, future studies with a large number of genes might be more informative.

CONCLUSION

The objective of this study is to identify genetic etiology among pediatric patients with non-syndromic congenital heart defects. We believe that one of the challenges is to interpret the VUS and give proper genetic counselling. To further identify the genetic background of the CHDs, genome-wide analysis done in larger populations, might be more effective than targeted tests.

Ethics

Ethics Committee Approval: The study was approved by the Non-interventional Clinical Research Ethics Committee of Eskişehir Osmangazi University (decision number: 18, date: 25.06.2019).

Informed Consent: Written informed consent was obtained from legal representatives before collecting blood samples in accordance with the Declaration of Helsinki.

Footnotes

Authorship Contributions

Surgical and Medical Practices: S.K., T.A., E.S., B.U., Concept: S.K., B.Ö., S.A., T.A., B.U., O.Ç., Design: E.S., Supervision: E.E.G., B.D.A., Resources: B.Ö., S.A., Material: S.A., Data Collection or Processing: S.K., S.A., Analysis or Interpretation: E.E.G., Literature Search: B.D.A., S.A., Writing: T.A., E.S., Critical Review: O.Ç., B.U.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Pierpont ME, Brueckner M, Chung WK, Garg V, Lacro R V, McGuire AL, et al. Genetic basis for congenital heart disease: revisited: a scientific statement from the American Heart Association. Circulation. 2018;138: 653-711.
2. Saliba A, Figueiredo ACV, Baroneza JE, Afiune JY, Pic-Taylor A, Oliveira SF de, et al. Genetic and genomics in congenital heart disease: a clinical review. J Pediatr (Rio J). 2020; 96: 279-88.
3. Digilio MC, Marino B. What is new in genetics of congenital heart defects? Front Pediatr. 2016; 4: 120.
4. Meester JAN, Verstraeten A, Alaerts M, Schepers D, Van Laer L, Loeys BL. Overlapping but distinct roles for NOTCH receptors in human cardiovascular disease. Clin Genet. 2019; 95: 85-94.
5. Øyen N, Poulsen G, Boyd HA, Wohlfahrt J, Jensen PKA, Melbye M. Recurrence of congenital heart defects in families. Circulation. 2009; 120: 295-301.
6. Farber S, Diamond LK, Mercer RD, Sylvester RF, Wolff JA. The New England Journal of Medicine Downloaded from nejm.org at Houston Academy of Medicine on January 10, 2012. For personal use only. No other uses without permission. From the NEJM Archive. Copyright © 2010 Massachusetts Medical Society. All rights res. Nejm. 1978;299:230-4.Available from: https://pubmed.ncbi.nlm.nih.gov/36202051/
7. Shieh JTC, Bittles AH, Hudgins L. Consanguinity and the risk of congenital heart disease. Am J Med Genet Part A. 2012; 158: 1236-41.
8. Jerves T, Beaton A, Kruszka P. The genetic workup for structural congenital heart disease. Am J Med Genet Part C Semin Med Genet. 2020; 184: 178-86.
9. Nees SN, Chung WK. The genetics of isolated congenital heart disease. Am J Med Genet Part C Semin Med Genet. 2020; 184: 97-106.
10. Chung IM, Rajakumar G. Genetics of congenital heart defects: The *NKX2-5* gene, a key player. Genes (Basel). 2016; 7: 6.

11. Tomita-Mitchell A, Maslen CL, Morris CD, Garg V, Goldmuntz E. GATA4 sequence variants in patients with congenital heart disease. J Med Genet. 2007; 44: 779-83.
12. Edwards JJ, Gelb BD. Genetics of congenital heart disease. Curr Opin Cardiol. 2016
13. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015; 17: 405-24.
14. 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 :245-57.
15. Fagerberg L, Hallstrom BM, Oksvold P, Kampf C, Djureinovic D, Odeberg J, et al. Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. Mol Cell Proteomics. 2014; 13: 397-406.
16. Lalani SR, Belmont JW. Genetic basis of congenital cardiovascular malformations. Eur J Med Genet. 2014; 57: 402-13.
17. Shabana N, Shahid SU, Irfan U. Genetic contribution to congenital heart disease (CHD). Pediatr Cardiol. 2020; 41: 12-23.
18. Syrmou A, Tzetis M, Fryssira H, Kosma K, Oikonomakis V, Giannikou K, et al. Array comparative genomic hybridization as a clinical diagnostic tool in syndromic and nonsyndromic congenital heart disease. Pediatr Res. 2013; 73: 772-6.
19. Lander J, Ware SM. Copy number variation in congenital heart defects. Curr Genet Med Rep. 2014; 2: 168-78.
20. Erdogan F, Larsen LA, Zhang L, Tümer Z, Tummerup 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: 704-9.
21. Greenway SC, Pereira AC, Lin JC, DePalma SR, Israel SJ, Mesquita SM, et al. *De novo* copy number variants-tetralogy of Fallot. 2010; 41:9 31-5.
22. Breckpot J, Thienpont B, Arens Y, Tranchevent LC, Vermeesch JR, Moreau Y, et al. Challenges of interpreting copy number variation in syndromic and non-syndromic congenital heart defects. Cytogenet Genome Res. 2011; 135: 251-9.
23. Goldmuntz E, Paluru P, Glessner J, Hakonarson H, Biegel JA, White PS, et al. Microdeletions and microduplications in patients with congenital heart disease and multiple congenital anomalies. Congenit Heart Dis. 2011; 6: 592-602.
24. Tomita-Mitchell A, Mahnke DK, Struble CA, Tuffnell ME, Stamm KD, Hidestrand M, et al. Human gene copy number spectra analysis in congenital heart malformations. Physiol Genomics. 2012; 44: 518-41.
25. Kim DS, Kim JH, Burt AA, Crosslin DR, Burnham N, Kim CE, et al. Burden of potentially pathologic copy number variants is higher in children with isolated congenital heart disease and significantly impairs covariate-adjusted transplant-free survival. J Thorac Cardiovasc Surg. 2017; 151: 1147-51.e4.
26. Soemedi R, Wilson IJ, Bentham J, Darlay R, Töpf 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: 489-501.
27. Qiao F, Hu P, Xu Z. Application of next-generation sequencing for the diagnosis of fetuses with congenital heart defects. Curr Opin Obstet Gynecol. 2019; 31: 132-8.
28. Reuter MS, Chaturvedi RR, Liston E, Manshaei R, Aul RB, Bowdin S, et al. The Cardiac Genome Clinic: implementing genome sequencing in pediatric heart disease. Genet Med. 2020; 22: 1015-24.
29. Jin SC, Homsy J, Zaidi S, Lu Q, Morton S, Depalma SR, et al. Contribution of rare inherited and de novo variants in 2,871 congenital heart disease probands. Nat Genet. 2017; 49: 1593-601.
30. El Malti R, Liu H, Doray B, Thauvin C, Maltret A, Dauphin C, et al. A systematic variant screening in familial cases of congenital heart defects demonstrates the usefulness of molecular genetics in this field. Eur J Hum Genet. 2016; 24: 228-36.
31. Zhang M, Li FX, Liu XY, Hou JY, Ni SH, Wang J, et al. TBX1 loss-of-function mutation contributes to congenital conotruncal defects. Exp Ther Med. 2018; 15: 447-53.
32. Dong W, Kaymakcalan H, Jin SC, Diab NS, Tanıdır C, Yalcin ASY, et al. Mutation spectrum of congenital heart disease in a consanguineous Turkish population. Mol Genet Genomic Med. 2022; 10: 1-12.
33. Blue GM, Kirk EP, Giannoulatou E, Dunwoodie SL, Ho JWK, Hilton DCK, et al. Targeted next-generation sequencing identifies pathogenic variants in familial congenital heart disease. J Am Coll Cardiol. 2014; 64: 2498-506.
34. Jia Y, Louw JJ, Breckpot J, Callewaert B, Barrea C, Sznajer Y, et al. The diagnostic value of next generation sequencing in familial nonsyndromic congenital heart defects. Am J Med Genet Part A. 2015; 167: 1822-9.
35. Pulignani S, Vecoli C, Borghini A, Foffa I, Ait-Alì L, Andreassi MG. Targeted next-generation sequencing in patients with non-syndromic congenital heart disease. Pediatr Cardiol. 2018; 39: 682-9.
36. Macgrogan D, Luna-Zurita L, de la Pompa JL. Notch signaling in cardiac valve development and disease. Birth Defects Res A Clin Mol Teratol. 2011; 91: 449-59.
37. MacGrogan D, D'Amato G, Travisano S, Martinez-Poveda B, Luxán G, Del Monte-Nieto G, et al. Sequential ligand-dependent notch signaling activation regulates valve primordium formation and morphogenesis. Circ Res. 2016; 118: 1480-97.
38. Krassowski M, Pellegrina D, Mee MW, Fradet-Turcotte A, Bhat M, Reimand J. ActiveDriverDB: interpreting genetic variation in human and cancer genomes using post-translational modification sites and signaling networks (2021 Update). Front Cell Dev Biol. 2021; 9: 1-11.