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Clinical, demographic and genetic features of patients with congenital heart disease : A single center experience

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ABSTRACT

Objective: We aimed to evaluate the demographic and clinical characteristics of children with congenital heart disease (CHD) in a private pediatric cardiovascular genetics clinic in Istanbul from January 2016 to July 2018 and increase the awareness and emphasize the importance of genetic counseling in CHD.

Patients and Methods: One hundred and seventeen patients (50 female, 67 male) from 3 days of age to 25 years of age in 17 months period (January 2016 to July 2018) were retrospectively analyzed. Data included age, sex, echocardiography results, extracardiac features, genetic test results, consanguinity and any family member with heart disease. Pearson's chi-squared test with 1 degree of freedom and 5% significance was used for correlations.

Results: Consanguinity rate was 23.9%. Most common diagnosis was Tetralogy of Fallot (TOF) followed by atrial septal defect (ASD) and ventricular septal defect (VSD) equally. 30 patients had genetic testing which revealed a diagnosis in 36.6 % of the patients. 6 patients had DiGeorge, one had Renpenning,one had Kabuki syndrome. We had one NODAL, one MYH7 and one MYH6 variant.

Conclusion: Genetic testing in CHD has a high diagnostic yield. Genetic counseling can help diagnostic, prognostic, and therapeutic and family planning decision making.

Keywords: Congenital heart disease, Genetics, Genetic counseling

1. INTRODUCTION

The spectrum of pediatric cardiology clinic referrals in children ranges from common complaints of chest pain, palpitations, syncope, shortness of breath, to more serious congenital heart diseases (CHDs).

Congenital heart disease is one of the most common type of birth defect and occurs in 7/1000 among live births. It is the leading cause of infant and perinatal mortality from a birth defect and the most common reason for pediatric cardiology consultations [1].

Over 400 CHD genes have been discovered so far and nearly 90% of CHD cases have a suspected genetic contribution [2-4].

Congenital heart disease can occur as an isolated finding or as part of a syndrome or as a result of a teratogenic exposure. Genetic testing can help in accurately diagnosing and counseling these patients. Also, patients with CHD now reach adulthood and have an increased risk of having infants with CHD [5]. Therefore, genetic counseling can have an impact on diagnostic, prognostic, and therapeutic decision making [6]. It is also important for family planning.

In this study, we aimed to evaluate the demographic and clinical characteristics of children with CHD seen in a private pediatric cardiovascular genetics clinic in Istanbul from January 2016

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to July 2018 and increase the awareness and emphasize the importance of genetic counseling in CHD.

2. PATIENTS and METHODS

After ethical approval by Demiroglu Bilim University Ethics Commitee (approval number: 22.12.2020/ 2020-24-04) we retrospectively analyzed the clinical data of 117 patients from 3 days of age to 25 years of age in 17 months period (January 2016 to July 2018) who were referred to a private pediatric cardiovascular genetics clinic in Istanbul, Turkey for CHD. Patients records included age,sex, reason for referral, consanguinity, any previous genetic evaluation, diagnosis, family history of heart disease and other coexisting health problems. Evaluations were done by the same pediatric cardiologist and pediatric geneticist. Pediatric cardiology exam included family history and echocardiography. Pediatric genetics exam included dysmorphology exam, family history and pedigree.

Statistical Analysis

We did Pearson's chi-squared test with 1 degree of freedom and 5% significance to determine any statistically significant correlation between genetic disease prevalence determined through genetic testing and sex, consanguinity and presence of family members with additional cardiac diseases.

3. RESULTS

Among the 117 patients in our study, 28 patients (23.9%) were children of consanguinious marriages. All were first cousin marriages. None of these 117 patients had a genetic diagnosis nor had a genetic consultation before. We excluded seven patients with no congenital heart disease from the study (these patients were referred because of a murmur heard on physical exam and thought to have a CHD by their pediatricians. Echocardiography showed no structural and functional changes) and remaining 110 patients were composed of 46 female and 64 male with 10:7 ratio. The most common heart defect in our patients was Tetralogy of Fallot (TOF) followed by atrial septal defect (ASD) and ventricular septal defect (VSD) equally. Clinical and demographic characteristics of these patients are given in Table I. We offered genetic counseling to all patients with CHD and 37 of them agreed to have genetic consultation. (33.6%). Main reasons for families who did not want to have genetic consultation were; they did not believe that genetic information would add any benefit to the disease management, they are already dealing with a chronic health problem and do not want to spend time in another clinic, and they did not want to spend money on genetic tests. Attitudes of families toward genetic counseling is another research topic.

We offered karyotype analysis to a patient with aort coarctation to look for Turner syndrome and we offered fluorescence in situ hybridization (FISH) (to look for 22q11.2 deletion) to patients with conotruncal lesions with or without extra cardiac features first and if the results are normal, we offered microarray analysis. For patients with consanguineous parents, we first offered microarray analysis and for patients who could not do microarray analysis and for patients with more complex heart diseases like dextrocardia, hypoplastic left heart we offered whole exome sequencing (WES). We could only do one WES in an accredited genetic diagnosis laboratory in Istanbul and for the rest, we sent the samples to a research laboratory at Yale University Prof.Gunel laboratory. We did not have CHD genetics panel testing at our hospital, so we could not offer this test to patients before WES. Also WES has a higher yield than the panel and cost is similar, so we prefer WES over CHD panel when possible.

Table I. *Characteristics of patient population*

Condition Name	# of Patients	Sex	Consanguinity
Dilated cardiomyopathy	1	1M	1N
Brugada	1	1M	1Y
Dextrocardia	5	4M/1F	2Y/2N/1NA
Ventricular septal defect	19	11M/8F	3Y/16N
Atrial septal defect	19	9M/10F	2Y/16N/1NA
Tetralogy of Fallot	20	13M/7F	8Y/10N/2NA
Aort Coarctation	6	4M/2F	6N
Double Arcus Aorta	1	1M	1Y
Truncus Arteriosus	3	3M	3N
Interrupted aortic arch	1	1M	1N
Hypoplastic left heart	4	3M/1F	3Y/1N
Pulmonary Atresia	4	3M/1F	4N
Tricuspid anomaly	1	1F	1Y
Tricuspid atresia	1	1F	1Y
Total anomolus pulmonary venous return	1	1M	1N
Transposition of great arteries	1	1M	1N
Bicuspid Aortic Valve	4	4M	4N
Biscuspid aorta	1	1F	1N
Hypertension	2	1M/1F	2N
Noncompaction Cardiomyopathy	1	1M	1N
Transposition of great arteries	4	1M/3F	2Y/2N
Double Inlet Single Ventricle	1	1M	1N
Pulmonary stenosis	2	1M/1F	2N
Aort Regurgitation	1	1F	1N
Aort stenosis	2	1M/1F	2N
Hypoplastic right heart	1	1M	1N
Double outlet right ventricle	1	1F	1N
Atrioventricular septal defect	1	1M	1N
Right ventricle outflow tract obstruction	1	1F	1NA
Patent ductus arteriosus	2	2F	2N
Anomalous left coronary artery from the pulmonary artery	1	1M	1N
Left ventricle outflow tract obstruction	1	1F	1N
Ascending aorta hypoplasia	1	1F	1N

F: Female, M: Male, N: No, Y: Yes

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Out of 37 patients who had genetic consultations, 30 patients (24 M, 6 F) had either one or two of the following genetic tests; WES, FISH, and karyotype. 14 patients had WES (3 of these patients had negative FISH test for DiGeorge syndrome (OMIM# 188400), 18 patients had FISH for DiGeorge syndrome, and one patient had karyotype analysis. Among patients who had genetic testing, 11 of them had positive genetic test result (36.6%). Most common diagnosis was DiGeorge syndrome (22q11.2 deletion syndrome) with 6 patients affected. In addition, genetic tests revealed one patient with Renpenning syndrome (OMIM#309500), one patient with Kabuki syndrome (OMIM: 147920) with a p.Gln2004Ter stopcodon variant in *KMT2D* gene. Also exome sequencing of probands have revealed a novel stop codon variant p.Arg237X in *NODAL* gene in one patient, a missense variant p.(Thr70Ser) with uncertain significance in *MYH7* gene and a novel splice variant variant (c.1474-2A>C) in *NEXN* gene in one patient, and a missense variant p.(Met90Thr) in *MYH6* gene in another patient. (Table II).

Table II. *Characteristics of patients with genetic diagnoses*

Heart Disease	Gene	Mutation	Novelty/ClinVar Accession number	Syndrome	Age	Sex	Extra cardiac manifestation	Consanguinity	Performed Genetic Test
TGA	Nodal	p.Arg237Ter	Novel		2 years	M	N	N	WES
Hypoplastic left heart	*KMT2D*	p.Gln2004Ter	Reported (VCV000692015.2)	Kabuki	1 week	M	Anal atresia	Y	WES
TOF with pulmonary atresia	*PQBP1*	p.Arg143fs	Novel	Renpenning	17 years	M	ID, short stature, scoliosis, microcephaly	Y	WES
Hypertrophic cardiomyopathy	*MYH7*	p.Thr70Ser	Reported (VCV000042877)		9 years	M	N	Y	WES
ASD	*MYH6*	MYH6 c.1962+1G>A	Novel		17 years	M	N	Y	WES
Aort coarctation		22q11.2 deletion	Reported	DiGeorge	2 month	M	N	N	FISH
TOF		22q11.2 deletion	Reported	DiGeorge	2 weeks	M	N	Y	FISH
ASD/VSD		22q11.2 deletion	Reported	DiGeorge	4 years	M	N	N	FISH
Truncus arteriosus		22q11.2 deletion	Reported	DiGeorge	1 month	M	Anal atresia	N	FISH
TOF		22q11.2 deletion	Reported	DiGeorge	12 years	M	ID, growth delay	Y	FISH
TOF		22q11.2 deletion		DiGeorge	1 year	M	N	Y	FISH

ASD: Atrial septal defect, ID: Intellectual deficiency, M: Male, N: No, TOF: Tetralogy of Fallot, TGA: Transposition of great arteries VSD: Ventricular septal defect , Y: Yes, WES: whole exome sequencing, FISH: Fluorescence in situ hybridization

As a result of the Pearson's chi-squared tests conducted, the only statistically significant correlation observed was the prevalence of any positive genetic test result and sex with a p-value of 0.03767 where 0.05 is the threshold, nevertheless, with diseases independent from each other, this result was found to be obsolete and potentially a result of sex bias. Additionally, as the samples were too small, a conclusive statistical result cannot be obtained with high reliability.

Congenital heart diseases were tabulated against the presence of any genetic condition. However, our sample is small to merit any statistical analysis of correlation, and prevents us from reaching any statistically-significant conclusions about the genetic condition and CHD occurring simultaneously. The only exception was the occurrence of TOF and DiGeorge Syndrome simultaneously in 3 individuals, whose occurrences also revealed a statistically significant correlation of the two diseases occurring simultaneously when tested through Pearson's chi-squared test.

The heart disease occurrences were also tabulated against consanguinity. However, to be able to reach to statistical significance, the number of patients with heart disease were low in our cohort. Furthermore, 6 patients did not know and/or did not want to talk about consanguinity, reducing the data available for an already small sample, so that no further statistical analysis was conducted. It is noteworthy, however, that out of 19 individuals with ASD, 16 had no consanguinity and similarly, out of 19 individuals with VSD, 16 had no consanguinity. For

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TOF, the yes/no ratio for consanguinity was much closer to 1, as was for dextrocardia.

Extra cardiac manifestations were reported only in 15 patients. (Table II) Among those, only 8 had genetic testing and 4 had genetic diagnosis; 2 by FISH and 2 by WES. These were one patient with Renpenning syndrome, one patient with Kabuki syndrome and 2 patients with DiGeorge syndrome.

4. DISCUSSION

The consanguinity rate in Turkey is 23.5 % according to a study conducted by Hacettepe University [7]. In our cohort, consanguinity rate was similar; 23.9%. We could have found more patients with recessively inhereted variants, but unfortunately we could not do WES analysis nor CHD genetics panel to most of our patients with consanguineous parents and complex CHD because of budget restraints and negative attitude of patients towards genetic testing. In our cohort, we did not have patients with more common syndromes like Down syndrome, Turner syndrome nor Williams syndrome. The fact that we did not see any patients with more common genetic syndromes with CHD like Down, Turner or Williams syndrome may be because these patients already had a genetic diagnosis and preferred to be followed up at a public hospital pediatric cardiology clinic.

The best first line genetic assessment for most patients with CHD is a microarray analysis [8]. Next step is WES. One study showed that involving a geneticist increased the diagnosis rate of infants with CHD by 7–13%, after excluding Down syndrome [6]. TOF is the most common cyanotic CHD, that accounts for 7-10% of all CHD. In our cohort 17 % of patients had TOF.

KMT2D related Kabuki syndrome is an autosomal dominant disorder, and most cases occur de novo. It is characterized by typical facial features, infantile hypotonia, developmental delay and/or intellectual deficiency (ID) and congenital heart defects. The majority of these defects are isolated shunt lesions, conotruncal abnormalities, or various forms of arch obstruction. Hypoplastic left heart defects are seen less commonly [9] and this was the case in our patient. As targeted therapies for Kabuki syndrome are being developed, it is important to be able to make the correct diagnosis.

MYH6 can cause an autosomal dominant form of ASD and variants of the same gene in patients with hypertrophic and dilated cardiomyopathy are also reported [10]. We report a missense p.(Met90Thr) variant in a 17 year old patient with ASD. In our case patient's father had ASD and carried the same variant. If the father had a genetic diagnosis before, the family would have a genetic counseling and a more informed family planning in terms of prenatal genetic diagnosis would be made.

Our patient with hypertrophic cardiomyopathy (HC) carried a missense variant p.(Thr70Ser) with uncertain significance in *MYH7* gene and a novel splice variant variant (c.1474-2A>C) in *NEXN* gene. His father had died due to HC and never had a genetic test. HC is frequently described as a disease of the sarcomere and pathogenic variants are detected in almost all sarcomeric proteins, which are responsible for generating the molecular force of myocyte contraction. 70 % of identified variants are encoded by *MYBPC3* and *MYH7* genes [11]. *NEXN* is a filamentous actin binding protein and important in early heart development and differentiation of cardiomyocytes, and expression of contractile elements [12].

Variants in *NODAL* gene and its signaling pathways have been implicated to play a role in the pathogenetis of laterality defects. Our patient carried a heterozygous missense *NODAL* variant p.Arg237X and had transposition of great arteries (TGA) [13].

DiGeorge Syndrome also known as 22q11.2 deletion syndrome and velocardiofascial syndrome has a prevelance of 1 in 3-6000 live births [14]. Phenotype varies widely and more than 100 phenotypic features have been recorded so far. Also, most of these features may not be apparent in the neonatal period. Approximately 60-80% of patients have a cardiac malformation most commonly conotruncal defects (TOF, truncus arteriosus, interrupted aortic arch type B), conoventricular and/or ASD, and aortic arch anomalies [15]. New guidelines suggest screening for a 22q11.2 deletion in the patient with TOF, truncus arteriosus, interrupted aortic arch type B, conoventricular septal defects as well as those with an isolated aortic arch anomaly [16]. We had 3 patients with TOF out of 6. One patient had aort ic coarctation, one patient had ASD/VSD and one patient had truncus arteriosus. Early identification of a 22q11.2 deletion in the neonate or infant can be difficult when other syndromic features may not be apparent [17]. In our cohort, only 2 patients had extra cardiac features (Table III).

We had several limitations in our study. First one is this is a retrospective study and second is the small number of patients in our research. Fewer patients with congenital anomalies are treated at private hospitals than public hospitals since they require multi specialty clinics and most private hospitals have limited number of specialty clinics. Nevertheless, to the best of our knowledge, this is the first descriptive research in a private pediatric cardiovascular genetics clinic. Again, to the best of our knowledge this is the only pediatric cardiovascular genetics clinic in Turkey where pediatric cardiologist and pediatric geneticist see patients at the same time. Third limitation is that we could not do more genetic tests, especially we could not do microarray analysis which is now considered the first tier genetic testing in CHD since the patients did not want to pay for this test and most of them thought that genetic diagnosis was not necessary.

Many individuals with CHD are now of reproductive age and are at increased risk of having children with CHD and would benefit from genetic evaluation for family planning. Patients with certain types of syndromes have different survival outcomes after surgery and this is important for planning the right treatment plan as part of personalized medicine [18]. They also have different neurodevelopmental outcomes that require early and different interventions [19].

We hope that our study increases the awareness and shows the importance of genetic testing in CHD among pediatric cardiologists. Our next step is to increase our sample size by making a multicenter study and find a funding for genetic tests.

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Compliance with the Ethical Standards

Ethical Approval: Ethical approval was obtained from the Ethical Committee of Demiroglu Bilim University dated 22.12.2020 with protocol no 2020-24-04. All participant gave written informed consent.

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Author Contributions: WD and AGES: Did genetic analysis, reviewed the manuscipt, EN and LY: Performed data and statistical analysis, YY: Examined the patients, reviewed the manuscript, HK: Designed, supervised the article, examined the patients, took the consents, did literature review and wrote the manuscript.

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