

Washington University School of Medicine

Digital Commons@Becker

---

2020-Current year OA Pubs

Open Access Publications

---

6-1-2022

## Mutation spectrum of congenital heart disease in a consanguineous Turkish population

Weilai Dong  
*Yale University*

Hande Kaymakcalan  
*Demiroglu Bilim University*

Sheng Chih Jin  
*Washington University School of Medicine in St. Louis*

Nicholas S Diab  
*Yale University*

Cansaran Tanıdır  
*Mehmet Akif Ersoy Hospital*

*See next page for additional authors*

Follow this and additional works at: [https://digitalcommons.wustl.edu/oa\\_4](https://digitalcommons.wustl.edu/oa_4)



Part of the [Medicine and Health Sciences Commons](#)

Please let us know how this document benefits you.

---

### Recommended Citation

Dong, Weilai; Kaymakcalan, Hande; Jin, Sheng Chih; Diab, Nicholas S; Tanıdır, Cansaran; Yalcin, Ali Seyfi Yalim; Ercan-Sencicek, A Gulhan; Mane, Shrikant; Gunel, Murat; Lifton, Richard P; Bilguvar, Kaya; and Brueckner, Martina, "Mutation spectrum of congenital heart disease in a consanguineous Turkish population." *Molecular Genetics & Genomic Medicine*. 10, 6. e1944 (2022).  
[https://digitalcommons.wustl.edu/oa\\_4/38](https://digitalcommons.wustl.edu/oa_4/38)

This Open Access Publication is brought to you for free and open access by the Open Access Publications at Digital Commons@Becker. It has been accepted for inclusion in 2020-Current year OA Pubs by an authorized administrator of Digital Commons@Becker. For more information, please contact [vanam@wustl.edu](mailto:vanam@wustl.edu).




---

**Authors**

Weilai Dong, Hande Kaymakcalan, Sheng Chih Jin, Nicholas S Diab, Cansaran Tanidir, Ali Seyfi Yalim Yalcin, A Gulhan Ercan-Sencicek, Shrikant Mane, Murat Gunel, Richard P Lifton, Kaya Bilguvar, and Martina Brueckner

## ORIGINAL ARTICLE

# Mutation spectrum of congenital heart disease in a consanguineous Turkish population

Weilai Dong<sup>1,2</sup> | Hande Kaymakcalan<sup>3</sup>  | Sheng Chih Jin<sup>4</sup>  | Nicholas S. Diab<sup>1</sup> | Cansaran Tanidir<sup>5</sup> | Ali Seyfi Yalim Yalcin<sup>3</sup> | A. Gulhan Ercan-Sencicek<sup>1,6</sup>  | Shrikant Mane<sup>1</sup> | Murat Gunel<sup>1</sup> | Richard P. Lifton<sup>2</sup> | Kaya Bilguvar<sup>1,7</sup> | Martina Brueckner<sup>1</sup>

<sup>1</sup>Department of Genetics, Yale School of Medicine, New Haven, Connecticut, USA

<sup>2</sup>Laboratory of Human Genetics and Genomics, The Rockefeller University, New York, New York, USA

<sup>3</sup>Department of Pediatrics, Demiroglu Bilim University, Istanbul, Turkey

<sup>4</sup>Department of Genetics, Washington University School of Medicine, St. Louis, Missouri, USA

<sup>5</sup>Department of Pediatrics, Mehmet Akif Ersoy Hospital, Istanbul, Turkey

<sup>6</sup>Biomedical research and translational medicine, Masonic Medical Research Institute, Utica, New York, USA

<sup>7</sup>Department of Genetics, Yale Center for Genomic Analysis, New Haven, Connecticut, USA

## Correspondence

Hande Kaymakcalan, Demiroglu Bilim University, Istanbul, Turkey.  
Email: [doctorhande@yahoo.com](mailto:doctorhande@yahoo.com)

Martina Brueckner, Department of Genetics, Yale School of Medicine, New Haven, CT, USA.  
Email: [martina.brueckner@yale.edu](mailto:martina.brueckner@yale.edu)

## Funding information

This work is supported by The Yale Center for Mendelian Genomics (UM1HG006504) which is funded by the National Human Genome Research Institute and National Heart, Lung, and Blood Institute. The GSP Coordinating Center (U24 HG008956) contributed to cross-program scientific initiatives and provided logistical and general study coordination. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. W.D. is supported by the American Heart Association Predoctoral Fellowship (19PRE34380842)

## Abstract

**Backgrounds:** While many studies agree that consanguinity increases the rate of congenital heart disease (CHD), few genome analyses have been conducted with consanguineous CHD cohorts.

**Methods:** We recruited 73 CHD probands from consanguineous families in Turkey and used whole-exome sequencing (WES) to identify genetic lesions in these patients.

**Results:** On average, each patient had 6.95 rare damaging homozygous variants, 0.68 of which are loss-of-function (LoF) variants. Seven patients (9.6%) carried damaging homozygous variants in five causal CHD genes. Six of those patients exhibited laterality defects (six HTX and one D-TGA). Three additional patients (4.1%) harbored other types of CHD-associated genomic alterations, which overall explained 13.7% (10/73) of the cohort. The contribution from recessive variants in our cohort is higher than 1.8% reported from a cohort of 2871 CHD subjects where 5.6% of subjects met the criteria for consanguinity.

**Conclusions:** Our WES screen of a Turkish consanguineous population with structural CHD revealed its unique genetic architecture. Six of seven damaging homozygous variants in CHD causal genes occur in the setting of laterality defects implies a strong contribution from consanguinity to these defects specifically. Our

Weilai Dong, Hande Kaymakcalan, Sheng Chih Jin, and Nicholas S. Diab contributed equally to this work.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2022 The Authors. *Molecular Genetics & Genomic Medicine* published by Wiley Periodicals LLC.

study thus provided valuable information about the genetic landscape of CHD in consanguineous families in Turkey.

#### KEYWORDS

congenital heart disease, consanguinity, genetics, mutation

## 1 | INTRODUCTION

Congenital heart disease (CHD) is the most frequent birth defect among live births and the leading cause of infant and perinatal mortality from a birth defect (van der Linde et al., 2011). Dr. John Maurice Hardman Campbell published the first paper on CHD genetics in 1949, and since then over 600 CHD genes have been discovered (Blue et al., 2017; Campbell, 1949; Gelb, 2015). Today, about 34% of CHD cases can be explained by genetic risk factors, such as aneuploidy, copy number variations (CNVs), and de novo and transmitted variants, but in 56% of cases the nature of that genetic contribution is unknown (Jin et al., 2017; Zaidi & Brueckner, 2017).

The journey toward a genetic understanding of CHD began in 19th century London, where Dr. Thomas Beville Peacock suggested that families with multiple siblings affected by CHD were afflicted by a “hereditary predisposition” to defective cardiac development (Gelb, 2015). The 20th century pathologist Maude Abbott took the case for a hereditary component to CHD further when she noted that consanguineous families experienced an elevated frequency of CHD (Gelb, 2015).

Abbott’s observations on consanguineous families with CHD foreshadowed the importance that studies on these families would have in furthering knowledge on CHD genetics. The majority of consanguineous CHD studies have taken place in Middle Eastern countries or India, dating back to the 1980s. One such study in Northern Israel found that children of consanguineous marriages were more than twice as likely to have CHD, while children of first-cousin marriages were 2.5 times more likely to have CHD compared to children of unrelated parents (Gev et al., 1986). Another study, performed by Badaruddoza and colleagues on North Indian Muslims, found that consanguineous progeny experienced CHD 2.76 times more often than non-consanguineous progeny (Badaruddoza et al., 1994; Bassili et al., 2000).

While many studies agree that consanguinity boosts the rate of CHD, there is considerable debate on which cardiac lesions are most likely to be affected by this boost. There is mixed evidence for a positive correlation between consanguinity and ventricular septal defects (VSDs); most studies agree that consanguinity increases the likelihood

of both VSDs and atrial septal defects (ASDs), but a few studies have failed to find evidence of a positive correlation between consanguinity and VSD (Badaruddoza et al., 1994; Bassili et al., 2000; Becker et al., 2001; Chehab et al., 2007; Nabulsi et al., 2003). Both positive and negative correlations are reported between consanguinity and patent ductus arteriosus, atrioventricular septal defects, and Tetralogy of Fallot (TOF) (Bassili et al., 2000; Becker et al., 2001; Bittles, 2011; Chehab et al., 2007; Nabulsi et al., 2003; Roguin et al., 1995; Yunis et al., 2006). Lack of uniformity in study design, variable diagnostic criteria, and other potential confounders complicates the interpretation of CHD studies on consanguineous cohorts and may account for the variable relationships reported between consanguinity and specific CHD lesions (Bittles, 2011; Bittles & Black, 2010).

18.5% of marriages occurring in Turkey according to a study between October and December 2013 conducted by the ministry of health are reported as consanguineous (Kaplan et al., 2016). Additionally, 57.5% of consanguineous marriages in Turkey are between first cousins (Kaplan et al., 2016). First-cousin unions almost double the probability of childhood or neonatal death, increase the likelihood of spontaneous abortions and intellectual disabilities (Kaplan et al., 2016). Indeed, 37.3% of women in first-cousin marriages reported having a spontaneous abortion compared to 24.1% of non-consanguineously married women (Kaplan et al., 2016). The Turkish ministry of health questioned 4608 married women (who had children) about whether one or more of their children had a congenital abnormality. 2.0% of women in non-consanguineous marriages reported yes, compared to 5.1% of women married to their first cousin and 4.2% married to their second cousin or a more distant relative, suggesting that consanguinity increases the risk of a congenital anomaly in Turkey (Kaplan et al., 2016).

While the effect of consanguinity on the incidence of CHD has been described, few gene discovery efforts have been made with consanguineous CHD cohorts. To this end, we recruited a cohort of 73 CHD patients from consanguineous families in Turkey and used whole-exome sequencing (WES) to identify the underlying genetic contribution. In 13.7% of our cohort, a variant was identified in previously established gene mutations associated

with CHD. This represents an important advance in CHD genetics and in our understanding of the genetic consequences of consanguinity.

## 2 | MATERIALS AND METHODS

### 2.1 | Patients

Seventy-four patients from 73 unique families were recruited at two centers in İstanbul, Turkey (Mehmet Akif Ersoy and Florence Nightingale Hospitals Pediatric Cardiology Departments) for congenital cardiac problems. The approval was taken from Demiroglu Bilim University Clinical Research Ethics Committee, number 16.06.2015/32–282 and informed written consent forms were obtained from all involved subjects by their referring physician.

The patients have either isolated or syndromic CHD. Their cardiac phenotypes were classified into five major categories as described before<sup>5</sup>, including heterotaxy (HTX,  $n = 12$ ), D-transposition of the great arteries (D-TGA,  $n = 2$ ), non-TGA conotruncal defect (CTD,  $n = 22$ ), left ventricular outflow tract obstruction (LVO,  $n = 12$ ), and other ( $n = 25$ ) (Table 1).

### 2.2 | Whole-exome sequencing

Peripheral blood samples were collected from affected individuals. Genomic DNA was extracted using standard protocol of Gentra Puregene Blood Kit (Qiagen).

**TABLE 1** Clinical characteristics of 73 consanguineous CHD patients

Variables	Statistics
Inbreeding coefficient	0.0621 ± 0.0359
Longest HBD segment	37.5 ± 15.2
Male (%)	34 (46.6%)
Female (%)	39 (53.4%)
<b>Distribution of different subtypes of CHD</b>	
HTX	12 (16.4%)
D-TGA	2 (2.7%)
CTD	22 (30.1%)
LVO	12 (16.4%)
Other	25 (34.2%)
Total	73

Abbreviations: HTX, heterotaxy; D-TGA, D-transposition of great artery; CTD, conotruncal defect; LVO, left outflow track obstruction.

Genomic DNA was captured by either Nimblegen v.2 exome capture reagent, Nimblegen SeqxCap EZ MedExome Target Enrichment Kit, or IDT xGen target capture kit, followed by paired-end sequencing on Illumina HiSeq2500 or NovaSeq6000 platforms as described before<sup>5</sup> (Supplementary Table S1); all experiments were performed at the Yale Center for Genome Analysis. The sequence reads were mapped onto the GRCh37/hg19 reference genome using Burrow-Wheeler Aligner-MEM (BWA-MEM) software (H. Li & Durbin, 2009). Single nucleotide changes and small insertions/deletions (indels) were called following GATK Best Practices workflow (Van der Auwera et al., 2013) and annotated with ANNOVAR (Wang et al., 2010) for population minor allele frequencies in public databases including 1000 Genomes (August 2015) (Auton et al., 2015), NHLBI Exome Variant Server (EVS) (Server, 2014), and ExAC (v3) (Lek et al., 2016). The MetaSVM and the Combined Annotation Dependent Deletion (CADD v1.3) (Dong et al., 2015; Kircher et al., 2014).

### 2.3 | Variant filtering

Only high confident variants with a quality score recalibration (VQSR) “PASS,” read depth (DP) ≥ 8, genotype quality (GQ) score ≥ 20 were kept for the analysis. False positives were excluded by in silico visualization using Integrative Genomics Viewer (Robinson et al., 2011) and BLAT search.

For recessive variants, we filtered for homozygous and compound heterozygous variants that are rare (minor allele frequency [MAF] ≤ 10<sup>-3</sup> in 1000 Genomes, EVS, and ExAC databases) and have damaging effect on protein structure. Only damaging variants, including loss-of-function variants (LoF; canonical splice site, frameshift insertion/deletion, stop-gain, and stop-loss), damaging missense variants (D-Mis; missense variants predicted as deleterious by MetaSVM or with a CADD score at least 20), and non-frameshift indel variants were kept. For compound heterozygous variants, specifically, we only considered those with at least one LoF allele.

For hemizygous and heterozygous variants, we kept extremely rare (MAF ≤ 1 × 10<sup>-5</sup>) LoF variants.

### 2.4 | Copy number analysis

CNVs were called using the XHMM software (Fromer & Purcell, 2014). Briefly, GATK Depth Of Coverage was used to calculate mean read coverage from the aligned file. The output data were normalized by removing the variance component with variance > 70% and the z-score was

calculated. Then, the hidden Markov-based model called CNVs and calculated the quality scores. Only high quality CNVs (quality score  $\geq 90$ ) were kept for the analysis. CNVs were further annotated with frequencies in gnomAD v2.1 (Gudmundsson et al., 2021) controls-only samples when they have at least 50% of overlapping base pairs and only rare CNVs with frequency  $\leq 1 \times 10^{-3}$  were kept.

## 2.5 | Kinship analysis

Relatedness of the samples was investigated using the pairwise identity-by-descent (IBD) calculation in PLINK1.9 (Purcell et al., 2007). If the IBD sharing between a pair of samples is  $\geq 20\%$ , the sample that was sequenced using the latest capture reagent and with greater sequence coverage was kept in the analysis while the other was removed.

## 2.6 | Inbreeding coefficient calculation

Beagle v3.3.2 (Browning & Browning, 2007) was used to estimate inbreeding coefficients and the longest homozygosity-by-descent (HBD) fragments. Consanguinity was defined as (1) the longest HBD fragment is equal to or longer than 4 cM and (2) at least 0.35% of the genome being covered by HBD fragments  $\geq 2$  cM.

# 3 | RESULTS

## 3.1 | Cohort characteristics

We studied 74 consanguineous Turkish structural CHD cases from 73 families, including 72 singletons and one sibling pair. 46.6% of cases are males and 54.4% are females. HBD analysis suggested that all of them are truly consanguineous based on our criteria (Material and Methods). Our cohort's average inbreeding coefficient is  $0.0621 \pm 0.0359$ , which approximates the first-cousin marriage, and has a longest HBD segment of  $37.3 \pm 15.2$  cM. The cohort covers a broad phenotypic spectrum, including 12 (16.4%) HTX, 2 (2.7%) D-TGA, 22 (30.1%) CTD, 12 (16.4%) LVO, and 25 (34.2%) other (Table 1).

## 3.2 | Homozygous variants in CHD-related genes

Through WES analysis, 507 high-confidence homozygous damaging variants (LoF, D-Mis, or non-frameshift indels) were called from 73 unrelated CHD patients. Each patient carried on average 6.95 damaging homozygous

variants. Twelve of these damaging homozygous variants fall within known CHD genes. Notably, 7 of the 12 variant carriers had laterality defects (HTX or D-TGA). We examined the mutation enrichment in known CHD gene sets using a one-tailed binomial test in which the expectation was estimated by fitting a polynomial regression model as described previously (Jin et al., 2017). Even though no significant enrichment was seen when considering all 73 patients, significant enrichments for damaging variants ( $P = 2.27 \times 10^{-3}$ , Enrichment = 3.86) were observed in 14 patients with laterality defect (Table 2).

To define the potential causal variants and estimate what percent of the cohort can be explained by damaging homozygous variants in known CHD genes (Jin et al., 2017), we inspected patient phenotypes in OMIM Clinical Synopsis and published reports. We only considered the variants with a genotype-phenotype concordance. This resulted in seven likely causal variants from five known CHD genes: *MMP21*, *BBS1*, *PKD1*, *CCDC40*, and *CACNA1C* (Table 3).

*MMP21*, encoding Matrix Metalloproteinase 21, is the only gene that harbored more than one damaging homozygous variant. *MMP21* is known to cause visceral heterotaxy (OMIM #616749) in an autosomal recessive pattern and its biallelic mutations were most frequently reported in Middle East consanguineous families (Guimier et al., 2015; Perles et al., 2015). In our cohort, three rare homozygous mutations were identified in *MMP21*, including p.Gln499X carried by NG2607-1, p.Cys117X carried by NG2702-1, and p.Leu104Pro shared by a pair of siblings (NG2697-1 and NG2697-3). All three mutations are novel in ExAC and have not been previously reported. Both p.Leu104Pro and p.Cys117X were located in the autoinhibitory propeptide domain. p.Cys117X disrupted the cysteine switch motif that binds the inhibitory catalytic zinc ion. p.Gln499X was mapped to the metalloproteinase domain between two C-terminal hemopexin repeats, and thus may disrupt the hemopexin structure and affect the substrate specificity (Marchenko et al., 2003) (Supplementary Figure S1). All three patients exhibited features of laterality defects (defects of embryonic left-right axis patterning), such as situs inversus, dextrocardia, right atrial isomerism (RAI), L-TGA, and double-outlet right ventricle (DORV), which are consistent with reported phenotypes resulting from *MMP21* mutations (Akawi et al., 2015; Guimier et al., 2015; Perles et al., 2015).

Additionally, four subjects carried homozygous damaging variants in *BBS1*, *PKD1*, *CCDC40*, or *CACNA1C*. NG2608-1 carried a splice site mutation c.48-1G > A in *BBS1* which is associated with Bardet-Biedl syndrome 1 featuring developmental defects in eyes, limb, heart, and reproductive system (S. A. Khan et al., 2016). Laterality defects including dextrocardia and situs



TABLE 2 Significant enrichment of damaging homozygous variants in laterality defect patients

Gene set (# genes)	Observed		Expected		p-value
	# homo	# unique genes	# homo	Enrich	
<b>All 73 patients</b>					
All genes (19,347)	507	490	-	-	-
Recessive Known Human (96)	5	5	4	1,25	0,37
Recessive Known Mouse or Human (137)	8	6	5,83	1,37	0,23
Known Mouse or Human (255)	13	11	10,79	1,2	0,29
<b>Fourteen patients with laterality defect (HTX or D-TGA)</b>					
All genes (19,347)	71	68	-	-	-
Recessive Known Human (96)	3	3	0,59	5,13	0,02
Recessive Known Mouse or Human (137)	6	4	0,9	6,63	<b>3,02E-04</b>
Known Mouse or Human (255)	7	5	1,81	3,86	<b>2,27E-03</b>

inversus were also reported in *BBS1* mutant patients (I. Khan et al., 2015). NG2608-1 exhibited heterotaxy features such as left atrial isomerism and DORV, as well as polydactyly and strabismus which are typically reported in Bardet–Biedl syndrome patients. NG3194-1 harbored a homozygous missense variant p.Val3297Met in *PKD1*, which encodes the polycystin-1 and is associated with autosomal dominant adult polycystic kidney disease (OMIM# 173900). *Pkd1*<sup>del17–21betageo</sup> homozygous knock-out mice manifested DORV, disorganized myocardium, ASD and VSD, and died at E13.5–14.5 (Boulter et al., 2001). The patient exhibited VSD, pulmonary stenosis, patent foramen ovale (PFO), and D-TGA, which partially overlapped with the mouse phenotypes. The patient did not show renal dysfunction, which may be a result of late onset or varied expressivity of recessive *PKD1* variant. NG2781-1 harbored a damaging missense mutation p.Leu778Pro in *CCDC40* which is associated with biallelic ciliary dyskinesia. NG2781 exhibited RAI and DORV which are consistent with ciliary dyskinesia cardiac presentation (Antony et al., 2013; Becker-Heck et al., 2011). NG3283-1 carried a nonframeshift deletion (c.5261\_5281del; p.1754\_1761del) in *CACNA1C*, which is related to Timothy syndrome. Though NG3283-1 did not present with arrhythmia or extracardiac features of Timothy syndrome, he exhibited TOF and PFO which were previously reported in Timothy syndrome patients (Splawski et al., 2004).

In summary, 7 of 73 (9.6%) patients in the cohort can be explained by damaging homozygous mutations in the known CHD genes. Notably, six of the seven patients exhibited laterality defect (six HTX and one D-TGA). Thus, 6 of 14 (42.9%) of laterality defect patients can be accounted for by damaging homozygous variants in known CHD genes. In addition, we identified five damaging homozygous variants in known CHD genes including *EFTUD2*, *COL1A1*, *DOCK6*, and *FLNA*, but the patients either

lacked sufficient phenotypic information or did not possess phenotypic features of the syndromes caused by mutations in those genes.

### 3.3 | Other CHD-related genomic alterations

We also examined compound heterozygous, heterozygous, and hemizygous variants, as well as CNVs (Table 4).

We identified one potential compound heterozygous genotype in *DNAH11* (p.G4443fs/p.T210N) in patient NG2959-1 with heterotaxy. *DNAH11* encodes Dynein Axonemal Heavy Chain 11 and is an OMIM gene for autosomal recessive ciliary dyskinesia (OMIM:611884), with which situs inversus was observed in approximately half of the patients (El Zein et al., 2003). Analysis of hemizygous X-linked variants also revealed one rare LoF variant c.427\_430del (NM\_001032383: p.Arg143fs) in *PQBPI*. *PQBPI*, encoding Polyglutamine Binding Protein 1, leads to Renpenning syndrome (OMIM: 309500) which is associated with intellectual disability, dysmorphic features, short stature, and cardiac defects such as TOF, ASD, VSD, and situs inversus. Our patient (NG3016-1) exhibited TOF and other clinical features of Renpenning syndrome.

Additionally, we have called CNVs from all 74 families using the XHMM pipeline (Zhao et al., 2020). One hundred and eighty-three CNVs were identified from 41 probands, including 15 with CTD (nine are TOF), five with LVO, one with HTX, one with D-TGA, and 15 with other phenotypes. Notably, 90.0% (9 of 10) of TOF patients in the cohort carried CNVs while only 13.3% (2 of 15) of patients with laterality defects. Ninety-six CNVs are rare (MAF  $\leq 1 \times 10^{-3}$ ) in gnomAD and 1000 Genomes data bases. We classified CNVs following the ACMG guideline along with ClassifyCNV (Gurbich & Ilinsky, 2020; Riggs et al., 2020) and identified one as pathogenic, 116 of unknown

TABLE 3 Genotype and phenotype information for carriers of damaging homozygous variants in CHD-related genes

ID	Cardiac phenotypes [other phenotypes]	Extracardiac phenotypes	Phenotype classification	Gene	Genotypes	OMIM inheritance
NG2702-1	SITUS INVERSUS+DEXTROCARDIA+CCTGA	None	HTX	<i>MMP21</i>	Homo	AR
NG2697-1	RAI/CAVSD/DORV	None	HTX	<i>MMP21</i>	Homo	AR
NG2697-3	Heterotaxy	None	HTX	<i>MMP21</i>	Homo	AR
NG2607-1	Situs inversus/Dextrocardia/Tricuspid atresia/PA	None	HTX	<i>MMP21</i>	Homo	AR
NG2608-1	CASD/DORV/PS/LAI/	polydactyly/ strabismus, problem with night vision	HTX	<i>BBS1</i>	Homo	AD/AR
NG3283-1	Tetralogy of Fallot, PFO	None	CTD	<i>CACNA1C</i>	Homo	AD
NG2781-1	RAI/CAVSD/DORV/PS/TAPVD	None	HTX	<i>CCDC40</i>	Homo	AR
NG3194-1	D-Transposition of great arteries, Ventricular septal defect, Pulmonary stenosis, Patent foramen ovale	Growth delay. Height and weight were below 3%.	D-TGA	<i>PKD1</i>	Homo	AD

significance, and 66 as benign. The pathogenic CNV is a 22q11.21 deletion (chr22:18893887–21,386,101) in patient NG3207-1, who presented with TOF encompassing *TBX1*, which was previously associated with DiGeorge syndrome and TOF (Gao et al., 2015).

In addition to the three high-confidence causal alterations mentioned above, we found additional CNVs that may confer CHD risk (Supplementary Table S2). We observed one less rare recurrent 22q11.21 duplication (chr22:18893887–18,923,800) in three probands. The duplication has a MAF of 1.1% in gnomAD and has never been observed in 1000 Genomes. It does not cover *TBX1* but includes *DGCR6* and *PRODH*, the microduplications of which have previously been reported in conotruncal defect cases (Gao et al., 2015). Two of the probands exhibited TOF while the other one showed mid-muscular VSD. One proband (NG3016-1) also possessed a LoF variant in *PQBPI*, which is likely responsible for his Renpenning syndrome. Considering the relatively common occurrence and variable expressiveness of 22q11 CNVs (Zhao

et al., 2020), the recurrent 22q11.21 duplication might serve as the modifier to the phenotype.

The other potentially causal CNVs include a 15q11.2 duplication in NG3272-1 with complete AVSD. The overlapping duplications were identified in three cases with either ASD or VSD from the DECIPHER database (Firth et al., 2009).

In addition, we also found two CNVs spanning known CHD genes, including one 115kp deletion encompassing *C1orf127* from a TOF patient and one 1 Mb duplication covering the galactosyltransferase gene *B3GALT6* in a patient with hypoplastic left heart syndrome. *C1orf127* knockout mice presented laterality defect (Y. Li et al., 2015). *B3GALT6* mutation is associated with cardiac and joint defect. Patients with biallelic mutation presented atrial septal defect, mitral valve prolapse, ventricular septal defect, bicuspid aortic valve, etc. (Ritelli et al., 2019).

Based on the allele fractions of mutations on the CNV regions, all the candidate CNVs mentioned above are likely single copy deletion or heterozygous duplication,



TABLE 3 (Continued)

OMIM diseases: Cardiac phenotypes [other phenotypes matching our patients]	Transcript	cDNA change	Amino acid change	Class	ExAC	MetaSVM	CADD
Visceral heterotaxy (OMIM:616749): Dextrocardia, Transposition of the great arteries, Pulmonary atresia, Bilateral superior vena cava, Aortic arch abnormalities, Septal defects, Atrioventricular canal defect, Total anomalous pulmonary venous return, Pulmonary trunk defects, Valvular stenosis	NM_147191	c.C351A	p.Cys117X	Stop-gain	Novel	NA	34
	NM_147191	c.T311C	p.Leu104Pro	D-Mis	Novel	T	24.1
	NM_147191	c.T311C	p.Leu104Pro	D-Mis	Novel	T	24.1
	NM_147191	c.C1495T	p.Gln499X	Stop-gain	Novel	NA	36
Bardet–Biedl syndrome 1 (OMIM:209900): hypertrophy of left ventricle, dilated cardiomyopathy ASD, BAV, PS, AV canal, dextrocardia, situs inversus, [polydactyly], [strabismus]	NM_024649	c.48-1G > A	N/A	Splicing	8.24E-06	NA	23.9
Timothy syndrome (OMIM:601005): Cardiac arrhythmias, Long QT interval, Ventricular tachyarrhythmia, Bradycardia, atrioventricular block, PFO, VSD, TOF, Cardiomegaly, PDA, Pulmonary hypertension	NM_199460	c.5261_5281del	p.1754_1761del	Nonframeshift deletion	Novel	NA	NA
Ciliary dyskinesia (OMIM:613808): heterotaxy, situs inversus	NM_017950	c.T2333C	p.Leu778Pro	D-Mis	Novel	T	25
Polycystic kidney disease 1 (OMIM:173900): valvular disease, intracranial aneurysm, left ventricular hypertension, early onset hypertension, aortic dissection/aneurysm	NM_001009944	c.G9889A	p.Val3297Met	D-Mis	2.00E-04	T	23.5

respectively, and thus were not resulted from regions of homozygosities.

## 4 | DISCUSSION

This study represents the first whole-exome screen of a Turkish consanguineous cohort with structural CHD and reveals its unique genetic architecture. Our data show that 7 of 73 (9.6%) patients carried damaging homozygous variants in known CHD genes and 3 of 73 (4.1%) of them harbored other types of CHD-related genomic alterations, which overall accounts for 13.7% of the cohort. As expected, the contribution from recessive variants (8/73, 11.0%) is greater than previously reported 1.8% from a cohort of 2871 CHD subjects with lower consanguinity (5.6% of the subjects are consanguineous) (Jin et al., 2017).

Specifically, we found three rare damaging homozygous variants in *MMP21* causing heterotaxy. *MMP21* biallelic variants were most commonly reported in the

consanguineous Middle East populations leading to heterotaxy. The previously published cases with available ethnicity information contained one Arabian, three Turkish, two North African, two European, one Hispanic, and one Caribbean individuals (Guimier et al., 2015; Perles et al., 2015). Out of the 10 cases, the six Middle East cases all harbored homozygous damaging mutations resulting from consanguineous marriage. Considering the previous reports that Ashkenazi Jewish founder mutation in *GDF1* led to heterotaxy (Jin et al., 2017) and Lebanon founder mutation in *BBS* gene families caused Bardet–Biedl syndrome (Zlotogora, 2007), the *MMP21* homozygous mutations observed in our cohort might also be population specific. Future studies are needed to investigate whether ethnicity background may alter the susceptibility to *MMP21* biallelic mutations.

Fifty percent (7 of 14) of laterality defect (HTX and D-TGA) patients can be explained by detected genomic alterations, while the ratio stays low at only 6.8% (4 of 59) for non-laterality defect patients. Six of seven events

TABLE 4 Genotype and phenotype information for carriers of other CHD-related genomic alterations

ID	Cardiac phenotypes	Phenotype classification	Gene	Genotype	OMIM inheritance	OMIM diseases: Cardiac phenotypes	cDNA change	Amino acid change	Class	ExAC	MetaSVM	CADD
NG2959-1	Heterotaxy	HTX	<i>DNAH11</i>	Potential compound heterozygote	AR	Ciliary dyskinesia (OMIM:611884); situs inversus	c.13327_13328insGAACCAT/c. C629A	p.Glu4443fs/p. Thr210Asn	Frameshift insertion/ D-Mis	Novel/ Novel	NA/T	NA/27.7
NG3016-1	Tetralogy of Fallot, DiGeorge negative	CTD	<i>PQBPI</i>	Hemizygote	XLR	Renpenning syndrome (OMIM:309500); CHD, TOF, ASD, VSD, Situs inversus	c.427_430del	p.Arg143fs	Frameshift deletion	Novel	NA	NA
NG3207-1	Tetralogy of fallot	CTD	<i>TBX1</i>	22q11.21 one copy deletion	AD	DiGeorge syndrome (OMIM:188400); TOF, Truncus arteriosus, Interrupted aortic arch, Right aortic arch, VSD, PDA; Conotruncal anomaly face syndrome (OMIM:217095), Tetralogy of Fallot (OMIM:187500), Velocardiofacial syndrome (OMIM:192430); VSD, TOF, Right aortic arch, Aberrant left subclavian, Internal carotid artery abnormalities	/	/	/	/	/	/

detected for laterality defects resulted from damaging homozygous variants, suggesting a strong contribution from consanguinity to laterality defect specifically. Although few studies have examined the association of consanguinity and laterality defects as they are relatively rare, a higher incidence of heterotaxy was reported in consanguineous Lebanese and Asian Muslim populations compared to low consanguinity controls (Chehab et al., 2007; Gatrad et al., 1984). These results, together with our findings, support the hypothesis that high consanguinity is more likely to be associated with recessive cardiac abnormalities. Additionally, our findings fit the general perception that severe cardiac defects, such as laterality defects, have higher heritability than milder malformations such as ASD and VSD. Based on Jin et al., 2017, even in the apparently non-consanguineous cohort of CHD, recessive genotypes are enriched (especially genes of laterality defects) along with the possibility of cryptic or overt parental consanguinity (Jin et al., 2017).

On the contrary, other genomic alterations including hemizygous variants and CNVs are more commonly found in patients with conotruncal defect, especially TOF. This might be because CTD cases generally adopt a dominant inheritance pattern and TOF is less associated with homozygous variants resulting from consanguinity (Yunis et al., 2006), but future analysis is needed to compare CNVs in TOF patients from consanguineous versus non-consanguineous cases.

It is possible that CNVs, alongside SNPs and indels play a role in the inherited disease risk burden in consanguineous populations (Fakhro et al., 2015). Although CNVs are most commonly results of de novo mutations and appear in the heterozygous state (Inoue & Lupski, 2002), in consanguineous populations they may appear in the homozygous state and may affect disease gene dosage that are transmitted with Mendelian inheritance; therefore, for counseling and family planning, we should also check parents for same CNVs.

We expect to see less X linked disorders in consanguineous families (Monies et al., 2019), but it can occur in consanguineous families as in our case with Renpenning syndrome. Keeping and maintaining good family record is important because it can guide us in finding the inheritance pattern.

The majority of the cases (86.3%) remained unexplained by an identified genetic factor. First, our analysis only focused on variants in the exonic regions and copy number changes. It is possible that noncoding variants with regulatory effect and complex structural variations may contribute to disease pathogenesis. Second, we adopted stringent filtering criteria to prioritize the rare damaging variants which are more likely to contribute to

disease. However, common and less frequent damaging variants may also exert the effect leading to CHD through modifying disease penetrance or expressivity. Third, the cohort size of the current study is still very small, which limits the power to identify novel, recurrent risk genes. Lastly, nongenetic factors including maternal illnesses and lifestyles may also play an essential role but were not considered in this study.

In conclusion, our study systematically examined a Turkish consanguineous cohort for the contribution of exonic genetic factors in the pathogenesis of CHD. The results revealed a greater contribution from recessive variants compared to the other studies with lower consanguinity (Jin et al., 2017). Such contribution is more significant in patients with laterality defects. Our data provide a more complete understanding of genetic architectures in consanguineous CHD patients in Turkish and will help implement better genetic counseling and disease preventions in consanguineous unions.

## ACKNOWLEDGMENTS

None.

## CONFLICT OF INTEREST

The authors have no competing interest.

## AUTHOR CONTRIBUTIONS


W.D, S.J, and N.D did genetic analysis, performed data, and statistical analysis, did literature review and wrote the manuscript. H.K examined the patients, took the consents, collected peripheral blood sample, did literature review and wrote the manuscript. C.T and A.Y made the cardiac diagnosis and reviewed the manuscript, A.G.E.S performed genetic analysis and reviewed the manuscript, S.M, M.G, R.L, K.B, and M.B designed, supervised, assisted in analyzing the genetic data, and reviewed the manuscript.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## ORCID

Hande Kaymakcalan  <https://orcid.org/0000-0001-7736-7634>

Sheng Chih Jin  <https://orcid.org/0000-0002-5777-7262>

A. Gulhan Ercan-Sencicek  <https://orcid.org/0000-0003-2838-3341>

## REFERENCES

- Akawi, N., McRae, J., Ansari, M., Balasubramanian, M., Blyth, M., Brady, A. F., Clayton, S., Cole, T., Deshpande, C., Fitzgerald, T. W.,

- Foulds, N., Francis, R., Gabriel, G., Gerety, S. S., Goodship, J., Hobson, E., Jones, W. D., Joss, S., King, D., ... Hurles, M. E. (2015). Discovery of four recessive developmental disorders using probabilistic genotype and phenotype matching among 4,125 families. *Nature Genetics*, *47*(11), 1363–1369.
- Antony, D., Becker-Heck, A., Zariwala, M. A., Schmidts, M., Onoufriadis, A., Forouhan, M., Wilson, R., Taylor-Cox, T., Dewar, A., Jackson, C., Goggin, P., Loges, N. T., Olbrich, H., Jaspers, M., Jorissen, M., Leigh, M. W., Wolf, W. E., Daniels, M. L., Noone, P. G., ... Mitchison, H. M. (2013). Mutations in CCDC39 and CCDC40 are the major cause of primary ciliary dyskinesia with axonemal disorganization and absent inner dynein arms. *Human Mutation*, *34*(3), 462–472. <https://doi.org/10.1002/humu.22261>
- Auton, A., Brooks, L. D., Durbin, R. M., Garrison, E. P., Kang, H. M., Korbel, J. O., Marchini, J. L., McCarthy, S., McVean, G. A., & Abecasis, G. R. (2015). A global reference for human genetic variation. *Nature*, *526*(7571), 68–74. <https://doi.org/10.1038/nature15393>
- Badaruddoza, Afzal, M., & Akhtaruzzaman. (1994). Inbreeding and congenital heart diseases in a north Indian population. *Clinical Genetics*, *45*(6), 288–291. <https://doi.org/10.1111/j.1399-0004.1994.tb04032.x>
- Bassili, A., Mokhtar, S. A., Dabous, N. I., Zaher, S. R., Mokhtar, M. M., & Zaki, A. (2000). Risk factors for congenital heart diseases in Alexandria, Egypt. *European Journal of Epidemiology*, *16*(9), 805–814. <https://doi.org/10.1023/a:1007601919164>
- Becker, S. M., Al Halees, Z., Molina, C., & Paterson, R. M. (2001). Consanguinity and congenital heart disease in Saudi Arabia. *American Journal of Medical Genetics*, *99*(1), 8–13. [https://doi.org/10.1002/1096-8628\(20010215\)99:1<8::aid-ajmg1116>3.0.co;2-u](https://doi.org/10.1002/1096-8628(20010215)99:1<8::aid-ajmg1116>3.0.co;2-u)
- Becker-Heck, A., Zohn, I., Okabe, N., Pollock, A., Lenhart, K. B., Sullivan-Brown, J., McSheene, J., Loges, N. T., Olbrich, H., Haefner, K., Fliegau, M., Horvath, J., Reinhardt, R., Nielsen, K. G., Marthin, J. K., Baktai, G., Anderson, K. V., Geisler, R., Niswander, L., ... Burdine, R. D. (2011). The coiled-coil domain containing protein CCDC40 is essential for motile cilia function and left-right axis formation. *Nature Genetics*, *43*(1), 79–84.
- Bittles, A. H. (2011). Assessing the influence of consanguinity on congenital heart disease. *Annals of Pediatric Cardiology*, *4*(2), 111–116. <https://doi.org/10.4103/0974-2069.84637>
- Bittles, A. H., & Black, M. L. (2010). Evolution in health and medicine Sackler colloquium: Consanguinity, human evolution, and complex diseases. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(Suppl 1) 1091–6490 [Electronic], 1779–1786. <https://doi.org/10.1073/pnas.0906079106>
- Blue, G. M., Kirk, E. P., Giannoulatou, E., Sholler, G. F., Dunwoodie, S. L., Harvey, R. P., & Winlaw, D. S. (2017). Advances in the genetics of congenital heart disease: A Clinician's guide. *Journal of the American College of Cardiology*, *69*(7), 859–870. <https://doi.org/10.1016/j.jacc.2016.11.060>
- Boulter, C., Mulroy, S., Webb, S., Fleming, S., Brindle, K., & Sandford, R. (2001). Cardiovascular, skeletal, and renal defects in mice with a targeted disruption of the Pkd1 gene. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(21), 12174–12179. <https://doi.org/10.1073/pnas.211191098>
- Browning, S. R., & Browning, B. L. (2007). Rapid and accurate haplotype phasing and missing-data inference for whole-genome association studies by use of localized haplotype clustering. *American Journal of Human Genetics*, *81*(5), 1084–1097.
- Campbell, M. (1949). Genetic and environmental factors in congenital heart disease. *The Quarterly Journal of Medicine*, *18*(72), 379–391 D - CLML: 5018:443p:6 OTO - NLM.
- Chehab, G., Chedid, P., Saliba, Z., & Bouvagnet, P. (2007). Congenital cardiac disease and inbreeding: Specific defects escape higher risk due to parental consanguinity. *Cardiology in the Young*, *17*(4), 414–422. <https://doi.org/10.1017/s1047951107000704>
- Dong, C., Wei, P., Jian, X., Gibbs, R., Boerwinkle, E., Wang, K., & Liu, X. (2015). Comparison and integration of deleteriousness prediction methods for nonsynonymous SNVs in whole exome sequencing studies. *Human Molecular Genetics*, *24*(8), 2125–2137. <https://doi.org/10.1093/hmg/ddu733>
- El Zein, L., Omran, H., & Bouvagnet, P. (2003). Lateralization defects and ciliary dyskinesia: Lessons from algae. *Trends in Genetics*, *19*(3), 162–167. [https://doi.org/10.1016/S0168-9525\(03\)00026-X](https://doi.org/10.1016/S0168-9525(03)00026-X)
- Fakhro, K. A., Yousri, N. A., Rodriguez-Flores, J. L., Robay, A., Staudt, M. R., Agosto-Perez, F., Salit, J., Malek, J. A., Suhre, K., Jayyousi, A., Zirie, M., Stadler, D., Mezey, J. G., & Crystal, R. G. (2015). Copy number variations in the genome of the Qatari population. *BMC Genomics*, *16*, 834. <https://doi.org/10.1186/s12864-015-1991-5>
- Firth, H. V., Sm Fau, R., Bevan, A. P., Ap Fau, B., Clayton, S., S Fau, C., Corpas, M., M Fau, C., Rajan, D., D Fau, R., Van Vooren, S., & Carter, N. P. (2009). DECIPHER: Database of chromosomal imbalance and phenotype in humans using Ensembl resources. *American Journal of Human Genetics*, *84*(4), 524–533.
- Fromer, M., & Purcell, S. M. (2014). Using XHMM software to detect copy number variation in whole-exome sequencing data. *Current Protocols in Human Genetics*, *81*(1934-8258 [Electronic]), 7–23.
- Gao, W., Higaki, T., Eguchi-Ishimae, M., Iwabuki, H., Wu, Z., Yamamoto, E., Takata, H., Ohta, M., Imoto, I., Ishii, E., & Eguchi, M. (2015). DGCR6 at the proximal part of the DiGeorge critical region is involved in conotruncal heart defects. *Human Genome Variation*, *2*, 15004.
- Gatrad, A. R., Read, A. P., & Watson, G. H. (1984). Consanguinity and complex cardiac anomalies with situs ambiguus. *Archives of Disease in Childhood*, *59*(3), 242–245.
- Gelb, B. D. (2015). History of our understanding of the causes of congenital heart disease. *Circulation. Cardiovascular Genetics*, *8*, 529–536.
- Gev, D. F., Roguin, N., Roguin, N. F., Freundlich, E., & Freundlich, E. (1986). Consanguinity and congenital heart disease in the rural Arab population in northern Israel. *Human Heredity*, *36*(4), 213–217. <https://doi.org/10.1159/000153628>
- Gudmundsson, S., Karczewski, K. J., Francioli, L. C., Tiao, G., Cummings, B. B., Alfoldi, J., Wang, Q., Collins, R. L., Laricchia, K. M., Ganna, A., Birnbaum, D. P., Gauthier, L. D., Brand, H., Solomonson, M., Watts, N. A., Rhodes, D., Singer-Berk, M., England, E. M., Seaby, E. G., ... MacArthur, D. G. (2021). Addendum: The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature*, *597*(7874), E3–E4. <https://doi.org/10.1038/s41586-021-03758-y>. Erratum for: Nature. 2020 May;581(7809):434-443
- Guimier, A., Gabriel, G. C., Bajolle, F., Tsang, M., Liu, H., Noll, A., Schwartz, M., El Malti, R., Smith, L. D., Klena, N. T., Jimenez, G., Miller, N. A., Oufadem, M., de Bellaing, A. M., Yagi, H., Saunders, C. J., Baker, C. N., Di Filippo, S., Peterson, K. A., ...



- Gordon, C. T. (2015). MMP21 is mutated in human heterotaxy and is required for normal left-right asymmetry in vertebrates. *Nature Genetics*, *47*(11), 1260–1263.
- Gurbich, T. A., & Ilinsky, V. V. (2020). ClassifyCNV: A tool for clinical annotation of copy-number variants. *Scientific Reports*, *10*(1), 20375. <https://doi.org/10.1038/s41598-020-76425-3>
- Inoue, K., & Lupski, J. R. (2002). Molecular mechanisms for genomic disorders. *Annual Review of Genomics and Human Genetics*, *3*, 199–242. <https://doi.org/10.1146/annurev.genom.3.032802.120023>
- Jin, S. C., Homsy, J., Zaidi, S., Lu, Q., Morton, S., DePalma, S. R., Zeng, X., Qi, H., Chang, W., Sierant, M. C., Hung, W. C., Haider, S., Zhang, J., Knight, J., Bjornson, R. D., Castaldi, C., Tikhonova, I. R., Bilguvar, K., Mane, S. M., ... Brueckner, M. (2017). Contribution of rare inherited and de novo variants in 2,871 congenital heart disease probands. *Nature Genetics*, *49*(11), 1593–1601. <https://doi.org/10.1038/ng.3970>
- Kaplan, S., Pinar, G., Kaplan, B., Aslantekin, F., Karabulut, E., Ayar, B., & Dilmen, U. (2016). The prevalence of consanguineous marriages and affecting factors in Turkey: A national survey. *Journal of Biosocial Science*, *48*(5), 616–630. <https://doi.org/10.1017/S0021932016000055>
- Khan, I., Butt, M., Ashfaq, W., Khan, S., & Batoool, M. (2015). Bardet-Biedl syndrome associated with Dextrocardia and Situs inversus. *Journal of Islamabad Medical and Dental College*, *4*(1), 37–39.
- Khan, S. A., Muhammad, N., Khan, M. A., Kamal, A., Rehman, Z. U., & Khan, S. (2016). Genetics of human Bardet-Biedl syndrome, an updates. *Clinical Genetics*, *90*(1), 3–15. <https://doi.org/10.1111/cge.12737>
- Kircher, M., Witten, D. M., Jain, P., O’Roak, B. J., Cooper, G. M., & Shendure, J. (2014). A general framework for estimating the relative pathogenicity of human genetic variants. *Nature Genetics*, *46*(3), 310–315. <https://doi.org/10.1038/ng.2892>
- Lek, M., Karczewski, K. J., Minikel, E. V., Samocha, K. E., Banks, E., Fennell, T., O’Donnell-Luria, A. H., Ware, J. S., Hill, A. J., Cummings, B. B., Tukiainen, T., Birnbaum, D. P., Kosmicki, J. A., Duncan, L. E., Estrada, K., Zhao, F., Zou, J., Pierce-Hoffman, E., Berghout, J., ... MacArthur, D. G. (2016). Analysis of protein-coding genetic variation in 60,706 humans. *Nature*, *536*(7616), 285–291. <https://doi.org/10.1038/nature19057>
- Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with burrows-wheeler transform. *Bioinformatics*, *25*(14), 1754–1760. <https://doi.org/10.1093/bioinformatics/btp324>
- Li, Y., Klena, N. T., Gabriel, G. C., Liu, X., Kim, A. J., Lemke, K., Chen, Y., Chatterjee, B., Devine, W., Damerla, R. R., Chang, C., Yagi, H., San Agustin, J. T., Thahir, M., Anderton, S., Lawhead, C., Vescovi, A., Pratt, H., Morgan, J., ... Lo, C. W. (2015). Global genetic analysis in mice unveils central role for cilia in congenital heart disease. *Nature*, *521*(7553), 520–524.
- Marchenko, G. N., Marchenko, N. D., & Strongin, A. Y. (2003). The structure and regulation of the human and mouse matrix metalloproteinase-21 gene and protein. *The Biochemical Journal*, *372*(Pt 2), 503–515. <https://doi.org/10.1042/BJ20030174>
- Monies, D., Abouelhoda, M., Assoum, M., Moghrabi, N., Rafiullah, R., Almontashiri, N., Alowain, M., Alzaidan, H., Alsayed, M., Subhani, S., Cupler, E., Faden, M., Alhashem, A., Qari, A., Chedrawi, A., Aldhalaan, H., Kurdi, W., Khan, S., Rahbeeni, Z., ... Alkuraya, F. S. (2019). Lessons learned from large-scale, first-tier clinical exome sequencing in a highly consanguineous population. *American Journal of Human Genetics*, *104*(6), 1182–1201. <https://doi.org/10.1016/j.ajhg.2019.04.011>
- Nabulsi, M. M., Tamim, H., Sabbagh, M., Obeid, M. Y., Yunis, K. A., & Bitar, F. F. (2003). Parental consanguinity and congenital heart malformations in a developing country. *American Journal of Medical Genetics. Part A*, *116A*(4), 342–347. <https://doi.org/10.1002/ajmg.a.10020>
- Perles, Z., Moon, S., Ta-Shma, A., Yaacov, B., Francescato, L., Edvardson, S., Rein, A. J. T., Elpeleg, O., & Katsanis, N. (2015). A human laterality disorder caused by a homozygous deleterious mutation in MMP21. *Journal of Medical Genetics*, *52*(12), 840–847. <https://doi.org/10.1136/jmedgenet-2015-103336>
- Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A., Bender, D., Maller, J., Sklar, P., de Bakker, P. I. W., Daly, M. J., & Sham, P. C. (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics*, *81*(3), 559–575. <https://doi.org/10.1086/519795>
- Riggs, E. R., Andersen, E. F., Cherry, A. M., Kantarci, S., Kearney, H., Patel, A., Raca, G., Ritter, D. I., South, S. T., Thorland, E. C., Pineda-Alvarez, D., Aradhya, S., & Martin, C. L. (2020). 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). *Genetics in Medicine*, *22*(2), 245–257. <https://doi.org/10.1038/s41436-019-0686-8>
- Ritelli, M., Cinquina, V., Giacomuzzi, E., Venturini, M., Chiarelli, N., & Colombi, M. (2019). Further defining the phenotypic Spectrum of B3GAT3 mutations and literature review on Linkeropathy syndromes. *Genes (Basel)*, *10*(9), 631. <https://doi.org/10.3390/genes10090631>
- Robinson, J. T., Thorvaldsdottir, H., H Fau, T., Winckler, W., W Fau, W., Guttman, M., M Fau, G., Lander, E. S., Es Fau, L., Getz, G., G Fau, G., Mesirov, J. P., & Mesirov, J. P. (2011). Integrative genomics viewer. *Nature Biotechnology*, *29*(1), 24–26.
- Roguin, N., Du, Z. D., Barak, M., Nasser, N., Hershkowitz, S., & Milgram, E. (1995). High prevalence of muscular ventricular septal defect in neonates. *Journal of the American College of Cardiology*, *26*(6), 1545–1548. [https://doi.org/10.1016/0735-1097\(95\)00358-4](https://doi.org/10.1016/0735-1097(95)00358-4)
- Server, E. V. (2014). *NHLBI GO exome sequencing project (ESP)*. <http://evs.gs.washington.edu/EVS/>. Retrieved 2020 <http://evs.gs.washington.edu/EVS/>
- Splawski, I., Timothy, K. W., Sharpe, L. M., Decher, N., Kumar, P., Bloise, R., Napolitano, C., Schwartz, P. J., Joseph, R. M., Condouris, K., Tager-Flusberg, H., Priori, S. G., Sanguinetti, M. C., & Keating, M. T. (2004). Ca(V)<sub>1.2</sub> calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism. *Cell*, *119*(1), 19–31. <https://doi.org/10.1016/j.cell.2004.09.011>
- Van der Auwera, G. A., Carneiro, M. O., Hartl, C., Poplin, R., Del Angel, G., Levy-Moonshine, A., Jordan, T., Shakir, K., Roazen, D., Thibault, J., Banks, E., Garimella, K. V., Altshuler, D., Gabriel, S., & DePristo, M. A. (2013). From FastQ data to high confidence variant calls: The genome analysis toolkit best practices pipeline. *Current Protocols in Bioinformatics*, *43*, 11.10–11.33. <https://doi.org/10.1002/0471250953.bi1110s43>
- van der Linde, D., Konings, E. E., Slager, M. A., Witsenburg, M., Helbing, W. A., Takkenberg, J. J., & Roos-Hesselink, J. W.

- (2011). Birth prevalence of congenital heart disease worldwide: A systematic review and meta-analysis. *Journal of the American College of Cardiology*, 58(21), 2241–2247. <https://doi.org/10.1016/j.jacc.2011.08.025>
- Wang, K., Li, M., & Hakonarson, H. (2010). ANNOVAR: Functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Research*, 38(16), e164. <https://doi.org/10.1093/nar/gkq603>
- Yunis, K., Mumtaz, G., Bitar, F., Chamseddine, F., Kassar, M., Rashkidi, J., Makhoul, G., & Tamim, H. (2006). Consanguineous marriage and congenital heart defects: A case-control study in the neonatal period. *American Journal of Medical Genetics. Part A*, 140(14), 1524–1530. <https://doi.org/10.1002/ajmg.a.31309>
- Zaidi, S., & Brueckner, M. (2017). Genetics and genomics of congenital heart disease. *Circulation Research*, 120(6), 923–940. <https://doi.org/10.1161/circresaha.116.309140>
- Zhao, Y., Diacou, A., Johnston, H. R., Musfee, F. I., McDonald-McGinn, D. M., McGinn, D., Crowley, T. B., Repetto, G. M., Swillen, A., Breckpot, J., Vermeesch, J. R., Kates, W. R., Digilio, M. C., Unolt, M., Marino, B., Pontillo, M., Armando, M., Di Fabio, F., Vicari, S., ... Morrow, B. E. (2020). Complete sequence of the 22q11.2 allele in 1,053 subjects with 22q11.2 deletion syndrome reveals modifiers of conotruncal heart defects. *American Journal of Human Genetics*, 106(1), 26–40. <https://doi.org/10.1016/j.ajhg.2019.11.010>
- Zlotogora, J. (2007). Multiple mutations responsible for frequent genetic diseases in isolated populations. *European Journal of Human Genetics*, 15(3), 272–278. <https://doi.org/10.1038/sj.ejhg.5201760>

## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

**How to cite this article:** Dong, W., Kaymakcalan, H., Jin, S. C., Diab, N. S., Tanıdır, C., Yalcin, A. S., Ercan-Sencicek, A. G., Mane, S., Gunel, M., Lifton, R. P., Bilguvar, K., Brueckner, M. (2022). Mutation spectrum of congenital heart disease in a consanguineous Turkish population. *Molecular Genetics & Genomic Medicine*, 10, e1944. <https://doi.org/10.1002/mgg3.1944>