Congenital Heart Disease

Tech Science Press

DOI: 10.32604/chd.2022.021580

check for updates

ARTICLE

Factors Affecting the Genetic Diagnostic Rate in Congenital Heart Disease

Jun Sung Park[1], Go Hun Seo[2], Yunha Choi[1], Soojin Hwang[1], Minji Kang[3], Hyo-Sang Do[3], Young-Hwue Kim[4], Jeong Jin Yu[4], Ellen Ai-Rhan Kim[5], Euiseok Jung[5], Byong Sop Lee[5], Jae Suk Baek[4] and Beom Hee Lee[1,6,*]

[1]Department of Pediatrics, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

[2]3 Billion, Inc., Seoul, Korea

[3]Genome Research Center for Birth Defects and Genetic Diseases, Asan Institute for Life Sciences, Asan Medical Center, Seoul, Korea

[4]Division of Cardiology, Department of Pediatrics, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

[5]Division of Neonatology, Department of Pediatrics, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

[6]Medical Genetics Center, Asan Medical Center Children's Hospital, University of Ulsan College of Medicine, Seoul, Korea

[*]Corresponding Author: Beom Hee Lee. Email: bhlee@amc.seoul.kr

Received: 24 February 2022 Accepted: 20 June 2022

ABSTRACT

Background: Over 400 genes contribute to the development of congenital heart disease (CHD). Additionally, multisystemic manifestations accompanying syndromic CHD pose a higher risk of genetic diseases. This study investigated the diagnostic yield of whole-exome sequencing (WES) in patients with sporadic syndromic CHD and the phenotypic factors affecting the genetic diagnostic rate. **Methods:** Sixty-four patients with sporadic syndromic CHD aged <18 years underwent WES between May 2018 and December 2020 in a single tertiary center, and the association between genetic testing data and extracardiac phenotypes was analyzed. **Results:** Extracardiac phenotypes were measured as 3.66 ± 3.05 (standard deviation, interquartile range: 2–5) items per patient. WES detected diagnostic variants in 19 (29.7%) patients: seven (36.8%), seven (36.8%), and five (26.3%) with pathogenic variants, likely pathogenic variants, and variants of unknown significance, respectively. Post-diagnosis surveillance identified the extracardiac phenotype in 54.5% (6/11) of patients. *De novo* variants accounted for 76.2% (15/19) of variants and autosomal dominant inheritance for 94.7% (18/19). Most diseases were ultra-rare. No significant differences were noted in cardiac and extracardiac phenotypes, single or combined (all $P > 0.05$), between the groups with and without a diagnostic variant. However, patients with ≥3 extracardiac phenotypes had a significantly higher likelihood of having a diagnostic variant than those with ≤2 (38.3% *vs.* 5.9%, odds ratio = 9.93, 95% confidence interval = 1.21–81.44, $P = 0.013$). **Conclusions:** The number of extracardiac phenotypes is important in predicting the possibility of genetic diagnosis. Physicians will be able to select patients with a high probability of genetic diagnosis and provide appropriate genetic counseling based on the results of this study.

KEYWORDS

Heart defects; congenital; whole-exome sequencing; genetic testing; phenotype

1 Introduction

Congenital heart disease (CHD) develops because of abnormalities in the structure of the heart that arise before birth. It is the most frequently occurring congenital disorder in newborns, with an estimated incidence of 8 per 1000 live births per year (range 3–10) [1]. Despite progress in medical and surgical treatments, CHD remains the leading cause of mortality among all congenital anomalies.

The etiology of CHD has been proposed as both genetic and environmental, with evidence increasingly supporting the role of a genetic contribution [2]. Chromosome disorders, including Down syndrome (OMIM 190685) and Edwards syndrome (OMIM 300484); microdeletion syndrome, including Williams syndrome (OMIM 194050) and 22q11.2 microdeletion syndrome (OMIM 188400, 192430, and 182212); and monogenic diseases, including Noonan syndrome (OMIM 164757, 164760, 164790, etc.) and Kabuki syndrome (OMIM 147920 and 300867), have been revealed to underlie syndromic CHD. To date, approximately 400 genes have been suggested to be involved in CHD development [3].

Karyotyping and chromosome microarray (CMA) can detect chromosome disorders and copy number variants (CNVs). Overall, karyotyping and CNV-related molecular diagnosis can each be made approximately in 10%–25% of patients with CHD [2–4]. However, genomic sequencing, such as whole-exome sequencing (WES) using the next-generation sequencing technique can be effectively applied to diagnose monogenic diseases, considering their genetic heterogeneity [2]. Elucidating the genetic etiology of CHD provides essential information on patient care by surveillance of unpredicted extracardiac manifestations and preemptive prevention of delayed-onset manifestations [5–8]. Furthermore, genetic diagnosis significantly contributes to the provision of appropriate genetic counseling for patients and their family members. Conversely, reckless and over-extensive genetic testing can incur high medical costs and offset its effectiveness [9,10]. Therefore, it is important to determine the clinical features that would enhance the diagnostic yield of the genetic test in terms of cost-effectiveness and understanding the genetic contribution to CHD.

A recent study reported a significant difference between the number of body systems affected and the likelihood of having a pathogenic variant in patients with multiple anomalies [11]. Importantly, extracardiac congenital anomalies are frequently observed in newborns with CHD, with almost twice the prevalence observed in non-CHD individuals [12,13]. Therefore, it is necessary to assess the contribution of these accompanying extracardiac manifestations to the genetic diagnostic rate in patients with CHD. However, there is no research reporting on the association between extracardiac manifestations and the likelihood of detecting the genetic variant in syndromic CHD.

In this study, we performed WES in pediatric patients with sporadic syndromic CHD and assessed the factors affecting diagnostic yield.

2 Material and Methods

2.1 Study Design and Population

We conducted WES in probands aged ≤18 years with sporadic syndromic CHD, which accompanied the diagnosis of at least one extracardiac phenotype at Asan Medical Center Children's Hospital, Seoul, South Korea, between May 2018 and December 2020. All types of hemodynamically significant CHD were included. Hemodynamically insignificant phenotypes, including mild pulmonary stenosis, spontaneously closure of small atrial septal defect (ASD), left superior vena cava, and so on were excluded. Moreover, patients with cardiomyopathy and isolated transient fetal circulation with spontaneous resolution were excluded; patients with all other physiological abnormalities, including arrhythmia combined with CHD, were included. Patients with positive results in other genetic tests, including chromosome analysis, gene panel, multiplex ligation-dependent probe amplification, and CMA, were excluded. The patients' detailed demographics and clinical characteristics were reviewed, including age, sex, initial presentation, family

history, and cardiac and extracardiac manifestations. Informed consent was obtained from the parents of the patients for genetic testing. The Institutional Review Board for Human Research at Asan Medical Center approved this study (IRB numbers: 2018-0574 and 2018-0180).

2.2 Classification of Manifestations

Cardiac phenotypes are classified according to the International Pediatric and Congenital Cardiac Code (IPCCC) and the Eleventh Iteration of the International Classification of Diseases (ICD-11) [14], and extracardiac phenotypes are classified according to the Human Phenotype Ontology (HPO) [15,16]. Cardiac manifestations were assessed using the latest echocardiography or computed tomography or via surgical diagnosis by pediatric cardiologists, pediatric cardiac surgeons, or radiologists. All abnormalities of the morphology and the vasculature, including abnormality of the aorta, coronary artery, vena cava, and pulmonary vasculature, were included (Appendix A).

Patients with CHD underwent routine surveillance, including brain and abdominal ultrasound, tandem mass spectrometry, and complete inspection after birth. Extracardiac manifestations were described by a physician with at least 1 year of experience as a medical geneticist after specializing in pediatrics. Confirmation of findings was provided by a senior geneticist with 10 years of experience. Each phenotype was classified based on the highest level of organ system: head and neck, nervous system, ear, genitourinary system, abdomen, growth, limbs, skeletal system, respiratory system, eye, integument, endocrine system, musculature, prenatal, breast, cardiovascular system, metabolism, and homeostasis. The number of extracardiac phenotypes was counted as the number of systems, not all the individual phenotypes. Multiple phenotypes belonging to the same system were counted as one extracardiac phenotype. For example, the three phenotypes of hypertelorism, high-arched palate, and cryptorchidism were counted as two extracardiac phenotypes: the former two are an abnormality of the head and neck, and the latter is an abnormality of the genitourinary tract.

2.3 Variant Annotation and Interpretation by WES Analysis

We performed WES using genomic DNA isolated from the patient's whole blood or a buccal swab sample. All exons of all human genes (approximately 22 000) were captured using the Twist Human Core Exome Kit (Twist Bioscience, San Francisco, CA, USA). The captured genomic regions were sequenced using a NovaSeq 6000 platform (Illumina, San Diego, CA, USA). Raw genome sequencing data analysis included alignment to the reference sequence (NCBI genome assembly GRCh37; accessed February 2009). The mean depth of coverage was 100-fold with 99.2% higher coverage than that of 10-fold. Each variant's pathogenicity was assessed using the automated variant interpretation system EVIDENCE, in line with the American College of Medical Genetics (ACMG) guidelines 12 and relevant patient phenotypes [17]. Candidate variants based on EVIDENCE were reviewed and selected by expert medical geneticists. Sanger sequencing of the variant identified via exome sequencing was performed for patients and their parents. "Diagnostic variant" was defined as the variant that was pathogenic, likely pathogenic, or variant of unknown significance (VUS) noted as a result of "de novo" events or of which the phenotype determined by a gene was fully matched with a patient's phenotype after complete parental tests.

2.4 Outcomes

The primary goal was to investigate the usefulness of WES for identifying diagnostic variants and evaluate the phenotypes after the genetic diagnosis in patients with CHD. The secondary goal was to assess the factors affecting the diagnostic rate of WES in terms of the number and the cardiac and extracardiac phenotypes.

2.5 Statistical Analysis

Descriptive data were expressed using a graph and table created using Excel 365 (Microsoft Corp., Redmond, WA, USA). For comparison between the groups, the $\chi 2$ test or Fisher's exact test was performed. These analyses were performed using IBM-SPSS for Windows software, version 21 (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

3 Results

WES was conducted for 101 pediatric patients with cardiac abnormalities during the study period. Excluding 19 patients with cardiomyopathy, six with isolated arrhythmia, four with isolated persistent fetal circulation, and eight with CNV in CMA, 64 patients with both syndromic features and cardiac abnormalities were included (Fig. 1).

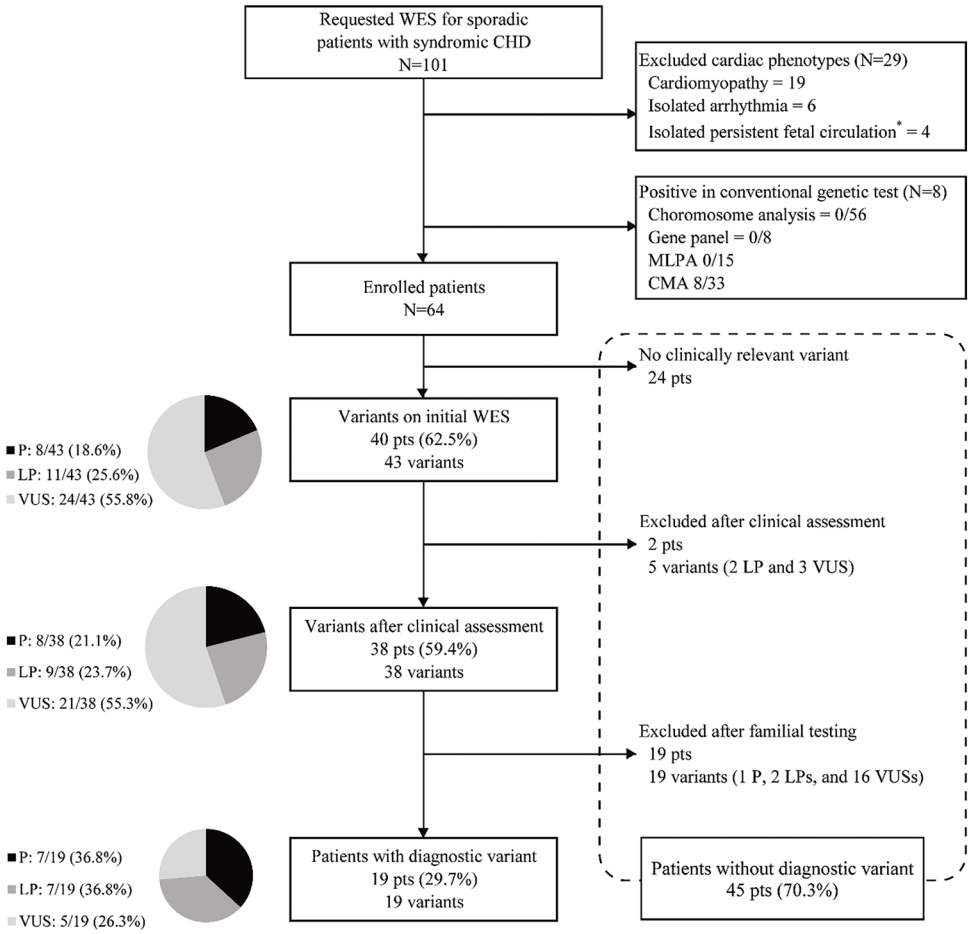


Figure 1: Flow chart of genetic diagnosis of a patient with congenital heart disease using whole-exome sequencing

Abbreviation: WES, whole-exome sequencing; CHD, congenital heart disease; MLPA, multiplex ligation-dependent probe amplification; CMA, chromosome microarray; P, pathogenic; LP, likely pathogenic; VUS, variant of unknown significance.

**Persistent fetal circulations included patent foramen ovale and patent ductus arteriosus.*

3.1 Clinical Features of the Patients

Basic demographics and clinical manifestations of the patients are summarized in Table 1. There were 34 (53.1%) males and 30 (47.9%) females, most of whom were requested for WES in their neonatal periods.

Ventricle or ventricular septal defects, atrium or atrial septal defects, and great artery defects were found in 33 (51.6%), 32 (50.0%), and 15 (23.4%) patients, respectively. Among individual phenotypes, ASD (33 patients, 51.6%) and ventricular septal defect (VSD) (31 patients, 48.4%) were the most common cardiac phenotypes (Table 2).

Table 1: Clinical characteristics of the patients with sporadic syndromic congenital heart disease with or without a diagnostic variant

Variables	Patients without diagnostic variant N = 45 (70.3%)	Patients with diagnostic variant N = 19 (29.7%)	Total N = 64	*P*
Sex, Male	25 (55.6)	9 (47.4)	34 (53.1)	0.593
Diagnosis age, y	0 (0–0)	0 (0–0)	0 (0–0)	0.524
Current age, y	2.4 (1.8–3.9)	2.9 (1.4–3.8)	2.7 (1.8–3.9)	0.629
Mortality	4 (6.3)	2 (4.4)	2 (10.5)	0.576
Cardiac Phenotypes				
Ventricle or ventricular septum	24 (53.3)	9 (47.4)	33 (51.6)	0.786
Atrium or atrial septum	22 (48.9)	10 (52.6)	32 (50)	0.784
Great artery	13 (28.9)	2 (10.5)	15 (23.4)	0.113
VA valve	9 (20)	5 (26.3)	14 (21.9)	0.577
AV or VA connection	7 (15.6)	2 (10.5)	9 (14.1)	0.597
AV valve or AV septum	4 (8.9)	2 (10.5)	6 (9.4)	0.837
Mediastinal vein	1 (2.2)	2 (10.5)	3 (4.7)	0.151
Extracardiac Phenotypes				
Head and neck	37 (82.2)	14 (73.7)	51 (79.7)	0.503
Nervous system	25 (55.6)	12 (63.2)	37 (57.8)	0.782
Ear	15 (33.3)	8 (42.1)	23 (35.9)	0.574
Genitourinary system	15 (33.3)	5 (26.3)	20 (31.3)	0.769
Abdomen	10 (22.2)	5 (26.3)	15 (23.4)	0.753
Growth	11 (24.4)	1 (5.3)	12 (18.8)	0.090
Limbs	9 (20)	4 (21.1)	13 (20.3)	1.000
Skeletal system	8 (17.8)	6 (31.6)	14 (21.9)	0.321
Respiratory system	8 (17.8)	3 (15.8)	11 (17.2)	1.000
Eye	4 (8.9)	5 (26.3)	9 (14.1)	0.111
Integument	2 (4.4)	4 (21.1)	6 (9.4)	0.059
Endocrine system	4 (8.9)	2 (10.5)	6 (9.4)	1.000
Musculature	3 (6.7)	2 (10.5)	5 (7.8)	0.629
Prenatal	2 (4.4)	1 (5.3)	3 (4.7)	1.000
Breast	1 (2.2)	1 (5.3)	2 (3.1)	0.509

(Continued)

Table 1 (continued)

Variables	Patients without diagnostic variant N = 45 (70.3%)	Patients with diagnostic variant N = 19 (29.7%)	Total N = 64	*P*
Cardiovascular system	1 (2.2)	2 (10.5)	3 (4.7)	0.208
Metabolism and homeostasis	3 (6.7)	0 (0)	3 (4.7)	N/A

Note: Results are presented as number (%) and median (interquartile range).
Abbreviations: VA, ventriculoarterial; AV, atrioventricular.
Cardiac phenotypes are classified according to the International Pediatric and Congenital Cardiac Code (IPCCC) and the Eleventh Iteration of the International Classification of Diseases (ICD-11) [13]. Extracardiac manifestations are classified according to the Human Phenotype Ontology (HPO) [14,15].

Table 2: Common cardiac and extracardiac phenotypes of the patients with sporadic syndromic congenital heart disease with or without a diagnostic variant

	Patients without diagnostic variant N = 45 (70.3%)	Patients with diagnostic variant N = 19 (29.7%)	Total N = 64	*P*
Cardiac Phenotype				
ASD	24 (53.3)	9 (47.4)	33 (51.6)	0.786
VSD	23 (51.1)	8 (42.1)	31 (48.4)	0.590
PDA	11 (24.4)	2 (10.5)	13 (20.3)	0.312
PS	7 (15.6)	4 (21.1)	11 (17.2)	0.719
TOF	4 (8.9)	2 (10.5)	6 (9.4)	1.000
DORV	4 (8.9)	1 (5.3)	5 (7.8)	1.000
CoA	3 (6.7)	2 (10.5)	5 (7.8)	0.629
AVSD	3 (6.7)	0 (0)	3 (4.7)	0.549
Other[a]	14 (31.1)	4 (20)	20 (27.4)	N/A
Extracardiac Phenotype				
Developmental delay	14 (31.1)	5 (26.3)	19 (29.7)	0.773
Hypertelorism	9 (20)	3 (15.8)	12 (18.8)	1.000
Macrocephaly	6 (13.3)	4 (21.1)	10 (15.6)	0.466
Low-set ear	5 (11.1)	4 (21.1)	9 (14.1)	0.432
High-arched palate	6 (13.3)	1 (5.3)	7 (10.9)	0.664
Microcephaly	6 (13.3)	2 (10.5)	8 (12.5)	1.000
Micrognathia	7 (15.6)	0 (0)	7 (10.9)	0.094
Hypotonia	4 (8.9)	2 (10.5)	6 (9.4)	1.000

Note: [a]Other less common cardiac phenotypes included transposition of great arteries, pulmonary atresia, major anomalous pulmonary collateral arteries, bicuspid aortic valve, truncus arteriosus, partial anomalous pulmonary venous return, aortic regurgitation, aortic stenosis, and tricuspid regurgitation.
Abbreviations: ASD, atrial septal defect; VSD, ventricular septal defect; PDA, patent ductus arteriosus; PS, pulmonary stenosis; TOF, tetralogy of Fallot; DORV, double outlet right ventricle; CoA, coarctation of aorta; AVSD, atrioventricular septal defect.

Extracardiac phenotypes were measured as 3.66 ± 3.05 (standard deviation, interquartile range, 2–5) items per patient. The most commonly observed system of phenotypic abnormality was the head and neck (51 patients, 79.7%), followed by the nervous system (37 patients, 57.8%), ear (23 patients, 35.9%), genitourinary system (20 patients, 31.3%), and abdomen (15 patients, 23.4%). Regarding individual

phenotypes, developmental delay (19 patients, 29.7%) was the most common, followed by dysmorphic features, such as macrocephaly, microcephaly, hypertelorism, low-set ear, high-arched palate, and micrognathia (Table 2).

The detailed phenotypic information of the individual patients is described in Table 3 (patients with diagnostic variant) and Appendix B (patients without diagnostic variant).

3.2 Genetic Diagnosis

WES identified 43 variants in 40 (63.1%) patients (Fig. 1). According to the ACMG guidelines [18], among the 43 variants, eight (18.6%), 11 (25.6%), and 24 (55.8%) were categorized as pathogenic, likely pathogenic, and VUS, respectively. After clinical reassessment, excluding two variants (*RYR2,* OMIM 180902; and *AP4M1,* OMIM 612936) that are highly pathogenic and cause multiple anomalies, but not a cardiac structural abnormality, eight (21.1%), nine (23.7%), and 21 (55.3%) variants were categorized as pathogenic, likely pathogenic, and VUS, respectively. After asymptomatic parental testing, 19 (29.7%) patients were finally genetically confirmed: seven (36.8%), seven (36.8%), and five (26.3%) patients with pathogenic variants, likely pathogenic variants, and VUS, respectively. No patients with more than one diagnostic variant were noted. Detailed information on the variants and phenotypes of each patient are described in Table 3. Among the total number of 19 diagnostic variants found in our study, 15 (78.9%) were de novo variants. With the exception of Patient 12 who had Adams–Oliver syndrome 2 with an autosomal recessive inheritance, all other patients (18/19 patients, 94.7%) had genetic diseases with autosomal dominant inheritance. Nine variants in nine genes were not previously reported (bold text in Table 3, searched by December 24, 2021).

A pathogenic variant of *PTPN11*, a likely pathogenic variant of *COL1A2* or *DOCK6,* or a VUS in *MYH11*, was inherited from an asymptomatic parent (4/19, 21.1%, Patients 5, 8, 12, and 18). However, the phenotypes of these variants were highly consistent with associated clinical symptoms and were therefore regarded as a diagnostic variant of probands exhibiting reduced penetrance in the asymptomatic parent. Surveillance for hidden phenotypes was conducted in asymptomatic parents after genetic confirmation, which did not show any affected systems.

3.3 Post-Diagnosis Clinical Evaluation

Post-diagnosis surveillance for the extracardiac phenotypes was conducted in 11 (57.9%) patients. An additional phenotype was identified in six (54.5%) patients: epilepsy in Patient 2 with congenital heart defects, dysmorphic facial features, and intellectual developmental disorder; polymicrogyria in Patient 9 with megalencephaly–capillary malformation–polymicrogyria syndrome, somatic; exudative vitreoretinopathy in Patient 12 with Adams–Oliver syndrome 2; esophoria in Patient 14 with KBG syndrome; multiple hyperechoic foci of the liver and spleen in Patient 17 with Adams–Oliver syndrome 6; and dysphagia in Patient 19 with Kabuki syndrome.

3.4 Assessment of the Factors Affecting the Genetic Diagnostic Rate

No significant difference was noted in the detection rate according to cardiac or extracardiac phenotypes (all $P > 0.05$, Tables 1 and 2, and Appendix C). Among the various cardiac phenotypes, the genetic diagnostic rate was higher in patients with coarctation of aorta (2/5 patients, 40%), pulmonary stenosis (4/11 patients, 36.4%), and tetralogy of Fallot (2/6 patients, 33.3%). A higher genetic diagnostic rate was observed in patients with extracardiac phenotypes of low-set ear (4/9 patients, 44.4%), macrocephaly (4/10 patients, 40%), and hypotonia (2/6 patients, 33.3%). According to the organ system categorization, a higher genetic diagnostic rate was noted in the eye (5/9 patients, 55.6%), skeletal system (6/14 patients, 42.9%), and ear (8/23 patients, 34.8%).

Table 3: Clinical and genetic characteristics of the patients with sporadic syndromic congenital heart disease

ID	Sex	Cardiac phenotype	Extracardiac phenotype		Gene name	MIM number[a]	Diagnosis	Inheritance	Prevalence	Position/GenBank number	Nucleotide change/Protein change	Pathogenicity[c]	Genetic origin
			Details	No.									
1	F	AS, PS, BAV	hypertelorism, low-set ear, ear abnormality, low nasal bridge, thin lips, short neck, polysplenia, medullary kidney disease, DD	5	***ZEB2***	235730	Mowat-Wilson syndrome	AD	1/50,000–70,000	**chr2:145156698 NM_001171653.1**	**c.1984G > T p. E686Ter**	P	de novo
2	M	ASD, PFO	narrow frontal head, hypotelorism, short palpebral fissure, bulbous nose, abundant nape, low-set ear, sacral dimple, DD	4	*CDK13*	617360	Congenital heart defects, dysmorphic facial features, and intellectual developmental disorder[b]	AD	≈20 cases	chr7:40085606 NM_003718	c.2525A > G p. N842S	P	de novo
3	M	ASD	macrocephaly, prominent forehead, hypertelorism, low nasal bridge, thick tongue, short, long bone, finger abnormalities, simian crease, sacral dimple	4	***DVL1***	616331	Robinow syndrome, autosomal dominant 2[b]	AD	≈10 cases	**chr1:1273476 NM_001330311.1**	**c.1411CA > C**	P	de novo
4	M	ASD, VSD	ectipic kidney, sensorineural hearing loss	4	*CSNK2A1*	617062	Okur-Chung neurodevelopmental syndrome[b]	AD	≈30 cases	chr20:485835 NM_001895.3	c.140G > A p. W532GfsTer142	P	de novo
5	F	CoA, VSD, ASD	low-set ear, thin lip, webbed neck, pectus excavatum, widely spaced nipple	3	*PTPN11*	163950	Noonan syndrome 1	AD	1/1,000–2,500	chr12:112888172 NM_001330437	c.188A > G p. Y63C	P	mother
6	F	DORV, MAPCA	microcephaly, poor eye fixation, long eyelashes, thin ear helix, lateral eversion of lower palpebrae, microcephaly, sparse eyebrow, broad thumb, hypotonia, DD	6	*CTNNB1*	615075	Neurodevelopmental disorder with spastic diplegia and visual defects[b]	AD	≈20 cases	chr3:41275648 NM_001904.3	c.1543C > T p. R515Ter	P	de novo
7	M	PS, ASD	Low-set ear, thin lip, webbed neck, pectus excavatum, widely spaced nipple	4	*PTPN11*	163950	Noonan syndrome 1	AD	1/1,000–2,500	chr12:112888168 NM_001330437	c.184 T > G p. Y62D	P	de novo
8	F	ASD	asthma, DD, hyperextensibility of joint, blue sclera	4	***COL1A2***	617821	Ehlers-Danlos syndrome, arthrochalasia type, 2[b]	AD	≈30 cases	**chr7:94045793 NM_001330437**	**c.1841_1848C p. P615LfsTer65**	LP	mother
9	F	ASD	polydactyly, hypertrichosis, broad	6	*PIK3CA*	602501	Megalencephaly-capillary	AD	≈150 cases	chr3:178936074 NM_006218.3	c.1616C > G p. P539R	LP	de novo

(Continued)

Table 3 (continued)

ID	Sex	Cardiac phenotype	Extracardiac phenotype		Gene name	MIM number[a]	Diagnosis	Inheritance	Prevalence	Position/GenBank number	Nucleotide change/Protein change	Pathogenicity[c]	Genetic origin
			Details	No.									
			forehead, hypertelorism, low-set ear, short palpebral fissure, thick lip, small chin, macrocephaly, polymicrogyria, eye lens defect				malformation-polymicrogyria syndrome, somatic[b]						
10	M	ccTGA, ASD	DD, hypotonia, infantile spasms	2	***NR2F1***	615722	Bosch-Boonstra-Schaaf optic atrophy syndrome[b]	AD	≈55 cases	**chr5:92923656 NM_005654.5**	**c.497C > T p. P166L**	LP	de novo
11	F	CoA	hemivertebrae, IA, DD	3	***KMT2D***	147920	Kabuki syndrome 1	AD	1/32,000	**chr12:49434145 NM_003482.3**	**c.7407_7408insT p.P2470SfsTer7**	LP	de novo
12	M	TOF	developmental delay, plagiocephaly, microcephaly, IVH, exudative vitreoretinopathy	4	*DOCK6*	614219	Adams-Oliver syndrome 2[b]	AR	≈100 cases	chr19:11353819 NM_020812.3	c.1396C > T p. Q434RfsTer21	LP	father
13	F	TOF	megalencephaly, low-set ear, hypotonia, outer ear shape abnormality, webbed neck, increased nuchal skin fold, ventriculomegaly, hydronephrosis, sparse eyebrow, sparse hair	5	*BRAF*	115150	Cardiofaciocutaneous syndrome[b]	AD	≈300 cases	chr7:140477806 NM_004333.4	c.1502A > G p. R466Ter	LP	de novo
14	F	VSD	prominent ears, bulbous nose, anteverted nares, long philtrum, thin vermilion of the upper lip, arched eyebrows	3	***ANKRD11***	148050	KBG syndrome[b]	AD	≈150 cases	**chr16:89357420 NM_001256182.1**	**c.397 + 1G > A-**	LP	de novo
15	M	VSD, BAV	congenital megacolon, multicystic dysplastic kidney, inguinal hernia, DD	4	***ACTB***	243310	Baraitser-Winter syndrome 1[b]	AD	< 1/ 1,000,000	**chr7:5569013 NM_001101.3**	**c.142G > T p. G48C**	VUS	de novo
16	M	ASD, PS	macrocephaly, thyroglossal duct cyst, hemangioma, chronic lung disease, congenital hypothyroidism, hypertelorism, small mouth, micrognathia, short neck	5	*SHOC2*	607721	Noonan syndrome-like with loose anagen hair 1[b]	AD	≈50 cases	chr10:112724120 NM_001269039.2	c.4A > G p.S2G	VUS	de novo
17	F			3	***DLL4***	616589	Adams-Oliver syndrome 6[b]	AD	≈15 cases	**chr15:41224579 NM_019074.3**	**c.784G > A p. G262S**	VUS	de novo

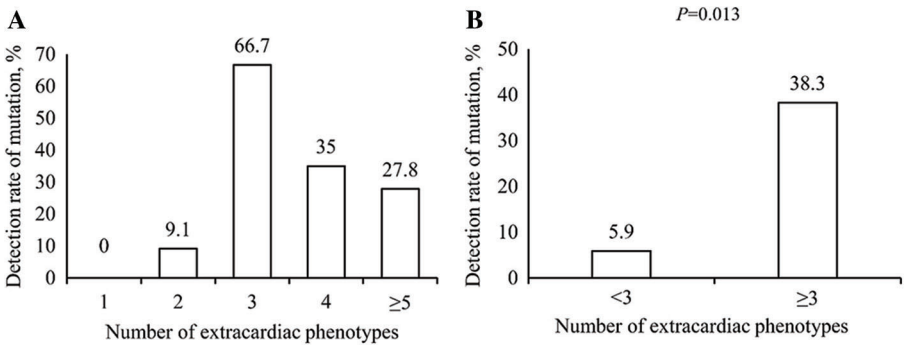
(Continued)

Table 3 (continued)

ID	Sex	Cardiac phenotype	Extracardiac phenotype		Gene name	MIM number[a]	Diagnosis	Inheritance	Prevalence	Position/GenBank number	Nucleotide change/Protein change	Pathogenicity[c]	Genetic origin
			Details	No.									
		ASD, VSD, PAPVR	growth delay, inguinal hernia, partial scalp agenesis, skull defect										
18	F	MR	joint hyperextensibility, pectus carinatum, intermittent exotropia, long fingers	3	***MYH11***	132900	Aortic aneurysm, familial thoracic 4[b]	AD	≈10 cases	**chr16:15808850 NM_002474.2**	**c.5702G > A p. R1908 K**	VUS	father
19	F	VSD, PFO	hemivertebrae, IA, DD	3	*KMT2D*	147920	Kabuki syndrome 1	AD	1/32,000	chr12:49420645 NM_003482.3	c.15104G > A p. C5035Y	VUS	de novo

Note: [a]www.omim.org
[b]Ultra-rare diseases.
[c]As presented in the consensus statement of the ACMG (American College of Medical Genetics). (P, pathogenic; LP, likely pathogenic; VUS, variant unknown significance).
Bold: novel variant.
Abbreviations: WES, whole exome sequencing; AS, aortic stenosis; PS, pulmonary stenosis; BAV, bicuspid aortic valve; PFO, patent foramen ovale; ASD, atrial septal defect; VSD, ventricular septal defect; CoA, coarctation of aorta; DORV, double outlet right ventricle; MAPCA, major aortopulmonary collateral artery; MR, mitral regurgitation; PDA, patent ductus arteriosus; ccTGA, congenitally corrected transposition of great arteries; TOF, tetralogy of Fallot; PAPVR, partial anomalous pulmonary venous return; DD, developmental delay; IA, imperforated anus; IVH, intraventricular hemorrhage; OPH, ophthalmology; AD, autosomal dominant; AR, autosomal recessive.

The correlation of the number of phenotype items according to the extracardiac organ system was analyzed with the genetic diagnostic rate in each patient. Despite no linear correlation of the genetic diagnostic rate with the number of extracardiac phenotypes (Fig. 2A), the genetic diagnostic rate was higher in patients with three or more items in an extracardiac phenotype than in patients with two or fewer (Fig. 2B) (38.3% *vs.* 5.9%, odds ratio [OR] = 9.93, 95% confidence interval [CI] = 1.21–81.44, $P = 0.013$). No statistical comparison was noted among the patients with each number of extracardiac phenotypes owing to the small number of patients.



	Number of extracardiac phenotypes					Total
	1	2	3	4	≥5	
Patients with diagnostic variant	0 (0)	1 (9.1)	6 (66.7)	7 (35)	5 (27.8)	19
Patients without diagnostic variant	6	10	3	13	13	45
Total	6	11	9	20	18	64

Figure 2: The detection rate of a diagnostic variant according to the number of extracardiac phenotypes

Among those patients with three or more extracardiac phenotypes, common combinations of extracardiac phenotypes and their diagnostic variant detection rates are summarized in Appendix D. Each combination showed a similar detection rate compared with the overall number of patients with three or more extracardiac phenotypes.

3.5 Ultra-Rare Diseases

The genetic spectra of the patients were diverse, the details of which are described in Table 3. Some genetic diseases were common in the cohort: Noonan syndrome was found in three patients (Patients 5 and 7 with a diagnostic variant of *PTPN11*; Patient 16 with a diagnostic variant of *SHOC2*), Kabuki syndrome in two patients (Patients 11 and 19 with a diagnostic variant of *KMT2D*), and Mowat–Wilson syndrome (Patient 1 with a diagnostic variant of *ZEB2*); however, no predominant disease phenotype was identified. Meanwhile, most diseases found in the patient cohort were ultra-rare diseases (Table 3).

4 Discussion

The current study demonstrated that WES identified a monogenic defect in 29.7% of the patients with sporadic syndromic CHD and normal CMA, which is comparable to that of the previous studies (28%–29%) [12]. Considering that the previous studies included familial cases besides sporadic cases, our similar diagnostic rate in patients with purely sporadic syndromic CHD becomes more meaningful. Furthermore, our study highlights the existence of extracardiac phenotypes and their quantity of items, which will enhance or improve the predictability of genetic diagnosis (patients with three or more extracardiac phenotypes, 38.3%; the others, 5.9%, $P = 0.013$). These data indicate that genomic sequencing is less

likely to detect a genetic alteration in those with isolated CHD, which is consistent with the reports of previous studies [19–21]. However, no study has yet demonstrated the association between the probability of detecting genetic diagnostic variants and the number of extracardiac phenotypes using a specific cut-off value. Thus, our results are important in guiding physicians when discussing the necessity of genomic sequencing and the likelihood of finding a genetic alteration in patients or their guardians.

The results of our study are consistent with several recent reports, indicating that genomic sequencing techniques, such as WES or whole-genome sequencing, would be recommended for the genetic diagnosis of patients with CHD, either familial or sporadic, with normal CMA [22,23]. However, karyotyping and CMA are recommended in general for patients with sporadic CHD as the first-tier test, which reveals genetic alterations in up to 20% of the tested patients [22–24]. Furthermore, although WES can detect CNVs through various algorithms [25], CMA is still an irreplaceable genetic test to detect CNVs. CMA has yielded a diagnostic result in 10%–25% of patients tested [2,4]. In fact, considering there were eight (8/33, 24.2%) patients with positive CMA results who were excluded from our study, WES and CMA exert synergistic effects on each other in terms of the genetic diagnosis of syndromic CHD [26]. Notably, conventional chromosome analysis and gene panel are strongly recommended if the patient shows a typical phenotype, such as Noonan and Down syndrome.

Encouraging genetic elucidation of CHD through WES can help predict and prepare for long-term disease progression. Confirming genetic diagnosis allows the identification of individuals at higher risk of cardiac comorbidities, such as heart failure or arrhythmias, who will benefit from early screening and intervention [27–29]. Preemptive surveillance and intervention for extracardiac manifestations can also promote clinical progress. For example, genetic confirmation of ciliopathy, which decreases mucociliary clearance and causes postoperative respiratory complications [30], can help physicians prepare for augmented respiratory support. Screening and early intervention for neurodevelopmental delay can also contribute to the long-term quality of life for patients [31,32]. Additionally, screening of the families of identified patients for disease is possible [27].

Although most variants were de novo and consistent with those of a previous large cohort study [20], diagnostic variants inherited from asymptomatic parents were detected. These variants are indicative of the confounding genetic inquiry into the genetic diversity of human patients and the heterogeneity associated with CHD [2–5]. In our study, two likely pathogenic, protein-truncating variants were inherited from an asymptomatic parent (Patients 8 and 12). Such reduced penetrance is poorly understood; however, it is hypothetically associated with the genomic context, maternal–fetal environment [33], cardiac biomechanics [34], cell history, microenvironment, cellular states in situ, and other unknown factors impacting the clinical consequences of variants [35–37]. Further additional genomic and clinical data must be accumulated to obtain a more appropriate solution for factors affecting penetrance.

This study had some limitations. The small number of patients caused difficulty in performing a subgroup analysis by phenotype. A previous study has reported that specific phenotypes are associated with a genetic mutation in patients with CHD [21,38]. However, no statistically significant phenotype was noted in our results as detected in those studies. Similarly, despite noting a significantly higher likelihood of diagnostic variants in patients with three or more extracardiac phenotypes, no linear association between the number of extracardiac phenotypes and the detection rate of diagnostic variants was observed. Moreover, structural extracardiac anomalies, such as brain and genitourinary anomalies, would be missed unless appropriate imaging studies have been performed. Phenotypic evaluation was conducted when CHD was detected, and WES was performed; therefore, considering phenotypic progression according to a patient's age, serial reassessment of a patient's phenotype is required to improve genetic diagnosis. Finally, despite the possibility of detecting multiple variants of different genes in a single patient [39], only a single or few genetic variants were selected as responsible for the patient's

phenotype in our study. The application of an artificial intelligence-based pipeline to evaluate genomic data may have caused the criteria for the filtration to not detect some lower impact variants.

Authorship: Study conception and design: BHL; data collection: YC, SH, Y-HK, JJY, EA-RK, EJ, BSL, and JSB; analysis and interpretation of results: GHS, MK, H-SD; draft manuscript preparation: JSP. All authors reviewed the results and approved the final version of the manuscript.

Availability of Data and Materials: The datasets generated and analyzed during the current study are not publicly available because the IRB of the Asan Medical Center (IRB Nos. 2018-0574 and 2018-0180) does not allow data to be shared with out-of-hospital facilities due to ethical consideration. However, the datasets are available upon request.

Funding Statement: This work was supported by an Institute for Information and Communications Technology Promotion (IITP) grant funded by the Korean Government (MSIT) (2018-0-00861, Intelligent SW Technology Development for Medical Data Analysis).

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

References

1. van der Bom, T., Zomer, A. C., Zwinderman, A. H., Meijboom, F. J., Bouma, B. J. et al. (2011). The changing epidemiology of congenital heart disease. *Nature Reviews Cardiology, 8(1),* 50–60. DOI 10.1038/nrcardio.2010.166.
2. Zaidi, S., Brueckner, M. (2017). Genetics and genomics of congenital heart disease. *Circulation Research, 120(6),* 923–940. DOI 10.1161/CIRCRESAHA.116.309140.
3. Blue, G. M., Kirk, E. P., Giannoulatou, E., Sholler, G. F., Dunwoodie, S. L. et al. (2017). Advances in the genetics of congenital heart disease: A clinician's guide. *Journal of the American College of Cardiology, 69(7),* 859–870. DOI 10.1016/j.jacc.2016.11.060.
4. Costain, G., Silversides, C. K., Bassett, A. S. (2016). The importance of copy number variation in congenital heart disease. *NPJ Genomic Medicine, 1,* 16031. DOI 10.1038/npjgenmed.2016.31.
5. Williams, K., Carson, J., Lo, C. (2019). Genetics of congenital heart disease. *Biomolecules, 9(12),* 879. DOI 10.3390/biom9120879.
6. Shabana, N. A., Shahid, S. U., Irfan, U. (2020). Genetic contribution to congenital heart disease (CHD). *Pediatric Cardiology, 41(1),* 12–23. DOI 10.1007/s00246-019-02271-4.
7. Saliba, A., Figueiredo, A., Baroneza, J. E., Afiune, J. Y., Pic-Taylor, A. et al. (2020). Genetic and genomics in congenital heart disease: A clinical review. *Jornal de Pediatria, 96(3),* 279–288. DOI 10.1016/j.jped.2019.07.004.
8. Gilboa, S. M., Devine, O. J., Kucik, J. E., Oster, M. E., Riehle-Colarusso, T. et al. (2016). Congenital heart defects in the United States: Estimating the magnitude of the affected population in 2010. *Circulation, 134(2),* 101–109. DOI 10.1161/CIRCULATIONAHA.115.019307.
9. Landis, B. J., Ware, S. M. (2016). The current landscape of genetic testing in cardiovascular malformations: Opportunities and challenges. *Frontiers in Cardiovascular Medicine, 3,* 22. DOI 10.3389/fcvm.2016.00022.
10. Schwarze, K., Buchanan, J., Taylor, J. C., Wordsworth, S. (2018). Are whole-exome and whole-genome sequencing approaches cost-effective: A systematic review of the literature. *Genetics in Medicine, 20(10),* 1122–1130. DOI 10.1038/gim.2017.247.
11. Ziats, M. N., Ahmad, A., Bernat, J. A., Fisher, R., Glassford, M. et al. (2020). Genotype-phenotype analysis of 523 patients by genetics evaluation and clinical exome sequencing. *Pediatric Research, 87(4),* 735–739. DOI 10.1038/s41390-019-0611-5.
12. Jin, S. C., Homsy, J., Zaidi, S., Lu, Q., Morton, S. et al. (2017). Contribution of rare inherited and *de novo* variants in 2,871 congenital heart disease probands. *Nature Genetics, 49(11),* 1593–1601. DOI 10.1038/ng.3970.

13. Egbe, A., Lee, S., Ho, D., Uppu, S., Srivastava, S. (2014). Prevalence of congenital anomalies in newborns with congenital heart disease diagnosis. *Annals of Pediatric Cardiology, 7(2),* 86–91. DOI 10.4103/0974-2069.132474.
14. Franklin, R. C. G., Béland, M. J., Colan, S. D., Walters, H. L., Aiello, V. D. et al. (2017). Nomenclature for congenital and paediatric cardiac disease: The International Paediatric and Congenital Cardiac Code (IPCCC) and the eleventh iteration of the International Classification of Diseases (ICD-11). *Cardiology in the Young, 27(10),* 1872–1938. DOI 10.1017/S1047951117002244.
15. Köhler, S., Schulz, M. H., Krawitz, P., Bauer, S., Dölken, S. et al. (2009). Clinical diagnostics in human genetics with semantic similarity searches in ontologies. *The American Journal of Human Genetics, 85(4),* 457–464. DOI 10.1016/j.ajhg.2009.09.003.
16. Köhler, S., Vasilevsky, N. A., Engelstad, M., Foster, E., McMurry, J. et al. (2017). The human phenotype ontology in 2017. *Nucleic Acids Research, 45,* D865–D876. DOI 10.1093/nar/gkw1039.
17. Seo, G. H., Kim, T., Choi, I. H., Park, J. Y., Lee, J. et al. (2020). Diagnostic yield and clinical utility of whole exome sequencing using an automated variant prioritization system, EVIDENCE. *Clinical Genetics, 98(6),* 562–570. DOI 10.1111/cge.13848.
18. Richards, S., Aziz, N., Bale, S., Bick, D., Das, S. et al. (2015). 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. *Genetics in Medicine, 17(5),* 405–424. DOI 10.1038/gim.2015.30.
19. Cowan, J. R., Ware, S. M. (2015). Genetics and genetic testing in congenital heart disease. *Clinics in Perinatology, 42(2),* 373–393. DOI 10.1016/j.clp.2015.02.009.
20. Sifrim, A., Hitz, M. P., Wilsdon, A., Breckpot, J., Turki, S. H. et al. (2016). Distinct genetic architectures for syndromic and nonsyndromic congenital heart defects identified by exome sequencing. *Nature Genetics, 48(9),* 1060–1065. DOI 10.1038/ng.3627.
21. Mone, F., Eberhardt, R. Y., Morris, R. K., Hurles, M. E., McMullan, D. J. et al. (2021). Congenital heart disease and the diagnostic yield with exome sequencing (CODE) study: Prospective cohort study and systematic review. *Ultrasound in Obstetrics & Gynecology, 57(1),* 43–51.
22. Qiao, F., Hu, P., Xu, Z. (2019). Application of next-generation sequencing for the diagnosis of fetuses with congenital heart defects. *Current Opinion in Obstetrics and Gynecology, 31(2),* 132–138.
23. Clark, M. M., Stark, Z., Farnaes, L., Tan, T. Y., White, S. M. et al. (2018). Meta-analysis of the diagnostic and clinical utility of genome and exome sequencing and chromosomal microarray in children with suspected genetic diseases. *NPJ Genomic Medicine, 3,* 16.
24. Turan, S., Asoglu, M. R., Gabbay-Benziv, R., Doyle, L., Harman, C. et al. (2018). Yield rate of chromosomal microarray analysis in fetuses with congenital heart defects. *European Journal of Obstetrics, Gynecology, and Reproductive Biology, 221,* 172–176.
25. Suzuki, H., Yamada, M., Uehara, T., Takenouchi, T., Kosaki, K. (2020). Parallel detection of single nucleotide variants and copy number variants with exome analysis: Validation in a cohort of 700 undiagnosed patients. *American Journal of Medical Genetics Part A, 182(11),* 2529–2532.
26. Hu, X., Guo, R., Guo, J., Qi, Z., Li, W. et al. (2020). Parallel tests of whole exome sequencing and copy number variant sequencing increase the diagnosis yields of rare pediatric disorders. *Frontiers in Genetics, 11,* 473.
27. de Backer, J., Bondue, A., Budts, W., Evangelista, A., Gallego, P. et al. (2020). Genetic counselling and testing in adults with congenital heart disease: A consensus document of the ESC working group of grown-up congenital heart disease, the ESC working group on aorta and peripheral vascular disease and the european society of human genetics. *European Journal of Preventive Cardiology, 27(13),* 1423–1435.
28. Fahed, A. C., Roberts, A. E., Mital, S., Lakdawala, N. K. (2014). Heart failure in congenital heart disease: A confluence of acquired and congenital. *Heart Failure Clinics, 10(1),* 219–227.
29. Theis, J. L., Zimmermann, M. T., Evans, J. M., Eckloff, B. W., Wieben, E. D. et al. (2015). Recessive MYH6 mutations in hypoplastic left heart with reduced ejection fraction. *Circulation: Cardiovascular Genetics, 8(4),* 564–571.
30. Kennedy, M. P., Omran, H., Leigh, M. W., Dell, S., Morgan, L. et al. (2007). Congenital heart disease and other heterotaxic defects in a large cohort of patients with primary ciliary dyskinesia. *Circulation, 115(22),* 2814–2821.

31. Marino, B. S., Lipkin, P. H., Newburger, J. W., Peacock, G., Gerdes, M. et al. (2012). Neurodevelopmental outcomes in children with congenital heart disease: Evaluation and management: A scientific statement from the American heart association. *Circulation, 126(9),* 1143–1172. DOI 10.1161/CIR.0b013e318265ee8a.
32. Diamond, A., Barnett, W. S., Thomas, J., Munro, S. (2007). Preschool program improves cognitive control. *Science, 318(5855),* 1387–1388. DOI 10.1126/science.1151148.
33. Zhu, H., Kartiko, S., Finnell, R. H. (2009). Importance of gene-environment interactions in the etiology of selected birth defects. *Clinical Genetics, 75(5),* 409–423. DOI 10.1111/j.1399-0004.2009.01174.x.
34. Goenezen, S., Rennie, M. Y., Rugonyi, S. (2012). Biomechanics of early cardiac development. *Biomechanics and Modeling in Mechanobiology, 11(8),* 1187–1204. DOI 10.1007/s10237-012-0414-7.
35. Fahed, A. C., Gelb, B. D., Seidman, J. G., Seidman, C. E. (2013). Genetics of congenital heart disease: The glass half empty. *Circulation Research, 112(4),* 707–720. DOI 10.1161/CIRCRESAHA.112.300853.
36. Camp, J. G., Platt, R., Treutlein, B. (2019). Mapping human cell phenotypes to genotypes with single-cell genomics. *Science, 365(6460),* 1401–1405. DOI 10.1126/science.aax6648.
37. Cooper, D. N., Krawczak, M., Polychronakos, C., Tyler-Smith, C., Kehrer-Sawatzki, H. (2013). Where genotype is not predictive of phenotype: Towards an understanding of the molecular basis of reduced penetrance in human inherited disease. *Human Genetics, 132(10),* 1077–1130. DOI 10.1007/s00439-013-1331-2.
38. Shikany, A. R., Landis, B. J., Parrott, A., Miller, E. M., Coyan, A. et al. (2020). A comprehensive clinical genetics approach to critical congenital heart disease in infancy. *The Journal of Pediatrics, 227,* 231–238.e14. DOI 10.1016/j.jpeds.2020.07.065.
39. Bales, N. D., Johnson, N. M., Judge, D. P., Murphy, A. M. (2016). Comprehensive versus targeted genetic testing in children with hypertrophic cardiomyopathy. *Pediatric Cardiology, 37(5),* 845–851. DOI 10.1007/s00246-016-1358-y.

Appendix A: List of ontology-based classification of cardiac and extracardiac phenotypes

Cardiac Phenotypes	
Ventricle or ventricular septum	Ventricular septal defect, Tetralogy of Fallot
Atrium or atrial septum	Atrial septal defect
Great artery	Patent ductus arteriosus, Coarctation of aorta, Major aortopulmonary collateral artery
VA valve	Aortic stenosis, Aortic regurgitation, Bicuspid aortic valve, Pulmonary stenosis, Pulmonary atresia
AV or VA connection	Transposition of great arteries, Congenitally corrected transposition of great arteries, Double outlet right ventricle
AV valve or AV septum	Atrioventricular septal defect, Mitral regurgitation, Mitral stenosis, Mitral valve prolapse
Mediastinal vein	Total anomalous pulmonary venous return, Partial anomalous pulmonary venous return
Extracardiac Phenotypes	
Head and neck	Micrognathia, Retrognathia, Macrocephaly, Plagiocephaly, Microcephaly, Craniosynostosis, Frontal bossing, Skull defect, Hemifacial hypoplasia, Long face, Triangular face, Round face, Malar flattening, Hypoplasia of the midface, Cleft palate, Cleft lip, Thick upper lip vermilion, Wide mouth, Dental crowding, Small mouth, Lower lip hypoplasia, High narrow palate, Long philtrum, Bifid uvula, Macroglossia, Tented philtrum, Depressed nasal bridge, Choanal stenosis, Wide nasal bridge, High nasal bridge. Bulbous nose, Anteverted nares, Pointed chin, Broad forehead, Narrow forehead, Hypotelorism, Deeply set eye, Hypertelorism, Microphthalmia, Proptosis
Nervous system	Leukoencephalopathy, Polymicrogyria, Tubulinopathy, Lissencephaly, Agenesis corpus callosum, Hypodysplasia of the corpus callosum, Arnold–Chiari malformation, Cerebellar vermis hypoplasia, Cerebellar hypoplasia, Hydrocephalus, Ventriculomegaly, Seizure, Infantile spasms, Febrile seizure, Global developmental delay
Ear	Sensorineural hearing impairment, Otitis media, Low-set ear, Prominent ear helix, Elfin ear, Thin ear helix, Small ear, Overfolded helix, Macrotia
Genitourinary system	Cryptorchidism, Penoscrotal transposition, Micropenis, Hydrocele testis, Hypospadias, Hydronephrosis, Ectopic kidney, Multicystic kidney dysplasia, Renal dysplasia, Nephrotic syndrome, Cloacal abnormality
Abdomen	Esophageal atresia, Tracheoesophageal fistula, Anal atresia, Aganglionic megacolon, Meconium ileus, Duodenal atresia, Cholestasis, Biliary atresia, Hepatomegaly, Inguinal hernia, Omphalocele, Abnormality of abdominal situs
Growth	Intrauterine growth retardation, Small for gestational age, Tall stature, Short stature

(Continued)

(continued)	
Limbs	Polydactyly, Syndactyly, Overlapping fingers, Prominent fingertip pads, Arachnodactyly, Broad toe, Big toe, Broad thumb, Clinodactyly, Absent radius, Hemihypertrophy
Skeletal system	Hemivertebrae, Scoliosis, Sacral dimple, Butterfly vertebra, Pectus excavatum. Pectus carinatum, Joint hypermobility, Camptodactyly, Arthrogryposis multiplex congenita
Respiratory system	Pleural effusion, Bronchogenic cyst, Pulmonary hypoplasia, Chronic lung disease, Congenital cystic adenomatoid malformation, Laryngomalacia, Subglottic stenosis, Tracheobronchial malacia, Congenital diaphragmatic hernia, Central apnea
Eye	Deeply set eye, Microphthalmia, Proptosis, Proptosis astigmatism, Ectopia lentis, Exotropia, Esotropia, Abnormal conjugate eye movement, Nystagmus, Setting-sun eye phenomenon, Ptosis
Integument	Skin tag, Single transverse palmar crease (simian crease), Café-au-lait spot, Hypopigmentation of the skin, Sparse scalp hair, Brittle hair
Endocrine system	Thyroiglossal cyst, Hypothyroidism, Hyperinsulinemia
Musculature	Hypotonia
Prenatal	Hydrops fetalis
Breast	Wide intermammillary distance
Cardiovascular system	Hemangioma
Metabolism and homeostasis	Lymphedema, Cystic hygroma, Lactic acidosis

Abbreviations: VA, ventriculoarterial; AV, atrioventricular.

Appendix B: Cardiac and extracardiac phenotypes of a patient without a diagnostic variant

Cardiac Phenotype	Extracardiac Phenotype
ASD, PDA	Polydactyly, Triphalangeal thumb, Camptodactyly, Undescended testis, Pleural effusion, DD
ASD	EA, TEF, IA, Hydronephrosis, Hypoplastic sacrum
ASD	DD, Ptosis, Short palpebral fissure, Flat nasal root, Prominent upper lip, Short philtrum, Prominent ears, Ventriculomegaly, Bronchogenic cyst
ASD	Fetal hydrops, Plagiocephaly, Micrognathia
ASD	DD, Growth delay, Hypertelorism, Flat nasal root, Mild facial asymmetry, Low-set ear, Thin ear helix, Micrognathia, Small labium major
ASD	DD, Polymicrogyria, Microcephaly
MAPCA, ASD	Lung hypoplasia, GN, Seizure, CCAM, Fetal hydrops
ASD	Cleft palate, DD

(Continued)

(continued)

Cardiac Phenotype	Extracardiac Phenotype
PDA, ASD	Cleft palate, Craniosynostosis, Hypoplastic corpus callosum, DD
PDA, ASD	Hydronephrosis, Cloaca anomaly, Hypothyroidism, Short stature, Microcephaly, Hypotelorism, Frontal bossing, Esotropia, Prominent ears, Overcrowded teeth, Teeth eruption
ASD	Hemifacial microsomia, Cleft lip, Ear tag
ASD, VSD, PDA	Radius agenesis, Hypospadia
ASD	Microcephaly, Ventriculomegaly, Cleft palate, Vertebral anomaly
AVSD, DORV, PS	Biliary atresia
AVSD, TGA, PA	DD, Situs ambiguous, Webbed neck, HN
CoA, ASD	Macrocephaly, Prominent forehead, Triangular face, Hypertelorism, Flat nasal root, Growth delay, Tracheobronchomalacia, Macrocytic anemia
CoA, PDA	IA, Ventriculomegaly, Cryptorchidism, Lactic acidosis, Hypotonia
CoA, VSD	DD, Hypotonia, Micrognathia, Ventriculomegaly
DORV	Vertebra anomaly, Horseshoe kidney, SNHL
DORV, VSD, PS	Cleft palate, Round face, Hypertelorism, Low-set ear, Micrognathia, Overlapping fingers, Both foot inversion, Cleft palate, DD
DORV	Central apnea, Arthrogryposis, Cryptorchidism, Hypothyroidism, Small thoracic cage, Overlapping fingers, Low-set ears, Small chin, Cryptorchidism
ASD, PDA	Polydactyly, Hypotonia, Clinodactyly, Brain vasculopathy
VSD, ASD, PDA	Corpus callosum hypoplasia, Small cerebellum, Cryptorchidism, Hydronephrosis, SNHL
VSD, ASD	Long eyelashes, Thick or arched eyebrows, Wide nasal bridge, Down slanting and vertically narrow palpebral fissures, DD
PS	Chiari malformation, Both hearing loss, Craniosynostosis, DD, Thoracolumbar scoliosis, Macrocephaly, Low-set ear, Down slanting palpebral fissure, Broad nasal root, Hypertelorism, Thick lower lip, Midfacial hypoplasia, Pectus carinatum, sparse eyebrow, Short and broad fingers, Strabismus and nystagmus, Myopic astigmatism
TOF	High-arched palate, Bulbous nose, Prominent ear, Micrognathia, DD
TOF	Low-set ear, Flat nasal bridge, Long philtrum, Thin upper lip, Large mouth, Short neck, Hypotonia, MCDK, SNHL
TOF	Bifid uvula, Broad nasal root, Small lip, Velopharyngeal insufficiency, Recurrent otitis media, SNHL
TOF, LPA interruption	Asymmetric limb, Growth delay, DD, CP, Omphalocele, Inguinal hernia, Seizure, Microcephaly
TA, ASD, VSD, PAPVR	EA, TEF, IA, Subglottic stenosis, Syndactyly
TA, AR, AS	Cleft palate, Lower lip hypoplasia, Micrognathia

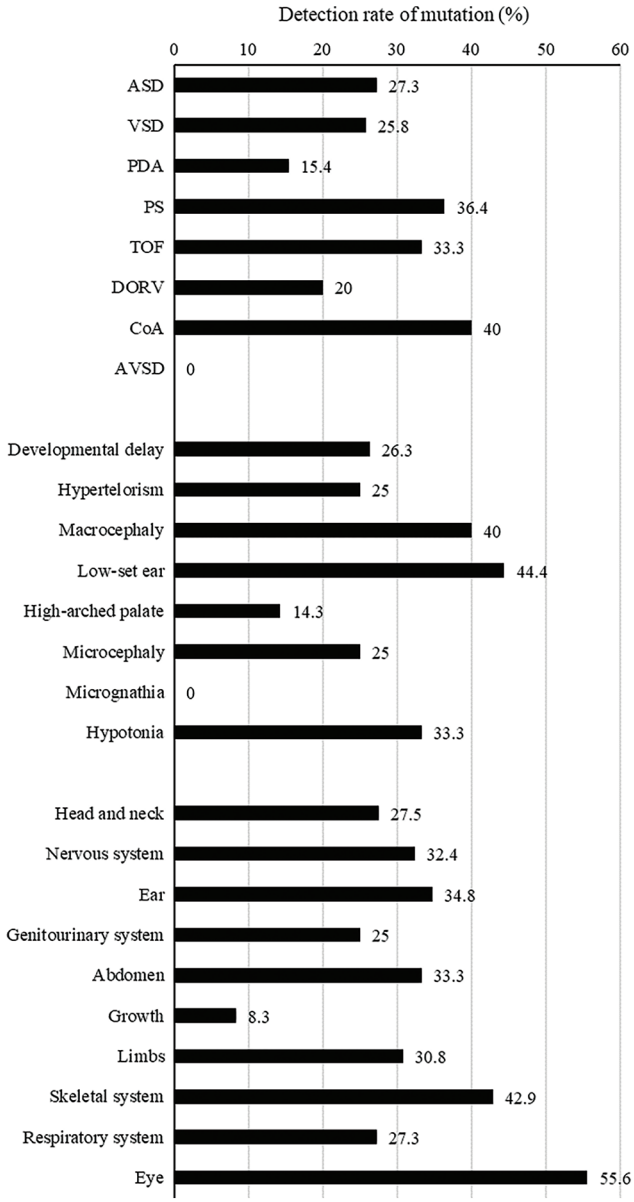
(Continued)

(continued)

Cardiac Phenotype	Extracardiac Phenotype
VSD	Hypoplastic finger, Ptosis, Prominent ears, Hypertelorism, Flat nasal root
VSD	DD, Leukomalacia, Macrocephaly, Prominent forehead, High-arched palate
VSD	Hypertelorism, Down slanting palpebral fissure, Prominent nasal root, Cleft palate, Coloboma
VSD	Hypertelorism, Blephalophimosis, High-arched palate, Bifid uvula, Micrognathia, Small lip, IUGR, DD, Hypothyroidism
VSD, TR, PS, dysmorphic TV	Hypertelorism, Downward palpebral fissure, Short neck
VSD, PDA	EA, TEF, Cystic hygroma, Eyelid fullness, Down slanting palpebral fissure, Low-set ear, Short neck, Narrow forehead, Wide spaced nipples
VSD ASD PDA BAV AS, AR	Frontal bossing, Low-set ear, Short neck, Hypertelorism, High-arched palate, Prominent philtrum, Retrognathia mild, Micropenis, Penoscrotal transposition, Hydrocele, ACC
VSD, ASD, BAV	Hypertelorism, High-arched palate, Micrognathia, Polydactyly, Deep sacral dimple
VSD PFO	Arched eyebrows, Frontal bossing, Flat nasal root, Epicanthal folds, Lateral eversion of lower eyelids, Long philtrum, Prominent ears, Sparse eyebrows, Vermis hypoplasia, DD
VSD	Lower lip palsy, Vascular ring, Vertebral anomaly
VSD, ASD	Duodenal atresia, Ectopic kidney, Inguinal hernia, Congenital hypoplasia of depressor angularis oris
VSD	CDH, Hypothyroidism, Coloboma, Hemivertebrae, IUGR, GMH, Short palpebral fissure
VSD, PS	Meconium plug, Inguinal hernia, Hypospadia, Triangular face

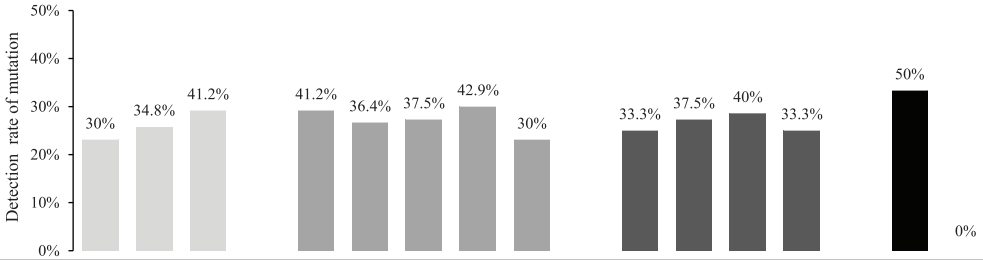
Abbreviations: ASD, atrial septal defect; PDA, patent ductus arteriosus; MAPCA, major aortopulmonary collateral artery; VSD, ventricular septal defect; AVSD, atrioventricular septal defect; PS, pulmonary stenosis; TGA, transposition of great arteries; PA, pulmonary atresia; BAV, bicuspid aortic valve; DORV, double outlet right ventricle; CoA, coarctation of aorta; TOF, tetralogy of Fallot; LPA, left pulmonary artery; TA, truncus arteriosus; PAPVR, partial anomalous pulmonary venous return; AR, aortic regurgitation; AS, aortic stenosis; TR, tricuspid regurgitation; TV, tricuspid valve; BAV, bicuspid aortic valve; DD, developmental delay; IA, imperforated anus; EA, esophageal atresia; TEF, tracheoesophageal fistula; GN, glomerulonephritis; CCAM, congenital cystic adenomatoid malformation; CLD, chronic lung disease; SGA, small for gestational age; SNHL, sensorineural hearing loss; CDH, congenital diaphragmatic hernia; MCDK, multicystic dysplastic kidney; CP, cerebral palsy; IUGR, intrauterine growth retardation; ACC, agenesis corpus callosum; GMH, germinal matrix hemorrhage.

Appendix C: Genetic diagnostic rate according to an individual cardiac or extracardiac phenotype



Abbreviations: ASD, atrial septal defect; VSD, ventricular septal defect; PDA, patent ductus arteriosus; PS, pulmonary stenosis; TOF, tetralogy of Fallot; DORV, double outlet right ventricle; CoA, coarctation of aorta; AVSD, atrioventricular septal defect.

Appendix D: The detection rate of a genetic variant according to the combination of extracardiac phenotypes among patients with three or more extracardiac phenotypes



Combinations of extracardiac phenotypes	2 systems			3 systems					4 systems				5 systems	
Head and neck	●	●		●	●		●	●	●	●	●	●	●	●
Nervous system	●		●	●	●	●	●		●	●	●	●	●	●
Ear		●	●	●		●		●	●	●	●	●	●	●
Genitourinary system					●	●		●		●				●
Limbs											●		●	
Skeletal system									●					
Eye							●					●	●	●
Endocrine system														●
patients with diagnostic variant	9	8	7	7	4	3	3	3	1	3	2	2	2	0
Total patients	30	23	17	17	11	8	7	10	3	8	5	6	4	2
Detection rate of diagnostic variant	30%	34.8%	41.2%	41.2%	36.4%	37.5%	42.9%	30%	33.3%	37.5%	40%	33.3%	50%	0%
P value	0.211	0.766	0.766	0.766	1.000	1.000	1.000	0.719	1.000	1.000	1.000	1.000	0.631	N/A