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ORIGINAL ARTICLE

Evidence-Based Assessment of Congenital Heart Disease Genes to Enable Returning Results in a Genomic Study

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BACKGROUND: Congenital heart disease (CHD) is the most common major congenital anomaly and causes significant morbidity and mortality. Epidemiologic evidence supports a role of genetics in the development of CHD. Genetic diagnoses can inform prognosis and clinical management. However, genetic testing is not standardized among individuals with CHD. We sought to develop a list of validated CHD genes using established methods and to evaluate the process of returning genetic results to research participants in a large genomic study.

METHODS: Two-hundred ninety-five candidate CHD genes were evaluated using a ClinGen framework. Sequence and copy number variants involving genes in the CHD gene list were analyzed in Pediatric Cardiac Genomics Consortium participants. Pathogenic/likely pathogenic results were confirmed on a new sample in a clinical laboratory improvement amendments-certified laboratory and disclosed to eligible participants. Adult probands and parents of probands who received results were asked to complete a post-disclosure survey.

RESULTS: A total of 99 genes had a strong or definitive clinical validity classification. Diagnostic yields for copy number variants and exome sequencing were 1.8% and 3.8%, respectively. Thirty-one probands completed clinical laboratory improvement amendments-confirmation and received results. Participants who completed postdisclosure surveys reported high personal utility and no decision regret after receiving genetic results.

CONCLUSIONS: The application of ClinGen criteria to CHD candidate genes yielded a list that can be used to interpret clinical genetic testing for CHD. Applying this gene list to one of the largest research cohorts of CHD participants provides a lower bound for the yield of genetic testing in CHD.

Key Words: hospitalizations ■ live birth ■ morbidity ■ mortality ■ survey

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Congenital heart disease (CHD) is the most common, major congenital anomaly, occurring in about 1% of all live births.[1–3] Patients with CHD are diverse in lesion severity and the presence of comorbidities; however, collectively, they experience increased morbidity and mortality compared to the general population.[4]

Epidemiologic evidence supports the role of genetic factors in CHD.[5] The risk of CHD recurrence in the offspring of an affected parent is between 3% and

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Nonstandard Abbreviations and Acronyms

CHD	congenital heart disease
CNV	copy number variant
PCGC	Pediatric Cardiac Genomics Consortium

20% depending on the lesion, and the recurrence risk in siblings is about 3% when parents are unaffected.[6,7] Identifying the underlying genetic causes of CHD has clinical utility to guide medical care, educational support, and reproductive planning for the proband and their families.[8,9] Individuals with an identifiable genetic cause for CHD experience differences in mortality, length of postoperative hospitalizations, and other associated morbidities.[10–12] Individuals with CHD report personal utility in genetic testing results for self-knowledge as well as to address causation for medicolegal liability.

Despite the importance of genetic testing, we lack a standardized testing approach for individuals with CHD, and to date, the fraction of individuals with CHD who receive genetic testing remains low.[12,13] Furthermore, genetic testing is often limited to chromosome microarray and/or karyotype. This low utilization of genetic testing for CHD can be explained in part due to the historically low diagnostic yield, especially in individuals without obvious extracardiac features.

Over 500 genes have been identified in humans and mice that are involved in cardiac development, and variants in many of these genes may contribute to the risk of CHD.[8,14–19] As genomic research in CHD advances, evidence is accumulating to identify CHD-related genes, especially in individuals with isolated CHD. However, many of these new genes have only case reports or limited functional data to support their association with CHD. We sought to systematically and broadly evaluate CHD candidate genes in which predicted pathogenic human variants have been identified. We independently applied the ClinGen framework to create a list of genes likely to be associated with CHD to advance the utility and application of clinical genetic testing for patients with CHD.[20]

The Pediatric Cardiac Genomics Consortium (PCGC) was established in 2010 to accelerate the discovery of genetic etiologies of CHD and over 13 000 probands have been enrolled to date.[5] When the PCGC was launched, the standard was not to return individual genetic research results, and only one clinical site obtained consent at the time of enrollment to return clinically relevant genetic results. Thus, most participants across the consortium were not enrolled with the intention of returning genetic research results. With improved knowledge of genetic risks for CHD as well as a paradigm shift in the practice of returning individual research results, it became increasingly important to the PCGC to return individual genetic results to participants.[21] The PCGC, therefore, established a working group to develop a list of genes with strong evidence supporting association with CHD and then identify PCGC participants with likely pathogenic/pathogenic variants according to American College of Medical Genetics criteria in those CHD genes.[22] We determined the clinical diagnostic yield of exome sequencing or chromosome microarray in a diverse group with CHD and the impact of returning results on participants.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request. Participants were consented to the Congenital Heart Disease GEne NEtwork Study of the PCGC (CHD GENES: **REGISTRATION:** URL: https://www.clinicaltrials.gov; Unique identifier: NCT01196182). All participants provided written informed consent prior to participation. A full description of the methods is available in Supplemental Material.

RESULTS

Gene List

A total of 558 genes were identified as candidate CHD genes (Table S1). After initial limited curation, 263 genes were excluded based on insufficient evidence for human CHD association (Figure 1). Of the remaining 295 genes, 75 were previously curated by a ClinGen working group for association with syndromes, not isolated CHD. After reviewing the literature for CHD association, 35 of the 75 ClinGen-curated genes (46.7%) had strong or definitive clinical validity classification for CHD and were included in the CHD gene list. The remaining 40 genes had either moderate (N=8, 10.7%) or limited/no (N=32, 42.7%) reported evidence for association with CHD (full curation results in Table S2).

Of the 295 genes that underwent full review, 220 had not been previously curated by ClinGen (Table S2). Based on our curation, 60 (27.3%) of these genes had strong or definitive clinical validity classification and were included in the CHD gene list. An additional 46 (20.9%) genes had moderate clinical validity classification and of these, 4 genes (*KDM6A, NPHP3, RAB23, ROR2*) were included in the final CHD gene list based on strong association with a syndrome characterized by CHD as well as consensus for inclusion among the Return of Results committee. The remaining 114 genes had either limited or no evidence of CHD association. In total, 99 genes were retained on the final CHD gene list, of which 18 were associated with isolated CHD and 81 were associated with syndromic CHD (Table 1).

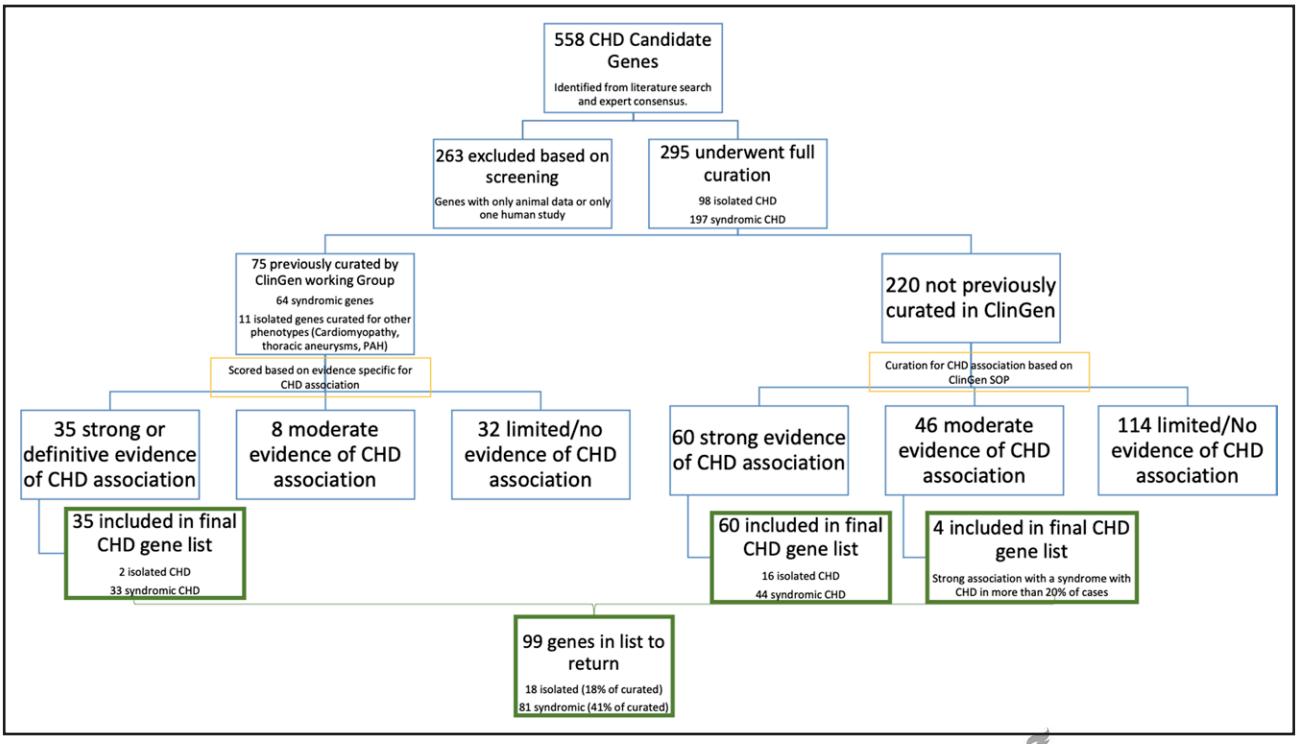

Figure 1. Candidate gene curation flowchart.
The progression of candidate gene curation is demonstrated in the below flowchart. Genes were initially filtered based on preliminary screening of the relevant literature in order to determine which required full curation. Evidence was quantified according to the ClinGen criteria and led to categorization of definitive, strong, moderate, or limited evidence of congenital heart disease (CHD) association.

Variant Curation

A total of 6767 probands had existing genomic data that was assessed for returnable results, which included 2172 probands with exome sequencing only, 2949 probands with copy number variant (CNV) analysis only, and 1646 probands with both. Demographic characteristics of ethnicity and age were representative of the PCGC cohort (Table 2).

Filtering for missense or loss-of-function de novo variants in genes from the final CHD gene list identified 142 variants in the probands of 3506 trios. Among those, 81 were classified as pathogenic or likely pathogenic based on available clinical data in the PCGC database. An additional 14 variants were initially classified as variants of unknown significance (VUSs), but after additional phenotype data were obtained from the sites, they were reclassified as likely pathogenic or pathogenic. A total of 95 de novo variants were retained for possible return of results (Table S3). There were 133 rare loss-of-function variants identified that were either inherited or identified in 312 probands without parental sequencing. After American College of Medical Genetics classification, 37 variants were classified as likely pathogenic or pathogenic (Table S4). Recessive genotypes in genes from the final CHD gene list were identified from previous PCGC publications.[14,16] There were 43 probands with 2 variants in the same gene. Each of the 2 variants were classified using American College of Medical Genetics criteria. A total of 12 probands had 2 likely pathogenic or pathogenic variants (Table S5). A total of 81 CNVs were classified as likely pathogenic or pathogenic (Table S6) from 4595 probands with CNV analysis available. Combining these variant types, a total of 225 results were classified as returnable. The CNV diagnostic yield was 1.8%, and the exome sequencing diagnostic yield was 3.8%, a combined diagnostic yield of 3.3% (Figure 2).

Obtaining Consent for Return of Results

A total of 18771 letters were mailed to elicit preferences for participants enrolled in PCGC who had not previously had the option of receiving results. The mailings yielded 2213 (12%) that were returned with participant's return of results preferences; of those returned, 2125 (96%) participants opted in to return of results (Figure S1). Additionally, 5404 of 5768 (94%) Columbia University Irving Medical Center participants had previously consented to receiving results at initial consent. Of all the probands consented to return of results, 1316 had available genomic data (Figure 2).

Results Available for Return

After matching returnable results to probands who were consented to receive results, 94 results were eligible for return. In the Columbia University Irving Medical Center cohort that opted in to return of results at the time of

Table 1. Final Congenital Heart Disease Gene List for Return

A. Gene list for isolated congenital heart disease						
Gene	**Loci**	**Mode of inheritance**	**Associated cardiac disease**	**Previously curated in ClinGen**	**Total points**	**ClinGen category**
CITED2	6q24.1	AD	ASD, VSD	No	13.5	Definitive
CRELD1	3p25.3	AD	ASD, AVSD	No	12.5	Definitive
ETS1	11q24.3	AD	ASD, VSD, aortic arch anomalies	No	13.5	Definitive
FLT4	5q35.3	AD	TOF	No	15.5	Strong
GATA4	8p23.1	AD	ASD, VSD, AVSD, PVS, TOF	No	13.5	Definitive
GATA5	20q13.33	AD/AR	ASD, BAV, DORV, TOF, VSD	No	12	Definitive
GATA6	18q11.2	AD	ASD, AVSD, TA, TOF	No	12	Definitive
HAND1	5q33.2	AD	SV, VSD	No	12	Definitive
HAND2	4q34.1	AD	PVS, TOF, VSD	No	12	Definitive
MEIS2	15q14	AD	ASD, VSD, COA	No	12.5	Definitive
MYH6	14q11.2	AD/AR	ASD, HCM, DCM, HLHS	Yes	13	Definitive
NFATC1	18q23	AD	AVSD, ASD, TA	No	13.5	Definitive
NKX2-5	5q35.1	AD	ASD, TOF, HLHS	No	12	Definitive
NR2F2	15q26.2	AD	AS, AVSD, COA, DORV, HLHS, TOF, VSD	No	16	Definitive
SMAD2	18q21.1	AD	AVSD, DEX, PVS	Yes	14	Definitive
SMAD6	15q22.31	AD	AS, BAV, COA	No	13	Definitive
TAB2	6q25.1	AD	AS, BAV, TOF	No	12	Definitive
TBX20	7p14.2	AD	ASD, DCM, MS, VSD	No	12	Definitive
B. Gene List for Syndromic Congenital Heart Disease						
Gene	**Loci**	**Mode of inheritance**	**Syndrome**	**Cardiac disease**	**Previously curated in ClinGen**	**Total points**	**ClinGen Cat**
ABCC9	12p12.1	AD	Cantu	PDA, BAV, HCM, COA, AS	Yes		Definitive
ADAMTS10	19p13.2	AR	Weill-Marchesani syndrome I	PS, AS, dysplastic valves, VSD, MR	No	13	Definitive
ARID1B	6q25	AD	Coffin Siris	ASD, AVSD, VSD, MR, PDA, PS, DEX, AS	No	12	Strong
B3GAT3	11q12.3	AR	B3GAT3 related Linkeropathies	ASD, VSD, PDA, BAV	No	13	Definitive
B3GLCT/ B3GALTL	13q12.3	AR	Peter's Plus	ASD, VSD, PS, Subvalvular AS	No	12	Definitive
BBS2	16q13	AR	Bardet-Biedl	ASD, BAV	No	14	Definitive
BBS6/MKKS	20p12.2	AR	McKusick-Kaufman Syndrome and Bardet-Biedl	AS, PS, PDA	No	13	Definitive
BCOR	Xp11.4	XLD	Microphthalmia, syndromic 2 (oculofaciocardiodental syndrome)	ASD, VSD, PDA, AS, DORV, DEX, PS	No	13.5	Definitive
BRAF	7q34	AD	Cardiofaciocutaneous (CFC), Noonan	PVS, ASD, HCM	Yes		Definitive
CCDC39	3q26.33	AR	Primary Ciliary Dyskinesia	Heterotaxy	No	16	Strong
CCDC40	17q25.3	AR	Primary Ciliary Dyskinesia	Heterotaxy	No	13.5	Strong
CDK13	7p14.1	AD	Congenital heart defects, dysmorphic facial features, and intellectual developmental disorder	ASD, VSD, PS	No	12	Strong
CHD4	12p13.31	AD	Sifrim-Hitz-Weiss syndrome	ASD, COA, TOF, VSD	No	14	Strong
CHD7	8q12	AD	CHARGE	TOF, PDA, DORV, AVSD, VSD	Yes		Definitive
CREBBP/ CBP	16p13.3	AD	Rubinstein-Taybi syndrome	ASD, BAV, COA, PDA, PS, VSD	Yes		Definitive
DHCR7	11q13.4	AR	Smith-Lemli-Opitz	AVSD, HLHS, ASD, PDA, VSD	Yes		Definitive
DLL4	15q15.1	AD	Adams-Oliver	ASD, VSD, COA, HLHS, DORV	No	13	Definitive
DNAAF1/ LRRC50	16q24.1	AR	Primary Ciliary Dyskinesia	PCD	No	12	Strong

(*Continued*)

Table 1. Continued

B. Gene List for Syndromic Congenital Heart Disease							
Gene	Loci	Mode of inheritance	Syndrome	Cardiac disease	Previously curated in ClinGen	Total points	ClinGen Cat
DNAH5	5p15.2	AR	Primary Ciliary Dyskinesia	PCD	No	14	Definitive
DNAI1	9p13.3	AR	Primary Ciliary Dyskinesia	AVSD, L-TGA	No	12.5	Definitive
DOCK6	19p13.2	AR	Adams-Oliver	ASD, VSD, COA, HLHS, DORV, TOF	No	13	Definitive
EFTUD2	17q21.31	AR	Mandibulofacial dysostosis, Guion-Almeida type	ASD, PDA, VSD	No	12	Strong
EHMT1	9q34.3	AD	Kleefstra syndrome	VSD, asymmetric aortic valve	Yes		Definitive
ELN	7q11.23	AD	Williams-Beuren	SVAS, PAS, VSD, ASD	No	14	Definitive
EOGT	3p14.1	AR	Adams-Oliver	ASD, VSD, CA, HLHS, DORV	No	14.5	Strong
EP300	22q13.2	AD	Rubinstein Taybi	PDA, VSD, ASD, HLHS, BAV	No	13	Definitive
ESCO2	8p21.1	AR	Roberts	ASD, AS	No	12	Strong
EVC	4p16.2	AR	Ellis-van Creveld	CA	Yes		Definitive
EVC2	4p16.2	AR	Ellis-van Creveld	CA	Yes		Definitive
FOXC1	6p25.3	AD	Axenfeld-Rieger syndrome, type 3	ASD, TOF	No	12	Definitive
FOXC2	16q24.1	AD	Lymphedema-distichiasis syndrome (with or without renal disease and diabetes mellitus)	PDA, TOF, VSD	No	13	Definitive
FOXF1	16q24.1	AD	Alveolar capillary dysplasia with misalignment of pulmonary veins	HLHS, ACDMVP	No	15	Definitive
G6PC3	17q21.31	AR	Dursun syndrome, congenital neutropenia	ASD, PS, PDA, cor triatum	No	13	Definitive
GDF1	19p13.11	AD/AR	Heterotaxy	DORV, PA, PS, TGA, TOF, DEX, CA	No	16.5	Definitive
HOXA1	7p15.2	AR	Bosley-Salih-Alorainy syndrome	ASD, VSD, conotruncal	Yes	17.5	Definitive
HRAS	11p15.5	AD	Costello	PS, ASD, VSD, HCM, Arrhythmias	Yes		Definitive
JAG 1	20p12.2	AD	Alagille	PPS, TOF, PA	No	17	Definitive
KANSL1	17q21.31	AD	Koolen-De Vries syndrome	ASD, BAV, PS, VSD, MRy, anomalous right subclavian artery	No	14.5	Definitive
KAT6A	8p11.21	AD	Mental retardation, autosomal dominant 32	ASD, MVP, PDA, PFO, VSD	No	15	Definitive
KAT6B	10q22.2	AD	Genitopatellar syndrome, SBBYSS syndrome	ASD, PDA, PFO, VSD	No	12.5	Strong
KDM6A	Xp11.3	XLD	Kabuki	COA, BAV, VSD, TOF, TGA, HLHS	No	11.5	Moderate
KMT2D	12q13	AD	Kabuki	COA, BAV, VSD, TOF, TGA, HLHS	No	15.5	Definitive
KRAS	12p12.1	AD	Cardiofaciocutaneous (CFC), Noonan	PVS, ASD, HCM	Yes		Definitive
MAP2K1	15q22.31	AD	Cardiofaciocutaneous (CFC), Noonan	PVS, ASD, HCM	Yes		Definitive
MAP2K2	19p13.3	AD	Cardiofaciocutaneous (CFC), Noonan	PVS, ASD, HCM	Yes		Definitive
NEK8	17q11.2	AR	Nephronopthisis, renal-hepatic-pancreatic dysplasia 2	TA, AS, PVS, HCM	No	12	Definitive
NF1	17q11.2	AD	Neurofibromatosis	ASD, CoA, PVS, VSD, MS	Yes		Definitive
NIPBL	5p13	AD	Cornelia de Lange	PVS, VSD, ASD, PDA	Yes		Definitive
NODAL	10q22.1	AD	Heterotaxy	ASD, AVSD, COA, DORV, PA, PAPVR, PDA, TAPVR, TGA, VSD, SV, CA	No	13	Definitive
NOTCH1	9q34.3	AD	Adams-Oliver, BAV	ASD, VSD, CoA, HLHS, DORV	Yes	15	Definitive
NPHP3	3q22.1	AR	Meckel syndrome 7, Nephronophthisis 3, Renal-hepatic-pancreatic dysplasia 1	AS, ASD, PDA, MR	No	11	Moderate

(*Continued*)

Table 1. Continued

B. Gene List for Syndromic Congenital Heart Disease							
Gene	Loci	Mode of inheritance	Syndrome	Cardiac disease	Previously curated in ClinGen	Total points	ClinGen Cat
NRAS	1p13.2	AD	Noonan	PVS, ASD, TOF, AVSD, HCM, VSD, PDA	Yes		Definitive
NSD1	5q35.3	AD	Sotos	ASD, PDA, VSD	Yes		Definitive
OFD1	Xp22.2	XLD/XLR	Oro-facial-digital syndrome, Simpson-Golabi-Behmel	ASD, VSD, PVS, COA, TGA, PDA, PFO	Yes		Definitive
PBX1	1q23.3	AR	Pancreatic agenesis	TOF, TA, VSD	No	13	Definitive
PKD1	16p13.3	AD	Polycystic Kidney disease	MVP, AR, TR	Yes		Definitive
PTPN11	12q24.13	AD	Noonan	PVS, ASD, TOF, AVSD, HCM, VSD, PDA	Yes		Definitive
RAB23	6p11.2	AR	Carpenter	VSD, ASD, PDA, PVS, TOF, TGA	No	7.5	Moderate
RAF1	3p25.2	AD	Noonan	PVS, ASD, TOF, AVSD, HCM, VSD, PDA	Yes		Definitive
RAI1	17p11.2 deletion	AD	Smith Magenis	ASD, AS, PVS, TOF, TAPVR	Yes		Definitive
RBM10	Xp11.3	AD	TARP syndrome	ASD, persistent left superior vena cava	No	12.5	Definitive
RECQL4	8q24.3	AR	Baller-Gerold	VSD, TOF, Subaortic disease	Yes		Definitive
RIT1	1q22	AD	Noonan	PVS, ASD, TOF, AVSD, HCM, VSD, PDA	Yes		Definitive
ROR2	9q22.31	AR	Robinow, Brachydactyly	RVOTO	No	10.5	Moderate
RPL5	1p2.1	AD	Diamond-Blackfan Anemia	ASD, VSD, AVSD, COA	No	12	strong
RPS19	19q13.2	AD	Diamond-Blackfan Anemia	ASD, VSD, AVSD, COA	No	12	Definitive
SALL1	16p12.1	AD	Townes-Brocks	ASD, TOF, VSD, TA, PA, PDA	No	13.5	Definitive
SHOC2	10q25.2	AD	Noonan	PVS, ASD, TOF, AVSD, HCM, VSD, PDA	Yes		Definitive
SMAD3	15q22.33	AD	Loeys-Dietz syndrome 3	HLHS, MVS, PDA, PVS, VSD, AI	Yes		Definitive
SMAD4	18q21.2	AD	Myhre syndrome	AS, ASD, MS, PDA, PVS, VSD, AS, COA	Yes		Definitive
SMARCA4	19p13.2	AD	Coffin Siris	ASD, AVSD, VSD, MR, PDA, PVS, DEX, AS	Yes		Definitive
SON	21q22.11	AD	ZTTK syndrome	ASD, PDA, VSD, AR	No	12.5	Definitive
SOS1	2p22.1	AD	Noonan	PVS, ASD, TOF, AVSD, HCM, VSD, PDA	Yes		Definitive
STRA6	15q24.1	AR	Microphthalmia, syndromic 9	ASD, COA, HLHS, PDA, TOF, VSD, PA, Right aortic arch	No	16.5	Definitive
TBX5	12q24.1	AD	Holt Oran	VSD, ASD, AVSD, conduction defects	No	18	Definitive
TFAP2B	6p12.3	AD	Char	PDA, VSD	No	12.5	Definitive
TGFBR1	9q22.33	AD	Loeys-Dietz syndrome 1	ASD, MVP, PDA	Yes		Definitive
TGFBR2	3p24.1	AD	Loeys-Dietz syndrome 2	ASD, BAV, MVP, PDA	Yes		Definitive
UBR1	15q15.2	AR	Johanson-Blizzard syndrome	ASD, PDA, TOF, VSD	No	12.5	Definitive
ZEB2	2q22.3	AD	Mowat Wilson	VSD, CoA, ASD, PDA, PAS	Yes		Definitive
ZIC3	Xq26.3	XLR	Heterotaxy, VACTERL association, X-linked	ASD, TGA, PVS	No	15.5	Definitive

ACDMVP indicates alveolar capillary dysplasia with misalignment of pulmonary veins; AD, autosomal dominant; AI, aortic insufficiency; AR, autosomal recessive; AS, aortic stenosis; ASD, atrial septal defect; AVSD, atrioventricular septal defect; BAV, bicuspid aortic valve; CA, common atrium; COA, coarctation of the aorta; DCM, dilated cardiomyopathy; DEX, dextrocardia; DORV, double outlet right ventricle; HCM, hypertrophic cardiomyopathy; HLHS, hypoplastic left heart syndrome; L-TGA, L-looped transposition of the great arteries; MR, Mitral regurgitation; MS, mitral stenosis; MVP, Mitral valve prolapse; PA, pulmonary atresia; PAPVR, partial anomalous pulmonary venous return; PAS, pulmonary artery stenosis; PCD, Primary ciliary dyskinesia; PDA, Patent ductus arteriosus; PPS, peripheral pulmonary stenosis; PVS, pulmonary valve stenosis; RVOTO, right ventricular outflow tract obstruction; SV, single ventricle; SVAS, supravalvar aortic stenosis; TA, truncus arteriosus; TAPVR, total anomalous pulmonary venous return; TOF, tetralogy of Fallot; TS, tricuspid stenosis; VSD, ventricular septal defect; XLD, X-linked dominant; and XLR, X-linked recessive.

Table 2. Demographic Characteristics of Probands With Genomic Data in the CHD GENES Cohort

	Probands with sequencing data		
Number	6767		
Percentage male	46%		
Enrollment status			
Trio enrollment	65%		
Isolated congenital heart disease	70%		
Existing genetic diagnosis	5%		
Age at consent in years			
Mean	6.4		
SD	13		
Percentage <1 y	33%		
Age now in years (approximated by birth year)			
Mean	19.5		
SD	12.9		
Ethnicity			
Asian	5.8%		
American Indian or Alaskan Native	0.1%		
Black	7.0%		
Native Hawaiian or other Pacific Islander	0.3%		
White	78.7%		
Mixed ethnicity	5.6%		
Information incomplete or unknown	2.5%		
Hispanic	15%		
Non-Hispanic	85%		
Education level	Maternal	Paternal	Proband
Kindergarten to 6th grade	0.9%	1.1%	1.5%
7–9th grade	1.7%	2.1%	0.6%
10–11th grade	3.1%	3.1%	0.5%
High school	18.4%	23.2%	0.5%
Partial college	21.0%	18.2%	0.4%
College graduate	25.6%	23.1%	0.4%
Post graduate degree	16.7%	14.7%	0.2%
Information incomplete, not applicable, or unknown	12.6%	14.5%	95.9%

Demographic data, including a medical record review, was conducted at the time of enrolment. CHD indicates congenital heart disease.

initial consent, 67 of the 71 variants eligible for return were found in probands who had consented to return of results. Conversely, in the cohort requiring recontact for return of results, 24 of 152 variants eligible for return were found in those re-consented and hence, eligible participants (Figure S2). Within the collective group with returnable results, 37 probands (40%) were already aware of their genetic diagnosis and 6 probands (6.3%) were deceased. Twenty probands (21%) did not follow through with return of results after initial consent due to loss to follow-up, subsequent active decline, or failure to submit samples for clinical laboratory improvement amendments-confirmation. Thirty-one probands (33%) completed clinical laboratory improvement amendments-confirmation and received their results (Figure 2). One trio was submitted for clinical laboratory improvement amendments-confirmation, but results were not returned as the expected variant was not identified; this was determined to be due to a mismatch of research identification numbers and the enrolling center informed the family that no new research results were available.

Trio confirmatory testing was performed for 29 of the 31 probands; one confirmation was completed as a duo and one as a singleton as parental samples were not provided (Table S7). All variants that were submitted as a trio or duo confirmation received a classification of likely pathogenic or pathogenic which was congruent with that of the research classification. The proband submitted as a singleton confirmation received a classification of VUS; this was determined to be incongruent with the research classification of likely pathogenic because parental samples were available in research testing but not for clinical laboratory improvement amendments-confirmation. Of the 10 probands found to have inherited autosomal dominant variants, 4 were inherited from parents affected with similar diagnoses of CHD. Of the 7 probands with inherited *FLT4* variants, 2 were inherited from an affected parent, 2 were inherited without any family history of congenital heart disease, and 3 were inherited from an unaffected parent in a family in which a sibling of the proband also had CHD; only one of these families moved forward with variant confirmation in the affected sibling (Table S7).

Postdisclosure Survey

Forty-seven participants were invited to complete an optional survey one month after receiving their results. Three parents opted out of participation, 25 did not respond to the invitation, and 19 (14 parents and 5 unique adult probands) completed the survey online. A total of 5 participants (2 adult probands and 3 parents), completed the phone interview. Demographic characteristics demonstrated a population that was mostly White and highly educated (Table S8).

The mean decision regret score about their choice to participate in the study and receive results was low at 1.5 (SD 2.6, 0–5) for parents and 5.0 (SD 8.7, 0–20) for probands. During the interview, one adult proband mentioned that learning that the cause for her CHD was de novo was "settling" for her parents, specifically her father who was "hesitant" to learn of the results for fear that he had caused her CHD.

The majority of parents and adult probands indicated that they understood the results and strongly agreed that they understood the chance of the variant being passed down in the family. For adult probands, the results were perceived to be very or extremely useful in life planning,

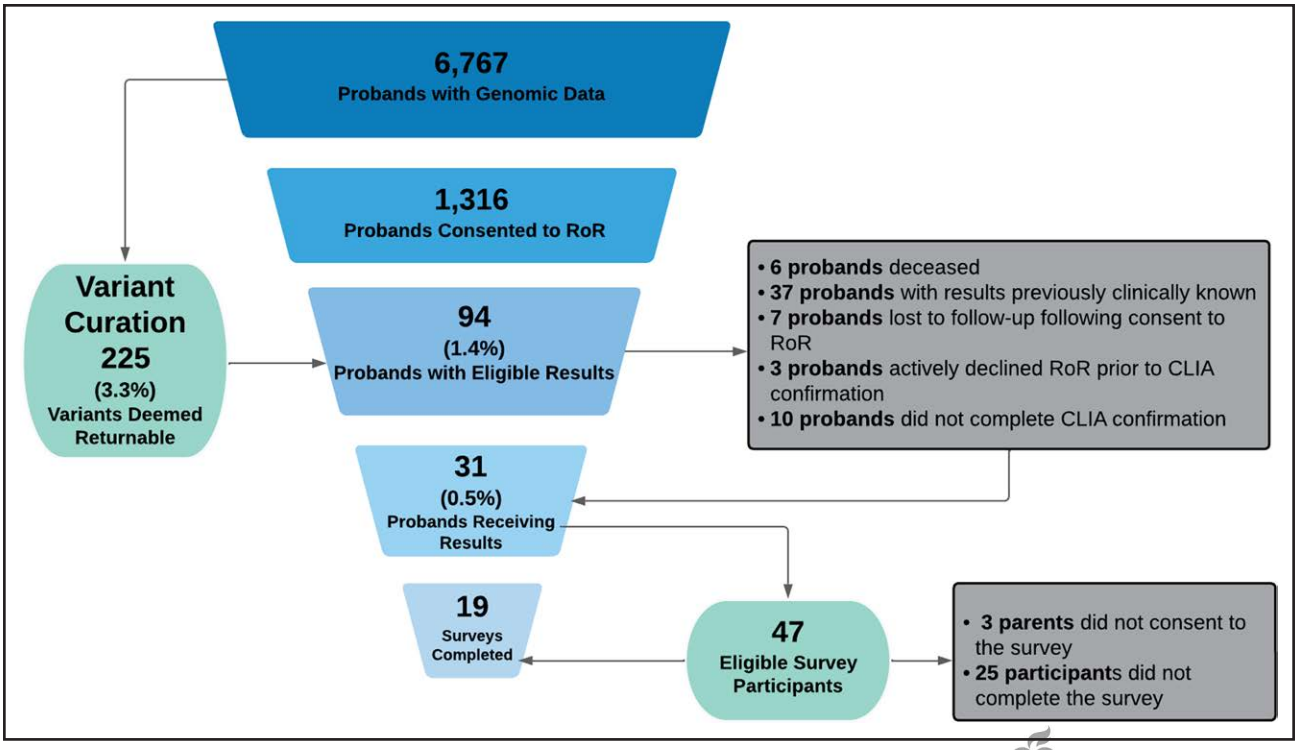

Figure 2. The sequence of returning results across the consortia.
This flowchart demonstrates the sequence of the return of results across the consortia with the corresponding number of probands eligible at each stage. Percentages demonstrate the fraction of probands that proceeded through the process. Participants who were eligible for the impact survey, including parents of probands and adult probands, are included as the final data point.

testing for a future pregnancy, and to improve their self-knowledge (Figure 3). One adult proband interviewee reported that "it was a feeling of relief" when thinking about using this information for future planning. Parents reported the results helped them to better understand their child's health, taking responsibility for their child's health, and to feel good about providing information to family members (Figure 4). An interviewed parent phrased it as, "…it just helps to have the whole picture." During the interview, another parent reported knowing her child's results reduced her feelings of shame regarding the origin of her child's CHD: "…you always wonder if somebody thought, 'Did she have a drink during her pregnancy or something like that?' So [these results helped me] to be able to just say, 'You know, I didn't do anything wrong."

DISCUSSION

Gene List

The embryologic development of the heart is an intricate process that involves complex interactions between many proteins and other molecules.[23] The genetics of CHD is complicated due to genetic heterogeneity, variable expressivity, and decreased penetrance. In recent years, the availability of exome and genome sequencing has accelerated the discovery of new CHD genes, and there are hundreds of potential candidate genes, but many of these are found in single cases or small groups of participants.[24]

Genetic testing is important for clinical care of patients with CHD. In some cases, knowledge of a genetic cause of CHD can have a significant impact on the prognosis or clinical management.[25] Our prior work has demonstrated that pathogenic CNVs and de novo variants are associated with poorer clinical outcomes.[11,26] Syndromic forms of CHD can have associated extracardiac findings and neurodevelopmental delays that may not be apparent in infancy.[26] In these cases, a genetic diagnosis can prompt appropriate screening and intervention.

Gene panels for CHD are clinically available, but there is variability in the genes included and no agreed upon list of validated CHD genes. Among 188 genes included in CHD panels in 3 diagnostic laboratories, only 16% (31 genes) are assessed by all 3 laboratories, and 72% (136 genes) are unique to individual panels. Some of this variation is due to a lack of specific ClinGen curation for most CHD genes.

Starting with a broadly inclusive list of over 500 candidate CHD genes, we identified 99 validated CHD genes. For 54 genes, there was moderate evidence with human genetic studies but limited experimental support for gene relevance to CHD. Experimental evidence from models of cardiac development is essential for evaluating

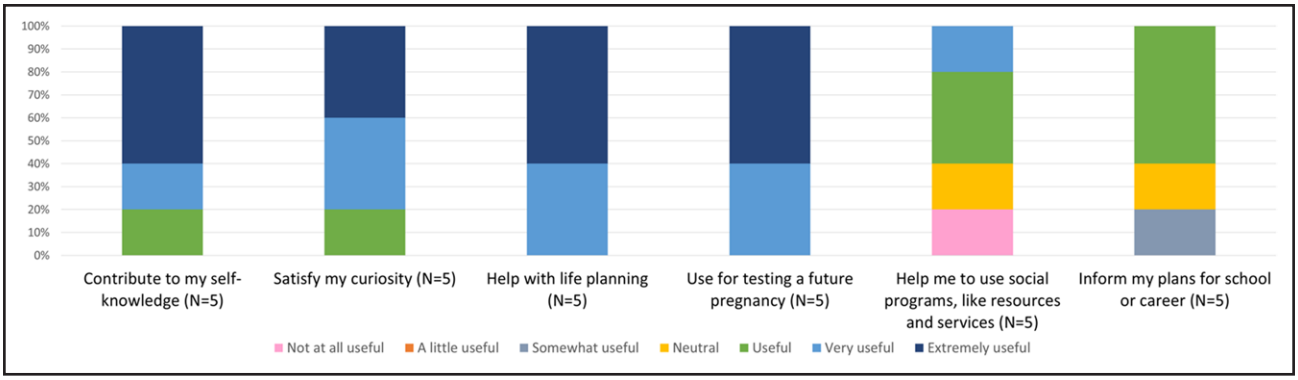

Figure 3. Utility of results for adult probands.
Results from surveys taken by probands who were adults at the time of result return regarding their perception of the utility of genetic results.

candidate CHD genes and variants, but many trait-specific issues were raised during evaluation that are unique to CHD.[12,13,27]

Given the complexities of validating genes for a diverse phenotype such as CHD, a ClinGen expert curation panel to establish a CHD-specific curation protocol and validate additional genes will be critical for the field. Evidence for many of these genes continues to accumulate so it is also important to regularly update the curation as more data become available.

Diagnostic Yield

The CNV diagnostic yield of 1.8% and the exome sequencing diagnostic yield of 3.8% was much lower than other publications which report yields as high as 30% to 40% when CNV analysis and exome/genome sequencing were performed.[8,23] Though the PCGC cohort was inclusive of all individuals with CHD, enrollment and subsequent genomic analysis were skewed toward participants who did not have a previous clinical genetic diagnosis as the aim of this investigation was to identify novel genetic contributions to CHD. Furthermore, 40% of participants with returnable results were found to have received these results clinically since the time of study enrollment; the majority of these diagnoses were well-known syndromes including Noonan, CHARGE, and Williams syndromes. Of the participants who were not aware of their eligible result, 58% had results that could have been identified by available clinical testing, such as chromosomal microarray or CHD gene panels currently in use. These results demonstrate that genetic testing remains underutilized in patients with CHD. As many CHD patients are identified prenatally or at birth with what appears to be isolated CHD, they do not raise suspicion for a genetic etiology at the time of initial evaluation. Extracardiac features, including neurodevelopmental disabilities, may only come to attention after cardiac surgery, and genetic testing may not be routinely considered if features are not severe. Opportunities for early intervention are therefore missed.

Current guidelines recommend genetic testing be offered for all fetuses diagnosed with CHD since a positive test may help identify additional anomalies and affect pregnancy management.[25,28,29] Postnatally, patients with CHD and extracardiac finding as well as those with a family history of congenital anomalies or multiple miscarriages should be offered genetic testing.[8,30] There are no clear guidelines for genetic testing in patients with isolated CHD.[31] Genetic testing will potentially have greater benefit to the patient with presumably isolated CHD and their family if performed early in life to guide medical care, educational support, and reproductive planning.

Surveyed participants expressed that knowing a genetic etiology earlier in their care could have increased personal and clinical utility. Though formal guidelines do

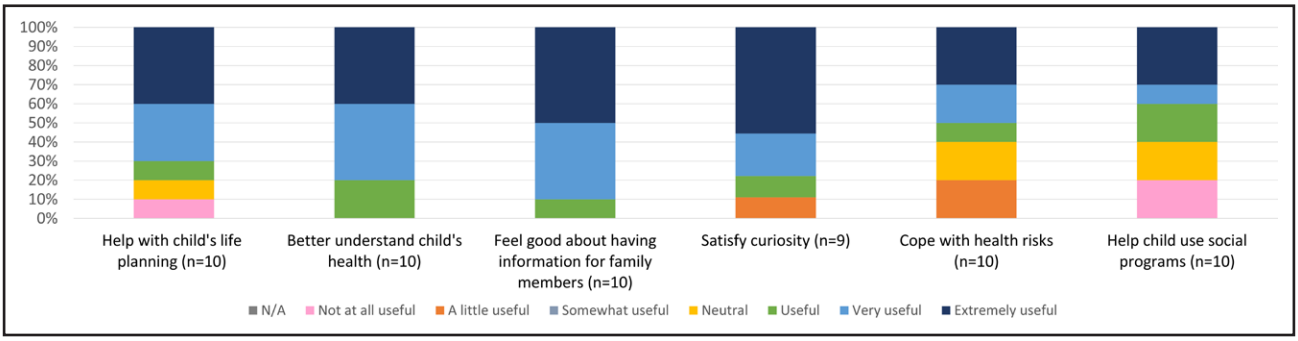

Figure 4. Utility of results for parents of probands.
Results from surveys taken by parents of probands regarding their perception of the utility of genetic results.

not yet exist, recent practice guidelines suggest that the field is moving to include exome and genome sequencing, often in addition to chromosomal microarray, as first-tier testing strategies.[23]

Best Practice for Return of Results

Though it requires immense resources in a large research study, the ability to return clinically confirmed genetic results helps translate research advancements to clinical care and is respectful of participants and their contribution to research. Participants who consented but did not have results to return communicated a strong interest and excitement for the advances in research to returning results.

The intra-consortia protocol differences demonstrated 2 critical findings in the ability to return research results: first, the importance of collecting consent for return of results at the time of original consent and second, the importance of ongoing engagement with research participants. The single site that gathered preferences/consent on return of results at the time of enrollment was able to return more results than all other 9 sites combined (Figure S2); those sites seeking consent for return of results could only communicate 15% of variants deemed returnable due to a lack of participant consent. Many participants were also lost to follow-up due to the lack of contact between consent and results availability, demonstrating the importance of consistent patient engagement in ongoing research studies. Fortunately, it is now standard practice to incorporate return of results into the study design and protocol for most genetic studies. Our results highlight the importance of maintaining contact with participants throughout the study and ensuring accurate contact information is maintained in order to return results. which may be available months or years after the study is initiated and a participant is enrolled.

Though the survey to assess the impact of the genetic diagnosis was limited, the responses suggest a modest benefit and, importantly, do not suggest harm. Though utility is likely impacted by the delay in receiving their results, participants reported these genetic results having utility in many areas of their lives, from reducing personal guilt to informing family planning and future care (Figures S3 and S4). The decision regret scale demonstrated that no harm was done as well as demonstrated less regret than other studies that have returned research results.[32,33] It is possible that regret was low because of self-selection for those opting in and because most probands already manifested the symptoms of their condition at the age they received results.

Limitations

Due to the length of time between the initial and return of results consent, we were not able to make contact with all participants, and most did not respond. As such, there is likely significant bias in our results, over-representing participants who are highly motivated to stay engaged in research. The majority of this population was White with high education levels which affects the generalizability of these findings. The interest in and the impact of these results may be skewed toward those with lower mortality from their CHD and higher educational attainment.

The COVID-19 pandemic interfered with the return of results process. The procedure for clinical confirmation was not modified by the pandemic, but many more results were reported over the phone or video than intended. From the participants' perspective, many communicated an increased accessibility to completing the confirmation process as many parent/proband trios unexpectedly had more time or were cohabitating (working remotely, home from college, furloughed from their jobs, etc). Others communicated an increased burden from the process as they had new challenges or uncertainties that may have kept them preoccupied or uninterested in completing clinical confirmation for a research study.

In conclusion, patients and families can benefit from information about the genetic etiology of their CHD. Genetic results have clinical utility and personal utility. Testing at an early age, including prenatally and neonatally, will help to clarify prognosis and identify associated features. Even when results are returned at older ages, there is high satisfaction in understanding the genetic risk of CHD and being able to use this information to inform family planning. In order to best care for children and adults with CHD, access to genetic testing should be expanded and standardized protocols developed to define the appropriate patient, test, and gene. Clinical testing relies on validated gene lists to efficiently identify genetic risk factors. This study offers an evidence-based gene list to improve the diagnostic yield of clinical testing for CHD and should be regularly reassessed as new evidence emerges.

ARTICLE INFORMATION

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Disclosures

None.

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