SUPPLEMENTAL MATERIAL FOR:

Identification of clinically actionable variants from genome sequencing of families with CHD

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TABLE OF CONTENTS

SUPPLEMENTAL METHODS

Calling and annotation of sequencing data

Kinship and gender validation of samples were performed using King[1] and Plink v1.07-x86_64[2], respectively. Bedtools v2.26[3] and Picard Tools v2.17.8 (http://broadinstitute.github.io/picard/) were used to provide quality-control checks on coverage.

Variant prioritization and analysis

A rare exonic variant is defined as one that has a minor allele frequency (MAF) less than 1% (MAF < 0.01) within the ExAC[4] and 1000 Genomes[5] databases. A predicted-damaging variant (nonsense, frameshift, splicing, or missense) is defined as having a Polyphen-2 HVAR[6] score of >0.446 and a scaled CADD[7] score of ≥15. ANNOVAR-annotated variant files, per family, were analyzed using the VarSifter tool.[8] Manual verification of variant calls was performed by visual inspection of the variant locus using the Integrative Genomics Viewer (IGV).[9] Potential disease-causal variants were evaluated according to the updated standards and guidelines for the clinical interpretation of sequence variants established by the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) (ACMG-AMP Guidelines).[10] Web based tools, InterVar[11] and Genetic Variant Interpretation Tool,[12] were used to assign concordant ACMG-AMP variant classifications. Each denomination (pathogenic, likely-pathogenic, benign, likely-benign, uncertain significance) is provided accompanied by a Roman numeral (i, ii, iii, iv, v) indicating evidence criteria used for classification. The proceeding Roman numeral in pathogenic classifications (a, b, c) relates to the strength of the evidence used to arrive at the pathogenic classification. The criteria used to determine variant classifications are provided in **Supplementary Tables S4**, **S5**, **S10**, and **S11**.

Curation and expansion of the hcCHD genes list

Curation of a dynamic high-confidence list of genes known to reproducibly cause CHD in humans (hcCHD) has been previously described.[13] In brief, genes were originally included in this list if sufficient published data supported their role in causing either isolated CHD or CHD as part of a syndrome in humans. First, searches in public repositories including NCBI MedGen (https://www.ncbi.nlm.nih.gov/medgen), ClinVar,[14] AmiGO gene ontology database,[15] ENSEMBL BioMart (http://www.ensembl.org/biomart/martview/), OMIM (https://www.omim.org/),[16] Human Phenotype Ontology Browser (http://compbio.charite.de/hpoweb/showterm?id=HP:0000118),[17] and published literature were performed to obtain a list of candidate genes from which the following stringent criteria were applied: Genes were only included in the hcCHD list if variants in the respective gene had been reported as the monogenic cause for CHD (isolated or in the context of a syndrome) in at least three independent familial or sporadic cases in at least two separate publications. In a few cases, single publications reporting multiple individual *de novo* or autosomal dominant cases with monogenic causative variants were deemed sufficient for inclusion of the respective gene. In contrast, genes were excluded whose evidence for disease causation was solely based on animal models, or genes which were only predicted to cause CHD based on expression and known protein function, or non-cardiac phenotypes resembling those of known CHD genes. Since its first iteration, comprising 90 genes, this list has been expanded to 101 genes (**Supplementary Table S2**) with the inclusion of the following 11 genes: *ADAMTS10, ANKRD11, ARID1A, ARID1B, BCOR, DOCK6, KMT2A, MESP1, SMARCA4, SMARCB1,* and *SMARCE1* (http://CHDgene.victorchang.edu.au).

hcCHD Gene Screen

In this first approach (hcCHD Gene Screen), exonic regions of affected probands were screened for rare and predicted-damaging variants within hcCHD genes **(Supplementary Tables S4** and **S5**).

Comprehensive analysis

In a second approach, unbiased analysis considering all inheritance models and genes, was performed on a per-family basis. MAF thresholds in ExAC and 1000 Genomes were set to < 0.001 (<0.1%) for autosomal dominant inheritance models, and <0.01 (1%) for autosomal recessive models. In addition, upper thresholds for the homozygous variant count in ExAC and our cohort (with respect to 338 whole genome sequenced individuals of this cohort) were set to <1 for *de novo* and autosomal dominant, <5 and <15 respectively, for autosomal recessive. The maximum number of heterozygotes present in our own cohort per variant was set to <10 for *de novo*, autosomal dominant, and compound heterozygous inheritance models. Variants in highly polymorphic genes which have notoriously high false-positive variant calls (e.g. mucins) were removed irrespective of read quality.

Regarding genes without previous association with CHD, only variants that were rare, predicted-damaging and disease-segregating, with a high intolerance for variation (Residual Variation Intolerance Score < 50%)[18] for heterozygous missense variants, and high intolerance for loss-of-function (pLI > 0.9) for heterozygous loss-of-function variants in ExAC, were considered for their role in CHD causality. To supplement association of tentative human CHD genes, a list of 1326 genes for which loss-of-function mouse models result in abnormal heart phenotypes was curated from the Mouse Genome Database (www.informatics.jax.org; search terms: "abnormal cardiovascular development" [MP:0002925] and "abnormal heart morphology" [MP:0000266]; **Supplementary Table S12**). Rare, disease-segregating, predicted-damaging variants identified in these genes were considered. Incomplete penetrance was considered in cases of loss-of-function variants, or for variants shared by multiple affected individuals per family.

Copy number variant (CNV) analysis

After initial identification of CNVs, calls were merged into a unified site list which was used to re-genotype samples. At this stage, variants that were either "LowQual" or had genotype quality less than 25 in a given sample were set to no-call ("."). Calls were once again merged into a single VCF file and germline filtering was applied with the minimum allele frequency set to 0 and the minimum genotype ratio set to 0.5. For identification of CNVs larger than 1 Mb, the data were transformed to estimated copy number by dividing by sample median depth and multiplying by two. Z-scores were calculated first for each sample and then across each bin. Bin regions with large copy number deviation and absolute z-score greater than 10 were investigated further and visually screened for CNVs.

Principal Component Analysis

Principal Component Analysis (PCA) was performed using Illumina Ancestry and Kinship Tools with reference to its wgs.hg38.vcf file, containing 32136 "reliable" bi-allelic SNPs in 1000 Genomes phase 3 with global MAF ≥ 5%. The principal components of the samples were projected onto pre-calculated components from 1000 Genomes phase 3 (hg19 coordinates converted to hg38). PCA plots using the first two principal components were created using the standard plot function in R (https://www.R-project.org).

Validation of the *NOTCH1* CNV deletion in Family 3173

50 ng of patient DNA was PCR amplified using the KAPA HiFi PCR Kit (KAPA Biosystems) according to manufacturer's instructions with primers spanning the *NOTCH1* deletion locus:

Forward primer: ccatgcatgtcctgaaccca

Reverse primer: cacgcgtgacatggatgaac

PCR conditions were as follows:

Initial Denaturation	95°C 3 min
Denaturation	98°C 20 sec
Annealing	60°C 15 sec
Extension	72°C 1 min
Repeat *Denaturation, Annealing, Extension* ×17	
Final Extension	72°C 10 min

The expected PCR product size in carriers of the 22 kb *NOTCH1* deletion was 550 bp.

SUPPLEMENTAL TABLES

Table S1. Phenotypic, ethnic, and familial relationships of the 97 genome-sequenced families in the study cohort.

Family	Individual ID	Relationship	Sex	Cardiac lesion [a]	Cardiac phenotypes	Extra-cardiac anomalies	Ethnicity [b]	Family history	Extended family history
44	44.1	Proband	F	Functional single ventricle	HLHS	Vertebral fusion defects, small dysplastic right kidney, small left kidney, left ear sensorineural hearing loss, left vocal cord palsy, sacral tethered cord with terminal lipoma/ lipomyelomeningocele, spinal dysraphism.	West Asian	Sporadic	N
44	44.4	Sibling	M						
44	44.2	Mother	F						
44	44.3	Father	M						
195 [c]	195	Proband	F	Functional single ventricle	DORV, PS, VSD, PDA, ASD, coronary artery anomaly	none	Caucasian	Familial	Y
195 [c]	619	Sibling	M	Other	MVR	none			
195 [c]	620	Sibling	M	Septal defect	VSD (spontaneous closure)	none			
195 [c]	622	Mother	F	Other	MVR (trivial)	none			
195 [c]	621	Father	M						
575	575	Proband	M	Malformation of outflow tracts	DORV-TGA, VSD, AS	Non-functioning gallbladder, gallbladder stone, severe prawn allergy	Caucasian	Familial	Y
575	3870	Sibling	M	Septal defect	VSD (spontaneous closure)	none			
575	3871	Sibling	F						
575	3868	Mother	F						
575	3869	Father	M						
742	742	Proband	F	Other	Acute dilated cardiomyopathy	none	Caucasian	Sporadic	Y

742	877	Mother	F						
742	878	Father	M						
821	821	Proband	M	Functional single ventricle	TGA, TA	Premature birth	Caucasian	Sporadic	N
821	3857	Sibling	F						
821	3858	Sibling	M		none	Palpitations			
821	3856	Sibling	F						
821	830	Mother	F						
821	829	Father	M						
835 [c]	835	Proband	F	Functional single ventricle	Dextrocardia, DORV, AVSD, PS, RAA, PDA, left SVC persisting to coronary sinus	Asplenia	Caucasian	Familial	Y
835 [c]	841	Mother	F						
835 [c]	842	Father	M	Septal defect	'Hole' in heart	none			
881	881	Proband	M	Septal defect with minor abnormalities	ASD (secundum), VSD (perimembranous)	Unknown	Caucasian	Sporadic	N
881	887	Mother	F						
881	888	Father	M						
894	894	Proband	F	Septal defect	ASD (secundum)	none	Caucasian	Familial	Y
894	895	Mother	F		none	Heart murmur, leaky valve			
894	896	Father	M						
939	939	Proband	F	Septal defect	ASD (secundum)	Esotropia, vascular tumor (removed), ADHD and oppositional defiance disorder	Caucasian	Familial	N
939	3812	Sibling	M	Septal defect	VSD (spontaneous closure)	none			
939	940	Mother	F						
939	941	Father	M						
959	959	Proband	M	Septal defect	ASD (secundum)	Unknown	Caucasian	Sporadic	N
959	960	Mother	F						
959	961	Father	M						

1071[d]	1071	Proband	F	Septal defect	ASD (secundum)	none	Caucasian	Familial	Y
1071 [d]	3835	Sibling	M	Other	ASD (secundum)/PFO	none			
1071 [d]	1072	Mother	F		none	Mild scoliosis			
1071 [d]	1073	Father	M						
1085	1085	Proband	M	Septal defect	ASD (secundum)	none	Caucasian	Sporadic	Y
1085	1086	Mother	F						
1085	1087	Father	M						
1125	1125	Proband	F	Septal defect	ASD (secundum)	Bilateral squint	Caucasian	Familial	N
1125	1126	Mother	F	Septal defect	ASD (surgically closed)	none			
1125	1127	Father	M						
1167	1167	Proband	M	Septal defect	VSD (perimembranous)	none	Caucasian	Sporadic	N
1167	1168	Mother	F		none	Nephritis, epilepsy			
1167	1169	Father	M						
1179	1179	Proband	M	Malformation of outflow tracts	TOF, PVR	Failure to thrive	Caucasian	Familial	N
1179	1180	Mother	F	Septal defect	VSD	none			
1179	1181	Father	M						
1236	1236	Proband	F	Septal defect	ASD (secundum)	none	Caucasian	Sporadic	Y
1236	1237	Mother	F						
1236	1238	Father	M						
1302	1302	Proband	F	Septal defect with minor abnormalities	VSD, PFO	none	Caucasian	Sporadic	Y
1302	1303	Mother	F						
1302	1304	Father	M						
1384	1384	Proband	M	Obstructive lesions	CoA, PDA	none	Caucasian	Sporadic	N
1384	3702	Sibling	M		none	Autism			
1384	1385	Mother	F						
1384	1386	Father	M						
1449	1449	Proband	M	Septal defect	ASD (secundum)	Cleft palate	Caucasian	Familial	Y

1449	1450	Mother	F	Septal defect	ASD (secundum, surgically closed), cardiomyopathy	Cleft palate			
1449	1451	Father	M						
1456	1456	Proband	M	AVSD and variants	AVSD AV valvar abnormality	Renal impairment, disconjugate eye movements, non-epileptic non-focal movements	Other	Familial	N
1456	1457	Mother	F	Other	MVI	none			
1456	1458	Father	M						
1658	1658	Proband	F	Obstructive lesions	AS, AAD, VSD, LV hypertrophy	none	Caucasian	Familial	N
1658	1659	Mother	F	Malformation of outflow tracts	AS	none			
1658	1660	Father	M						
1746	1746	Proband	F	Septal defect	ASD (secundum)	none	Caucasian	Sporadic	N
1746	1747	Mother	F						
1746	1748	Father	M						
1767	1767	Proband	M	Septal defect with minor abnormalities	VSD, PS, PFO, RVOTO, RV hypertrophy	Unknown	South Asian	Sporadic	N
1767	1768	Mother	F			Gestational diabetes			
1767	1769	Father	M						
1852	1852	Proband	F	Functional single ventricle	PA, VSD, HRH, PDA, PFO, TVS	Autism, chylous effusion, polyneuropathy, seasonal asthma	Caucasian	Familial	N
1852	3513	Sibling	M	Other	PFO	Hypochondriasis, mild Tourette's syndrome, ADHD, stroke			
1852	3514	Sibling	F	Malformation of outflow tracts	AS, CoA, AVR, PFO	none			
1852	1853	Mother	F						
1852	1854	Father	M						
2020	2020	Proband	F	Other	Ebstein's anomaly, PS	none	Other	Sporadic	N
2020	2021	Mother	F						

2020	2022	Father	M						
2252	2252	Proband	M	Malformation of outflow tracts	Vascular ring, DAA	none	Caucasian	Sporadic	N
2252	2253	Mother	F						
2252	2254	Father	M						
2306	2306	Proband	F	Functional single ventricle	HLHS	none	Caucasian	Sporadic	N
2306	2309	Mother	F						
2306	2310	Father	M						
2324	2324	Proband	M	Malformation of outflow tracts	TOF	anosmia	Caucasian	Sporadic	N
2324	2325	Mother	F			Gestational diabetes			
2324	2326	Father	M						
2606	2606	Proband	F	Functional single ventricle	HLHS	none	Caucasian	Sporadic	N
2606	2618	Mother	F						
2606	2619	Father	M						
2632	2632	Proband	M	Functional single ventricle	HLHS	none	Caucasian	Familial	Y
2632	1646	Sibling	F	Obstructive lesions	CoA, PDA, VSD	Severe developmental delay, seizures, agenesis of corpus callosum, Cerebral Palsy, cortical blindness, GORD			
2632	2643	Mother	F		none	Intramyocardial bridge			
2632	2644	Father	M						
2636	2636	Proband	M	Functional single ventricle	HLHS	Developmental delay, snoring, allergic rhinitis, asthma	Caucasian	Sporadic	Y
2636	2645	Mother	F						
2636	2646	Father	M						
2775	3041	Sibling	M	Septal defect with minor abnormalities	ASD (secundum), PS	none	West Asian	Familial	Y
2775	3289	Sibling	M						
2775	3040	Sibling	M	Septal defect with minor abnormalities	ASD (secundum), PS	none			

2775	3291	Sibling	F		none	Flow murmur			
2775	3282	Sibling	M	Septal defect	ASD (secundum)	none			
2775	3287	Mother	F			Gestational diabetes			
2775	3288	Father	M						
2825	2825	Proband	M	Functional single ventricle	HLHS	none	Caucasian	Sporadic	Y
2825	3685	Sibling	M						
2825	3684	Sibling	M						
2825	3686	Sibling	F						
2825	2826	Mother	F						
2825	2827	Father	M						
2831	2831	Proband	F	Malformation of outflow tracts	DORV, PVR, AVR, RV dilation	none	Caucasian	Familial	N
2831	2832	Mother	F						
2831	2833	Father	M						
2944	3255	Offspring	F	Septal defect with minor abnormalities	ASD (secundum), PS	none			
2944	3256	Offspring	M	Septal defect with minor abnormalities	ASD (secundum), PS	Left torticollis			
2944	3257	Offspring	F	Septal defect with minor abnormalities	ASD (secundum)	none			
2944	2944	Mother/proband	F	Septal defect with minor abnormalities	ASD, PS, SVT	none	Caucasian	Familial	Y
2985	2985	Proband	M	Functional single ventricle	HLHS, MVS, AVA, ASD (secundum)	none	Caucasian	Familial	N
2985	2986	Mother	F						
2985	2987	Father	M	Malformation of outflow tracts	TOF, infundibular stenosis, VSD, PFO	none			
3009	3009	Proband	F	AVSD and variants	ASD, AV canal (primum ASD)	none	Caucasian	Familial	N
3009	3625	Sibling	M		none	Arrhythmia			
3009	3626	Sibling	M						
3009	3010	Mother	F	AVSD and variants	ASD, AV canal (primum ASD)	none			
3009	3011	Father	M						

3129	3129	Proband	F	Functional single ventricle	HLHS	Bladder hematoma, right femoral deep vein thrombosis, hydrocephalus, immature development, delayed motor skills	Caucasian	Sporadic	Y
3129	3865	Sibling	F						
3129	3130	Mother	F						
3129	3131	Father	M						
3173	3173	Proband	M	Septal defect with minor abnormalities	VSD, malaligned outlet septum	Spherocytosis	Caucasian	Familial	N
3173	3925	Sibling	M	Malformation of outflow tracts	BAV	none			
3173	3920	Mother	F						
3173	3921	Father	M		none	Spherocytosis			
3191	3191	Proband	F	Functional single ventricle	HLHS, PAPVR	Unknown	Caucasian	Sporadic	N
3191	3219	Mother	F			Gestational diabetes			
3191	3220	Father	M						
3208	3208	Proband	M	Malformation of outflow tracts	TA, VSD, ASD/PFO	Unknown	Caucasian	Familial	N
3208	3587	Sibling	M	Septal defect	Hole' in heart	none			
3208	3585	Mother	F			Gestational diabetes			
3208	3586	Father	M						
3225	3225	Proband	M	Septal defect with minor abnormalities	VSD (perimembranous), PS	Hyperkalaemia, diffuse white matter disease, mild global developmental delay at 2yrs, focal weakness of left leg	Other	Familial	Y
3225	3241	Mother	F	Septal defect with minor abnormalities	ASD (secundum), VSD (perimembranous)	Gestational diabetes			
3225	3242	Father	M	Septal defect with minor abnormalities	CoA, VSD	none			
3225	3243	Grandparent	F	Septal defect	ASD	none			

3254	3254	Proband	F	Functional single ventricle	Dextrocardia, atrial situs inversus, SVC abnormality, AVSD with ventricular imbalance, Subpulmonary stenosis	Congenital asplenia, motor delay, hypotonia, feeding difficulties	Caucasian	Sporadic	N
3254	3876	Mother	F						
3254	3877	Father	M						
3320	3320	Proband	M	AVSD and variants	AVSD AV valvar abnormality	Gross motor developmental delay, contact dermatitis, anemia	Caucasian	Familial	Y
3320	3907	Sibling	F						
3320	3906	Sibling	F		none	Spontaneous fainting, asthma, eczema, hay fever			
3320	3692	Mother	F	Septal defect	ASD	none			
3320	3919	Father	M						
3370	3370	Proband	F	Septal defect with minor abnormalities	VSD, ASD	Dysmorphic features, global development delay, prominent coccyx, small lower sacral blind ending dimple, congenital hip dysplasia	Caucasian	Sporadic	Y
3370	3371	Mother	F						
3370	3372	Father	M						
3376	3376	Proband	F	Septal defect	ASD (fenestrated septum), RV dilation	none	Caucasian	Sporadic	N
3376	3377	Mother	F						
3376	3378	Father	M						
3394	3394	Proband	M	Functional single ventricle	HLHS	none	Caucasian	Sporadic	N
3394	3395	Mother	F						
3394	3396	Father	M						
3397	3397	Proband	F	Septal defect with minor abnormalities	ASD, VSD, PDA	none	Caucasian	Familial	N
3397	3398	Mother	F	Septal defect with minor abnormalities	VSD, ASD	none			

3397	3399	Father	M						
3400	3400	Proband	F	Obstructive lesions	PS, VSD, RV hypoplasia	none	Caucasian	Sporadic	N
3400	3401	Mother	F						
3400	3402	Father	M						
3424	3424	Proband	F	Malformation of outflow tracts	RVOT, TOF, VSD, RAA, abnormal left subclavian artery	Spinal scoliosis, hypokalemia	Caucasian	Sporadic	N
3424	3425	Mother	F						
3424	3426	Father	M						
3427	3427	Proband	F	Septal defect	VSD	Protein S deficiency, iron deficiency	Other	Sporadic	N
3427	3428	Mother	F						
3427	3429	Father	M						
3430	3430	Proband	F	AVSD and variants	AVSD (primum ASD), VSD	Hypokalemia	Caucasian	Sporadic	Y
3430	3431	Mother	F			Gestational diabetes			
3430	3432	Father	M						
3438	3438	Proband	F	Septal defect	ASD	Growth delay	South Asian	Sporadic	N
3438	3439	Mother	F						
3438	3440	Father	M						
3464	3464	Proband	F	AVSD and variants	AVSD (common AV junction)	none	Other	Sporadic	N
3464	3465	Mother	F						
3464	3466	Father	M						
3470	3470	Proband	M	Malformation of outflow tracts	SAM	Unknown	Caucasian	Sporadic	Y
3470	3471	Mother	F						
3470	3472	Father	M						
3473	3473	Proband	F	AVSD and variants	AVSD (common AV junction)	Growth delay	Other	Sporadic	N
3473	3474	Mother	F						
3473	3475	Father	M						

3485	3485	Proband	M	Septal defect	ASD	Spider naevus, mild developmental delay, Hypoxic Ischemic Encephalopathy (HIE), neonatal seizures, premature birth	Caucasian	Familial	Y
3485	3486	Mother	F		none	SVT			
3485	3487	Father	M						
3527	3527	Proband	F	Functional single ventricle	PA, tricuspid valve atresia, small arteriovenous malformation	none	Caucasian	Sporadic	N
3527	3528	Mother	F						
3527	3529	Father	M						
3564	3564	Proband	M	Obstructive lesions	CoA	Unknown	South Asian	Sporadic	N
3564	3565	Mother	F						
3564	3566	Father	M						
3568	3568	Proband	M	Malformation of outflow tracts	Vascular ring, DAA	Unknown	Caucasian	Sporadic	N
3568	3569	Mother	F						
3568	3570	Father	M						
3571	3571	Proband	M	Malformation of outflow tracts	CoA, subaortic ridge, BAV	Unknown	Caucasian	Sporadic	N
3571	3572	Mother	F						
3571	3573	Father	M						
3590	3590	Proband	M	Malformation of outflow tracts	AVS	none	Caucasian	Familial	Y
3590	3735	Sibling	F	Septal defect	‘Hole’ in heart	Antisocial behavior			
3590	3736	Mother	F						
3590	3661	Father	M						
3595	3595	Proband	F	Obstructive lesions	CoA, BAV, SAM	Aspergers syndrome	Caucasian	Sporadic	N
3595	3596	Mother	F						
3595	3597	Father	M						
3601	3601	Proband	M	Malformation of outflow tracts	BAV, CoA, dysplastic mitral valve	Unknown	Caucasian	Sporadic	N

3601	3602	Mother	F		none	Breast cancer			
3601	3603	Father	M						
3610	3610	Proband	M	Malformation of outflow tracts	CoA, BAV	none	Other	Sporadic	N
3610	3611	Mother	F						
3610	3612	Father	M						
3630	3630	Proband	F	Septal defect with minor abnormalities	ASD, VSD (perimembranous), MVR (mild)	Failure to thrive	Caucasian	Sporadic	Y
3630	3631	Mother	F						
3630	3632	Father	M						
3642	3642	Proband	F	Functional single ventricle	Dextrocardia, right isomerism, TGA, AVSD, PS, PVS	none	Other	Sporadic	N
3642	3643	Mother	F						
3642	3644	Father	M						
3645	3645	Proband	M	Septal defect	ASD	Unknown	South Asian	Sporadic	Y
3645	3646	Mother	F						
3645	3647	Father	M						
3648	3648	Proband	M	AVSD and variants	AVSD (primum ASD), AVR	Macrocephaly	Other	Sporadic	N
3648	3649	Mother	F						
3648	3650	Father	M						
3651	3651	Proband	M	Malformation of outflow tracts	TOF	Unknown	South Asian	Sporadic	N
3651	3652	Mother	F			Gestational diabetes			
3651	3653	Father	M						
3654	3654	Proband	M	Obstructive lesions	CoA, L-SVC, MVS	Unknown	South Asian	Sporadic	N
3654	3655	Mother	F						
3654	3656	Father	M						
3657	3657	Proband	M	Malformation of outflow tracts	TGA, VSD	Jaundice	Caucasian	Familial	N
3657	3781	Sibling	M	Septal defect	VSD (spontaneous closure)	none			

3657	3782	Sibling	F						
3657	3783	Sibling	F						
3657	3780	Mother	F						
3657	3784	Father	M						
3672	3672	Proband	M	Septal defect	VSD (perimembranous)	Unknown	Other	Sporadic	N
3672	3673	Mother	F						
3672	3674	Father	M						
3675	3675	Proband	M	Obstructive lesions	CoA	Unknown	Caucasian	Sporadic	N
3675	3676	Mother	F						
3675	3677	Father	M						
3678	3678	Proband	M	Malformation of outflow tracts	PVA	Unknown	Caucasian	Familial	Y
3678	3679	Mother	F	Malformation of outflow tracts	PVS	none			
3678	3680	Father	M						
3681	3681	Proband	F	Septal defect	VSD	Unknown	East Asian	Sporadic	N
3681	3682	Mother	F						
3681	3683	Father	M						
3695	3695	Proband	M	Malformation of outflow tracts	Subaortic stenosis, BAV, MVS, MVR, CoA	none	Caucasian	Familial	Y
3695	3739	Mother	F	Other	PFO	none			
3695	3740	Father	M						
3712	3712	Proband	F	Functional single ventricle	TGA, PA, VSD, ASD	Protein losing enteropathy	Caucasian	Sporadic	Y
3712	3713	Mother	F						
3712	3714	Father	M						
3712	3859	Paternal cousin	F	Septal defect	VSD	none			
3712	3830	Paternal uncle	M	Malformation of outflow tracts	TOF	none			
3717	3717	Proband	F	Septal defect	ASD	Failure to thrive (food aversion)	Caucasian	Sporadic	N
3717	3718	Mother	F						

3717	3719	Father	M						
3755	3755	Proband	F	Obstructive lesions	PS, PDA, PFO	Broad nasal bridge and anteverted nares	Caucasian	Familial	N
3755	3756	Sibling	M	Obstructive lesions	PS, ASD, PFO	Large fontanelle and sparse wispy hair, tight foreskin, absent moro reflex			
3755	3759	Sibling	M						
3755	3757	Mother	F						
3755	3758	Father	M						
3766	3766	Proband	M	Malformation of outflow tracts	TGA, VSD and PDA	Unknown	Caucasian	Familial	N
3766	3767	Mother	F		none	Heart murmur (diagnosed at 30yrs)			
3766	3768	Father	M						
3769	3769	Proband	M	Malformation of outflow tracts	TOF	Unknown	Caucasian	Familial	N
3769	3770	Mother	F						
3769	3771	Father	M	Malformation of outflow tracts	TOF	Unknown			
3792	3792	Proband	M	Malformation of outflow tracts	PA, IVS, RV dependent coronary artery fistula	none	Caucasian	Familial	Y
3792	3210	Sibling	M	Other	TAPVR	none			
3792	3799	Mother	F						
3792	3798	Father	M						
3843	3843	Proband	M	Malformation of outflow tracts	DORV, CoA, TGA, VSD	Sensory hearing loss, scoliosis	Caucasian	Familial	Y
3843	3846	Sibling	M	Septal defect with minor abnormalities	ASD, VSD	Scoliosis			
3843	3847	Sibling	F						
3843	3844	Mother	F						
3843	3845	Father	M						
3843	3992	Paternal grandparent	F	Malformation of outflow tracts	PAPVR, ASD	Duplex kidney, diabetes type I			
3932	3932	Proband	F	Functional single ventricle	PA, RV hypoplasia	none	Caucasian	Familial	N

3932	3965	Mother	F	Malformation of outflow tracts	PS	none			
3932	3966	Father	M						
3933	3933	Proband	F	Septal defect	ASD (secundum)	Congenital cataract glaucoma, global developmental delay, amblyopia	Caucasian	Familial	N
3933	3990	Mother	F	Other	'Hole' in heart	Glaucoma, gestational diabetes			
3933	3991	Father	M						
3953 [d]	3953	Proband	M	Malformation of outflow tracts	TOF	none	Caucasian	Sporadic	Y
3953 [d]	3956	Mother	F						
3953 [d]	3957	Father	M						
3967	3967	Proband	F	Other	TAPVR	none	Other	Sporadic	Y
3967	3968	Mother	F						
3967	3969	Father	M		none	Benign brain tumor			
3967	3970	Paternal uncle	M	Other	TAPVR, ASD/PFO, PDA	none			
38223	38223	Proband	F	Septal defect with minor abnormalities	VSD, restrictive cardiomyopathy	Congenital chylothoraces, short stature, dysmorphic features	Caucasian	Sporadic	N
38223	42837	Mother	F						
38223	43592	Father	M						

141550648	141550648	Proband	M	Septal defect	VSD	Vertebral anomalies, tracheoesophageal fistula with esophageal atresia, tracheomalacia, single transverse palmar crease of the right hand, overlapping toes on the right foot, right ear posteriorly rotated with moderate conductive hearing loss, filum terminale lipoma, prominent thoracolumbar central canal, left plagiocephaly.	West Asian	Sporadic	N
141550648	150570493	Mother	F						
141550648	150570492	Father	M						
150650086	150650086	Proband	M	Malformation of outflow tracts	TOF	Esophageal atresia, duodenal atresia, tracheoesophageal fistula, imperforate anus with mucous fistula, right multi-cystic dysplastic kidney with bilateral hydronephrosis, severe penoscrotal hypospadias, vertebral defects, bilateral fixed talipes deformities	Polynesian	Sporadic	N
150650086	152600356	Mother	F		Gestational diabetes				
150650086	152600354	Father	M						

152900216	152900216	Proband	F	Malformation of outflow tracts	DORV, pulmonary arteriosus, VSD	Right foot: 2nd 3rd toes, absent middle and distal phalanges; 4th toe, hypoplastic middle phalanges, absent distal phalanges; 5th toe, hypoplastic middle and distal phalanges. Left foot: 2nd and 3rd toes, hypoplastic middle and distal phalanges	Caucasian	Familial	N
152900216	153130510	Mother	F						
152900216	153130507	Father	M	Malformation of outflow tracts	Aortopulmonary window	Bilateral camptodactyly of third toe			
169036865	169036865	Proband	F	Malformation of outflow tracts	DORV, PAH, PS, AI	Thromboembolism	Other	Familial	Y
169036865	181678906	Sibling	F	Malformation of outflow tracts	AI/ AR, PAH	none			
169036865	181678937	Sibling	F						
169036865	181678913	Sibling	M	Malformation of outflow tracts	VSD, ASD, PAH, PS	Epilepsy, neurological symptoms (syncope, parasthesia, weakness), asthma			
169036865	181678920	Sibling	F	Malformation of outflow tracts	Left PAH	Abnormal liver function			
169036865	181678876	Mother	F						
169036865	181678890	Father	M	Septal defect	VSD	Bilateral campodactyly of 5th finger, reflux nephropathy, bilateral sensorineural hearing loss, intussusception at 6 months			
15R414475M	15R414475M	Proband	M	Septal defect with minor abnormalities	VSD, PDA	Single kidney, butterfly vertebrae, tracheoesophageal fistula, esophageal atresia, speech delay	Caucasian	Sporadic	N
15R414475M	15R416142F	Mother	F						
15R414475M	15R416148L	Father	M						

15Y05641	15Y05641	Proband	M	Malformation of outflow tracts	DORV, VSD, ASD	Esophageal atresia/trachea-esophageal fistula, single right kidney, vertebral defects, lymphangioma of left index finger, persistently patent posterior fontanelle, gastro-esophageal reflux disease with Gastric Metaplasia, high arch palate	Caucasian	Sporadic	N
15Y05641	15Y02605	Mother	F						
15Y05641	15Y02607	Father	M						
CVM31	31.1	Proband	M	Other	PDA	Multiple thoracic AVS, anal atresia, pelvic solitary right kidney, oligohydramnios and dilated bowel loops at 18 weeks gestation	Polynesian	Sporadic	N
CVM31	31.2	Mother	F		none	Hypertension at 30 weeks gestation			
CVM31	31.3	Father	M						
Trio9	668	Proband	M	Malformation of outflow tracts	TOF	none	Caucasian	Sporadic	Y
Trio9	883	Mother	F						
Trio9	884	Father	M						

All relationships are described relative to the proband. [a] Other: Includes all CHD subtypes not covered by the other categories, such as patent ductus arteriosus, total anomalous pulmonary venous return, aberrant subclavian artery, mitral valve incompetence, Ebstein's anomaly. [b] Other: Melanesian, Polynesian, South American, Caribbean; African, Mixed ethnicities. [c] 842 (father of 835) and 621 (father of 195) are paternal half-brothers. [d] 1073 (father of 1071) and 3956 (mother of 3953) are first cousins. Extended family history refers to the presence of an individual with CHD outside of the immediate family unit. AAD: ascending aorta dilation; AI: aortic incompetence; AR: aortic regurgitation; AS: aortic stenosis; ASD: atrial septal defect; AV canal: atrioventricular septal defect; AVA: aortic valve atresia; AVR: aortic valve regurgitation; AVS: aortic valve stenosis; AVSD: atrioventricular septal defect; BAV: bicuspid aortic valve; CoA: coarctation of the aorta; DAA: double aortic arch;

DORV: double outlet right ventricle; HLHS: hypoplastic left heart syndrome; HRH: hypoplastic right heart; IPS: infundibular pulmonary stenosis; IVS: intact ventricular septum; LV: left ventricle; MVI: mitral valve incompetence; MVR: mitral valve regurgitation; MVS: mitral valve stenosis; PA: pulmonary atresia; PAH: pulmonary artery hypoplasia; PAPVR: partial anomalous pulmonary venous return; PDA: persistent ductus arteriosus; PFO: patent foramen ovale; PS: pulmonary stenosis; PVA: pulmonary valve atresia; PVR: pulmonary valve regurgitation; PVS: pulmonary valve stenosis; RAA: right aortic arch; RV: right ventricle; RVD: right ventricular dilation; RVOTO: right ventricular outflow tract obstruction; SAM: subaortic membrane; SVC: superior vena cava; SVT: supraventricular tachycardia; TA: truncus arteriosus; TAPVR: total anomalous pulmonary venous return; TGA: transposition of the great arteries; TOF: Tetralogy of Fallot; TVS: tricuspid valve stenosis; VSD: ventricular septal defect.

Table S2. High-confidence CHD (hcCHD) genes list.

Gene[a]	HGNC Gene ID[b]	Human CHD phenotype[c]	Syndrome with associated CHD[d]	Animal models[e]	References
ACTC1	143	ASD, VSD	–	Homozygous null mouse has CHD	19-21
ACVR1	171	ASD, AVSD, DORV, TGA	–	Mouse with endothelial-specific conditional deletion has CHD	22,23
ACVR2B	174	ASD, AVSD, CAVC, DORV, MA, PA, PS, TAPVR, TGA, VSD, right-sided aortic arch, common atrium	Heterotaxy	Homozygous null mouse has CHD	24,25
ADAMTS10	13201	PS, aortic stenosis, dysplastic valves, VSD, mitral regurgitation	Weill-Marchesani syndrome 1, recessive (277600, AR)	MGI: no cardiovascular defect recorded	26-30
ANKRD11	21316	AS, MS, mitral regurgitation, VSD, AVSD	KBG syndrome (148050, AD)	MGI: no cardiovascular defect recorded	31-33
ARID1A	11110	CoA, ASD, VSD, AS	Coffin-Siris syndrome 2 (614607, AD)	Mice with a homozygous single point mutation (at pos. c.3203, p.V1068G) have CHD. Two different conditional transgenic mice have CHD	34
ARID1B	18040	Mitral insufficiency, PFO, ASD, AVSD	Coffin-Siris syndrome 1 (135900, AD)	MGI: no cardiovascular defect recorded	35-38
BCOR	20893	ASD, VSD, PDA, tricuspid valve insufficiency, pentalogy of Fallot, aortic valve stenosis, DORV, dextrocardia, pulmonary valve stenosis, floppy mitral valve	Microphthalmia, syndromic 2 (300166, XLD)	MGI: hemizygous male mice have CHD (heart looping defects)	39,40
BMPR2	1078	ASD, PAPVR, PDA, TGA, VSD, atrioventricular canal	–	Mice with homozygous single-base mutation or homozygous deletion of exon 2 have CHD	41,42
BRAF	1097	ASD, BAV, PS, mitral valve anomaly	Cardiofaciocutaneous syndrome (115150, AD), LEOPARD syndrome 3 (613707, AD), Noonan syndrome 7 (613706, AD)	Homozygous null mouse has CHD	43-46
CDK13	1733	ASD, VSD, outflow tract defects	Congenital heart defects, dysmorphic facial features, and intellectual developmental disorder (617360, AD)	no cardiovascular defects reported	47,48
CFC1	18292	AVSD, DORV, IAA, TGA, TOF, tricuspid atresia,	Heterotaxy	Mice with homozygous single-base mutations or homozygous null allele have CHD	49-52
CHD4	1919	ASD, CoA, TOF, VSD	Sifrim-Hitz-Weiss syndrome (617159, AD)	no cardiovascular defects reported	48
CHD7	20626	ASD, aberrant supraclavicular artery	CHARGE syndrome (214800, AD)	Mice with heterozygous stopgain mutation or heterozygous null allele have CHD	53-55
CITED2	1987	ASD, VSD	–	Heterozygous and homozygous null mice have CHD	56-58
CREBBP	2348	ASD, BAV, CoA, PDA, PS, VSD	Rubinstein-Taybi syndrome (180849, AD)	Heterozygous null mouse has CHD	59-63

CRELD1	14630	AVSD, dextrocardia	–	no cardiovascular defects reported	64,65
DOCK6	19189	PFO, VSD	Adams-Oliver syndrome 2 (614219, AR)	MGI: no cardiovascular defect recorded	66,67
EFTUD2	30858	ASD, PDA, VSD	Mandibulofacial dysostosis, Guion-Almeida type (610536, AD)	no cardiovascular defects reported	68,69
EHMT1	24650	VSD, asymmetric aortic valve	Kleefstra syndrome (610253, AD)	Mouse with cardiomyocyte-specific conditional knockout has CHD	70,71
ELN	3327	ASD, supravalvular aortic stenosis	–	Heterozygous and homozygous null mice have CHD	72,73
EVC	3497	ASD, PS, TGA, VSD, common atrium, atrioventricular canal defect	Ellis-van Creveld syndrome (225500, AR), Weyers acrodental dysostosis (193530, AD)	no cardiovascular defects reported	74-77
EVC2	19747	ASD, PS, TGA, VSD, common atrium, atrioventricular canal defect	Ellis-van Creveld syndrome (225500, AR), Weyers acrodental dysostosis (193530, AD)	no cardiovascular defects reported	74-77
FBN1	3603	AS, MVP, Mitral insufficiency, tricuspid stenosis, aortic valve insufficiency	Marfan syndrome (154700, AD)	Heterozygous and homozygous null mice have CHD	78,79
FLNA	3754	ASD, CoA, DORV, HLV, MA, PDA, valve insufficiency, mono-atrium	–	Female mice heterozygous and male mice hemizygous for a single-base mutation or null allele have CHD	80-82
FOXC1	3800	ASD, TOF	Axenfeld-Rieger syndrome, type 3 (602482, AD)	Heterozygous and homozygous null mice have CHD	83-85
FOXC2	3801	PDA, TOF, VSD	Lymphedema-distichiasis syndrome (with or without renal disease and diabetes mellitus) (153400, AD)	Homozygous null mouse has CHD	86,87
FOXH1	3814	TGA, TOF	–	Mice homozygous for a single point mutation or null allele have CHD	52,88
GATA4	4173	ASD, AVSD, PAPVR, PS, TOF, VSD	–	Homozygous null mouse has CHD, mice homozygous or heterozygous for single-base mutations have CHD	89-92
GATA5	15802	ASD, BAV, TOF, VSD	–	Homozygous null mouse has CHD	93-95
GATA6	4174	ASD, PDA, PS, PTA, TOF	–	Mouse with conditional deletion has CHD	96-99
GDF1	4214	DORV, MAPCAs, PA, PS, TGA, TOF, dextrocardia, common atrium,	Heterotaxy	Homozygous null mouse has CHD	100,101
GJA1	4274	HLHS, PA, VSD, right ventricular hypoplasia, tricuspid stenosis	Oculodentodigital dysplasia (164200, AD), heterotaxy	Homozygous null mouse has CHD	102,103
GPC3	4451	ASD, PDA, PFO, VSD, tricuspid valve hypoplasia, thickened pulmonary valve, hypoplastic left pulmonary artery	Simpson-Golabi-Behmel syndrome, type 1 (312870, XLR)	Male mice with hemizygous deletion have CHD	104-106
HAND1	4807	Hypoplastic ventricle, VSD	–	Heterozygous and homozygous null mice	107-109

				have CHD	
HAND2	4808	PS, TOF, VSD	–	Homozygous null mouse has CHD	110,111
HRAS	5173	ASD, BAV, MVS, PS	Costello syndrome (218040, AD)	Mouse with homozygous single-base mutation has CHD	46,112,113
INVS	17870	PFO, PVS, VSD, mitral insufficiency	Nephronophthisis 2, infantile (602088, AR)	Homozygous null mouse has CHD	114,115
JAG1	6188	PS, TOF, VSD, aortic dextroposition	Alagille syndrome 1 (118450, AD)	Mouse with conditional endothelial-specific deletion has CHD	116,117
KANSL1	24565	ASD, BAV, PFO, PS, VSD, mitral insufficiency, anomalous right subclavian artery	Koolen-De Vries syndrome (610443, AD)	no cardiovascular defects reported	118,119
KAT6A	13013	ASD, MVP, PDA, PFO, VSD	Mental retardation, autosomal dominant 32 (616268, AD)	Heterozygous and homozygous null mice have CHD	120-122
KAT6B	17582	ASD, PDA, PFO, VSD	Genitopatellar syndrome (606170, AD), SBBYSS syndrome (603736)	no cardiovascular defects reported	123-126
KDM6A	12637	ASD, PS, VSD, hypoplastic right ventricle	Kabuki syndrome 2 (300867, XLD)	Homozygous null and male mice with hemizygous deletion have CHD	127-129
KMT2A	7132	PDA, MVP, ASD	Leukemia, myeloid/lymphoid or mixed-lineage (159555, AD), Wiedemann-Steiner syndrome (605130, AD)	Homozygotes for targeted null mutations die at embryonic day 11.5-14.5	67,130
KMT2D	7133	ASD, BAV, CoA, VSD	Kabuki syndrome 1 (147920, AD)	Mice with conditional deletion in different cell types have CHD	131-134
KRAS	6407	ASD, PS, VSD, tricuspid valve prolapse, cleft mitral valve, dysplastic mitral valve	Noonan syndrome 3 (609942), Cardiofaciocutaneous syndrome 2 (615278)	Mice homozygous for single-base mutation or null allele have CHD	44,135,136
MAP2K1	6840	ASD, PS	Cardiofaciocutaneous syndrome 3 (615279)	Homozygous null mouse has vascular defects but no CHD	45,137,138
MAP2K2	6842	ASD, BAV, PS, PVS, pulmonary valve dysplasia	Cardiofaciocutaneous syndrome 4 (615280)	no cardiovascular defects reported	45,137,138
MED13L	22962	PFO, TGA, TOF, VSD	Mental retardation and distinctive facial features with or without cardiac defects (616789, AD)	no cardiovascular defects reported	139-142
MESP1	29658	VSD, TOF	Not linked to a disease	mouse has CHD	143,144
MYBPC3	7551	ASD, PDA, PFO, VSD, mitral valve regurgitation	–	Homozygous null mouse has CHD	145,146
MYH11	7569	PDA, aortic aneurysm	–	Homozygous null mouse has CHD	147,148
MYH6	7576	AS, ASD, PFO, TGA, VSD, tricuspid atresia	–	Mice heterozygous or homozygous for a null allele or single-base mutation have CHD	149,150
MYH7	7577	ASD, Ebstein anomaly, left ventricular	–	no cardiovascular defects reported	151-153

		noncompaction			
NF1	7765	ASD, CoA, PS, PVS, VSD, mitral valve thickening,	Neurofibromatosis-Noonan syndrome (601321, AD)	Homozygous null mouse has CHD	154-156
NIPBL	28862	ASD, MVP, VSD	Cornelia de Lange syndrome 1 (122470, AD)	Heterozygous null mouse has CHD, morpholino knockdown in zebrafish leads to CHD	157-160
NKX2-5	2488	ASD, AVSD, CoA, DORV, HLHS, IAA, TGA, TOF, VSD, Ebstein anomaly	Heterotaxy	Heterozygous and homozygous null mice have CHD, mouse homozygous for a single-base mutation has CHD	161,162
NKX2-6	32940	DORV, PTA, TOF, VSD, complex conotruncal defect	–	no cardiovascular defects reported	163-165
NODAL	7865	ASD, AVSD, CoA, DORV, PA, PAPVR, PDA, TAPVR, TGA, VSD, double-inlet left ventricle, single ventricle, single atrium	Heterotaxy	Heterozygous and homozygous null mice have CHD	166
NOTCH1	7881	AS, BAV, CoA, HLHS, LVOTO, TOF	–	Heterozygous and homozygous null mice have CHD	167,168
NOTCH2	7882	ASD, PDA, PS, TOF	Alagille syndrome 2 (610205, AD), Hajdu-Cheney syndrome, 102500, AD)	Mouse homozygous for a hypomorphic allele has CHD	169-171
NPHP3	7907	AS, ASD, PDA, dysplasia of valve cusps, mitral insufficiency	Meckel syndrome 7 (267010), Nephronophthisis 3 (604387), Renal-hepatic-pancreatic dysplasia 1 (208540)	Homozygous null mouse has CHD	115,172
NPHP4	19104	AVSD, DORV, TGA, dextrocardia	Heterotaxy	Mouse with homozygous stopgain mutation has subtle vascular defects but no CHD	173
NR2F2	7976	ASD, AVSD	–	Homozygous null mouse has CHD	174,175
NRAS	7989	PS, mitral valve dysplasia	Noonan syndrome 6 (613224, AD)	no cardiovascular defects reported	176,177
NSD1	14234	ASD, BAV, PDA, PFO, PS, VSD, Epstein anomaly	Sotos syndrome 1 (117550, AD)	no cardiovascular defects reported	178,179
PITX2	9005	ASD, DORV, TGA, TOF, VSD, mitral valve cleft, right-sided aortic arch	Axenfeld-Rieger syndrome, type 1 (180500, AD)	Homozygous null mouse has CHD	180-183
PRDM6	9350	PDA	–	Homozygous null mouse has mild CHD (thin myocardium)	184
PRKD1	9407	AVSD, TA, pulmonary valve abnormality	–	Mouse with cardiac-specific conditional deletion has CHD	48,185
PTPN11	9644	ASD, PS, mitral valve anomaly	LEOPARD syndrome 1 (151100, AD), Noonan syndrome 1 (163950, AD)	Mice heterozygous or homozygous for a single-base mutation or homozygous for a null allele have CHD	43,46,186,187
RAB23	14263	ASD, DORV, PDA, TOF	Carpenter syndrome (201000, AR)	Homozygous null mouse has CHD and	188-190

				morpholino knockdown in zebrafish leads to laterality defects	
RAD21	9811	PFO, TOF	Cornelia de Lange syndrome 4 (614701, AD)	no cardiovascular defects reported	191,192
RAF1	9829	ASD, PFO, PS, TOF, VSD, pulmonary valve dysplasia	LEOPARD syndrome 2 (611554), Noonan syndrome 5 (611553)	Mouse heterozygous for a single-base mutation has CHD	135,193,194
RIT1	10023	ASD, MVP, PDA, PS, VSD, mitral valve regurgitation	Noonan syndrome 8 (615355, AD)	No cardiovascular defects reported for mice, zebrafish injected with mutant transcripts have CHD	195-197
SALL1	10524	ASD, TA, VSD, absent pulmonary valve	Townes-Brocks syndrome (107480, AD)	No cardiovascular defects reported	198,199
SALL4	15924	PDA, TOF, VSD	Duane-radial ray syndrome (607323, AD)	Heterozygous null mouse has CHD	200-202
SF3B4	10771	AVSD, PDA, TOF	Acrofacial dysostosis 1, Nager type (154400, AD)	No cardiovascular defects reported	203-205
SHOC2	15454	ASD, PS, VSD, mitral dysplasia, tricuspid dysplasia	Noonan-like syndrome with loose anagen hair (607721, AD)	No cardiovascular defects reported	46,186,206
SMAD3	6769	HLHS, MVS, PDA, PS, VSD, aortic insufficiency,	Loeys-Dietz syndrome 3 (613795, AD)	Homozygous null mouse has subtle vascular defects but no CHD	207-209
SMAD4	6770	AS, ASD, MVS, PDA, PS, VSD, aortic valve stenosis, juxtaductal coarctation, polyvalvar dysplasia	Myhre syndrome (139210, AD)	Mouse with myocardial-specific conditional deletion has CHD	186,210
SMAD6	6772	AS, BAV, CoA, mitral valve regurgitation	–	Homozygous null mouse has CHD	211
SMARCA4	11100	VSD, PDA, MA, PA, ASD, single right ventricle	Coffin-Siris syndrome 4 (614609, AD)	Mouse homozygous for a point mutation has CHD, heterozygous null mouse has CHD	37,212
SMARCB1	11103	VSD, ASD, PS, dextrocardia	Coffin-Siris syndrome 3 (614608, AD)	Homozygous inactivation of this gene leads to peri-implantation lethality	35,37
SMARCE1	11109	ASD, dextrocardia, MS, PDA, AS, tricuspid stenosis, coronary artery anomaly	Coffin-Siris syndrome 5 (616938, AD)	Mice homozygous for a knock-out allele exhibit prenatal lethality	35,213,214
SMC1A	11111	ASD, PS, VSD	Cornelia de Lange syndrome 2 (300590, XLD)	No cardiovascular defects reported	158,215,216
SMC3	2468	AS, ASD, BAV, PDA, PS, TOF, VSD, pulmonary artery dysplasia and hypoplasia	Cornelia de Lange syndrome 3 (610759, AD)	No cardiovascular defects reported	158,217
SON	11183	ASD, PDA, VSD, aortic valve regurgitation	ZTTK syndrome (617140, AD)	No cardiovascular defects reported	218-220
SOS1	11187	ASD, PS, VSD	Noonan syndrome 4 (610733, AD)	Mice heterozygous or homozygous for a single-base mutation or homozygous for a null allele have CHD	43,46,135,186
STRA6	30650	ASD, CoA, HLHS, HLV, PDA, TOF, VSD, pulmonary trunk and pulmonary artery absence, dilated ductus arteriosus, right-sided aortic arch, dextroposed aorta	Microphthalmia, syndromic 9 (601186, AR)	Homozygous null mice have isolated cardiovascular defects, but no CHD	221-223

TAB2	17075	ASD, PDA, PS, TOF, VSD, aortic root dilatation, polyvalvular syndrome	–	Mouse with endothelial-specific conditional deletion has cardiovascular defects but no CHD	224,225
TBX1	11592	DORV, IAA, VSD	DiGeorge syndrome (188400, AD), Velocardiofacial syndrome (192430, AD)	Mice heterozygous or homozygous for single-base mutations or a null allele have CHD	226-228
TBX20	11598	ASD, CoA, DORV, HLV, MVS, PDA, PFO, VSD	–	Mice heterozygous or homozygous for a null allele have CHD	229,230
TBX5	11604	ASD, AVSD, VSD	Holt-Oram syndrome (142900, AD)	Mice heterozygous or homozygous for a null allele have CHD	231-233
TFAP2B	11743	PDA	Char syndrome (169100, AD)	Mice heterozygous or homozygous for a null allele have CHD	234-236
TGFBR1	11772	ASD, MVP, PDA	Loeys-Dietz syndrome 1 (609192, AD)	Homozygous null mouse has cardiovascular defects but no CHD	237,238
TGFBR2	11773	ASD, BAV, MVP, PDA, bicuspid pulmonary valve	Loeys-Dietz syndrome 2 (610168, AD)	Homozygous null mouse has cardiovascular defects, mouse with neural crest-specific conditional deletion has CHD	237,238
TLL1	11843	ASD, PDA, VSD	–	Mice homozygous for a single-base mutation or homozygous for a null allele have CHD	239-241
UBR1	16808	ASD, PDA, TOF, VSD	Johanson-Blizzard syndrome (243800, AR)	No cardiovascular defects reported	242-244
ZEB2	14881	ASD, PDA, PS, VSD	Mowat-Wilson syndrome (235730, AD)	Mouse with neural crest-specific conditional deletion has CHD	245-247
ZFPM2	16700	DORV, TGA, TOF	–	Mice homozygous for a single-base mutation or homozygous for a null allele have CHD	248,249
ZIC3	12874	ASD, TGA, PS	Heterotaxy, VACTERL association, X-linked (314390, XLR)	Mice heterozygous or homozygous for a null allele or homozygous for a single-base mutation have CHD	250-252

A list of genes, currently totaling 101, known to reproducibly cause human CHD when mutated. [a] This list is actively updated as novel results become available and can be found at http://CHDgene.victorchang.edu.au. [b] The gene ID refers to the HUGO Gene Nomenclature Committee, which is responsible for approving unique symbols and names for human loci, including protein-coding genes, non-coding RNA genes and pseudogenes, to allow unambiguous scientific communication (http://www.genenames.org/).[253] [c] Abbreviations of structural heart defects: AS: Aortic stenosis; ASD: Atrial septal defect; AVSD: Atrioventricular septal defect; BAV: Bicuspid aortic valve; CAVC: Complete atrioventricular canal defect; CoA: Coarctation of the aorta; DORV: Double-outlet right ventricle; HLHS: Hypoplastic left heart syndrome; HLV: Hypoplastic

left ventricle: IAA: Interrupted aortic arch; LVOTO: Left ventricular outflow tract obstruction; MA: Mitral atresia; MAPCAs: Major aortopulmonary collateral arteries; MVS: Mitral valve stenosis; MVP: Mitral valve prolapse; PA: Pulmonary atresia; PAPVR: Partial anomalous pulmonary venous return; PDA: Patent ductus arteriosus; PFO: Patent foramen ovale; PS: Pulmonary stenosis; PTA: Persistent truncus arteriosus; PVS: Pulmonary valve stenosis; TA: Truncus arteriosus; TAPVR: Total anomalous pulmonary venous return; TGA: Transposition of the great arteries; TOF: Tetralogy of Fallot; VSD: Ventricular septal defect. All other abbreviations: AD, autosomal dominant; AR, autosomal recessive; CHARGE, coloboma, heart anomaly, choanal atresia, retardation, genital and ear anomalies; KBG, initials of the first three families reported with the syndrome; LEOPARD, multiple lentigines, electrocardiographic conduction abnormalities, ocular hypertelorism, pulmonic stenosis, abnormal genitalia, retardation of growth, and sensorineural deafness; SBBYSS, Say-Barber-Biesecker-Young-Simpson; VACTERL, vertebral anomalies, anal atresia, congenital cardiac disease, tracheoesophageal fistula, renal anomalies, radial dysplasia, and other limb defects; XLD, X-linked dominant; XLR, X-linked recessive; ZTTK, zhu-tokita-takenouchi-kim. [d] The numbers refer to the Phenotype MIM number in the OMIM database (https://omim.org/). [e] Phenotype data was collected from the Mouse Genome Informatics database (http://www.informatics.jax.org/) and complemented with data from publications in selected cases. [f] Selected references reporting human patient cases and linking the observed congenital defects to the respective genes.

Table S3. Exonic and gene-regulatory variation in whole genome sequencing data.

		Exonic				ncRNA			Regulatory features					
	Genomic	**Exonic**	**Filtered variant types**	**MAF <0.01**	**MAF <0.001**	**Intronic**	**Exonic**	**Splicing**	**Promoter**	**Proximal enhancer**	**Distal enhancer**	**Open chromatin**	**TF binding sites**	**CTCF binding**
All variants	32882377	1016515	111353	70346	48538	1618375	92522	587	312467	680467	211273	676060	160282	1032869
hcCHD genes	263705	4997	545	398	278	0	0	0	2963	8282	2729	6473	1243	5149
Mouse Heart genes	3224971	65077	7373	4804	3215	0	0	0	30761	89268	31024	77370	12172	79733
Segregation	3507700	117788	15101	10808	7665	182076	11259	70	34282	70752	18969	69842	22552	136712
hcCHD genes	28388	591	73	54	36	0	0	0	384	954	373	643	109	559
Mouse Heart genes	304960	6614	755	559	354	0	0	0	3221	8751	2548	6738	1238	8287

Variants that fall within genomic regions (intronic, 5' and 3' UTRs, flanking regions, 1 kb upstream and downstream), regulatory regions, ncRNA (non-coding RNA) and exonic regions, are presented as total number of variants, and those that segregate with disease after removing variants that are present in > 2% of the cohort (142/143 affected individuals). Variants are further classified by genes lists (hcCHD and Mouse heart, **Supplementary Tables S2 and S12**, respectively). Variants that fall within non-exonic regions are further sub-classified into transcriptional regulatory regions, generic transcription factor (TF) binding sites or CTCF transcriptional repressor binding sites, as defined by Ensembl Regulatory Build.[254] Exonic variants are further prioritized by variant type and minor allele frequency cut-offs. Exonic variants considered in subsequent hcCHD and comprehensive analyses include: frameshifts, nonsynonymous SNVs, stopgains, splicing, and unknown/unclassified variants. All variant positions are annotated with respect to human reference genome hg38. Minor allele frequencies of exonic variants are presented with respect to ExAC, gnomAD and 1000 Genomes.

Table S4. Rare and predicted-damaging variants identified in hcCHD genes in probands with sporadic CHD.

Family ID	Gene	Model	Familial CHD	ECA	Genomic position	Variant type	Nucleotide variant	Amino acid variant	ExAC MAF	PP2 HVAR	CADD	ACMG class.	ACMG criteria	Cardiac lesion
3191	*SMAD6*	AD[a]	N	U	chr15:66703343	Frameshift deletion	NM_005585.4: c.86del	Gly29Alafs*35	0	NA	NA	P (Ic)	PP3, PM2, PVS1, BS2	FSV
3642	*INVS*	AR	N	N	chr9:100300652	Frameshift insertion	NM_014425.4: c.3182dup	Asn1061Lysfs*20	0.0002	NA	NA	P (Ic)	BS2, PVS1, PP3, PM3	FSV
3712	*GATA6*	AD[a]	N	Y	chr18:22183015	Frameshift deletion	NM_005257.5: c.1595_1596del	Pro532Hisfs*100	0	NA	NA	P (Ic)	PM2, PVS1, PP3, BS2	FSV
1384	*MYH6*	CH	N	N	chr14:23382430	Stopgain	NM_002471.3: c.5794A>T	Lys1932*	0.00001647	NA	43	P (Id)	PVS1, PP3, PP2, PM3	OL
3648	*JAG1* [255]	AD[a]	N	Y	chr20:10643807	Missense	NM_000214.2: c.2429C>T	Pro810Leu	0.000004062	0.955	34	P (II)	BS2, PS1, PP3, PS3, PP2, PM1	AVSD+
3953	*NF1*	DN	N	N	chr17:31233065	Missense	NM_000267.3: c.3560T>G	Leu1187Arg	0	0.994	27.6	LP (II)	PM2, PP3, PS2, PM1, BP1	MOT
1384	*MYH6*	CH	N	N	chr14:23404300	Missense	NM_002471.3: c.731G>A	Arg244His	0.000008237	0.994	34	LP (IV)	PP3, PP2, PM1, PM3, PM2	OL
150650086	*ACVR2B*	AD[a]	N	Y	chr3:38481448	Missense	NM_001106.3: c.1057G>T	Gly353Trp	0	0.995	34	LP (V)	PM2, BS2, PP3, PM1, PP2	MOT
150650086	*DOCK6*	AR	N	Y	chr19:11222812	Missense	NM_020812.3: c.3163G>A	Val1055Met	0.0015	0.995	33	LP (V)	PM2, PP3, PP2, PM3	MOT

821	*INVS*	AD[a]	N	U	chr9:100240090	Missense	NM_014425.4: c.646T>G	Trp216Gly	0	0.91	27.8	VUS	PM2, PP3	FSV
881	*NOTCH1*	AD[a]	N	U	chr9:136508919	Missense	NM_017617.4: c.3122G>A	Gly1041Asp	0	1	23.8	VUS	BS2, PP3, PP2	SDMA
1085	*CDK13*	AD[a]	N	N	chr7:39999498	Missense	NM_003718.4: c.2180C>T	Thr727Ile	0.001	0.998	32	VUS	PM2, PP3, PP2, BS4	SD
1085	*FLNA*	XLR	N	N	chrX:154365465	Missense	NM_001456.3: c.1451G>A	Arg484Gln	0.00004887	0.871	26.8	VUS	BS2, PM1, PP3, PP2	SD
1085	*PRDM6*	AD[a]	N	N	chr5:123170913	Missense	NM_001136239.3: c.1301A>T	Asp434Val	0.0001	0.598	23.4	VUS	BS2, PP3, PP2	SD
1167	*NOTCH1*	AD[a]	N	N	chr9:136509059	Missense	NM_017617.4: c.2982C>G	Asn994Lys	0	1	24.8	VUS	PM2, PP3, PP2	SD
1302	*FOXH1*	AD[a]	N	N	chr8:144475209	Missense	NM_003923.2: c.227G>A	Gly76Asp	0.00001716	0.997	29.7	VUS	PM1, PP3, BS2	SDMA
1767	*FLNA*	XLR	N	U	chrX:154349560	Missense	NM_001456.3: c.7534C>T	Arg2512Cys	0.0001	0.947	32	VUS	BS2, PM1, PP3, PP2	SDMA
1767	*NOTCH1*	AD[a]	N	U	chr9:136502003	Missense	NM_017617.4: c.5470C>T	Arg1824Trp	0.000008407	0.999	34	VUS	BS2, PP3, PP2	SDMA
3400	*ZEB2*	AD[a]	N	N	chr2:144399801	Missense	NM_014795.3: c.1386G>T	Lys462Asn	0.00E+00	0.597	22.6	VUS	PM2, BP1, PP3	OL
3430	*ELN*	AD[a]	N	Y	chr7:74054770	Splicing	NM_000501.3: c.1150+1G>A	NA	0.00004943	NA	23.6	VUS	BS2, PVS1, PP3	AVSD+
3438	*SON*	AD[a]	N	Y	chr21:33552633	Missense	NM_032195.2: c.3402T>G	Asp1134Glu	0	0.991	22.9	VUS	PM2, PP3, BP1	SD
3438	*SOS1*	AD[a]	N	Y	chr2:38986117	Missense	NM_005633.3: c.3709C>A	Pro1237Thr	0.00009068	0.992	23.2	VUS	BS2, PP3, PP2	SD
3470	*TBX5*	AD[a]	N	U	chr12:114399586	Missense	NM_000192.3: c.289A>G	Lys97Glu	0.000008236	0.789	24.5	VUS	PP3, BS2, PP2	MOT

3473	*MYH11*	AD[a]	N	Y	chr16:15732655	Missense	NM_022844.2: c.3560C>T	Thr1187Met	0	0.871	23.6	VUS	PM1, PM2, PP3, BS2	AVSD+
3564	*CRELD1*	AD[a]	N	U	chr3:9944574	Missense	NM_015513.4: c.1258A>G	Arg420Gly	0	0.815	22.3	VUS	PM2, PP3, PP2	OL
3568	*INVS*	AD[a]	N	U	chr9:100292689	Missense	NM_014425.4: c.2432C>G	Pro811Arg	0	0.999	24	VUS	PM2, PP3	MOT
3610	*NOTCH1*	AD[a]	N	N	chr9:136523753	Missense	NM_017617.4: c.368C>T	Thr123Met	9.51E-04	0.873	22.6	VUS	BS2, PP3, PP2	MOT
3953	*CHD7*	AD[a]	N	N	chr8:60850621	Splicing	NM_017780.3: c.5533G>A	Gly1845Arg	0.00003545	0.663	26.3	VUS	PVS1, PP3, BS2	MOT
3953	*ELN*	AD[a]	N	N	chr7:74042984	Splicing	NM_000501.3: c.326G>A	Gly109Asp	0.0004	0.999	23.1	VUS	PVS1, PP3, BS2	MOT
3953	*GATA5*	AD[a]	N	N	chr20:62464852	Missense	NM_080473.4: c.1178C>T	Ala393Val	0.000008712	0.994	33	VUS	BS2, PP3, PP2	MOT
15Y05641	*CHD4*	AD[a]	N	Y	chr12:6601382	Missense	NM_001273.3: c.706G>A	Val236Met	0.000008247	0.748	24.3	VUS	BS2, PP3, PP2	MOT
CVM31	*TGFBR2*	AD[a]	N	Y	chr3:30650373	Missense	NM_003242.5: c.367A>T	Met123Leu	0.00006592	0.884	27.7	VUS	BS2, PP3, PP2	Other
150650086	*INVS*	AD[a]	N	Y	chr9:100300652	Frameshift insertion	NM_014425.4: c.3182dup	Asn1061Lysfs*20	0.0002	NA	NA	VUS	BS2, PVS1, PP3	MOT
959	*GATA4*[256]	AD[a]	N	U	chr8:11756974	Missense	NM_002052.4: c.1037C>T	Ala346Val	0.00149	0.039	12.2	LB (I)	BS2, PS1, BP4, PP2	SD
1384	*EVC2*	AD[a]	N	N	chr4:5631929	Missense	NM_147127.4: c.1574C>T	Ala525Val	8.24E-06	0.988	19.32	LB (I)	BS2, PP3, BP5	OL
2020	*EHMT1*	AD[a]	N	N	chr9:137743452	Missense	NM_024757.4: c.905A>G	Lys302Arg	0.0002	0.999	26.3	LB (I)	BS2, PP3, BP5	Other
2306	*KMT2D*	AD[a]	N	N	chr12:49034472	Missense	NM_003482.3: c.10445G>A	Arg3482Gln	6.80E-05	0.859	29.1	LB (I)	PP3, BP1, BS2	FSV

2324	*NOTCH1*	AD[a]	N	Y	chr9:136510749	Missense	NM_017617.4: c.2644G>A	Ala882Thr	0.00002028	0.977	29.3	LB (I)	BS2, PP3, PP2, BP5	MOT
3427	*ELN*	AD[a]	N	Y	chr7:74056336	Missense	NM_000501.3: c.1216G>A	Gly406Ser	0.00004118	0.972	20.8	LB (I)	BP1, BS2, PP3	SD
3438	*KAT6B*	AD[a]	N	Y	chr10:75028575	Missense	NM_012330.3: c.3751G>A	Gly1251Arg	0.000008237	1	32	LB (I)	PM1, BP1, PP3, BS2	SD
3717	*STRA6*	AD[a]	N	Y	chr15:74181327	Missense	NM_022369.3: c.1652T>G	Leu551Arg	0	0.999	26.1	LB (I)	PM2, PP3, BP2, BS2	SD
3967	*ARID1A*	AD[a]	N	N	chr1:26763150	Missense	NM_006015.5: c.2597G>A	Arg866Gln	0.0000413	0.99	23.5	LB (I)	BS2, PP3, BP1, PP4	Other
15R414475M	*ARID1B*	AD[a]	N	Y	chr6:156778005	Missense	NM_020732.3: c.76A>C	Lys26Gln	0	NA	15.83	LB (I)	PM2, BS2, PP3, BP1	SDMA
CVM31	*CHD7*	AD[a]	N	Y	chr8:60823879	Missense	NM_017780.3: c.3241A>G	Ile1081Val	0.000008309	0.894	26.8	LB (I)	PM1, BP1, PP3, BS2	Other
CVM31	*ZEB2*	AD[a]	N	Y	chr2:144399741	Missense	NM_014795.3: c.1446A>C	Glu482Asp	0	0.956	15.64	LB (I)	PM2, BS2, BP1, PP3	Other
2324	*NF1*	AD[a]	N	Y	chr17:31232821	Missense	NM_000267.3: c.3436G>A	Val1146Ile	0.0001	0.927	15.27	LB (II)	BS2, PP3, BP1, BP5	MOT
2636	*KANSL1*	AD[a]	N	N	chr17:46170955	Missense	NM_001193466.1: c.1189G>A	Ala397Thr	5.77E-05	0.985	24.9	LB (II)	PP3, BP1, BP6	FSV

Variants in all affected individuals per family are presented. Model: inheritance model. AD: autosomal dominant; AR: autosomal recessive; CH: compound heterozygous; DN: *de novo*; XLR: X-linked recessive. Familial CHD: (Y/N) Yes/No; refers to the presence/absence of an individual with

CHD within the immediate family of the proband, respectively. ECA: Extra-cardiac anomalies (not restricted to congenital defects) present in the proband; N: no; Y: yes; U: unknown. Genomic position: variant position in human reference genome hg38. ExAC MAF: minor allele frequency in the ExAC database. PP2 HVAR: PolyPhen-2 Hvar predictive score. Score ≥ 0.909: probably damaging; 0.908 ≤ score ≤ 0.447: possibly damaging; score ≤ 0.446: benign; NA: not applicable. CADD: scaled CADD Score ≥ 15: damaging; NA: not applicable. ACMG Class: pathogenicity interpretation according to the ACMG-AMP guidelines [10]: P, pathogenic; LP, likely-pathogenic; LB, likely-benign; VUS, variant of uncertain significance. ACMG criteria: evidence used to arrive at the ACMG-AMP pathogenicity classification.[10] Cardiac lesion: primary cardiac lesion of the proband. FSV: functional single ventricle; MOT: malformation of the outflow tract; SD: septal defect; OL: obstructive lesion; AVSD+: atrioventricular septal defect and variants; SDMA: septal defect with minor abnormalities. For detailed phenotype descriptions, including extra-cardiac phenotypes, see **Supplementary Table S1**. For pedigrees of families with pathogenic or likely-pathogenic variants, see **Supplementary Figure S3**. [a] Incomplete penetrance / variant present in unaffected individual.

Table S5. Rare and predicted-damaging variants identified in hcCHD genes in probands with familial CHD.

Family ID	Gene	Model	Familial CHD	ECA	Genomic position	Variant type	Nucleotide variant	Amino acid variant	ExAC MAF	PP2 HVAR	CADD	ACMG class.	ACMG criteria	Cardiac lesion
195	*NODAL*	AD[a]	Y	N	chr10:70441525	Frameshift insertion	NM_018055.4: c.123_142dup	Tyr48Trpfs*5	0	NA	NA	P (Ia)	BS2, PM2, PVS1, PP3, PP1-S	FSV
835	*NODAL*	AD	Y	Y	chr10:70441525	Frameshift insertion	NM_018055.4: c.123_142dup	Tyr48Trpfs*5	0	NA	NA	P (Ia)	PM2, PVS1, PP3, PP1-S	FSV
2985	*CFC1* [49,50]	AD	Y	N	chr2:130593026	Frameshift deletion	NM_032545.3: c.522del	Ala175Argfs*56	0	NA	NA	P (Ia)	PM2, PVS1, PP3, PP1, PS1, PS3	FSV
575	*CHD7*	AD[a]	Y	Y	chr8:60794987	Splicing	NM_017780.3: c.2098A>G	Asn700Asp	0	NA	18.13	P (Ic)	PM2, BS2, PVS1, PP3	MOT
2831	*NOTCH1*	AD[a]	Y	N	chr9:136498973	Frameshift deletion	NM_017617.4: c.6105del	Ala2036Profs*3	0	NA	NA	P (Ic)	PM2, PP3, PVS1, BS2	MOT
3766	*NODAL*	AD	Y	U	chr10:70433060	Stopgain	NM_018055.4: c.919C>T	Arg307*	0	NA	40	P (Ic)	PM2, PVS1, PP3	MOT
1852	*NOTCH1*	AD[a]	Y	Y	chr9:136500621	Frameshift deletion	NM_017617.4: c.5865del	Asn1955Lysfs*26	0	NA	NA	P (Id)	PM2, PVS1, PP3, BS4	FSV
3933	*BCOR*	AD	Y	Y	chrX:40072856	Frameshift deletion	NM_001123383.1: c.2488_2489del	Ser830Cysfs*6	0	NA	NA	P (Id)	PM2, PVS1, PP3, PP1	SD
3225	*GATA4*	AD	Y	Y	chr8:11755095	Missense	NM_002052.4: c.959G>A	Arg320Gln	0	0.99	35	P (IIIb)	PM2, PP3, PP2, PM1, PP1, PS3	SDMA

1.69E+08	*JAG1*	AD	Y	Y	chr20:10658540	Missense	NM_000214.2: c.622G>C	Gly208Arg	0	0.999	32	P (IIIb)	BP1, PM1, PP1-S, PM2, PP4, PP3	MOT
1449	*ACTC1*	AD	Y	Y	chr15:34793496	Missense	NM_005159.4: c.203C>T	Thr68Ile	0	0.974	25.5	P (IIIc)	PP3, PM2, PP2, PM1, PP1, PS1[b]	SD
3695	*TLL1*	AD	Y	N	chr4:166091263	Missense	NM_012464.4: c.2578A>G	Thr860Ala	0	0.992	23.1	LP (V)	PM2, PP3, PP1, PP2, PM1	MOT
1.53E+08	*NOTCH1*	AD	Y	Y	chr9:136505480	Missense	NM_017617.4: c.4416C>G	Cys1472Trp	0	1	24.4	LP (V)	PM2, PP3, PM1, PP2, PP1	MOT
2944	*ADAMTS10*	AD	Y	N	chr19:8596380	Missense	NM_030957.3: c.1117G>A	Glu373Lys	8.52E-06	0.958	27.4	VUS	PP3, PM1, PP1-M, BS2	SDMA
2831	*MYH11*	AD[a]	Y	N	chr16:15708836	Missense	NM_022844.2: c.5792C>A	Pro1931His	0	0.536	21.2	VUS	PM2, PP3, BS2	MOT
3208	*NOTCH1*	AD[a]	Y	U	chr9:136518651	Missense	NM_017617.4: c.1039G>A	Gly347Ser	0.00001738	0.999	34	VUS	PP3, PM1, PP2, BS2	MOT
3225	*STRA6*	AD	Y	Y	chr15:74189222	Missense	NM_022369.3: c.983G>T	Gly328Val	0	1	31	VUS	PM2, PP3, PP1, BP5, PP4	SDMA
3225	*TLL1*	AD	Y	Y	chr4:166014468	Missense	NM_012464.4: c.950G>A	Arg317His	0.000033	0.799	25.4	VUS	PP3, PP2, BP5, PM1, PP1-M, BS2	SDMA

3485	*ARID1A*	AD	Y	Y	chr1:26772501	Splicing	NM_006015.5: c.3408G>A	Ala1136Ala	0.0001	NA	NA	VUS	PP3, BS2	SD
3695	*FBN1*	AD	Y	N	chr15:48465797	Missense	NM_000138.4: c.4809A>G	Ile1603Met	8.26E-06	0.974	23.9	VUS	PP3, PP2, PP1, BS2	MOT
1852	*NKX2-5* *257-259*	AD[a]	Y	Y	chr5:173235023	Missense	NM_004387.3: c.61G>C	Glu21Gln	0.0008	0.975	26.4	LB (I)	BS2, PP3, PP2, BP6	FSV
2831	*MYBPC3*	AD[a]	Y	N	chr11:47332204	Missense	NM_000256.3: c.3682C>T	Arg1228Cys	0.0002	0.993	35	LB (I)	BS2, PP3, BP1	MOT

Variants in all affected individuals per family are presented. Model: inheritance model. AD: autosomal dominant; AR: autosomal recessive; CH: compound heterozygous; DN: *de novo*; XLR: X-linked recessive. Familial CHD: (Y/N) Yes/No; refers to the presence/absence of an individual with CHD within the immediate family of the proband, respectively. ECA: Extra-cardiac anomalies (not restricted to congenital defects) present in the proband; N: no; Y: yes; U: unknown. Genomic position: variant position in human reference genome hg38. ExAC MAF: minor allele frequency in the ExAC database. PP2 HVAR: PolyPhen-2 Hvar predictive score. Score ≥ 0.909: probably damaging; 0.908 ≤ score ≤ 0.447: possibly damaging; score ≤ 0.446: benign; NA: not applicable. CADD: scaled CADD Score ≥ 15: damaging; NA: not applicable. ACMG Class: pathogenicity interpretation according to the ACMG-AMP guidelines [10]: P, pathogenic; LP, likely-pathogenic; LB, likely-benign; VUS: variant of uncertain significance. Cardiac lesion: primary cardiac lesion of the proband. ACMG criteria: evidence used to arrive at the ACMG-AMP pathogenicity classification.[10] FSV: functional single ventricle; MOT: malformation of the outflow tract; SD: septal defect; OL: obstructive lesion; SDMA: septal defect with minor abnormalities. For detailed phenotype descriptions, including extra-cardiac phenotypes, see **Supplementary Table S1**. For pedigrees of families with pathogenic or likely-pathogenic variants, see **Supplementary Figure S3**. [a] Incomplete penetrance / variant present in unaffected individual. [b] A homologous Thr68Ile variant has been identified in alpha skeletal actin (ACTA1), in a patient with severe lethal hypotonia and minimal spontaneous movements of the legs and arms.

Table S6. CNVs identified in hcCHD genes in probands.

Family	Gene	Model	Familial CHD	ECA	Variant type	Chrom	CNV start	CNV end	Size	Frequency	Region affected	ACMG class.	Cardiac lesion
3173	*NOTCH1*	AD[a]	Y	Y	DEL	chr9	136537696	136560250	22.55 kb	0.0027	Exonic	P	SDMA
44	*BMPR2*	AD[a]	N	Y	DEL	chr2	202430702	202448193	17.49 kb	0.0068	Intronic	VUS	FSV
44	*TLL1*	AD[a]	N	Y	DEL	chr4	165974126	165974438	312 bp	0.0054	Intronic	VUS	FSV
742	*SOS1*	AD[a]	N	N	DEL	chr2	39089439	39090604	1.17 kb	0.0027	Intronic	VUS	Other
959	*ZFPM2*	DN	N	U	DEL	chr8	105658042	105658686	644 bp	0.0149	Intronic	VUS	SD
959	*PITX2*	AD[a]	N	U	DEL	chr4	110624350	110624422	72 bp	0.0122	Intronic	VUS	SD
1085	*RAF1*	AD[a]	N	N	DEL	chr3	12604731	12604808	77 bp	0.0027	Intronic	VUS	SD
1085	*PITX2*	AD[a]	N	N	DEL	chr4	110638295	110638356	61 bp	0.0054	Intronic	VUS	SD
1167	*MAP2K2*	AD[a]	N	N	DEL	chr19	4120853	4120948	95 bp	0.0149	Intronic	VUS	SD
2020	*GJA1*	AD[a]	N	N	DEL	chr6	121427635	121428318	683 bp	0.0027	Intergenic	VUS	Other
2831	*DOCK6*	DN	Y	N	DEL	chr19	11210497	11210594	97 bp	0.0041	Intronic	VUS	MOT
3173	*ZFPM2*	AD[a]	Y	Y	DEL	chr8	104968516	104968637	121 bp	0.0081	Intergenic	VUS	SDMA
3376	*ZFPM2*	AD[a]	N	N	DEL	chr8	104968516	104968637	121 bp	0.0081	Intronic	VUS	SD
3424	*GDF1, (CERS1)*	AD[a]	N	Y	DUP	chr19	18885852	18889621	3.77 kb	0.0027	Intronic	VUS	MOT
3473	*PITX2*	AD[a]	N	Y	DEL	chr4	110638295	110638356	61 bp	0.0054	Intronic	VUS	AVSD+
3473	*TLL1*	AD[a]	N	Y	DEL	chr4	165974126	165974438	312 bp	0.0054	Intronic	VUS	AVSD+
3485	*CDK13*	AD	Y	Y	DEL	chr7	40074176	40074235	59 bp	0.0068	Intronic	VUS	SD
3527	*FBN1*	AD[a]	N	N	DEL	chr15	48445118	48445209	91 bp	0.0257	Intronic	VUS	FSV
3527	*FBN1*	AD[a]	N	N	DEL	chr15	48453290	48453351	61 bp	0.0257	Intronic	VUS	FSV
3564	*FBN1*	AD[a]	N	U	DEL	chr15	48445118	48445209	91 bp	0.0257	Intronic	VUS	OL
3564	*FBN1*	AD[a]	N	U	DEL	chr15	48453290	48453351	61 bp	0.0257	Intronic	VUS	OL
3601	*CDK13*	AD[a]	N	U	DEL	chr7	40074176	40074235	59 bp	0.0068	Intronic	VUS	MOT
3610	*PITX2*	AD[a]	N	N	DEL	chr4	110624350	110624422	72 bp	0.0122	Intronic	VUS	MOT
3630	*FOXH1*	AD[a]	N	U	DEL	chr8	144484983	144488709	3.73 kb	0.0027	Intergenic	VUS	SDMA

3648	*ZFPM2, ZFPM2-AS*	AD[a]	N	Y	DEL	chr8	105784303	105785078	775 bp	0.019	Intronic	VUS	AVSD+
3651	*GATA4*	AD[a]	N	U	DEL	chr8	11739420	11739758	338 bp	0.019	Intronic	VUS	MOT
3654	*FBN1*	AD[a]	N	U	DEL	chr15	48445118	48445209	91 bp	0.0257	Intronic	VUS	OL
3654	*FBN1*	AD[a]	N	U	DEL	chr15	48453290	48453351	61 bp	0.0257	Intronic	VUS	OL
3654	*GATA4*	AD[a]	N	U	DEL	chr8	11739420	11739758	338 bp	0.019	Intronic	VUS	OL
3792	*NOTCH2*	AD[a]	Y	N	DUP	chr1	119901157	119979217	78.06kb	0.0041	Exonic	VUS	MOT
3933	*TLL1*	AD	Y	Y	DEL	chr4	165962891	165963095	204 bp	0.0027	Intronic	VUS	SD
141550648	*CHD4*	AD[a]	N	Y	DEL	chr12	6575003	6575131	128 bp	0.0095	Intronic	VUS	SD
15R414475M	*HAND2, HAND2-AS*	DN	N	Y	DEL	chr4	173538431	173538783	352 bp	0.0068	Intronic	VUS	SDMA
Trio9	*GPC3*	AD[a]	N	Y	DEL	chrX	133878296	133879585	1.29 kb	0.0041	Intronic	VUS	MOT

Variants in all affected individuals per family are presented. Model: inheritance model. AD: autosomal dominant; DN: de novo. Familial CHD: (Y/N) Yes/No; refers to the presence/absence of an individual with CHD within the immediate family of the proband, respectively. ECA: Extra-cardiac anomalies (not restricted to congenital defects) present in the proband; N: no; Y: yes; U: unknown. Variant type: DEL: deletion; DUP: duplication. CNV start/end: genomic position of the variant in human genome reference hg38. Frequency: frequency of variant within our cohort (n=338). Region affected: variant falls within an exon, intron, or intergenic region of the gene. ACMG Class: pathogenicity interpretation according to the ACMG-AMP guidelines [260]: P, pathogenic; VUS, variant of uncertain significance. Cardiac lesion: primary cardiac lesion of the proband. FSV: functional single ventricle; MOT: malformation of the outflow tract; SD: septal defect; OL: obstructive lesion; AVSD+: atrioventricular septal defect and variants; SDMA: septal defect with minor abnormalities. For detailed phenotype descriptions, including extra-cardiac phenotypes, see **Supplementary Table S1**. For pedigree of family 3173, see **Supplementary Figure S3**. [a] Incomplete penetrance / variant present in unaffected individual.

Table S7. Non-exonic, disease-segregating hcCHD variants in probands.

Family ID	Gene	Model	Familial CHD	ECA	Genomic position	Variant type	Nucleotide variant	Regulatory features	gnomAD MAF	Cardiac lesion
44	*EHMT1*	AR	N	Y	chr9:137740389	Intronic	C>T	Open chromatin	0.001132246	FSV
	NOTCH1	AR	N	Y	chr9:136533361	Intronic	A>T	Proximal enhancer	0.001172104	FSV
835	*ELN*	AD	Y	Y	chr7:73937685	Intergenic	G>A	Open chromatin	0.00013031	FSV
	ELN	AD	Y	Y	chr7:73909428	Intergenic	C>T	Proximal enhancer	0.000226684	FSV
	ELN	AD	Y	Y	chr7:74034547	Intronic	A>G	Enhancer	0.000484496	FSV
	FBN1	AD	Y	Y	chr15:48374823	Intergenic	G>A	Proximal enhancer	0	FSV
	GATA4	AD	Y	Y	chr8:11656702	Intergenic	C>A	CTCF-BS	3.23E-05	FSV
	KDM6A	AD	Y	Y	chrX:44900389	Intronic	ATTAT>A	Proximal enhancer	0.000276281	FSV
	PITX2	AD	Y	Y	chr4:110615285	Intergenic	G>GA	TF-binding	0	FSV
	SMAD3	AD	Y	Y	chr15:67076300	Intronic	A>G	Proximal enhancer	0.000290623	FSV
	SMAD3	AD	Y	Y	chr15:67138679	Intronic	A>G	Proximal enhancer	0.000484371	FSV
	SMAD4	AD	Y	Y	chr18:51051204	Intronic	TC>T	CTCF-BS, Open chromatin	0	FSV
1125	*EVC*	AD	Y	Y	chr4:5747602	Intronic	A>G	Distal enhancer	0	SD
	PRKD1	AD	Y	Y	chr14:29236818	Intergenic	C>T	Proximal enhancer	0.000518	SD
	SMAD6	AD	Y	Y	chr15:66734417	Intronic	G>A	Distal enhancer	6.46E-05	SD
	TBX3, MED13L	AD	Y	Y	chr12:114762734	Intergenic	C>CGT	Distal enhancer	6.59E-05	SD
	TGFBR1	AD	Y	Y	chr9:99090674	Intergenic	A>G	Open chromatin	0.000743	SD
	TGFBR2	AD	Y	Y	chr3:30292432	Intergenic	C>A	TF-binding	0.000129	SD
	ZEB2	AD	Y	Y	chr2:144362695	Intergenic	A>G	Open chromatin	0	SD
	ZFPM2	AD	Y	Y	chr8:105090844	Intergenic	A>T	Open chromatin	6.47E-05	SD
1179	*ANKRD11*	AD	Y	Y	chr16:89249304	Intergenic	C>G	CTCF-BS	0	MOT
	CDK13	AD	Y	Y	chr7:39950756	Exonic	C>T	Promoter	0.000484186	MOT
	FBN1	AD	Y	Y	chr15:48388677	Intergenic	G>A	Open chromatin	3.23E-05	MOT
	MAP2K2	AD	Y	Y	chr19:4084835	Intergenic	CCT>C	Proximal enhancer	0.000226669	MOT
	SMAD4	AD	Y	Y	chr18:51042547	Intronic	A>G	Distal enhancer	3.25E-05	MOT
	TAB2	AD	Y	Y	chr6:149157343	Intergenic	C>A	Open chromatin	0.000710548	MOT
	ZEB2	AD	Y	Y	chr2:144404640	Intronic	G>A	Open chromatin	0	MOT

1449	*EHMT1*	AD	Y	Y	chr9:137695680	Intronic	C>T	Distal enhancer	3.23E-05	SD
	ELN	AD	Y	Y	chr7:74010564	Intergenic	C>T	Open chromatin	0.000388	SD
	ELN	AD	Y	Y	chr7:74006226	Intergenic	C>T	Open chromatin	0.000937	SD
	HAND2	AD	Y	Y	chr4:173523191	Intergenic	C>T	Proximal enhancer	0.000582	SD
	KAT6B	AD	Y	Y	chr10:74911336	Intronic	T>C	Open chromatin	0	SD
	KMT2D	AD	Y	Y	chr12:49019393	3' UTR	G>A	Promoter	0.000784	SD
	MED13L	AD	Y	Y	chr12:116166052	Intronic	A>G	Distal enhancer	0	SD
	SMAD6, SMAD3	AD	Y	Y	chr15:67049526	Intergenic	G>C	Proximal enhancer	0.000517	SD
	ZEB2	AD	Y	Y	chr2:144457444	Intronic	A>C	Distal enhancer	0.000485	SD
1456	*ANKRD11*	AD	Y	Y	chr16:89361594	Intronic	G>A	Proximal enhancer	0	AVSD+
	ANKRD11	AD	Y	Y	chr16:89374408	Intronic	T>C	Distal enhancer	0	AVSD+
	ARID1B	AD	Y	Y	chr6:156964533	Intronic	T>G	Proximal enhancer	3.23E-05	AVSD+
	CHD7	AD	Y	Y	chr8:60676377	Intergenic	G>A	TF-binding	3.23E-05	AVSD+
	CREBBP	AD	Y	Y	chr16:3878612	Intronic	G>A	Promoter	0.000129132	AVSD+
	EHMT1	AD	Y	Y	chr9:137664621	Intronic	C>T	Open chromatin	0.000422792	AVSD+
	FBN1	AD	Y	Y	chr15:48494020	Intronic	C>T	Proximal enhancer	3.23E-05	AVSD+
	GPC3	XLR	Y	Y	chrX:133792680	Intronic	C>A	Proximal enhancer	0	AVSD+
	KAT6A	AD	Y	Y	chr8:42002613	Intronic	T>A	Distal enhancer	3.23E-05	AVSD+
	KDM6A	XLR	Y	Y	chrX:44911427	Intronic	C>T	CTCF-BS	0.000198955	AVSD+
	MAP2K1	AD	Y	Y	chr15:66472009	Intronic	G>A	Proximal enhancer	6.60E-05	AVSD+
	NOTCH1	AD	Y	Y	chr9:136493602	Downstream	C>T	Open chromatin	0.000905036	AVSD+
	SMAD3	AD	Y	Y	chr15:67078574	Intronic	G>A	Proximal enhancer	0.000419788	AVSD+
	TBX1	AD	Y	Y	chr22:19731757	Intergenic	C>T	Proximal enhancer	0.000259051	AVSD+
	TBX3, MED13L	AD	Y	Y	chr12:114747494	Intergenic	G>T	Open chromatin	9.68E-05	AVSD+
	ZEB2	AD	Y	Y	chr2:144377232	Intergenic	G>T	Open chromatin	0.000129141	AVSD+
1658	*EVC2*	AD	Y	N	chr4:5683777	Intronic	A>C	Open chromatin	0	OL
	KAT6B	AD	Y	N	chr10:74824830	Intergenic	A>G	Promoter	0	OL
	KAT6B	AD	Y	N	chr10:74822632	Intergenic	T>G	Distal enhancer	0.000935846	OL
	SMAD6, SMAD3	AD	Y	N	chr15:66961591	Intergenic	G>C	Open chromatin	0	OL

	SMAD6, SMAD3	AD	Y	N	chr15:67059561	Intergenic	C>T	Proximal enhancer	0	OL
	TBX3, MED13L	AD	Y	N	chr12:115922783	Intergenic	G>A	CTCF-BS	0.000129199	OL
	TGFBR2	AD	Y	N	chr3:30511876	Intergenic	G>A	Distal enhancer	0.000322768	OL
	TGFBR2	AD	Y	N	chr3:30630764	Intronic	G>C	Proximal enhancer	0.000872262	OL
	UBR1	AD	Y	N	chr15:43106696	Upstream	A>AG	Promoter	0.000678076	OL
2944	*EHMT1*	AD	Y	N	chr9:137705538	Intronic	T>C	Proximal enhancer	0.00058102	SDMA
	PRDM6	AD	Y	N	chr5:123090323	Exonic	C>T	Promoter	9.55E-05	SDMA
	SMAD6, SMAD3	AD	Y	N	chr15:66800730	Intergenic	A>ACACA-CTCG	Open chromatin	0	SDMA
	SMAD6, SMAD3	AD	Y	N	chr15:66850550	Intergenic	T>A	Proximal enhancer	6.46E-05	SDMA
	SMAD6, SMAD3	AD	Y	N	chr15:67059489	Intergenic	C>T	Proximal enhancer	0.000580908	SDMA
	TBX1	AD	Y	N	chr22:19762261	Intronic	C>G	Proximal enhancer	6.46E-05	SDMA
	TGFBR2	AD	Y	N	chr3:30518411	Intergenic	C>T	Distal enhancer	0.000225996	SDMA
2985	*ANKRD11*	AD	Y	N	chr16:89240564	Intergenic	G>A	Proximal enhancer	0	FSV
	ANKRD11	AD	Y	N	chr16:89406355	Intronic	C>A	Open chromatin	0	FSV
	SHOC2	AD	Y	N	chr10:110945368	Intronic	T>C	Open chromatin	0.000516662	FSV
	TBX1	AD	Y	N	chr22:19765134	Intronic	C>T	Proximal enhancer	4.48E-05	FSV
	TBX3, MED13L	AD	Y	N	chr12:115423483	Intergenic	C>T	TF-binding	0	FSV
3191	*HAND2*	AR	N	U	chr4:173500472	Intergenic	T>G	CTCF-BS	0	FSV
3208	*SMAD6*	AD[a]	Y	U	chr15:66774833	Intronic	AT>A	Proximal enhancer	0	MOT
3394	*TBX5*	DN	N	N	chr12:114242064	Intergenic	T>C	CTCF-BS, Open chromatin	0.000249	FSV
3397	*ACVR1*	AD	Y	N	chr2:157696514	Intergenic	C>T	Open chromatin	6.46E-05	SDMA
	ANKRD11	AD	Y	N	chr16:89320454	Intronic	GCTGAG-CGGCA>G	Proximal enhancer	0	SDMA
	ANKRD11	AD	Y	N	chr16:89320465	Intronic	A>G	Proximal enhancer	0	SDMA
	ARID1B	AD	Y	N	chr6:156396168	Intergenic	A>C	Distal enhancer	0	SDMA
	BCOR	XLD	Y	N	chrX:40127261	Intronic	T>A	Distal enhancer	0.000278914	SDMA
	CHD4	AD	Y	N	chr12:6605881	Intronic	C>T	Promoter	0.000323018	SDMA

	GPC3	XLD	Y	N	chrX:133543633	Intronic	A>G	Distal enhancer	0.000320704	SDMA
	KDM6A	XLD	Y	N	chrX:44911772	Intronic	A>C	CTCF-BS	0.000468296	SDMA
	MED13L	AD	Y	N	chr12:116236758	Intronic	A>T	Distal enhancer	0	SDMA
	NPHP4	AD	Y	N	chr1:5923301	Intronic	C>T	Open chromatin	9.69E-05	SDMA
	SMAD3	AD	Y	N	chr15:67098926	Intronic	A>G	Proximal enhancer	4.87E-05	SDMA
	SMAD6, SMAD3	AD	Y	N	chr15:66935303	Intergenic	G>T	Open chromatin	3.23E-05	SDMA
	TBX1	AD	Y	N	chr22:19759227	Intronic	G>A	Proximal enhancer	0	SDMA
	TGFBR2	AD	Y	N	chr3:30443805	Intergenic	C>G	Open chromatin	0	SDMA
	TGFBR2	AD	Y	N	chr3:30641375	Intronic	G>A	Proximal enhancer	0	SDMA
3400	*ACVR1*	DN	N	N	chr2:157769997	Intronic	C>G	Open chromatin	0	OL
3438	*HAND1*	DN	N	Y	chr5:154478752	Upstream	G>A	Proximal enhancer	0	SD
3464	*TBX3, MED13L*	AR	N	N	chr12:114903717	Intergenic	G>A	Proximal enhancer	0	AVSD+
3470	*BCOR*	XLR	N	U	chrX:39906310	Intergenic	CTG>C	Proximal enhancer	0	MOT
3564	*ELN*	DN	N	U	chr7:73886700	Intergenic	A>T	Distal enhancer	0	OL
3642	*INVS*	AR	N	N	chr9:100126153	Intronic	A>G	Open chromatin	0.0000323	FSV
	MAP2K1	AR	N	N	chr15:66374480	Intergenic	G>A	CTCF-BS	0.000549	FSV
3648	*TAB2*	DN	N	Y	chr6:149113466	Intergenic	A>C	CTCF-BS	0	AVSD+
3672	*SMAD6*	DN	N	U	chr15:66698378	Intergenic	G>A	CTCF-BS	0	SD
3678	*ANKRD11*	AD	Y	U	chr16:89344028	Intronic	C>A	Proximal enhancer	0	MOT
	ANKRD11	AD	Y	U	chr16:89411191	Intronic	G>C	Distal enhancer	0	MOT
	ARID1B	AD	Y	U	chr6:157148239	Intronic	C>G	Proximal enhancer	6.46E-05	MOT
	SMAD3	AD	Y	U	chr15:67073586	Intronic	G>A	Proximal enhancer	0.000129166	MOT
	SMAD3	AD	Y	U	chr15:67145678	Intronic	A>G	Proximal enhancer	0.00096893	MOT
	TBX5	AD	Y	U	chr12:114403265	Intronic	CGGAGG-AGGCA>C	TF-binding	0.00058147	MOT
	TGFBR1	AD	Y	U	chr9:99118834	Intronic	T>C	Proximal enhancer	3.24E-05	MOT
	TGFBR2	AD	Y	U	chr3:30256955	Intergenic	T>C	Open chromatin	0.000678952	MOT
	ZFPM2	AD	Y	U	chr8:105390100	Intronic	T>G	Open chromatin	0	MOT
3695	*ANKRD11*	AD	Y	N	chr16:89459351	Intronic	C>T	Distal enhancer	0.000258331	MOT

	ANKRD11	AD	Y	N	chr16:89454624	Intronic	G>C	Proximal enhancer	0.000685871	MOT
	CHD7	AD	Y	N	chr8:60780977	Intronic	C>T	TF-binding	0.000171624	MOT
	ELN	AD	Y	N	chr7:73886377	Intergenic	A>C	Distal enhancer	0.000290904	MOT
	FLNA	XLR	Y	N	chrX:154333929	Intergenic	G>C	Distal enhancer	0	MOT
	GATA4	AD	Y	N	chr8:11616595	Intergenic	G>T	Open chromatin	0	MOT
	HAND2	AD	Y	N	chr4:173501151	Intergenic	C>T	Open chromatin	0	MOT
	KAT6B	AD	Y	N	chr10:74898287	Intronic	C>G	Proximal enhancer	0	MOT
	KAT6B	AD	Y	N	chr10:74913855	Intronic	C>T	Proximal enhancer	0.000129199	MOT
	MED13L	AD	Y	N	chr12:116166704	Intronic	T>C	Open chromatin	0	MOT
	NF1	AD	Y	N	chr17:31314373	Intronic	T>A	Promoter	0	MOT
	NIPBL	AD	Y	N	chr5:36878481	Intronic	T>A	Promoter	9.69E-05	MOT
	NOTCH1	AD	Y	N	chr9:136522122	Intronic	C>T	Proximal enhancer	6.52E-05	MOT
	TBX3, MED13L	AD	Y	N	chr12:115502783	Intergenic	A>G	CTCF-BS	6.46E-05	MOT
	TBX3, MED13L	AD	Y	N	chr12:115502801	Intergenic	T>C	CTCF-BS	6.46E-05	MOT
	TGFBR1	AD	Y	N	chr9:99086751	Intergenic	T>C	Distal enhancer	0	MOT
	TGFBR2	AD	Y	N	chr3:30646784	Intronic	G>T	Proximal enhancer	3.23E-05	MOT
	TGFBR2	AD	Y	N	chr3:30644305	Intronic	C>T	Proximal enhancer	6.46E-05	MOT
	TLL1	AD	Y	N	chr4:165819063	Intergenic	G>A	CTCF-BS	0	MOT
3717	*KDM6A*	XLR	N	Y	chrX:44875852	Intronic	C>A	Promoter	0.000604876	SD
3769	*ACVR1*	AD	Y	U	chr2:157714173	Intergenic	C>T	CTCF-BS	6.46E-05	MOT
	EHMT1	AD	Y	U	chr9:137625748	Intronic	G>A	Proximal enhancer	3.24E-05	MOT
	ELN	AD	Y	U	chr7:74051964	Exonic	C>T	TF-binding	0.00019901	MOT
	GATA4	AD	Y	U	chr8:11654572	Intergenic	C>G	CTCF-BS	0	MOT
	GATA4	AD	Y	U	chr8:11710274	Intronic	G>T	Open chromatin	0	MOT
	GATA4	AD	Y	U	chr8:11746417	Intronic	A>G	CTCF-BS	3.23E-05	MOT
	GATA5	AD	Y	U	chr20:62476390	Upstream	G>C	TF-binding	0	MOT
	NOTCH1	AD	Y	U	chr9:136535463	Intronic	GC>G	Proximal enhancer	0.000626	MOT
	NR2F2	AD	Y	U	chr15:96337892	3' UTR	T>C	Promoter	0.000196425	MOT
	TAB2	AD	Y	U	chr6:149141093	Intergenic	A>G	Proximal enhancer	0	MOT

	TAB2	AD	Y	U	chr6:149231811	Intronic	G>C	Proximal enhancer	0	MOT
	TBX3, MED13L	AD	Y	U	chr12:114811258	Intergenic	A>G	CTCF-BS	0	MOT
	TBX3, MED13L	AD	Y	U	chr12:115141458	Intergenic	A>G	Open chromatin	0	MOT
	TBX3, MED13L	AD	Y	U	chr12:115141597	Intergenic	C>T	Open chromatin	0	MOT
	TBX3, MED13L	AD	Y	U	chr12:115227267	Intergenic	C>T	Distal enhancer	0	MOT
	TBX3, MED13L	AD	Y	U	chr12:115771370	Intergenic	C>G	CTCF-BS	0	MOT
	TBX3, MED13L	AD	Y	U	chr12:115322396	Intergenic	T>C	Open chromatin	0.000355228	MOT
	TBX3, MED13L	AD	Y	U	chr12:115309109	Intergenic	A>G	Open chromatin	0.000355366	MOT
	TBX3, MED13L	AD	Y	U	chr12:115312879	Intergenic	C>T	Open chromatin	0.000355826	MOT
	TBX5	AD	Y	U	chr12:114287874	Intergenic	A>AT	Open chromatin	3.23E-05	MOT
	TGFBR2	AD	Y	U	chr3:30597113	Intergenic	G>T	Distal enhancer	0	MOT
3932	*ARID1B*	AD	Y	N	chr6:156427596	Intergenic	C>T	Open chromatin	0	FSV
	BCOR	AD	Y	N	chrX:40157503	Intronic	A>G	Proximal enhancer	0.000183933	FSV
	INVS	AD	Y	N	chr9:100104588	Exonic	G>A	Open chromatin	0.000292208	FSV
	NOTCH1	AD	Y	N	chr9:136515468	Intronic	C>T	Proximal enhancer	4.17E-06	FSV
	RAD21	AD	Y	N	chr8:116873823	Intronic	TA>T	Promoter	0.000161614	FSV
	TBX5	AD	Y	N	chr12:114340238	Intronic	C>T	Open chromatin	0	FSV
	TGFBR1	AD	Y	N	chr9:99086745	Intergenic	C>T	Distal enhancer	6.46E-05	FSV
	TGFBR2	AD	Y	N	chr3:30641970	Intronic	T>A	Proximal enhancer	0.000387923	FSV
3933	*ANKRD11*	AD	Y	Y	chr16:89491146	Upstream	G>C	Promoter	3.24E-05	SD
	ARID1B	AD	Y	Y	chr6:156560536	Intergenic	C>T	CTCF-BS	0	SD
	ARID1B	AD	Y	Y	chr6:156780252	Intronic	A>G	Promoter	0	SD
	ARID1B	AD	Y	Y	chr6:157033457	Intronic	T>C	Proximal enhancer	0	SD
	ARID1B	AD	Y	Y	chr6:156880196	Intronic	G>A	Distal enhancer	3.23E-05	SD
	ARID1B	AD	Y	Y	chr6:156422427	Intergenic	G>T	CTCF-BS	6.46E-05	SD
	ARID1B	AD	Y	Y	chr6:157146723	Intronic	C>T	Proximal enhancer	6.46E-05	SD
	ARID1B	AD	Y	Y	chr6:156361786	Intergenic	C>T	CTCF-BS	0.000227909	SD

	CHD7	AD	Y	Y	chr8:60731461	Intronic	C>T	CTCF-BS	0.000484402	SD
	CITED2	AD	Y	Y	chr6:139324031	Intergenic	C>T	Proximal enhancer	0	SD
	GPC3	XLD	Y	Y	chrX:133461458	Intergenic	G>A	Open chromatin	0.000662314	SD
	INVS	AD	Y	Y	chr9:100137011	Intronic	G>A	CTCF-BS	0.000258565	SD
	NIPBL	AD	Y	Y	chr5:36877405	Intronic	G>A	Promoter	0.000550162	SD
	SMAD6, SMAD3	AD	Y	Y	chr15:66862299	Intergenic	G>A	Open chromatin	3.23E-05	SD
	TBX3, MED13L	AD	Y	Y	chr12:114936706	Intergenic	C>A	TF-binding	0.000968617	SD
	TGFBR2	AD	Y	Y	chr3:30646861	Intronic	T>C	Proximal enhancer	0	SD
	ZEB2	AD	Y	Y	chr2:144435135	Intronic	C>G	Open chromatin	0	SD
	ZEB2	AD	Y	Y	chr2:144444213	Intronic	T>A	Proximal enhancer	0	SD
150650086	*FBN1*	AR	N	Y	chr15:48403093	Intergenic	T>C	Open chromatin	9.68E-05	MOT
	FBN1	AR	N	Y	chr15:48448058	Intronic	G>A	Open chromatin	0.000904977	MOT
152900216	*TBX3, MED13L*	AD	Y	Y	chr12:114864956	Intergenic	G>C	Open chromatin	6.46E-05	MOT
	TBX5	AD	Y	Y	chr12:114168323	Intergenic	G>A	Open chromatin	6.46E-05	MOT
	TBX20	AD	Y	Y	chr7:35198543	Intergenic	T>C	Open chromatin	0.000129291	MOT

Model: inheritance model: AD; autosomal dominant; AR; autosomal recessive; XLD: X-linked dominant; XLR: X-linked recessive. Familial CHD: (Y/N) Yes/No; refers to the presence/absence of an individual with CHD within the immediate family of the proband, respectively. ECA: Extra-cardiac anomalies (not restricted to congenital defects) present in the proband; N: no; Y: yes; U: unknown. Variant type: variants fall within intronic, intergenic, upstream and downstream (1 kb upstream of TSS or 1 kb downstream of TES), 3' UTR regions relative to the coding region of hcCHD genes. Regulatory features are defined as per Ensembl Regulatory Build,[254] and are characterized by one or combination of DNase1 hypersensitivity sites, transcription factor (TF) binding sites, H3K27Ac sites, H3K4me3 sites, CpG islands or CTCF transcription repressor binding sites. gnomAD MAF: minor allele frequency in the gnomAD database. Cardiac lesion: primary cardiac lesion of the proband. FSV: functional single ventricle; MOT: malformation of the outflow tract; SD: septal defect; OL: obstructive lesion; AVSD+: atrioventricular septal defect and variants; SDMA: septal defect with minor abnormalities. For detailed phenotype descriptions, including extra-cardiac phenotypes, see **Supplementary Table S1**. For pedigrees of families 3400 and 3438, see **Supplementary Figure S3**. [a] Incomplete penetrance / variant present in unaffected individual.

Table S8. Variant numbers from comprehensive analysis of families with sporadic CHD.

Family ID	**Autosomal dominant**		***De novo***		**Autosomal recessive**		**Compound heterozygous**	
	n (all)	n (damaging)	n (all)	n (damaging)	n (all)	n (damaging)	n (all)	n (damaging)
44	398	109	1	1	70	16 *HAAO*	30	4
742	240	75	0	0	1	0	15	0
821	242	82	1	1	2	0	14	0
881	242	90	3	1	4	2	6	0
959	394	124	2	0	2	0	9	2
1085	337	109	1	1	0	0	27	0
1167	214	73	0	0	0	0	5	2
1236	301	98	0	0	4	0	13	2
1302	248	88	0	0	3	0	11	4
1384	247	88	0	0	7	0	9	2 *MYH6*
1746	266	85	3	1 *HNRNPK*	0	0	8	4
1767	491	144	0	0	10	2	48	7
2020	375	114	0	0	2	0	29	2
2252	247	87	1	1	4	1	10	0
2306	336	116	0	0	0	0	29	4
2324	272	94 *SEMA3D*	2	1	0	0	20	2
2606	253	94	1	0	6	1	4	0
2636	229	78	2	2	5	0	15	0
2825	254	90	3	2	1	0	8	0
3129	280	94	1	0	1	0	28	6
3191	294	97 *SMAD6*	1	1	3	0	17	0
3254	262	93	2	2	3	1	15	0
3370	339	105	0	0	4	0	20	2
3376	243	69	0	0	0	0	17	0
3394	242	101	2	1	4	1	10	0
3400	374	119	0	0	1	0	17	7
3424	261	88	1	0	3	1	6	2

3427	402	116	6	0	6	0	37	8
3430	381	120	1	0	1	0	16	0
3438	397	117	2	0	13	2	32	4
3464	584	132	12	1	5	1	27	0
3470	257	71	2	0	3	0	8	0
3473	873	194	0	0	18	8	98	7
3527	124	39	1	1	0	0	4	0
3564	534	153	1	0	3	0	54	2
3568	171	59	2	1	1	1	18	6
3571	93	31	0	0	3	0	2	0
3595	157	67	6	2 *KMT2C*	0	0	6	0
3601	238	61	0	0	1	0	4	0
3610	227	86	1	1	4	0	0	0
3630	247	82	2	1	1	1	16	2
3642	376	105	3	3	29	4 *INVS*	40	6
3645	330	94	6	2	1	1	21	2
3648	642	129 *JAG1*	18	2	24	8	109	1
3651	282	94	3	0	21	5 *KIAA0586*	9	1
3654	331	109	0	0	7	0	10	4
3672	195	70	1	0	1	0	12	4
3675	217	73	1	1	2	0	9	0
3681	350	107	5	0	6	2	16	0
3712	249	89 *GATA6*	1	1	3	0	12	0
3717	167	61	1	0	1	0	8	0
3953	252	83	2	1 *NF1*	0	0	3	0
3967	65	18	2	2	1	0	26	2
38223	271	98	2	0	2	0	10	4
141550648	407	129	1	0	3	1	27	0
150650086	687	183 *ACVR2B*	0	0	34	8 *DOCK6*	69	3
15R414475M	253	98	3	1	0	0	15	2

15Y05641	384	129	5	1	2	0	37	2
CVM31	584	164	1	1	23	6	50	5
Trio9	238	69	2	1	6	1	20	2

Four inheritance models were applied, with respective minor allele frequencies (MAF): autosomal dominant (MAF < 0.001); de novo (MAF < 0.001); autosomal recessive (MAF < 0.01); compound heterozygous (MAF < 0.01), and the number of IGV-verified ("all") and predicted-damaging ("damaging") variants are reported. A predicted-damaging variant is defined by CADD ≥ 15 and PolyPhen2-HVAR ≠ *benign*. Gene names are presented in cases where clinically actionable variants were identified.

Table S9. Variant numbers from comprehensive analysis of cases with familial CHD.

Family ID	**Autosomal dominant**		***De novo***		**Autosomal recessive**		**Compound heterozygous**	
	n (all)	n (damaging)	n (all)	n (damaging)	n (all)	n (damaging)	n (all)	n (damaging)
195	159	70 *NODAL*	0	0	0	0	10	0
575	254	89 *CHD7*	2	1	1	0	11	2
835	221	100 *NODAL*	0	0	1	1	9	2
894	334	101	7	0	8	0	21	2
939	462	164	1	1 *KMT2C*	0	0	20	0
1071	141	46	4	0	0	0	4	2
1125	278	88	0	0	0	0	25	4
1179	247	120	3	1	4	0	19	4
1449	263	111 *ACTC1*	1	1	0	0	14	0
1456	357	104 *chr10q22.3-q23.2*[a]	1	0	4	0	32	2
1658	249	80	1	0	0	0	9	0
1852	285	93 *NOTCH1*	3	1	1	1	17	2
2632	271	78 *DCHS1*	0	0	3	0	12	0
2775	84	23	0	0	0	0	4	0
2831	259	94 *NOTCH1*	0	0	2	0	11	3
2944	32	13	0	0	0	0	0	0
2985	269	82 *CFC1*	0	0	1	0	24	2
3009	261	89	3	1	1	0	20	0
3173	287	88 *NOTCH1*[a]	2	1	7	2	8	0
3208	283	93 *NOTCH1*	2	0	1	0	25	2
3225	545	143 *GATA4*	0	0	1	0	31	1
3320	228	84	4	3	1	1	24	4
3397	262	83 *TEK*	1	0	1	0	12	2
3485	270	112	0	0	2	0	14	0
3590	196	54	5	1	1	1	0	0
3657	110	34	0	0	2	0	9	2
3678	266	92	0	0	0	0	12	2

3695	264	77 *TLL1*	1	1	3	0	16	4
3755	74	31	0	0	3	0	17	0
3766	148	53 *NODAL*	3	3	2	2	4	0
3769	265	81	10	4	1	1	8	2
3792	99	24	10	1	5	3	13	0
3843	58	14	1	1	0	0	2	0
3932	243	76	1	1	1	0	15	2
3933	315	92 *BCOR*	1	0	0	0	9	0
1.53E+08	295	96 *NOTCH1*	5	3	1	0	6	2
1.69E+08	28	6 *JAG1*	0	0	0	0	0	0

Four inheritance models were applied, with respective minor allele frequencies (MAF): autosomal dominant (MAF < 0.001); *de novo* (MAF < 0.001); autosomal recessive (MAF < 0.01); compound heterozygous (MAF < 0.01), and the number of IGV-verified ("all") and predicted-damaging ("damaging") variants are reported. A predicted-damaging variant is defined by CADD ≥ 15 and PolyPhen2-HVAR ≠ *benign*. Gene names are presented in cases where clinically actionable variants were identified. [a] CNVs.

Table S10. Rare, predicted-damaging, disease-segregating variants identified by comprehensive analysis in families with sporadic CHD.

Family ID	Gene	Model	Familial CHD	ECA	Genomic position	Variant type	Nucleotide variant	Amino acid variant	ExAC MAF	PP2 HVAR	CADD	Human CHD	MGI	ACMG Class	ACMG criteria	Cardiac lesion
44	*HAAO* [261]	AR	N	Y	chr2:42769785	Stopgain	NM_012205.2: c.558G>A	Trp186*	0.000004067	NA	39	Y	Y	P (Ia)	PP3, PM2, PS3, PM3, PVS1	FSV
3651	*KIAA0586* [262]	AR	N	U	chr14:58547780	Splicing	NM_014749.4: c.4268-1G>A	NA	0.000004092	NA	24	Y	Y	P (Ib)	PM2, PVS1, PP3, PM3	MOT
2324	*SEMA3D* [263,264]	AD[a]	N	Y	chr7:85097926	Stopgain	NM_152754.2: c.191C>A	Ser64*	0	NA	39	Y	Y	P (Ic)	PM2, PVS1, PP3, BS2	MOT
1746	*HNRNPK* [265]	DN	N	N	chr9:83970264	Missense	NM_002140.4: c.1259C>T	Ser420Leu	0	0.977	22.6	Y	N	LP (II)	PM2, PP3, PS2, PM1, BP1	SD
3595	*KMT2C* [266-268]	DN	N	Y	chr7:152182223	Frameshift insertion	NM_170606.2: c.5636dup	Gln1880Alafs*9	0	NA	NA	Y	N	LP (II)	PM2, PP3, PS2, BS4	OL
44	*PTPRB*	AR	N	Y	chr12:70635976	Missense	NM_001109754.3: c.146A>C	Asn49Thr	0	0.783	22.7	N	Y	VUS	PM2, PP3, PM1, BP5	FSV
2252	*IFT140*	CH	N	N	chr16:1568260	Missense	NM_014714.3: c.1727G>T	Arg576Leu	0	0.413	23	N	Y	VUS	PM1, PM2, PP3	MOT
2252	*IFT140*	CH	N	N	chr16:1520618	Missense	NM_014714.3: c.3644C>G	Ala1215Gly	0	0.995	29.5	N	Y	VUS	PM1, PM2, PP3	MOT
2825	*DMD*	XLR	N	N	chrX:32386371	Missense	NM_000109.3: c.4589C>T	Pro1530Leu	0	0.999	32	N	Y	VUS	BP1, PP3, PM2	FSV
3191	*VEGFA*	AD[a]	N	U	chr6:43770708	Frameshift insertion	NM_003376.5: c.2_3insGACG	Leu1Lysfs*22	0	NA	NA	N	Y	VUS	PM2, PP3, BP5	FSV
3595	*PIKFYVE*	DN	N	Y	chr2:208326271	Missense	NM_015040.3: c.3460G>A	Asp1154Asn	3.47E-05	0.99	25.2	N	Y	VUS	BS2, PP3, PS2	OL
1085	*FZD10*	DN	N	N	chr12:130163255	Missense	NM_007197.3: c.313G>A	Ala105Thr	0	0.913	29	N	N	VUS	PM2, PP3, PM1	SD
2636	*PLXNB1*	DN	N	Y	chr3:48406839	Missense	NM_002673.5: c.6212T>G	Leu2071Arg	0	1	32	N	N	VUS	PM2, PP3,	FSV

															PM1	
3464	*HRG*	AR	N	N	chr3:186668978	Missense	NM_000412.4: c.227C>T	Ser76Leu	0	0.716	25.3	N	N	VUS	PM2, PP3, PM1	AVSD+
3630	*EHD1*	DN	N	U	chr11:64860253	Missense	NM_006795.3: c.586A>G	Ile196Val	0	0.531	24.8	N	N	VUS	PM2, PP3, PM1	SDMA
3675	*ANKRD12*	DN	N	U	chr18:9256046	Frameshift deletion	NM_001083625.2: c.2714del	Lys905Serfs*3	0	NA	NA	N	N	VUS	PM2, PP3	OL
3712	*PRDM1*	AD[a]	N	Y	chr6:106105534	Frameshift deletion	NM_001198.3: c.1377del	Met460Cysfs*46	0	NA	NA	N	Y	VUS	PM2, PP3	FSV
141550648	*HAVCR1*	AR	N	Y	chr5:157057923	Frameshift insertion	NM_012206.3: c.20_21insTA	Leu8Thrfs*2	0.0001	NA	NA	N	N	VUS	PM2, PP3	SD
1384	*DMD*	XLR	N	N	chrX:32345947	Missense	NM_000109.3: c.5558T>C	Leu1853Pro	0	0.974	27	N	Y	LB (I)	PM2, PP3, BP1, BP5	OL
3568	*DMD*	XLR	N	U	chrX:31968382	Missense	NM_000109.3: c.6547C>T	Arg2183Trp	0.0004	0.999	31	N	Y	LB (I)	PP3, BS2, BP6	MOT

hcCHD gene variants have been omitted. Model: inheritance model: AD: autosomal dominant; AR: autosomal recessive; CH: compound heterozygous; DN: *de novo*; XLR: X-linked recessive. Familial CHD: (Y/N) Yes/No; refers to the presence/absence of an individual with CHD within the immediate family of the proband, respectively. ECA: Extra-cardiac anomalies (not restricted to congenital defects) present in the proband; N: no; Y: yes; U: unknown. MAF: minor allele frequency with respect to ExAC. PP2 HVAR: PolyPhen-2 Hvar predictive score. Score ≥ 0.909: probably damaging; 0.908 ≤ score ≤ 0.447: possibly damaging; score ≤ 0.446: benign; NA: not applicable. CADD: scaled CADD Score ≥ 15: damaging; NA: not applicable. Human CHD: evidence of causing human CHD in the published literature. MGI: heart phenotypes in knock-out model (Mouse Genome Informatics: http://www.informatics.jax.org/). ACMG Class: pathogenicity interpretation according to the ACMG-AMP guidelines [10]: P, pathogenic; LP, likely-pathogenic; VUS: variant of uncertain significance; LB, likely-benign. ACMG criteria: evidence used to arrive at the ACMG-AMP pathogenicity classification.[10] Cardiac lesion: primary cardiac lesion of the proband. FSV: functional single ventricle; MOT: malformation of the outflow tract; SD: septal defect; OL: obstructive lesion; AVSD+: atrioventricular septal defect and variants; SDMA: septal defect with minor abnormalities. For detailed phenotype descriptions, including extra-cardiac phenotypes, see **Supplementary Table S1**. For pedigrees of families with pathogenic or likely-pathogenic variants, see **Supplementary Figure S3**. [a] Incomplete penetrance / variant present in unaffected individual.

Table S11. Rare, predicted-damaging, disease-segregating variants identified by comprehensive analysis in familial cases of CHD.

Family ID	Gene	Model	Familial CHD	ECA	Genomic position	Variant type	Nucleotide variant	Amino acid variant	ExAC MAF	PP2 HVAR	CADD	Human CHD	MGI	ACMG Class	ACMG criteria	Cardiac lesion
3397	*TEK* [269]	AD	Y	N	chr9:27212764	Missense	NM_000459.4: c.2744G>A	Arg915His	0	0.999	35	Y	Y	P (II)	PS1, PS3, PM2, PP3, PP1, PP2, PM1	SDMA
939	*KMT2C* [266-268]	DN	Y	Y	chr7:152177063	Frameshift insertion	NM_170606.2: c.8390dup	Glu2798Glyfs*11	0	NA	NA	Y	N	LP (II)	PM2, PP3, PS2, BS4	SD
2632	*DCHS1* [270]	AD[a]	Y	N	chr11:6632186	Missense	NM_003737.3: c.3326C>T	Pro1109Leu	0	0.991	17.85	Y	Y	LP (V)	PM2, PP3, PM1, PP2, BS2	FSV
939	*MAP3K3*	AD[a]	Y	Y	chr17:63691840	Missense	NM_002401.3: c.1452G>A	Met484Ile	8.25E-06	0.857	23.8	N	Y	VUS	BS2, PP3	SD
939	*PLCG1*	AD[a]	Y	Y	chr20:41172822	Missense	NM_002660.2: c.3224A>T	Asp1075Val	0.0003	0.749	25.6	N	Y	VUS	BS2, PP3	SD
1071	*NTN4*	AD[a]	Y	N	chr12:95710482	Missense	NM_021229.3: c.1139G>A	Arg380His	5.77E-05	0.941	34	N	Y	VUS	BS2, PP3	SD
1071	*RPS6KA2*	AD[a]	Y	N	chr6:166432421	Missense	NM_021135.5: c.1402A>G	Asn468Asp	0	0.992	28.6	N	Y	VUS	PM2, PP3	SD
1125	*GAB1*	AD	Y	Y	chr4:143440352	Missense	NM_002039.3: c.1555G>A	Val519Met	0	0.972	28.8	N	Y	VUS	PM2, PP3	SD
1125	*JUP*	AD	Y	Y	chr17:41769069	Missense	NM_002230.3: c.607C>T	Arg203Cys	4.58E-05	0.991	35	N	Y	VUS	PP3, BS2	SD
1125	*MEGF8* [271]	AD	Y	Y	chr19:42368542	Missense	NM_001410.2: c.6160T>C	Cys2054Arg	0	0.996	27.5	Y	Y	VUS	PM2, PP3, PP1, PP2	MOT
1125	*PDS5B*	AD	Y	Y	chr13:32770373	Missense	NM_015032.3: c.3877C>A	Pro1293Thr	1.68E-05	0.926	23.7	N	Y	VUS	BS2, PP3	SD
1179	*BIN1*	AD	Y	Y	chr2:127063935	Missense	NM_004305.3: c.603C>A	Asn201Lys	0.0004	0.969	28.1	N	Y	VUS	PP3, BS2	MOT
1179	*DNAH5* [272,273]	AD	Y	Y	chr5:13716583	Missense	NM_001369.2: c.12813G>T	Trp4271Cys	0	0.941	34	Y	Y	VUS	PM2, PP3, BP1, PM1, PP1	MOT
1179	*KDM5A*	AD	Y	Y	chr12:295629	Missense	NM_001042603.2: c.4399C>T	Arg1467Trp	0.00001062	0.996	32	N	N	VUS	BS2, PP3	MOT
1179	*KMT2C* [266-268]	AD	Y	Y	chr7:152144798	Missense	NM_170606.2: c.14258A>T	Gln4753Leu	0	0.57	29.8	Y	N	VUS	PM2, PP3, PP1,	MOT

															BP1	
1456	*LRP2* [274]	AD	Y	Y	chr2:169170568	Missense	NM_004525.2: c.11363C>T	Ser3788Leu	0	0.999	32	Y	Y	VUS	PM2, PP3, PP1, PM1, BP1, BP5	AVSD+
1456	*MMP9*	AD	Y	Y	chr20:46012540	Frameshift deletion	NM_004994.2: c.1283_1287del	Gly428Alafs*4	8.12E-06	NA	NA	N	Y	VUS	BS2, PP3	AVSD+
1456	*MTHFD2L*	AD	Y	Y	chr4:74201284	Missense	NM_001144978.1: c.626A>T	Asn209Ile	0	0.993	27.5	N	Y	VUS	PM2, PP3	AVSD+
1658	*PBRM1*	AD	Y	N	chr3:52589213	Missense	NM_018313.4: c.2822C>T	Ser941Leu	8.48E-05	0.744	25	N	Y	VUS	PP3	OL
1658	*PKD1* [275]	AD	Y	N	chr16:2091460	Missense	NM_000296.3: c.11672G>A	Arg3891His	0	0.998	28.1	Y	Y	VUS	PM2, PP3, PM1	OL
1658	*TAX1BP1*	AD	Y	N	chr7:27828762	Missense	NM_006024.6: c.2303A>C	Tyr768Ser	1.65E-05	0.676	23.2	N	Y	VUS	PP3	OL
2632	*CD36*	AD[a]	Y	N	chr7:80672791	Missense	NM_000072.3: c.1045G>C	Ala349Pro	5.79E-05	0.979	32	N	Y	VUS	BS2, PP3, PP2	FSV
2632	*EPB41L5*	AD[a]	Y	N	chr2:120019116	Missense	NM_020909.3: c.32G>A	Arg11His	2.47E-05	0.999	33	N	Y	VUS	BS2, PP3	FSV
2632	*ETV2*	AD[a]	Y	N	chr19:35643335	Missense	NM_014209.3: c.297G>C	Trp99Cys	3.78E-05	0.453	15.96	N	Y	VUS	BS2, PP3	FSV
2632	*XIRP2*	AD[a]	Y	N	chr2:167210822	Missense	NM_152381.5: c.650T>A	Val217Asp	8.28E-06	0.961	29	N	Y	VUS	BS2, PP3	FSV
2985	*PARVA*	AD	Y	N	chr11:12504379	Missense	NM_018222.4: c.727G>A	Ala243Thr	1.67E-05	0.906	28.2	N	Y	VUS	PP3, BP5	FSV
2985	*PLEC*	AD	Y	N	chr8:143919019	Missense	NM_000445.4: c.10883G>A	Arg3628Gln	6.73E-05	0.729	33	N	Y	VUS	PP3, BP5	FSV
3009	*CENPJ*	AD	Y	N	chr13:24883966	Missense	NM_018451.4: c.3821A>G	Gln1274Arg	0	0.86	25.4	N	Y	VUS	PM2, PP3	AVSD+
3009	*TMEM2*	AD	Y	N	chr9:71745087	Missense	NM_013390.2: c.964C>G	Leu322Val	0	0.992	24.8	N	N	VUS	PM2, PP3	AVSD+
3173	*PENK*	AD[a]	Y	Y	chr8:56441916	Missense	NM_006211.3: c.160G>A	Gly54Ser	0	0.987	25.6	N	Y	VUS	PM2, PP3, BP5	SDMA
3173	*ROBO1* [276]	AD[a]	Y	Y	chr3:78685829	Missense	NM_002941.3: c.1259G>A	Arg420Gln	0	0.84	27.5	Y	Y	VUS	PM2, PP3, PM1, BP5	SDMA
3225	*ACAN*	AD	Y	Y	chr15:88847363	Missense	NM_001135.3: c.1550A>G	Tyr517Cys	0	0.998	23.3	N	Y	VUS	PM2, PP3, BP5	SDMA
3225	*GYS2*	AD	Y	Y	chr12:21604586	Stopgain	NM_021957.3: c.7C>T	Arg3*	0	NA	36	N	Y	VUS	PM2, PP3, BP5	SDMA

3225	*PLAU*	AD	Y	Y	chr10:73916411	Missense	NM_002658.4: c.1142T>A	Val381Asp	0	1	26.1	N	Y	VUS	PM2, PP3, BP5	SDMA
3225	*PLCG1*	AD	Y	Y	chr20:41173702	Missense	NM_002660.2: c.3445A>T	Ile1149Phe	0	0.949	25.3	N	Y	VUS	PM2, PP3, BP5	SDMA
3225	*TTN*	AD	Y	Y	chr2:178584683	Missense	NM_003319.4: c.37763C>T	Ala12588Val	0	0.994	22	N	Y	VUS	PM2, PP3, BP5	SDMA
3225	*PXN*	AD	Y	Y	chr12:120215108	Missense	NM_002859.3: c.997G>A	Gly333Arg	0	1	34	N	Y	VUS	PM2, PP3, BP5	SDMA
3320	*SEMA3D* [263,264]	AD[a]	Y	Y	chr7:85068229	Missense	NM_152754.2: c.551C>G	Pro184Arg	0	1	28.2	Y	Y	VUS	PM2, PP3, PM1, PP1, BP1, BS2	FSV
3397	*FOXD3*	AD	Y	N	chr1:63324119	Missense	NM_012183.2: c.1061C>G	Ala354Gly	0	0.739	19.29	N	Y	VUS	PM2, PP3	SDMA
3590	*CD36*	AD[a]	Y	N	chr7:80673353	Splicing	NM_000072.3: c.1200-2A>G	NA	4.18E-05	NA	22.9	N	Y	VUS	BS2, PVS1, PP3	MOT
3590	*CDH2*	AD[a]	Y	N	chr18:27993525	Missense	NM_001792.4: c.1133A>G	Asn378Ser	8.25E-06	0.99	26.2	N	Y	VUS	BS2, PP3	MOT
3590	*FUZ*	AD[a]	Y	N	chr19:49807197	Missense	NM_025129.4: c.1211G>A	Arg404Gln	9.98E-05	0.99	26.2	N	Y	VUS	PP3	MOT
3590	*PPP3CB*	AD[a]	Y	N	chr10:73479494	Missense	NM_021132.3: c.109C>T	Arg37Cys	8.27E-06	0.995	23.5	N	Y	VUS	BS2, PP3	MOT
3590	*EXOC5*	DN	Y	N	chr14:57231701	Missense	NM_006544.3: c.953C>G	Ser318Cys	0	0.759	20.8	N	Y	VUS	PM2, PP3, BS4	MOT
3657	*ADAMTS6*	AD[a]	Y	Y	chr5:65224944	Missense	NM_197941.3: c.2171A>G	Asn724Ser	0	0.53	15.65	N	Y	VUS	PM2, PP3	MOT
3695	*COL1A1* [277]	AD	Y	N	chr17:50188540	Missense	NM_000088.3: c.3197G>A	Arg1066His	8.30E-06	0.997	31	Y	Y	VUS	BS2, PP3, PP2, PP1, PM1	MOT
3695	*CYBRD1*	AD	Y	N	chr2:171553412	Missense	NM_024843.3: c.469C>T	Pro157Ser	4.95E-05	1	32	N	Y	VUS	BS2, PP3	MOT
3695	*RBM20*	AD	Y	N	chr10:110781492	Missense	NM_001134363: c.883G>A	Gly295Arg	0	0.972	24.6	N	Y	VUS	PM2, PP3	MOT
3695	*THBS2*	AD	Y	N	chr6:169223389	Missense	NM_003247.3: c.2860A>G	Ile954Val	0	0.996	23.5	N	Y	VUS	PM2, PP3	MOT
3755	*DDX5*	AD[a]	Y	Y	chr17:64503052	Missense	NM_004396.4: c.857T>A	Val286Glu	0	1	31	N	Y	VUS	PM2, PP3	OL
3755	*MFGE8*	AD[a]	Y	Y	chr15:88899532	Splicing	NM_005928.3: c.1027A>T	Ile343Phe	4.94E-05	0.962	29.3	N	Y	VUS	BS2, PP3	OL

3755	*SEMA5A*	AD[a]	Y	Y	chr5:9054179	Missense	NM_003966.2: c.2597G>A	Arg866His	0	0.998	34	N	Y	VUS	PM2, PP3	OL
3755	*TLR2*	AD[a]	Y	Y	chr4:153703026	Missense	NM_003264.4: c.119C>T	Ser40Leu	0.0002	0.517	19.77	N	Y	VUS	BS2, PP3	OL
3769	*CC2D2A*	AD	Y	U	chr4:15601328	Missense	NM_001080522.2: c.4766C>G	Pro1589Arg	4.17E-05	0.687	23.7	N	Y	VUS	BS2, PP3	MOT
3769	*WHSC1*	AD	Y	U	chr4:1916994	Missense	NM_007331.1: c.884A>T	Gln295Leu	0.0004	0.843	24.2	N	Y	VUS	BS2, PP3	MOT
3769	*XIRP2*	AD	Y	U	chr2:167242881	Missense	NM_152381.5: c.1489G>C	Glu497Gln	0	1	26.3	N	Y	VUS	PM2, PP3	MOT
3769	*XIRP2*	AD	Y	U	chr2:167245635	Missense	NM_152381.5: c.4243G>A	Gly1415Ser	0.0006	1	23.4	N	Y	VUS	BS2, PP3	MOT
3792	*LMNA*	AD[a]	Y	N	chr1:156115079	Missense	NM_005572.3: c.161C>T	Thr54Met	0	0.473	23.1	N	Y	VUS	PM2, PP3, PP2, BS2	MOT
3792	*PDLIM5*	AD[a]	Y	N	chr4:94640428	Missense	NM_006457.4: c.1261G>A	Ala421Thr	6.62E-05	0.996	34	N	Y	VUS	BS2, PP3, PM1	MOT
3932	*ZFPM1*	AD	Y	N	chr16:88534806	Missense	NM_153813.2: c.2848C>G	Pro950Ala	6.43E-05	0.986	22.7	N	Y	VUS	BS2, PP3	FSV
3933	*ARID3B*	AD	Y	Y	chr15:74591701	Missense	NM_006465.3: c.1307A>G	Lys436Arg	0	0.985	24.1	N	Y	VUS	PM2, PP3, BP2	SD
152900216	*ANK1*	AD	Y	Y	chr8:41718113	Missense	NM_000037.3: c.1199T>C	Val400Ala	0	0.999	24.2	N	Y	VUS	PM2, PP3, BP1	MOT
195	*XIRP1*	AD[a]	Y	N	chr3:39189163	Missense	NM_194293.3: c.283C>T	Arg95Cys	8.25E-06	0.999	28.9	N	Y	LB (I)	PP3, BP2, BS2	FSV
575	*MYO10*	AD[a]	Y	Y	chr5:16681877	Missense	NM_012334.2: c.4183G>A	Val1395Met	0.0004	0.885	26.7	N	Y	LB (I)	BS2, PP3, BP5	MOT
575	*SPEN*	AD[a]	Y	Y	chr1:15934084	Missense	NM_015001.2: c.7844C>T	Ala2615Val	6.61E-05	0.714	24.9	N	Y	LB (I)	BS2, PP3, BP5	MOT
835	*PNMT*	AD	Y	Y	chr17:39669676	Missense	NM_002686.4: c.250G>A	Val84Met	0.0005	0.635	23.7	N	Y	LB (I)	BS2, PP3, BP5	FSV
1449	*FREM2*	AD	Y	Y	chr13:38856129	Missense	NM_207361.5: c.6929T>C	Ile2310Thr	6.62E-05	0.909	27.5	N	Y	LB (I)	BS2, PP3, BP5	SD
1449	*NFATC4*	AD	Y	Y	chr14:24370054	Missense	NM_004554.4: c.656G>C	Arg219Pro	8.82E-06	0.974	24.8	N	Y	LB (I)	BS2, PP3, BP5	SD
1449	*PDGFRA*	AD	Y	Y	chr4:54272430	Missense	NM_006206.5: c.1274A>G	His425Arg	1.65E-05	0.844	15.1	N	Y	LB (I)	BS2, PP3, BP5	SD
1449	*WHSC1*	AD	Y	Y	chr4:1916994	Missense	NM_007331.1:	Gln295Leu	0.0004	0.843	24.2	N	Y	LB (I)	BS2,	SD

							c.884A>T								PP3, BP5	
1449	*ROBO1* [276]	AD	Y	Y	chr3:78717374	Missense	NM_002941.3: c.818T>C	Val273Ala	0.0001	0.992	24.9	Y	Y	LB (I)	BS2, PP3, BP5	SD
1449	*RAD51D*	AD	Y	Y	chr17:35116884	Stopgain	NM_001142571.1: c.298C>T	Arg100*	3.18E-05	NA	23.5	N	Y	LB (I)	BS2, PP3, BP5	SD
1852	*AKAP13*	AD[a]	Y	Y	chr15:85543936	Missense	NM_006738.5: c.643C>G	Leu215Val	0.0004	0.851	26.8	N	Y	LB (I)	BS2, PP3, BP5	FSV
1852	*PRDM16*	AD[a]	Y	Y	chr1:3412079	Missense	NM_022114.3: c.1882G>A	Asp628Asn	0.0002	0.984	28	N	Y	LB (I)	BS2, PP3, PP2, BP5	FSV
3173	*NFAT5*	AD[a]	Y	Y	chr16:69647146	Missense	NM_006599.3: c.318G>C	Glu106Asp	8.26E-06	0.874	23.5	N	Y	LB (I)	BS2, PP3, BP5	SDMA
3225	*IFT122*	AD	Y	Y	chr3:129481617	Missense	NM_018262.3: c.1399C>T	Arg467Cys	0.0004	0.995	34	N	Y	LB (I)	BS2, PP3, PP2, BP5	SDMA
3225	*PINK1*	AD	Y	Y	chr1:20644515	Missense	NM_032409.2: c.802C>G	Leu268Val	9.89E-05	0.714	23.9	N	Y	LB (I)	BS2, PP3, BP5	SDMA
3225	*TIE1*	AD	Y	Y	chr1:43313378	Missense	NM_005424.4: c.2171G>A	Gly724Glu	8.26E-06	0.98	23.8	N	Y	LB (I)	BS2, PP3, BP5	SDMA
3397	*SCNN1B*	AD	Y	N	chr16:23380141	Missense	NM_000336.2: c.1514G>A	Arg505His	8.24E-05	0.996	34	N	Y	LB (I)	BS2, PP3, BP5	SDMA
3590	*DNAH11* [278]	AD[a]	Y	N	chr7:21638985	Missense	NM_001277115.1: c.4864C>T	Arg1622Cys	3.32E-05	0.92	35	Y	Y	LB (I)	BS2, PP3, BP1	MOT
3678	*COL18A1*	AD	Y	U	chr21:45478330	Missense	NM_030582.3: c.1765G>A	Asp589Asn	0.0003	0.97	23.4	N	Y	LB (I)	BS2, PP3, BP1	MOT
3678	*DNAH5* [272,273]	AD	Y	U	chr5:13840955	Missense	NM_001369.2: c.5660A>G	Glu1887Gly	8.24E-06	0.996	23.6	Y	Y	LB (I)	BS2, PP3, BP1	MOT
3792	*GAA*	AD[a]	Y	N	chr17:80107685	Missense	NM_000152.4: c.821C>T	Thr274Ile	1.67E-05	0.774	23.7	N	Y	LB (I)	BS2, PP3, PP2	MOT
3933	*MED23*	AD	Y	Y	chr6:131603130	Missense	NM_004830.3: c.1831C>T	Arg611Trp	8.24E-06	1	34	N	Y	LB (I)	BS2, PP3, PP2, BP5	SD
3933	*MYO10*	AD	Y	Y	chr5:16681877	Missense	NM_012334.2: c.4183G>A	Val1395Met	0.0004	0.885	26.7	N	Y	LB (I)	BS2, PP3,	SD

															BP5	
3933	*PLXND1* [279]	AD	Y	Y	chr3:129565899	Missense	NM_015103.2: c.4310C>T	Ala1437Val	8.31E-06	0.983	23.7	Y	Y	LB (I)	BS2, PP3, BP5	SD
3933	*UBE4B*	AD	Y	Y	chr1:10132442	Missense	NM_006048.4: c.1598C>T	Ala533Val	8.25E-06	0.985	35	N	Y	LB (I)	BS2, PP3, BP5	SD
1179	*ABCC6* [280]	AD	Y	Y	chr16:16154935	Missense	NM_001171.5: c.3979G>A	Gly1327Arg	0.0001377	1	32	Y	Y	B (II)	PM1, PP1, PP3, BS1, BS2	MOT
2632	*LHX1*	AD[a]	Y	N	chr17:36942845	Missense	NM_005568.4: c.935C>A	Pro312His	0.0002889	0.781	27.1	N	Y	B (II)	PP3, BS1, BS2	FSV

hcCHD gene variants have been omitted. Model: inheritance model: AD: autosomal dominant; AR: autosomal recessive; CH: compound heterozygous; DN: *de novo*; XLR: X-linked recessive. Familial CHD: (Y/N) Yes/No; refers to the presence/absence of an individual with CHD within the immediate family of the proband, respectively. ECA: Extra-cardiac anomalies (not restricted to congenital defects) present in the proband; N: no; Y: yes; U: unknown. MAF: minor allele frequency with respect to ExAC. PP2 HVAR: PolyPhen-2 Hvar predictive score. Score ≥ 0.909: probably damaging; 0.908 ≤ score ≤ 0.447: possibly damaging; score ≤ 0.446: benign; NA: not applicable. CADD: scaled CADD Score ≥ 15: damaging; NA: not applicable. Human CHD: evidence of causing human CHD in the published literature. Mouse heart phenotype: heart phenotypes in knock-out model (Mouse Genome Informatics: http://www.informatics.jax.org/). ACMG Class: pathogenicity interpretation according to the ACMG-AMP guidelines [10]: P, pathogenic; LP, likely-pathogenic; VUS: variant of uncertain significance; LB, likely-benign; B, benign. ACMG criteria: evidence used to arrive at the ACMG-AMP pathogenicity classification.[10] Cardiac lesion: primary cardiac lesion of the proband. FSV: functional single ventricle; MOT: malformation of the outflow tract; SD: septal defect; OL: obstructive lesion; AVSD+: atrioventricular septal defect and variants; SDMA: septal defect with minor abnormalities. For detailed phenotype descriptions, including extra-cardiac phenotypes, see **Supplementary Table S1**. For pedigrees of families with pathogenic or likely-pathogenic variants, see **Supplementary Figure S3**. [a] Incomplete penetrance / variant present in unaffected individual.

Table S12. List of genes where loss-of-function causes heart defects in mice. Genes were extracted from the Mouse Genome Database (Mouse Genome Informatics: www.informatics.jax.org).

A1cf, A4gnt, Aars, Aars2, Abca1, Abca5, Abcb8, Abcc6, Abcc9, Abcg5, Abhd5, Abi1, Abl1, Abra, Acacb, Acadl, Acadm, Acadvl, Acan, Ace, Ace2, Ackr3, Acsl1, Acsl4, Acsl5, Actc1, Acvr1, Acvr2b, Acvrl1, Adam10, Adam17, Adam19, Adamts12, Adamts6, Adamts7, Adamts9, Adgra2, Adgrf5, Adgrg6, Adh5, Adipoq, Adm, Adora2a, Adra1b, Adra2b, Afap1l2, Afdn, Ager, Ago2, Agt, Agtr1, Agtr2, Ahr, Aifm1, Aip, Akap12, Akap13, Akap6, Akt1, Aldh1a2, Alg10b, Alg13, Alpk3, Amer1, Amn, Amot, Angpt1, Angpt2, Angptl4, Angptl6, Ank1, Ankrd1, Ankrd17, Ankrd26, Anks6, Anxa1, Anxa2, Ap2b1, Ap4e1, Apc, Apex1, Aph1a, Aplnr, Apoe, Ar, Arap3, Arhgef1, Arhgef15, Arhgef4, Arid1a, Arid2, Arid3a, Arid3b, Arl5c, Armc4, Arnt, Arntl, Arsb, Asl, Asxl2, Ate1, Atg5, Atmin, Atp1a2, Atp1b1, Atp2a2, Atp6ap2, Atp7a, Atr, Atrx, Axin1, Axin2, B4galt1, B9d1, Bag3, Baz1b, Bbx, Bcar1, Bcl6, Bcor, Becn1, Bicc1, Bin1, Birc6, Bloc1s2, Bmp10, Bmp2, Bmp4, Bmp5, Bmpr1a, Bmpr2, Bnip3l, Braf, Brca1, Bscl2, Btaf1, Btc, Bves, C1galt1, C1orf127, C2cd3, C5orf42, Cab39l, Cacna1c, Cacna1h, Cacna1i, Cacnb3, Calcrl, Calr, Camk2d, Camta2, Capn1, Capn2, Capns1, Casp8, Casq2, Cast, Cav1, Cav2, Cav3, Cbfb, Cbsl, Cc2d2a, Ccdc151, Ccdc160, Ccdc39, Ccdc88a, Ccl2, Ccm2, Ccm2l, Ccna2, Ccnd1, Ccnd2, Ccnd3, Ccnf, Ccr5, Cd151, Cd2ap, Cd36, Cd44, Cd47, Cd82, Cdc20, Cdc73, Cdh2, Cdh5, Cdkn1a, Cdkn1b, Cenpa, Cenpf, Cenpj, Cep290, Cep41, Cfc1b, Cfl1, Cflar, Chd2, Chd7, Chga, Chic2, Chm, Chmp5, Chrd, Chrnb2, Chst14, Ciapin1, Cib2, Cisd2, Cited2, Ckm, Ckmt2, Clcn3, Clec4e, Clic4, Clu, Cluap1, Cnot3, Cntrl, Col18a1, Col1a1, Col1a2, Col2a1, Col3a1, Col4a3bp, Commd9, Coq9, Corin, Cox1, Cox7a1, Crb2, Crebbp, Crhr2, Crk, Crkl, Csk, Csnk1a1, Csnk2a1, Csrp2, Csrp3, Ctbp2, Ctdsp2, Ctgf, Ctla4, Ctnna1, Ctnna3, Ctnnb1, Ctnnbip1, Ctss, Cubn, Cul4b, Cul7, Cxadr, Cxcl12, Cxcr3, Cxcr4, Cybb, Cybrd1, Cyp11b2, Cyp11b2, Cyp26a1, Cyp27b1, Cyp51a1, Cyr61, Daam1, Dag1, Dagla, Dand5, Daw1, Dbh, Dbn1, Dcbld2, Dchs1, Dctn5, Ddc, Ddr2, Ddx11, Ddx17, Ddx3x, Ddx5, Dedd, Defb131, Des, Dgcr8, Dhrs3, Diaph2, Diaph3, Dicer1, Disp1, Dlc1, Dll1, Dll4, Dlx3, Dmd, Dmp1, Dnaaf2, Dnaaf3, Dnah11, Dnah5, Dnai1, Dnaja3, Dnase1l2, Dnm1l, Dnm2, Dnmt3b, Dnmt3l, Dock1, Dock4, Dot1l, Dpm2, Drc1, Drd5, Dsp, Dstn, Dtna, Dusp3, Dusp6, Dusp7, Dusp8, Dvl2, Dvl3, Dync2h1, Dync2li1, Dynll1, Dynlrb2, Dyrk1a, Dyx1c1, Eaf2, Ece1, Ecscr, Edn1, Ednra, Efemp2, Efna1, Efnb2, Egfl7, Egfr, Egln1, Egr1, Egr2, Ehmt1, Eif2ak1, Eif2b5, Elavl1, Elk3, Eln, Eloa, Emc10, Emd, Eng, Enpp1, Enpp2, Entpd1, Ep300, Epas1, Epb41l5, Epha2, Epha3, Ephb4, Ephx2, Epm2a, Epo, Epor, Erbb2, Erbb3, Erbb4, Erf, Erg, Esam, Esr1, Esr2, Ets2, Etv2, Etv6, Exoc5, Eya1, Ezh2, F10, F11r, F2, F2r, F3, F7, Fabp3, Fadd, Fam134c, Fam151a, Fas, Fat4, Fbln1, Fbln5, Fbn1, Fbxl5, Fbxw7, Fdx1, Fes, Fgf10, Fgf16, Fgf19, Fgf2, Fgf8, Fgf9, Fgfbp3, Fgfr1, Fgfr2, Fgfrl1, Fhl2, Fhod3, Fig4, Fkbp1a, Fkbp1b, Fkbpl, Fli1, Flna, Flnb, Flrt2, Flrt3, Flt1, Flt4, Flvcr1, Fn1, Fnbp1l, Folh1, Fosl1, Fosl2, Foxa2, Foxc1, Foxc2, Foxd3, Foxf1, Foxh1, Foxj1, Foxm1, Foxo1, Foxp1, Foxp3, Foxp4, Fras1, Frem2, Frs2, Fstl1, Fstl3, Fubp1, Furin, Fus, Fuz, Fxn, Fxr1, Fxyd1, Fzd4, Fzd5, Fzd7, G6pd, Gaa, Gab1, Galnt3, Gata1, Gata2, Gata4, Gata5, Gata6, Gatad2a, Gbe1, Gbx2, Gdf1, Ghr, Gimap6, Gipc1, Gja1, Gja5, Gjc1, Gla, Gli3, Glmn, Glrx3, Gltscr1l, Gna13, Gnaq, Gnas, Gng5, Gpc3, Gpi, Gpr4, Gps1, Gpx1, Gpx7, Grb14, Grb2, Greb1, Grhl2, Grin2d, Grk2, Grk5, Grm6, Gsto2, Gstz1, Gtf2i, Gtf2ird1, Gucy1a3, Gucy2c, Gyg1, Gys1, Gys2, H2afx, Hace1, Hadha, Hadhb, Hand1, Hand2, Has2, Hbegf, Hccs, Hdac2, Hdac3, Hdac5, Hdac7, Hdac9, Hectd1, Hectd3, Heg1, Herc4, Hexim1, Hey2, Hfe2, Hgs, Hhex, Hic2, Hif1a, Hif3a, Hira, Hla-Dqb1, Hmga1, Hmgcl, Hmox1, Hopx, Hoxa1, Hoxa13, Hoxa3, Hoxb4, Hoxb7, Hpgd, Hprt1, Hpse, Hras, Hsd11b2, Hsd17b12, Hsd17b7, Hsp90b1, Hspb11, Hspb8, Hspg2, Htr2b, Htra2, Hus1, Huwe1, Icam1, Ide, Idh2, Idua, Ier3, Ifngr1, Ift122, Ift140, Ift172, Ift27, Ift46, Ift57, Ift74, Ift88, Igf1r, Igf2, Igf2r, Igfbp2, Ighmbp2, Ihh, Ikbkap, Il11ra, Il12rb2, Il13, Il17rd, Il18, Il1a, Il1b, Il1rn, Il5, Il6, Il6st, Ilk, Ino80, Inpp5f, Ins, Intu, Invs, Ireb2, Irx4, Isl1, Itga4, Itga5, Itga6, Itgav, Itgb1, Itgb3, Itgb4, Itgb8, Itpa, Jag1, Jarid2, Jmjd6, Jph2, Jun, Jund, Jup, Kat2a, Kat6a, Kat7, Kcnh2, Kcnmb1, Kcnn4, Kcnq1, Kctd10, Kdm2a, Kdm2b, Kdm4a, Kdm5c, Kdm6a, Kdm8, Kdr, Kiaa0586, Kidins220, Kif3a, Kif3b, Kif7, Kifap3, Kiss1r, Kl, Klf15, Klf2, Klf3, Klf5, Klf6, Klhl40, Klk3, Kmt2b, Kmt2, Knop1, Kras, Krit1, Krt1, Lama4, Lama5, Lamp2, Large1, Lasp1, Lats2, Lbx1, Ldb1, Ldb3, Ldha, Lef1, Lefty1, Lefty2, Lemd2, Lemd3, Lep, Lepr, Leprotl1, Letm1, Lhcgr, Lhx1, Lias, Lig3, Lin28a, Lmna, Lmo2, Lmod2, Lnpep, Lox, Loxl2, Lpar4, Lpl, Lrp1, Lrp2, Lrpprc, Lta,

Ltbp1, Ltbp4, Luzp1, Ly6e, Mab21l2, Map1s, Map2k1, Map2k3, Map2k5, Map2k7, Map3k1, Map3k3, Map3k7, Mapk1, Mapk11, Mapk12, Mapk14, Mapk3, Mapk6, Mapk7, Mapk8, Mapk9, Mapkap1, Mark2, Marveld2, Matr3, Mb, Mbd4, Mbtd1, Mcam, Mcoln1, Mcu, Mdm2, Mdm4, Mecom, Mecp2, Med1, Med12, Med23, Med24, Med30, Mef2a, Mef2c, Mef2d, Megf8, Meis1, Memo1, Men1, Meox2, Mesp1, Mesp2, Mest, Metap2, Mfge8, Mfn2, Mfsd8, Mgat1, Mgp, Mgrn1, Mib1, Mid2, Mixl1, Mkl1, Mkl2, Mks1, Mlst8, Mmp13, Mmp14, Mmp2, Mmp21, Mmp9, Morf4l1, Mospd3, Mpi, Mpzl3, Mre11, Mrps34, Msantd1, Mstn, Mterf3, Mterf4, Mthfd1, Mthfd2l, Mtmr14, Mto1, Mtus1, Murc, Mus81, Myb, Mybbp1a, Mybpc3, Myc, Mycn, Myh10, Myh11, Myh6, Myh7, Myh9, Myl2, Myl7, Mylk3, Myo10, Myo18b, Myo6, Myocd, Myoz2, Naca, Ncf1, Nckap1, Nckap1l, Ncoa6, Ncor1, Ncor2, Ncstn, Ndst1, Ndufs3, Ndufs6, Nedd4l, Nek6, Nek8, Nexn, Nf1, Nfat5, Nfatc1, Nfatc4, Ngf, Ngfr, Nipbl, Nkx2-5, Nlrp9, Nodal, Nol3, Nos1, Nos1ap, Nos2, Nos3, Nostrin, Notch1, Notch2, Notch3, Nov, Nox4, Nphp3, Npm1, Nppa, Nppb, Npr1, Nprl3, Npy1r, Nr1d1, Nr2f2, Nr3c1, Nr3c2, Nr6a1, Nrarp, Nrg1, Nrp1, Nsdhl, Ntf3, Ntn4, Ntrk3, Numb, Nup133, Nxn, Opa3, Osr1, Otub1, Otulin, Otx2, Ovol2, Pak1, Pak4, Palb2, Pam, Pappa2, Pard3, Pard3b, Parva, Patz1, Pax3, Paxip1, Pbrm1, Pbx1, Pc, Pcnt, Pcsk5, Pcsk6, Pdc, Pdcd1, Pdcd10, Pde12, Pdgfa, Pdgfb, Pdgfra, Pdgfrb, Pdha1, Pdlim3, Pdlim5, Pdlim7, Pdpk1, Pdpn, Pds5a, Pds5b, Pdzrn3, Penk, Pepd, Pex7, Pfkfb2, Pfkfb3, Pfkm, Pfn1, Pgf, Phc1, Phip, Piezo1, Pifo, Pigv, Pik3c3, Pik3ca, Pik3cb, Pik3cg, Pik3r1, Pikfyve, Pink1, Pip5k1c, Pitx2, Pja2, Pkd1, Pkd1l1, Pkd2, Pknox1, Pkp2, Pla2g15, Pla2g4a, Plagl1, Plat, Plau, Plaur, Plce1, Plcg1, Pld1, Plec, Plekhg5, Plg, Plin4, Plin5, Pln, Plpp3, Plrg1, Plvap, Plxnd1, Pmm2, Pnmt, Pnn, Pnpla2, Pnpla6, Pofut1, Pofut2, Poglut1, Polg, Popdc2, Por, Postn, Ppara, Pparg, Ppargc1a, Ppm1a, Ppp1r13l, Ppp1r3c, Ppp2r5c, Ppp3ca, Ppp3cb, Ppp3r1, Prdm1, Prdm16, Prdm4, Prdm6, Prelid2, Prickle1, Prkab1, Prkar1a, Prkca, Prkci, Prkd1, Prokr1, Pros1, Prrx1, Psen1, Pskh1, Ptcd2, Ptch1, Pten, Ptger1, Ptger4, Ptgs2, Pth, Ptk2, Ptk2b, Ptk7, Ptpn11, Ptpn12, Ptprb, Ptprj, Ptrf, Pxn, Qki, Rab19, Rac1, Rac2, Rad17, Rad23b, Rad51d, Raf1, Ramp2, Rap1a, Rapgef2, Rasa1, Rasa3, Rasd1, Rasip1, Rassf1, Rb1, Rb1cc1, Rbl2, Rbm20, Rbp4, Rbpj, Rcan1, Rce1, Rdh10, Reck, Rela, Ren, Rere, Rev3l, Rfwd2, Rfx3, Rgcc, Rhbdf1, Rheb, Rhob, Rhobtb3, Rhoj, Ric1, Ric8b, Rictor, Ripk3, Ripply3, Rnf10, Rnf213, Rnf4, Rnf7, Rnls, Robo1, Robo4, Rock2, Ror2, Rora, Rpa1, Rpgrip1l, Rpl38, Rps6ka2, Rps6ka6, Rpsa, Rrad, Rras, Rrm2b, Rspo3, Rtel1, Rttn, Runx1, Runx2, Rxra, Ryr1, Ryr2, S100a1, S1pr1, S1pr2, Sall4, Sc5d, Scarb1, Scg5, Scn5a, Scn8a, Scnn1b, Scx, Scyl1, Sdc4, Sec24b, Sema3a, Sema3c Sema3d, Sema4d, Sema5a, Senp1, Senp2, Serf1b, Serpinc1, Serpine1, Serpinf1, Setd2, Sfrp1, Sgca, Sgcb, Sgcd, Sgcg, Sgpl1, Sgsh, Sgta, Sh3pxd2b, Sharpin, Shc1, Shh, Shoc2, Shox2, Sik1, Sirt1, Sirt7, Six1, Slc11a2, Slc20a1, Slc22a5, Slc25a4, Slc2a4, Slc31a1, Slc38a10, Slc39a4, Slc40a1, Slc4a1, Slc6a4, Slc6a6, Slc8a1, Slc9a1, Slco2a1, Slit3, Smad1, Smad2, Smad4, Smad5, Smad6, Smad7, Smarca1, Smarca4, Smarcad1, Smg1, Smg9, Smn2, Smo, Smtn, Smyd1, Snai1, Snai2, Snrk, Snrnp200, Snx17, Snx27, Sod1, Sod2, Sos1, Sox11, Sox4, Sox7, Sox9, Sp4, Sparc, Speg, Spen, Spint1, Spp1, Spry2, Spta1, Sptan1, Sptb, Sptbn1, Srd5a3, Srf, Srsf1, Srsf10, Srsf2, Ss18, Ssbp2, Ssr1, Stard13, Stat3, Steap3, Stk11, Strap, Sufu, Sumo2, Supt20h, T, Tab1, Tab2, Tal1, Tax1bp1, Tbc1d32, Tbc1d4, Tbx1, Tbx18, Tbx2, Tbx20, Tbx3, Tbx4, Tbx5, Tbx6, Tcap, Tcea1, Tcf21, Tcf7l1, Tctn2, Tdg, Tdgf1, Tead1, Tek, Tfam, Tfap2a, Tfap2b, Tfb1m, Tfpi, Tfrc, Tgfb1, Tgfb2, Tgfbr1, Tgfbr2, Tgfbr3, Tgm2, Th, Thbd, Thbs1, Thbs2, Thbs4, Thra, Tie1, Timp3, Timp4, Tjp1, Tk2, Tkt, Tll1, Tlr2, Tlr4, Tmed2, Tmem100, Tmem106b, Tmem255b, Tmem67, Tmod1, Tmprss6, Tmsb4x, Tnf, Tnfsf4, Tnni3, Tnnt2, Tp53, Tph1, Tpm1, Tra2b, Traf6, Trex1, Trim28, Trim37, Trim54, Trim55, Trim63, Trip11, Trpm2, Tsc1, Tsc2, Tsc22d1, Tslp, Ttbk2, Ttc7a, Ttn, Twnk, Txndc5, Txnip, Txnrd2, Ube2b, Ube2u, Ube3c, Ube4b, Ubp1, Ubr4, Ubr5, Unc5b, Usp12, Usp24, Usp54, Usp8, Usp9x, Utf1, Uvrag, Vac14, Vangl2, Vash1, Vash2, Vav2, Vav3, Vcam1, Vcan, Vcl, Vcp, Vdr, Vegfa, Vegfb, Vezf1, Vhl, Vim, Vip, Vps52, Vps54, Wasf2, Wasl, Wdpcp, Wdr1, Wdr35, Wdr83, Whrn, Whsc1, Wnk1, Wnt5a, Wrn, Wt1, Xbp1, Xiap, Xirp1, Xirp2, Yap1, Yipf5, Ywhae, Ywhaq, Zbtb14, Zdhhc2, Zfp36, Zfp36l1, Zfpm1, Zfpm2, Zic3, Zmiz1, Zmpste24, Znf148, Znf366, Znf521, Zscan10.

Table S13. All rare, disease-segregating, CNVs identified in genes with a knockout mouse heart phenotype within the cohort.

Family	Gene	Model	Familial CHD	ECA	CNV type	Chrom	CNV start	CNV end	Size	Frequency	Region affected	ACMG class.	Cardiac lesion
1456	*BMS1P21, MBL1P, SFTPD, TMEM254-AS1, TMEM254, PLAC9, ANXA11, LINC00857, LOC100130698, MAT1A, DYDC1, DYDC2, FAM213A, TSPAN14, LOC101929574, LOC102723703, SH2D4B, LOC105378387, LOC105378386, LOC105378389, LOC105378390, NRG3, LOC107984294, NRG3-AS1, LOC107984187, LOC105378392, LOC105378394, LOC105378393, LOC105378396, LOC105378397, LOC107984181, LOC105378398, LOC105378399, GHITM, HOST2, C10orf99, CDHR1, LRIT2, LRIT1, RGR, LINC00858, CCSER2, LOC105378400, LOC105378401, LOC107984249, LINC01519, LOC105378402, LOC105378403, LOC101929646, LOC101929662, LINC01520, LOC105378404, GRID1-AS1, GRID1, MIR346, WAPL, LOC105378408, LOC105378407, LOC105378406, OPN4, LOC105378409, LDB3, BMPR1A, MMRN2, SNCG, ADIRF, AGAP11, FAM25A, GLUD1, FAM35A, LOC105378410, NUTM2A, NUTM2A-AS1, LINC00863, NUTM2D*	AD	Y	Y	DEL	chr10	79859001	87375000	7.5 Mb	0.0027	Multiple genes	P	AVSD+
195	*RERE*	AD[a]	Y	N	DEL	chr1	8635120	8655822	20.7 kb	0.0054	Intronic	VUS	FSV
1125	*RB1*	AD	Y	Y	DEL	chr13	48307582	48308382	800 bp	0.0203	Intronic	VUS	SD
1125	*ARHGEF4*	AD	Y	Y	DEL	chr2	130902484	130902731	247 bp	0.0163	Intergenic	VUS	SD
1125	*C5orf42*	AD	Y	Y	DEL	chr5	37254529	37257666	3.14 kb	0.0461	Intergenic	VUS	SD
1125	*CD2AP*	AD	Y	Y	DEL	chr6	47596993	47599175	2.18 kb	0.0474	Intronic	VUS	SD
1179	*ZMIZ1*	AD	Y	Y	DEL	chr10	79175462	79176702	1.24 kb	0.0081	Intronic	VUS	MOT
1179	*TRIM37*	AD	Y	Y	DEL	chr17	59067737	59067852	115 bp	0.0285	Intronic	VUS	MOT
1449	*FAM151A*	AD	Y	Y	DEL	chr1	54610754	54610810	56 bp	0.0027	Intronic	VUS	SD

1449	*FLT1*	AD	Y	Y	DEL	chr13	28377409	28377476	67 bp	0.0447	Intronic	VUS	SD
1449	*RERE, (SLC45A1)*	AD	Y	Y	DUP	chr1	8053462	8419850	366.39 kb	0.0027	Exonic	VUS	SD
1456	*LTBP1*	AD	Y	Y	DEL	chr2	33057082	33057280	198 bp	0.042	Intronic	VUS	AVSD+
1456	*HMOX1*	AD	Y	Y	DEL	chr22	35378812	35378890	78 bp	0.0027	Intergenic	VUS	AVSD+
1456	*PPARGC1A*	AD	Y	Y	DEL	chr4	23909434	23910245	811 bp	0.0081	Intronic	VUS	AVSD+
1456	*SEMA5A*	AD	Y	Y	DEL	chr5	9512781	9514373	1.59 kb	0.0027	Intronic	VUS	AVSD+
1456	*SSBP2*	AD	Y	Y	DEL	chr5	81423515	81424197	682 bp	0.0068	Intronic	VUS	AVSD+
1658	*CTNNBIP1*	AD	Y	N	DEL	chr1	9850813	9851169	356 bp	0.0027	Intronic	VUS	OL
1852	*UBE4B*	AD[a]	Y	Y	DEL	chr1	10036020	10043597	7.58 kb	0.0054	Intronic	VUS	FSV
2252	*GTF2IRD1*	DN	N	N	DEL	chr7	74601654	74601979	325 bp	0.0014	Exonic	VUS	MOT
2944	*FXN*	AD	Y	N	DEL	chr9	69056773	69056983	210 bp	0.0366	Intronic	VUS	SDMA
2985	*NOS1AP*	AD	Y	N	DEL	chr1	162357534	162357975	441 bp	0.023	Intronic	VUS	FSV
2985	*TRIM37*	AD	Y	N	DEL	chr17	59067737	59067852	115 bp	0.0285	Intronic	VUS	FSV
2985	*FHOD3*	AD	Y	N	DEL	chr18	36353894	36353987	93 bp	0.0122	Intronic	VUS	FSV
2985	*ERBB4*	AD	Y	N	DEL	chr2	211768748	211768802	54 bp	0.019	Intronic	VUS	FSV
2985	*LAMA5*	AD	Y	N	DEL	chr20	62376895	62377027	132 bp	0.0352	Intergenic	VUS	FSV
2985	*ABRA*	AD	Y	N	DEL	chr8	106771144	106775756	4.61 kb	0.0108	Intergenic	VUS	FSV
3225	*CAPN1*	AD	Y	Y	DUP	chr11	65202237	65204309	2.07 kb	0.0027	Exonic	VUS	SDMA
3397	*PARD3B*	AD	Y	N	DEL	chr2	205263313	205265134	1.82 kb	0.0488	Intronic	VUS	SDMA
3397	*MTHFD2L*	AD	Y	N	DEL	chr4	74148633	74149495	862 bp	0.0136	Intronic	VUS	SDMA
3400	*PPP1R13L*	AR	N	N	DEL	chr19	45400768	45401275	507 bp	0.0217	Intronic	VUS	OL
3400	*SSBP2*	AR	N	N	DEL	chr5	81455338	81455843	505 bp	0.0325	Intronic	VUS	OL
3568	*DNMT3B*	DN	N	U	DEL	chr20	32797772	32798055	283 bp	0.0014	Intronic	VUS	MOT
3571	*PRDM16*	DN	N	U	DEL	chr1	3321785	3322080	295 bp	0.0068	Intronic	VUS	MOT
3610	*SSBP2*	AR	N	N	DEL	chr5	81455338	81455843	505 bp	0.0325	Intronic	VUS	MOT
3630	*TMEM106B*	AR	N	U	DEL	chr7	12241832	12242402	570 bp	0.0163	Exonic	VUS	SDMA
3678	*ROR2*	AD	Y	U	DEL	chr9	91870089	91870157	68 bp	0.0081	Intronic	VUS	MOT
3695	*HSPB11*	AD	Y	N	DEL	chr1	53907310	53916109	8.8 kb	0.0027	Intergenic	VUS	MOT
3695	*LAMA5*	AD	Y	N	DEL	chr20	62376895	62377027	132 bp	0.0352	Intergenic	VUS	MOT

3695	*POPDC2*	AD	Y	N	DEL	chr3	119631741	119635251	3.51 kb	0.0176	Intergenic	VUS	MOT
3695	*MTERF3*	AD	Y	N	DEL	chr8	96250299	96250541	242 bp	0.0312	Intronic	VUS	MOT
3695	*ABRA*	AD	Y	N	DEL	chr8	106771144	106775756	4.61 kb	0.0108	Intergenic	VUS	MOT
3769	*RPS6KA2*	AD	Y	U	DEL	chr6	166765042	166765182	140 bp	0.0325	Intronic	VUS	MOT
3932	*NPRL3*	AD	Y	N	DEL	chr16	131489	136640	5.15 kb	0.0027	Intronic	VUS	FSV
3933	*LTBP1*	AD	Y	Y	DEL	chr2	33057082	33057280	198 bp	0.042	Intronic	VUS	SD
3933	*PARD3B*	AD	Y	Y	DEL	chr2	205263313	205265134	1.82 kb	0.0488	Intronic	VUS	SD
3933	*TGM2*	AD	Y	Y	DEL	chr20	38122404	38122467	63 bp	0.0379	Intergenic	VUS	SD
3933	*RFX3*	AD	Y	Y	DEL	chr9	3393535	3393770	235 bp	0.0108	Intronic	VUS	SD
152900216	*FOXP1*	AD	Y	Y	DEL	chr3	70956607	70956869	262 bp	0.0027	Exonic	VUS	MOT
152900216	*MAPK6*	AD	Y	Y	DEL	chr15	51996442	51997319	877 bp	0.0027	Intergenic	VUS	MOT
152900216	*FXN*	AD	Y	Y	DEL	chr9	69056773	69056983	210 bp	0.0366	Intronic	VUS	MOT

Model: inheritance model: AD: autosomal dominant; AR: autosomal recessive; DN: *de novo*. Familial CHD: (Y/N) Yes/No; refers to the presence/absence of an individual with CHD within the immediate family of the proband, respectively. ECA: Extra-cardiac anomalies (not restricted to congenital defects) present in the proband; N: no; Y: yes; U: unknown. CNV type: DEL: deletion; DUP: duplication. CNV start/end: genomic position of the CNV in human genome reference hg38. Frequency: frequency of variant within our cohort. Region affected: variant falls within an exon, intron, or intergenic region of the gene. ACMG class: pathogenicity interpretation according to the ACMG guidelines [10]: P, pathogenic; VUS, variant of uncertain significance. Cardiac lesion: primary cardiac lesion of the proband. FSV: functional single ventricle; MOT: malformation of the outflow tract; SD: septal defect; OL: obstructive lesion; AVSD+: atrioventricular septal defect and variants; SDMA: septal defect with minor abnormalities. For detailed phenotype descriptions, including extra-cardiac phenotypes, see **Supplementary Table S1**. For pedigree of family 1456, see **Supplementary Figure S3**.
[a] Incomplete penetrance / variant present in unaffected individual.

Table S14. Findings from recent, notable studies of CHD cohorts.

Sequencing method	Publication	Cohort characteristics	Cohort size	ACMG class. used (Y/N)	Overall diagnostic rate	CHD+ECA/ isolated CHD diagnostic rate	Familial/ sporadic diagnostic rate	Number of genes considered in analysis
CMA	Turan *et al* (2018)[281]	Heterogeneous CHD	145 singletons	N	16%	24.5% (+ECA), 17.4% (isolated)	NA	All genes
ES	Blue *et al* (2014)[282]	Heterogeneous, familial CHD	16 families (16 familial)	N	31%	NA	31% (familial)	57 human CHD genes
	Jin *et al* (2017)[268]	Heterogeneous, sporadic CHD	2871 probands (2645 sporadic trios, 226 singletons)	N	10%	28% (+ECA), 3% (isolated)	10% (sporadic)	All genes
	Szot *et al* (2018)[13]	Heterogeneous CHD	30 families (16 familial, 14 sporadic)	Y	40%	44% (+ECA), 38% (isolated)	56% (familial), 29% (sporadic)	All genes
GS	Hauser *et al* (2018)[283]	Heterogeneous CHD	34 families (2 familial, 32 sporadic)	Y	6%	13% (+ECA)	6% (sporadic)	All genes
	Alankarage *et al* (2018)	Heterogeneous CHD	97 families (37 familial, 60 sporadic)	Y	31%	43% (+ECA), 27% (isolated)	49% (familial), 20% (sporadic)	All genes

The table summarizes the findings of recent, notable CHD cohort studies that have utilized various methodologies; chromosomal microarray (CMA), exome sequencing (ES) and genome sequencing (GS). Each study varies in terms of CHD subtype of the cohort, size of the cohort analyzed, classification of variant pathogenicity by internal or ACMG guidelines. The overall rate of genetic diagnosis as well as diagnostic rates for specific sub-cohorts; CHD+extra-cardiac anomalies (ECA) v isolated CHD, familial v sporadic CHD, are presented. All studies, apart from Blue *et al* (2014) who used a gene panel approach, considered variants in all genes during analysis.

Table S15. All rare variants in hcCHD genes found in probands of the cohort.

Family ID	Gene	Model	Familial CHD	ECA	Variant type	Genomic position	Nucleotide variant	Amino acid variant	MAF	PP2 HVAR	CADD	ACMG class.	Cardiac lesion
44	BMPR2	AD[a]	N	Y	Structural	chr2:202430702-202448193	17.49 KB-DEL	NA	0.0068	NA	NA	VUS	FSV
44	TLL1	AD[a]	N	Y	Structural	chr4:165974126-165974438	312 BP-DEL	NA	0.0054	NA	NA	VUS	FSV
44	EHMT1	AR	N	Y	Intronic	chr9:137740389	C>T	NA	0.001132246	NA	NA	NA	FSV
44	NOTCH1	AR	N	Y	Intronic	chr9:136533361	A>T	NA	0.001172104	NA	NA	NA	FSV
195	NODAL	AD[a]	Y	N	Frameshift insertion	chr10:70441525	NM_018055.4: c.123_142dup	Tyr48Trpfs*5	0	NA	NA	P (Ia)	FSV
575	CHD7	AD	Y	Y	Splicing	chr8:60794987	NM_017780.3: c.2098A>G	Asn700Asp	0	NA	NA	P (Ic)	MOT
742	SOS1	AD[a]	N	N	Structural	chr2:39089439-39090604	1.17 KB-DEL	NA	0.0027	NA	NA	VUS	Other
821	INVS	AD[a]	N	U	Missense	chr9:100240090	NM_014425.4: c.646T>G	Trp216Gly	0.00E+00	0.91	27.8	VUS	FSV
835	ELN	AD	Y	Y	Intergenic	chr7:73937685	G>A	NA	0.00013031	NA	NA	NA	FSV
835	ELN	AD	Y	Y	Intergenic	chr7:73909428	C>T	NA	0.000226684	NA	NA	NA	FSV
835	ELN	AD	Y	Y	Intronic	chr7:74034547	A>G	NA	0.000484496	NA	NA	NA	FSV
835	FBN1	AD	Y	Y	Intergenic	chr15:48374823	G>A	NA	0	NA	NA	NA	FSV
835	GATA4	AD	Y	Y	Intergenic	chr8:11656702	C>A	NA	3.23E-05	NA	NA	NA	FSV
835	KDM6A	AD	Y	Y	Intronic	chrX:44900389	ATTAT>A	NA	0.000276281	NA	NA	NA	FSV
835	NODAL	AD	Y	Y	Frameshift insertion	chr10:70441525	NM_018055.4: c.123_142dup	Tyr48Trpfs*5	0	NA	NA	P (Ia)	FSV
835	PITX2	AD	Y	Y	Intergenic	chr4:110615285	G>GA	NA	0	NA	NA	NA	FSV
835	SMAD3	AD	Y	Y	Intronic	chr15:67076300	A>G	NA	0.000290623	NA	NA	NA	FSV
835	SMAD3	AD	Y	Y	Intronic	chr15:67138679	A>G	NA	0.000484371	NA	NA	NA	FSV
835	SMAD4	AD	Y	Y	Intronic	chr18:51051204	TC>T	NA	0	NA	NA	NA	FSV
881	NOTCH1	AD[a]	N	U	Missense	chr9:136508919	NM_017617.4: c.3122G>A	Gly1041Asp	0	1	23.8	VUS	SDMA
959	GATA4 256	AD[a]	N	U	Missense	chr8:11756974	NM_002052.4: c.1037C>T	Ala346Val	0.00149	0.039	12.2	LB (I)	SD
959	PITX2	AD[a]	N	U	Structural	chr4:110624350-110624422	72 BP-DEL	NA	0.0122	NA	NA	VUS	SD
959	ZFPM2	DN	N	U	Structural	chr8:105658042-105658686	644 BP-DEL	NA	0.0149	NA	NA	VUS	SD

1085	CDK13	AD[a]	N	N	Missense	chr7:39999498	NM_003718.4: c.2180C>T	Thr727Ile	0.001	0.998	32	VUS	SD
1085	PITX2	AD[a]	N	N	Structural	chr4:110638295-110638356	61 BP-DEL	NA	0.0054	NA	NA	VUS	SD
1085	PRDM6	AD[a]	N	N	Missense	chr5:123170913	NM_001136239.3: c.1301A>T	Asp434Val	0.0001	0.598	23.4	VUS	SD
1085	RAF1	AD[a]	N	N	Structural	chr3:12604731-12604808	77 BP-DEL	NA	0.0027	NA	NA	NA	SD
1085	FLNA	XLR	N	N	Missense	chrX:154365465	NM_001456.3: c.1451G>A	Arg484Gln	0.00004887	0.871	26.8	VUS	SD
1167	MAP2K2	AD[a]	N	N	Structural	chr19:4120853-4120948	95 BP-DEL	NA	0.0149	NA	NA	VUS	SD
1167	NOTCH1	AD[a]	N	N	Missense	chr9:136509059	NM_017617.4: c.2982C>G	Asn994Lys	0	1	24.8	VUS	SD
1125	EVC	AD	Y	Y	Intronic	chr4:5747602	A>G	NA	0	NA	NA	NA	SD
1125	PRKD1	AD	Y	Y	Intergenic	chr14:29236818	C>T	NA	0.000518	NA	NA	NA	SD
1125	SMAD6	AD	Y	Y	Intronic	chr15:66734417	G>A	NA	6.46E-05	NA	NA	NA	SD
1125	TBX3, MED13L	AD	Y	Y	Intergenic	chr12:114762734	C>CGT	NA	6.59E-05	NA	NA	NA	SD
1125	TGFBR1	AD	Y	Y	Intergenic	chr9:99090674	A>G	NA	0.000743	NA	NA	NA	SD
1125	TGFBR2	AD	Y	Y	Intergenic	chr3:30292432	C>A	NA	0.000129	NA	NA	NA	SD
1125	ZEB2	AD	Y	Y	Intergenic	chr2:144362695	A>G	NA	0	NA	NA	NA	SD
1125	ZFPM2	AD	Y	Y	Intergenic	chr8:105090844	A>T	NA	6.47E-05	NA	NA	NA	SD
1179	ANKRD11	AD	Y	Y	Intergenic	chr16:89249304	C>G	NA	0	NA	NA	NA	MOT
1179	CDK13	AD	Y	Y	Exonic	chr7:39950756	C>T	NA	0.000484186	NA	NA	NA	MOT
1179	FBN1	AD	Y	Y	Intergenic	chr15:48388677	G>A	NA	3.23E-05	NA	NA	NA	MOT
1179	MAP2K2	AD	Y	Y	Intergenic	chr19:4084835	CCT>C	NA	0.000226669	NA	NA	NA	MOT
1179	SMAD4	AD	Y	Y	Intronic	chr18:51042547	A>G	NA	3.25E-05	NA	NA	NA	MOT
1179	TAB2	AD	Y	Y	Intergenic	chr6:149157343	C>A	NA	0.000710548	NA	NA	NA	MOT
1179	ZEB2	AD	Y	Y	Intronic	chr2:144404640	G>A	NA	0	NA	NA	NA	MOT
1302	FOXH1	AD[a]	N	N	Missense	chr8:144475209	NM_003923.2: c.227G>A	Gly76Asp	0.00001716	0.997	29.7	VUS	SDMA
1384	EVC2	AD[a]	N	N	Missense	chr4:5631929	NM_147127.4: c.1574C>T	Ala525Val	8.24E-06	0.988	19.32	LB (I)	OL
1384	MYH6	CH	N	N	Missense	chr14:23404300	NM_002471.3: c.731G>A	Arg244His	0.000008237	0.994	34	LP (IV)	OL
1384	MYH6	CH	N	N	Stopgain	chr14:23382430	NM_002471.3:	Lys1932*	0.00001647	NA	43	P (Id)	OL

							c.5794A>T						
1449	ACTC1	AD	Y	Y	Missense	chr15:34793496	NM_005159.4: c.203C>T	Thr68Ile	0	0.974	25.5	P (IIIc)	SD
1449	EHMT1	AD	Y	Y	Intronic	chr9:137695680	C>T	NA	3.23E-05	NA	NA	NA	SD
1449	ELN	AD	Y	Y	Intergenic	chr7:74010564	C>T	NA	0.000388	NA	NA	NA	SD
1449	ELN	AD	Y	Y	Intergenic	chr7:74006226	C>T	NA	0.000937	NA	NA	NA	SD
1449	HAND2	AD	Y	Y	Intergenic	chr4:173523191	C>T	NA	0.000582	NA	NA	NA	SD
1449	KAT6B	AD	Y	Y	Intronic	chr10:74911336	T>C	NA	0	NA	NA	NA	SD
1449	KMT2D	AD	Y	Y	3' UTR	chr12:49019393	G>A	NA	0.000784	NA	NA	NA	SD
1449	MED13L	AD	Y	Y	Intronic	chr12:116166052	A>G	NA	0	NA	NA	NA	SD
1449	SMAD6, SMAD3	AD	Y	Y	Intergenic	chr15:67049526	G>C	NA	0.000517	NA	NA	NA	SD
1449	ZEB2	AD	Y	Y	Intronic	chr2:144457444	A>C	NA	0.000485	NA	NA	NA	SD
1456	ANKRD11	AD	Y	Y	Intronic	chr16:89361594	G>A	NA	0	NA	NA	NA	AVSD+
1456	ANKRD11	AD	Y	Y	Intronic	chr16:89374408	T>C	NA	0	NA	NA	NA	AVSD+
1456	ARID1B	AD	Y	Y	Intronic	chr6:156964533	T>G	NA	3.23E-05	NA	NA	NA	AVSD+
1456	CHD7	AD	Y	Y	Intergenic	chr8:60676377	G>A	NA	3.23E-05	NA	NA	NA	AVSD+
1456	CREBBP	AD	Y	Y	Intronic	chr16:3878612	G>A	NA	0.000129132	NA	NA	NA	AVSD+
1456	EHMT1	AD	Y	Y	Intronic	chr9:137664621	C>T	NA	0.000422792	NA	NA	NA	AVSD+
1456	FBN1	AD	Y	Y	Intronic	chr15:48494020	C>T	NA	3.23E-05	NA	NA	NA	AVSD+
1456	KAT6A	AD	Y	Y	Intronic	chr8:42002613	T>A	NA	3.23E-05	NA	NA	NA	AVSD+
1456	MAP2K1	AD	Y	Y	Intronic	chr15:66472009	G>A	NA	6.60E-05	NA	NA	NA	AVSD+
1456	NOTCH1	AD	Y	Y	Downstream	chr9:136493602	C>T	NA	0.000905036	NA	NA	NA	AVSD+
1456	SMAD3	AD	Y	Y	Intronic	chr15:67078574	G>A	NA	0.000419788	NA	NA	NA	AVSD+
1456	TBX1	AD	Y	Y	Intergenic	chr22:19731757	C>T	NA	0.000259051	NA	NA	NA	AVSD+
1456	TBX3, MED13L	AD	Y	Y	Intergenic	chr12:114747494	G>T	NA	9.68E-05	NA	NA	NA	AVSD+
1456	ZEB2	AD	Y	Y	Intergenic	chr2:144377232	G>T	NA	0.000129141	NA	NA	NA	AVSD+
1456	GPC3	XLR	Y	Y	Intronic	chrX:133792680	C>A	NA	0	NA	NA	NA	AVSD+
1456	KDM6A	XLR	Y	Y	Intronic	chrX:44911427	C>T	NA	0.000198955	NA	NA	NA	AVSD+
1658	EVC2	AD	Y	N	Intronic	chr4:5683777	A>C	NA	0	NA	NA	NA	OL

1658	KAT6B	AD	Y	N	Intergenic	chr10:74824830	A>G	NA	0	NA	NA	NA	OL
1658	KAT6B	AD	Y	N	Intergenic	chr10:74822632	T>G	NA	0.000935846	NA	NA	NA	OL
1658	SMAD6, SMAD3	AD	Y	N	Intergenic	chr15:66961591	G>C	NA	0	NA	NA	NA	OL
1658	SMAD6, SMAD3	AD	Y	N	Intergenic	chr15:67059561	C>T	NA	0	NA	NA	NA	OL
1658	TBX3, MED13L	AD	Y	N	Intergenic	chr12:115922783	G>A	NA	0.000129199	NA	NA	NA	OL
1658	TGFBR2	AD	Y	N	Intergenic	chr3:30511876	G>A	NA	0.000322768	NA	NA	NA	OL
1658	TGFBR2	AD	Y	N	Intronic	chr3:30630764	G>C	NA	0.000872262	NA	NA	NA	OL
1658	UBR1	AD	Y	N	Upstream	chr15:43106696	A>AG	NA	0.000678076	NA	NA	NA	OL
1767	NOTCH1	AD[a]	N	U	Missense	chr9:136502003	NM_017617.4: c.5470C>T	Arg1824Trp	0.000008407	0.999	34	VUS	SDMA
1767	FLNA	XLR	N	U	Missense	chrX:154349560	NM_001456.3: c.7534C>T	Arg2512Cys	0.0001	0.947	32	VUS	SDMA
1852	NKX2-5[257-259]	AD[a]	Y	Y	Missense	chr5:173235023	NM_004387.3: c.61G>C	Glu21Gln	0.0008	0.975	26.4	LB (I)	FSV
1852	NOTCH1	AD[a]	Y	Y	Frameshift deletion	chr9:136500621	NM_017617.4: c.5865del	Asn1955Lysfs*26	0	NA	NA	P (Id)	FSV
2020	EHMT1	AD[a]	N	N	Missense	chr9:137743452	NM_024757.4: c.905A>G	Lys302Arg	0.0002	0.999	26.3	LB (I)	Other
2020	GJA1	AD[a]	N	N	Structural	chr6:121427635-121428318	683 BP-DEL	NA	0.0027	NA	NA	VUS	Other
2306	KMT2D	AD[a]	N	N	Missense	chr12:49034472	NM_003482.3: c.10445G>A	Arg3482Gln	6.80E-05	0.859	29.1	LB (I)	FSV
2324	NF1	AD[a]	N	Y	Missense	chr17:31232821	NM_000267.3: c.3436G>A	Val1146Ile	0.0001	0.927	15.27	LB(II)	MOT
2324	NOTCH1	AD[a]	N	Y	Missense	chr9:136510749	NM_017617.4: c.2644G>A	Ala882Thr	0.00002028	0.977	29.3	LB (I)	MOT
2636	KANSL1	AD[a]	N	N	Missense	chr17:46170955	NM_001193466.1: c.1189G>A	Ala397Thr	5.77E-05	0.985	24.9	LB (II)	FSV
2831	DOCK6	DN	Y	N	Structural	chr19:11210497-11210594	97 BP-DEL	NA	0.0041	NA	NA	VUS	MOT
2831	MYBPC3	AD[a]	Y	N	Missense	chr11:47332204	NM_000256.3: c.3682C>T	Arg1228Cys	0.0002	0.993	35	LB (I)	MOT
2831	MYH11	AD[a]	Y	N	Missense	chr16:15708836	NM_022844.2: c.5792C>A	Pro1931His	0	0.536	21.2	VUS	MOT
2831	NOTCHl	AD[a]	Y	N	Frameshift deletion	chr9:136498973	NM_017617.4: c.6105del	Ala2036Profs*3	0	NA	NA	P (Ic)	MOT
2944	ADAMTS10	AD	Y	N	Missense	chr19:8596380	NM_030957.3: c.1117G>A	Glu373Lys	8.52E-06	0.958	27.4	VUS	SDMA

2944	EHMT1	AD	Y	N	Intronic	chr9:137705538	T>C	NA	0.00058102	NA	NA	NA	SDMA
2944	PRDM6	AD	Y	N	Exonic	chr5:123090323	NM_001136239.3: c.309C>T	Ala103Ala	9.55E-05	NA	NA	NA	SDMA
2944	SMAD6, SMAD3	AD	Y	N	Intergenic	chr15:66800730	A>ACACACTCG	NA	0	NA	NA	NA	SDMA
2944	SMAD6, SMAD3	AD	Y	N	Intergenic	chr15:66850550	T>A	NA	6.46E-05	NA	NA	NA	SDMA
2944	SMAD6, SMAD3	AD	Y	N	Intergenic	chr15:67059489	C>T	NA	0.000580908	NA	NA	NA	SDMA
2944	TBX1	AD	Y	N	Intronic	chr22:19762261	C>G	NA	6.46E-05	NA	NA	NA	SDMA
2944	TGFBR2	AD	Y	N	Intergenic	chr3:30518411	C>T	NA	0.000225996	NA	NA	NA	SDMA
2985	ANKRD11	AD	Y	N	Intergenic	chr16:89240564	G>A	NA	0	NA	NA	NA	FSV
2985	ANKRD11	AD	Y	N	Intronic	chr16:89406355	C>A	NA	0	NA	NA	NA	FSV
2985	CFC1 [49,50]	AD	Y	N	Frameshift deletion	chr2:130593026	NM_032545.3: c.522del	Ala175Argfs*56	0	NA	NA	P (Ia)	FSV
2985	SHOC2	AD	Y	N	Intronic	chr10:110945368	T>C	NA	0.000516662	NA	NA	NA	FSV
2985	TBX1	AD	Y	N	Intronic	chr22:19765134	C>T	NA	4.48E-05	NA	NA	NA	FSV
2985	TBX3, MED13L	AD	Y	N	Intergenic	chr12:115423483	C>T	NA	0	NA	NA	NA	FSV
3173	NOTCH1	AD[a]	Y	Y	Structural	chr9:136537696-136560250	22.55 KB-DEL	NA	0.0027	NA	NA	P	SDMA
3173	ZFPM2	AD[a]	Y	Y	Structural	chr8:104968516-104968637	121 BP-DEL	NA	0.0081	NA	NA	VUS	SDMA
3191	SMAD6	AD[a]	N	U	Frameshift deletion	chr15:66703343	NM_005585.4: c.86del	Gly29Alafs*35	0	NA	NA	P (Ic)	FSV
3191	HAND2	AR	N	U	Intergenic	chr4:173500472	T>G	NA	0	NA	NA	NA	FSV
3208	NOTCH1	AD[a]	Y	U	Missense	chr9:136518651	NM_017617.4: c.1039G>A	Gly347Ser	0.00001738	0.999	34	VUS	MOT
3208	SMAD6	AD[a]	Y	U	Intronic	chr15:66774833	AT>A	NA	0	NA	NA	NA	MOT
3225	GATA4	AD	Y	Y	Missense	chr8:11755095	NM_002052.4: c.959G>A	Arg320Gln	0	0.99	35	P (IIIb)	SDMA
3225	STRA6	AD	Y	Y	Missense	chr15:74189222	NM_022369.3: c.983G>T	Gly328Val	0	1	31	VUS	SDMA
3225	TLL1	AD	Y	Y	Missense	chr4:166014468	NM_012464.4: c.950G>A	Arg317His	0.000033	0.799	25.4	VUS	SDMA
3376	ZFPM2	AD[a]	N	N	Structural	chr8:104968516-104968637	121 BP-DEL	NA	0.0081	NA	NA	VUS	SD
3394	TBX5	DN	N	N	Intergenic	chr12:114242064	T>C	NA	0.000249	NA	NA	NA	FSV

3394	TBX5	DN	N	N	Intergenic	chr12:114242064	T>C	NA	0.000249	NA	NA	NA	FSV
3397	ACVR1	AD	Y	N	Intergenic	chr2:157696514	C>T	NA	6.46E-05	NA	NA	NA	SDMA
3397	ANKRD11	AD	Y	N	Intronic	chr16:89320454	GCTGAGCGGCA>G	NA	0	NA	NA	NA	SDMA
3397	ANKRD11	AD	Y	N	Intronic	chr16:89320465	A>G	NA	0	NA	NA	NA	SDMA
3397	ARID1B	AD	Y	N	Intergenic	chr6:156396168	A>C	NA	0	NA	NA	NA	SDMA
3397	CHD4	AD	Y	N	Intronic	chr12:6605881	C>T	NA	0.000323018	NA	NA	NA	SDMA
3397	MED13L	AD	Y	N	Intronic	chr12:116236758	A>T	NA	0	NA	NA	NA	SDMA
3397	NPHP4	AD	Y	N	Intronic	chr1:5923301	C>T	NA	9.69E-05	NA	NA	NA	SDMA
3397	SMAD3	AD	Y	N	Intronic	chr15:67098926	A>G	NA	4.87E-05	NA	NA	NA	SDMA
3397	SMAD6, SMAD3	AD	Y	N	Intergenic	chr15:66935303	G>T	NA	3.23E-05	NA	NA	NA	SDMA
3397	TBX1	AD	Y	N	Intronic	chr22:19759227	G>A	NA	0	NA	NA	NA	SDMA
3397	TGFBR2	AD	Y	N	Intergenic	chr3:30443805	C>G	NA	0	NA	NA	NA	SDMA
3397	TGFBR2	AD	Y	N	Intronic	chr3:30641375	G>A	NA	0	NA	NA	NA	SDMA
3397	BCOR	XLD	Y	N	Intronic	chrX:40127261	T>A	NA	0.000278914	NA	NA	NA	SDMA
3397	GPC3	XLD	Y	N	Intronic	chrX:133543633	A>G	NA	0.000320704	NA	NA	NA	SDMA
3397	KDM6A	XLD	Y	N	Intronic	chrX:44911772	A>C	NA	0.000468296	NA	NA	NA	SDMA
3400	ZEB2	AD[a]	N	N	Missense	chr2:144399801	NM_014795.3: c.1386G>T	Lys462Asn	0.00E+00	0.597	22.6	VUS	OL
3400	ACVR1	DN	N	N	Intronic	chr2:157769997	C>G	NA	0	NA	NA	NA	OL
3424	GDF1, (CERS1)	AD[a]	N	Y	Structural	chr19:18885852-18889621	3.77 KB-DUP	NA	0.0027	NA	NA	VUS	MOT
3427	ELN	AD[a]	N	Y	Missense	chr7:74056336	NM_000501.3: c.1216G>A	Gly406Ser	0.00004118	0.972	20.8	LB(I)	SD
3430	ELN	AD[a]	N	Y	Splicing	chr7:74054770	NM_000501.3: c.1150+1G>A	NA	0.00004943	NA	23.6	VUS	AVSD+
3438	KAT6B	AD[a]	N	Y	Missense	chr10:75028575	NM_012330.3: c.3751G>A	Gly1251Arg	0.000008237	1	32	LB (I)	SD
3438	SON	AD[a]	N	Y	Missense	chr21:33552633	NM_032195.2: c.3402T>G	Asp1134Glu	0	0.991	22.9	VUS	SD
3438	SOS1	AD[a]	N	Y	Missense	chr2:38986117	NM_005633.3: c.3709C>A	Pro1237Thr	0.00009068	0.992	23.2	VUS	SD
3438	HAND1	DN	N	Y	Upstream	chr5:154478752	G>A	NA	0	NA	NA	NA	SD
3464	TBX3, MED13L	AR	N	N	Intergenic	chr12:114903717	G>A	NA	0	NA	NA	NA	AVSD+

3470	TBX5	AD[a]	N	U	Missense	chr12:114399586	NM_000192.3: c.289A>G	Lys97Glu	0.000008236	0.789	24.5	VUS	MOT
3470	BCOR	XLR	N	U	Intergenic	chrX:39906310	CTG>C	NA	0	NA	NA	NA	MOT
3473	MYH11	AD[a]	N	Y	Missense	chr16:15732655	NM_022844.2: c.3560C>T	Thr1187Met	0	0.871	23.6	VUS	AVSD+
3473	PITX2	AD[a]	N	Y	Structural	chr4:110638295-110638356	61 BP-DEL	NA	0.0054	NA	NA	VUS	AVSD+
3473	TLL1	AD[a]	N	Y	Structural	chr4:165974126-165974438	312 BP-DEL	NA	0.0054	NA	NA	VUS	AVSD+
3485	ARID1A	AD	Y	Y	Splicing	chr1:26772501	NM_006015.5: c.3408G>A	Ala1136Ala	0.0001	NA	NA	VUS	SD
3485	CDK13	AD	Y	Y	Structural	chr7:40074176-40074235	59 BP-DEL	NA	0.0068	NA	NA	VUS	SD
3527	FBN1	AD[a]	N	N	Structural	chr15:48445118-48445209	91 BP-DEL	NA	0.0257	NA	NA	VUS	FSV
3527	FBN1	AD[a]	N	N	Structural	chr15:48453290-48453351	61 BP-DEL	NA	0.0257	NA	NA	VUS	FSV
3564	CRELD1	AD[a]	N	U	Missense	chr3:9944574	NM_015513.4: c.1258A>G	Arg420Gly	0.00E+00	0.815	22.3	VUS	OL
3564	FBN1	AD[a]	N	U	Structural	chr15:48445118-48445209	91 BP-DEL	NA	0.0257	NA	NA	VUS	OL
3564	FBN1	AD[a]	N	U	Structural	chr15:48453290-48453351	61 BP-DEL	NA	0.0257	NA	NA	VUS	OL
3564	ELN	DN	N	U	Intergenic	chr7:73886700	A>T	NA	0	NA	NA	NA	OL
3568	INVS	AD[a]	N	U	Missense	chr9:100292689	NM_014425.4: c.2432C>G	Pro811Arg	0.00E+00	0.999	24	VUS	MOT
3601	CDK13	AD[a]	N	U	Structural	chr7:40074176-40074235	59 BP-DEL	NA	0.0068	NA	NA	VUS	MOT
3610	NOTCH1	AD[a]	N	N	Missense	chr9:136523753	NM_017617.4: c.368C>T	Thr123Met	9.51E-04	0.873	22.6	VUS	MOT
3610	PITX2	AD[a]	N	N	Structural	chr4:110624350-110624422	72 BP-DEL	NA	0.0122	NA	NA	VUS	MOT
3630	FOXH1	AD[a]	N	U	Structural	chr8:144484983-144488709	3.73 KB-DEL	NA	0.0027	NA	NA	VUS	SDMA
3642	INVS	AR	N	N	Frameshift insertion	chr9:100300652	NM_014425.4: c.3182dup	Asn1061Lysfs*20	0.0002	NA	NA	P (Ic)	FSV
3642	INVS	AR	N	N	Intronic	chr9:100126153	A>G	NA	0.0000323	NA	NA	NA	FSV
3642	MAP2K1	AR	N	N	Intergenic	chr15:66374480	G>A	NA	0.000549	NA	NA	NA	FSV
3648	JAG1 [255]	AD[a]	N	Y	Missense	chr20:10643807	NM_000214.2: c.2429C>T	Pro810Leu	0.000004062	0.955	34	P (II)	AVSD+
3648	ZFPM2	AD[a]	N	Y	Structural	chr8:105784303-105785078	775 BP-DEL	NA	0.019	NA	NA	VUS	AVSD+

3648	TAB2	DN	N	Y	Intergenic	chr6:149113466	A>C	NA	0	NA	NA	NA	AVSD+
3651	GATA4	AD[a]	N	U	Structural	chr8:11739420-11739758	338 BP-DEL	NA	0.019	NA	NA	VUS	MOT
3654	FBN1	AD[a]	N	U	Structural	chr15:48445118-48445209	91 BP-DEL	NA	0.0257	NA	NA	VUS	OL
3654	FBN1	AD[a]	N	U	Structural	chr15:48453290-48453351	61 BP-DEL	NA	0.0257	NA	NA	VUS	OL
3654	GATA4	AD[a]	N	U	Structural	chr8:11739420-11739758	338 BP-DEL	NA	0.019	NA	NA	VUS	OL
3672	SMAD6	DN	N	U	Intergenic	chr15:66698378	G>A	NA	0	NA	NA	NA	SD
3678	ANKRD11	AD	Y	U	Intronic	chr16:89344028	C>A	NA	0	NA	NA	NA	MOT
3678	ANKRD11	AD	Y	U	Intronic	chr16:89411191	G>C	NA	0	NA	NA	NA	MOT
3678	ARID1B	AD	Y	U	Intronic	chr6:157148239	C>G	NA	6.46E-05	NA	NA	NA	MOT
3678	SMAD3	AD	Y	U	Intronic	chr15:67073586	G>A	NA	0.000129166	NA	NA	NA	MOT
3678	SMAD3	AD	Y	U	Intronic	chr15:67145678	A>G	NA	0.00096893	NA	NA	NA	MOT
3678	TBX5	AD	Y	U	Intronic	chr12:114403265	CGGAGGAGGCA>C	NA	0.00058147	NA	NA	NA	MOT
3678	TGFBR1	AD	Y	U	Intronic	chr9:99118834	T>C	NA	3.24E-05	NA	NA	NA	MOT
3678	TGFBR2	AD	Y	U	Intergenic	chr3:30256955	T>C	NA	0.000678952	NA	NA	NA	MOT
3678	ZFPM2	AD	Y	U	Intronic	chr8:105390100	T>G	NA	0	NA	NA	NA	MOT
3695	ANKRD11	AD	Y	N	Intronic	chr16:89459351	C>T	NA	0.000258331	NA	NA	NA	MOT
3695	ANKRD11	AD	Y	N	Intronic	chr16:89454624	G>C	NA	0.000685871	NA	NA	NA	MOT
3695	CHD7	AD	Y	N	Intronic	chr8:60780977	C>T	NA	0.000171624	NA	NA	NA	MOT
3695	ELN	AD	Y	N	Intergenic	chr7:73886377	A>C	NA	0.000290904	NA	NA	NA	MOT
3695	FBN1	AD	Y	N	Missense	chr15:48465797	NM_000138.4: c.4809A>G	Ile1603Met	8.26E-06	0.974	23.9	VUS	MOT
3695	GATA4	AD	Y	N	Intergenic	chr8:11616595	G>T	NA	0	NA	NA	NA	MOT
3695	HAND2	AD	Y	N	Intergenic	chr4:173501151	C>T	NA	0	NA	NA	NA	MOT
3695	KAT6B	AD	Y	N	Intronic	chr10:74898287	C>G	NA	0	NA	NA	NA	MOT
3695	KAT6B	AD	Y	N	Intronic	chr10:74913855	C>T	NA	0.000129199	NA	NA	NA	MOT
3695	MED13L	AD	Y	N	Intronic	chr12:116166704	T>C	NA	0	NA	NA	NA	MOT
3695	NF1	AD	Y	N	Intronic	chr17:31314373	T>A	NA	0	NA	NA	NA	MOT
3695	NIPBL	AD	Y	N	Intronic	chr5:36878481	T>A	NA	9.69E-05	NA	NA	NA	MOT
3695	NOTCH1	AD	Y	N	Intronic	chr9:136522122	C>T	NA	6.52E-05	NA	NA	NA	MOT

3695	TBX3, MED13L	AD	Y	N	Intergenic	chr12:115502783	A>G	NA	6.46E-05	NA	NA	NA	MOT
3695	TBX3, MED13L	AD	Y	N	Intergenic	chr12:115502801	T>C	NA	6.46E-05	NA	NA	NA	MOT
3695	TGFBR1	AD	Y	N	Intergenic	chr9:99086751	T>C	NA	0	NA	NA	NA	MOT
3695	TGFBR2	AD	Y	N	Intronic	chr3:30646784	G>T	NA	3.23E-05	NA	NA	NA	MOT
3695	TGFBR2	AD	Y	N	Intronic	chr3:30644305	C>T	NA	6.46E-05	NA	NA	NA	MOT
3695	TLL1	AD	Y	N	Missense	chr4:166091263	NM_012464.4: c.2578A>G	Thr860Ala	0.00E+00	0.992	23.1	LP(V)	MOT
3695	TLL1	AD	Y	N	Intergenic	chr4:165819063	G>A	NA	0	NA	NA	NA	MOT
3695	FLNA	XLR	Y	N	Intergenic	chrX:154333929	G>C	NA	0	NA	NA	NA	MOT
3712	GATA6	AD[a]	N	Y	Frameshift deletion	chr18:22183015	NM_005257.5: c.1595_1596del	Pro532Hisfs*100	0.00E+00	NA	NA	P (Ic)	FSV
3717	STRA6	AD[a]	N	Y	Missense	chr15:74181327	NM_022369.3: c.1652T>G	Leu551Arg	0	0.999	26.1	LB(I)	SD
3717	KDM6A	XLR	N	Y	Intronic	chrX:44875852	C>A	NA	0.000604876	NA	NA	NA	SD
3766	NODAL	AD	Y	U	Stopgain	chr10:70433060	NM_018055.4: c.919C>T	Arg307*	0	NA	40	P (Ic)	MOT
3769	ACVR1	AD	Y	U	Intergenic	chr2:157714173	C>T	NA	6.46E-05	NA	NA	NA	MOT
3769	EHMT1	AD	Y	U	Intronic	chr9:137625748	G>A	NA	3.24E-05	NA	NA	NA	MOT
3769	ELN	AD	Y	U	Exonic	chr7:74051964	NM_000501.3: c.930C>T	Ala310Ala	0.00019901	NA	NA	NA	MOT
3769	GATA4	AD	Y	U	Intergenic	chr8:11654572	C>G	NA	0	NA	NA	NA	MOT
3769	GATA4	AD	Y	U	Intronic	chr8:11710274	G>T	NA	0	NA	NA	NA	MOT
3769	GATA4	AD	Y	U	Intronic	chr8:11746417	A>G	NA	3.23E-05	NA	NA	NA	MOT
3769	GATA5	AD	Y	U	Upstream	chr20:62476390	G>C	NA	0	NA	NA	NA	MOT
3769	NOTCH1	AD	Y	U	Intronic	chr9:136535463	GC>G	NA	0.000626	NA	NA	NA	MOT
3769	NR2F2	AD	Y	U	3' UTR	chr15:96337892	T>C	NA	0.000196425	NA	NA	NA	MOT
3769	TAB2	AD	Y	U	Intergenic	chr6:149141093	A>G	NA	0	NA	NA	NA	MOT
3769	TAB2	AD	Y	U	Intronic	chr6:149231811	G>C	NA	0	NA	NA	NA	MOT
3769	TBX3, MED13L	AD	Y	U	Intergenic	chr12:114811258	A>G	NA	0	NA	NA	NA	MOT
3769	TBX3, MED13L	AD	Y	U	Intergenic	chr12:115141458	A>G	NA	0	NA	NA	NA	MOT
3769	TBX3, MED13L	AD	Y	U	Intergenic	chr12:115141597	C>T	NA	0	NA	NA	NA	MOT

3769	TBX3, MED13L	AD	Y	U	Intergenic	chr12:115227267	C>T	NA	0	NA	NA	NA	MOT
3769	TBX3, MED13L	AD	Y	U	Intergenic	chr12:115771370	C>G	NA	0	NA	NA	NA	MOT
3769	TBX3, MED13L	AD	Y	U	Intergenic	chr12:115322396	T>C	NA	0.000355228	NA	NA	NA	MOT
3769	TBX3, MED13L	AD	Y	U	Intergenic	chr12:115309109	A>G	NA	0.000355366	NA	NA	NA	MOT
3769	TBX3, MED13L	AD	Y	U	Intergenic	chr12:115312879	C>T	NA	0.000355826	NA	NA	NA	MOT
3769	TBX5	AD	Y	U	Intergenic	chr12:114287874	A>AT	NA	3.23E-05	NA	NA	NA	MOT
3769	TGFBR2	AD	Y	U	Intergenic	chr3:30597113	G>T	NA	0	NA	NA	NA	MOT
3792	NOTCH2	AD[a]	Y	N	Structural	chr1:119901157-119979217	78.06 KB-DUP	NA	0	NA	NA	VUS	MOT
3932	ARID1B	AD	Y	N	Intergenic	chr6:156427596	C>T	NA	0	NA	NA	NA	FSV
3932	BCOR	AD	Y	N	Intronic	chrX:40157503	A>G	NA	0.000183933	NA	NA	NA	FSV
3932	INVS	AD	Y	N	Exonic	chr9:100104588	NM_014425.4: c.67G>A	Val23Ile	0.000292208	0.021	14.3	NA	FSV
3932	NOTCH1	AD	Y	N	Intronic	chr9:136515468	C>T	NA	4.17E-06	NA	NA	NA	FSV
3932	RAD21	AD	Y	N	Intronic	chr8:116873823	TA>T	NA	0.000161614	NA	NA	NA	FSV
3932	TBX5	AD	Y	N	Intronic	chr12:114340238	C>T	NA	0	NA	NA	NA	FSV
3932	TGFBR1	AD	Y	N	Intergenic	chr9:99086745	C>T	NA	6.46E-05	NA	NA	NA	FSV
3932	TGFBR2	AD	Y	N	Intronic	chr3:30641970	T>A	NA	0.000387923	NA	NA	NA	FSV
3933	ANKRD11	AD	Y	Y	Upstream	chr16:89491146	G>C	NA	3.24E-05	NA	NA	NA	SD
3933	ARID1B	AD	Y	Y	Intergenic	chr6:156560536	C>T	NA	0	NA	NA	NA	SD
3933	ARID1B	AD	Y	Y	Intronic	chr6:156780252	A>G	NA	0	NA	NA	NA	SD
3933	ARID1B	AD	Y	Y	Intronic	chr6:157033457	T>C	NA	0	NA	NA	NA	SD
3933	ARID1B	AD	Y	Y	Intronic	chr6:156880196	G>A	NA	3.23E-05	NA	NA	NA	SD
3933	ARID1B	AD	Y	Y	Intergenic	chr6:156422427	G>T	NA	6.46E-05	NA	NA	NA	SD
3933	ARID1B	AD	Y	Y	Intronic	chr6:157146723	C>T	NA	6.46E-05	NA	NA	NA	SD
3933	ARID1B	AD	Y	Y	Intergenic	chr6:156361786	C>T	NA	0.000227909	NA	NA	NA	SD
3933	BCOR	AD	Y	Y	Frameshift deletion	chrX:40072856	NM_001123383.1: c.2488_2489del	Ser830Cysfs*6	0	NA	NA	P (Id)	SD
3933	CHD7	AD	Y	Y	Intronic	chr8:60731461	C>T	NA	0.000484402	NA	NA	NA	SD
3933	CITED2	AD	Y	Y	Intergenic	chr6:139324031	C>T	NA	0	NA	NA	NA	SD

3933	INVS	AD	Y	Y	Intronic	chr9:100137011	G>A	NA	0.000258565	NA	NA	NA	SD
3933	NIPBL	AD	Y	Y	Intronic	chr5:36877405	G>A	NA	0.000550162	NA	NA	NA	SD
3933	SMAD6, SMAD3	AD	Y	Y	Intergenic	chr15:66862299	G>A	NA	3.23E-05	NA	NA	NA	SD
3933	TBX3, MED13L	AD	Y	Y	Intergenic	chr12:114936706	C>A	NA	0.000968617	NA	NA	NA	SD
3933	TGFBR2	AD	Y	Y	Intronic	chr3:30646861	T>C	NA	0	NA	NA	NA	SD
3933	TLL1	AD	Y	Y	Structural	chr4:165962891-165963095	204 BP-DEL	NA	0.0027	NA	NA	VUS	SD
3933	ZEB2	AD	Y	Y	Intronic	chr2:144435135	C>G	NA	0	NA	NA	NA	SD
3933	ZEB2	AD	Y	Y	Intronic	chr2:144444213	T>A	NA	0	NA	NA	NA	SD
3933	GPC3	XLD	Y	Y	Intergenic	chrX:133461458	G>A	NA	0.000662314	NA	NA	NA	SD
3953	CHD7	AD[a]	N	N	Splicing	chr8:60850621	NM_017780.3: c.5533G>A	Gly1845Arg	0.00003545	0.663	26.3	VUS	MOT
3953	ELN	AD[a]	N	N	Splicing	chr7:74042984	NM_000501.3: c.326G>A	Gly109Asp	0.0004	0.999	23.1	VUS	MOT
3953	GATA5	AD[a]	N	N	Missense	chr20:62464852	NM_080473.4: c.1178C>T	Ala393Val	0.000008712	0.994	33	VUS	MOT
3953	NF1	DN	N	N	Missense	chr17:31233065	NM_000267.3: c.3560T>G	Leu1187Arg	0	0.994	27.6	LP(II)	MOT
3967	ARID1A	AD[a]	N	N	Missense	chr1:26763150	NM_006015.5: c.2597G>A	Arg866Gln	0.0000413	0.99	23.5	LB (I)	Other
141550648	CHD4	AD[a]	N	Y	Structural	chr12:6575003-6575131	128 BP-DEL	NA	0.0095	NA	NA	VUS	SD
150650086	ACVR2B	AD[a]	N	Y	Missense	chr3:38481448	NM_001106.3: c.1057G>T	Gly353Trp	0	0.995	34	LP (V)	MOT
150650086	INVS	AD[a]	N	Y	Frameshift insertion	chr9:100300652	NM_014425.4: c.3182dup	Asn1061Lysfs*20	0.0002	NA	NA	VUS	MOT
150650086	DOCK6	AR	N	Y	Missense	chr19:11222812	NM_020812.3: c.3163G>A	Val1055Met	0.0015	0.995	33	LP(V)	MOT
150650086	FBN1	AR	N	Y	Intergenic	chr15:48403093	T>C	NA	9.68E-05	NA	NA	NA	MOT
150650086	FBN1	AR	N	Y	Intronic	chr15:48448058	G>A	NA	0.000904977	NA	NA	NA	MOT
152900216	NOTCH1	AD	Y	Y	Missense	chr9:136505480	NM_017617.4: c.4416C>G	Cys1472Trp	0	1	24.4	LP (V)	MOT
152900216	TBX20	AD	Y	Y	Intergenic	chr7:35198543	T>C	NA	0.000129291	NA	NA	NA	MOT
152900216	TBX3, MED13L	AD	Y	Y	Intergenic	chr12:114864956	G>C	NA	6.46E-05	NA	NA	NA	MOT
152900216	TBX5	AD	Y	Y	Intergenic	chr12:114168323	G>A	NA	6.46E-05	NA	NA	NA	MOT

169036865	JAG1	AD	Y	Y	Missense	chr20:10658540	NM_000214.2: c.622G>C	Gly208Arg	0	0.999	32	P (IIIb)	MOT
15R414475M	ARID1B	AD[a]	N	Y	Missense	chr6:156778005	NM_020732.3: c.76A>C	Lys26Gln	0.00E+00	NA	15.83	LB (I)	SDMA
15R414475M	HAND2	DN	N	Y	Structural	chr4:173538431-173538783	352 BP-DEL	NA	0.0068	NA	NA	VUS	SDMA
15Y05641	CHD4	AD[a]	N	Y	Missense	chr12:6601382	NM_001273.3: c.706G>A	Val236Met	0.000008247	0.748	24.3	VUS	MOT
CVM31	CHD7	AD[a]	N	Y	Missense	chr8:60823879	NM_017780.3: c.3241A>G	Ile1081Val	0.000008309	0.894	26.8	LB (I)	Other
CVM31	TGFBR2	AD[a]	N	Y	Missense	chr3:30650373	NM_003242.5: c.367A>T	Met123Leu	0.00006592	0.884	27.7	VUS	Other
CVM31	ZEB2	AD[a]	N	Y	Missense	chr2:144399741	NM_014795.3: c.1446A>C	Glu482Asp	0.00E+00	0.956	15.64	LB (I)	Other
Trio9	GPC3	AD[a]	N	Y	Structural	chrX:133878296-133879585	1.29 KB-DEL	NA	0.0041	NA	NA	VUS	MOT

Model: inheritance model: AD: autosomal dominant; AR: autosomal recessive; CH: compound heterozygous; DN: de novo; XLR: X-linked recessive. Familial CHD: (Y/N) Yes/No; refers to the presence/absence of an individual with CHD within the immediate family of the proband, respectively. ECA: Extra-cardiac anomalies (not restricted to congenital defects) present in the proband; N: no; Y: yes; U: unknown. Variant type: SNVs and indels found within protein-coding regions (missense, frameshift insertion/deletion, splicing, stopgain), structural variants, SNVs and indels found within regulatory regions (intergenic, intronic, exonic, 3' UTR, downstream, upstream). MAF: minor allele frequency with respect to ExAC (exonic variants); cohort frequency (for structural variants), and gnomAD (for non-coding variants). PP2 HVAR: PolyPhen-2 Hvar predictive score. Score ≥ 0.909: probably damaging; 0.908 ≤ score ≤ 0.447: possibly damaging; score ≤ 0.446: benign; NA: not applicable. CADD: scaled CADD Score ≥ 15: damaging; NA: not applicable. ACMG Class: pathogenicity interpretation according to the ACMG-AMP guidelines [10,260]: P: pathogenic; LP: likely-pathogenic; LB: likely-benign; NA: not applicable; VUS: variant of uncertain significance. Cardiac lesion: primary cardiac lesion of the proband. FSV: functional single ventricle; MOT: malformation of the outflow tract; SD: septal defect; OL: obstructive lesion; AVSD+: atrioventricular septal defect and variants; SDMA: septal defect with minor abnormalities. For detailed phenotype descriptions, including extra-cardiac phenotypes, see Supplementary Table S1. For pedigrees of families with pathogenic or likely-pathogenic variants, see Supplementary Figure S3. [a] Incomplete penetrance / variant present in unaffected individual.

SUPPLEMENTAL FIGURES

Figure S1. Principal Component Analysis of all individuals in the cohort. All subjects of this study (n = 338) were compared with 2504 individuals from the 1000 Genomes phase 3 release (hg19 coordinates converted to hg38) for the five super populations: European (EUR; red dots); East Asian (EAS; blue dots); American (AMR; green dots); South Asian (SAS; purple dots); African (AFR; orange dots). Study subjects (black dots) predominantly cluster with individuals of European descent. Study subjects also cluster with South Asian, East Asian, and African populations. There are also subjects that cluster outside of the super populations, indicative of mixed ethnicities (refer to **Supplementary Table S1** for self-declared ethnicities).

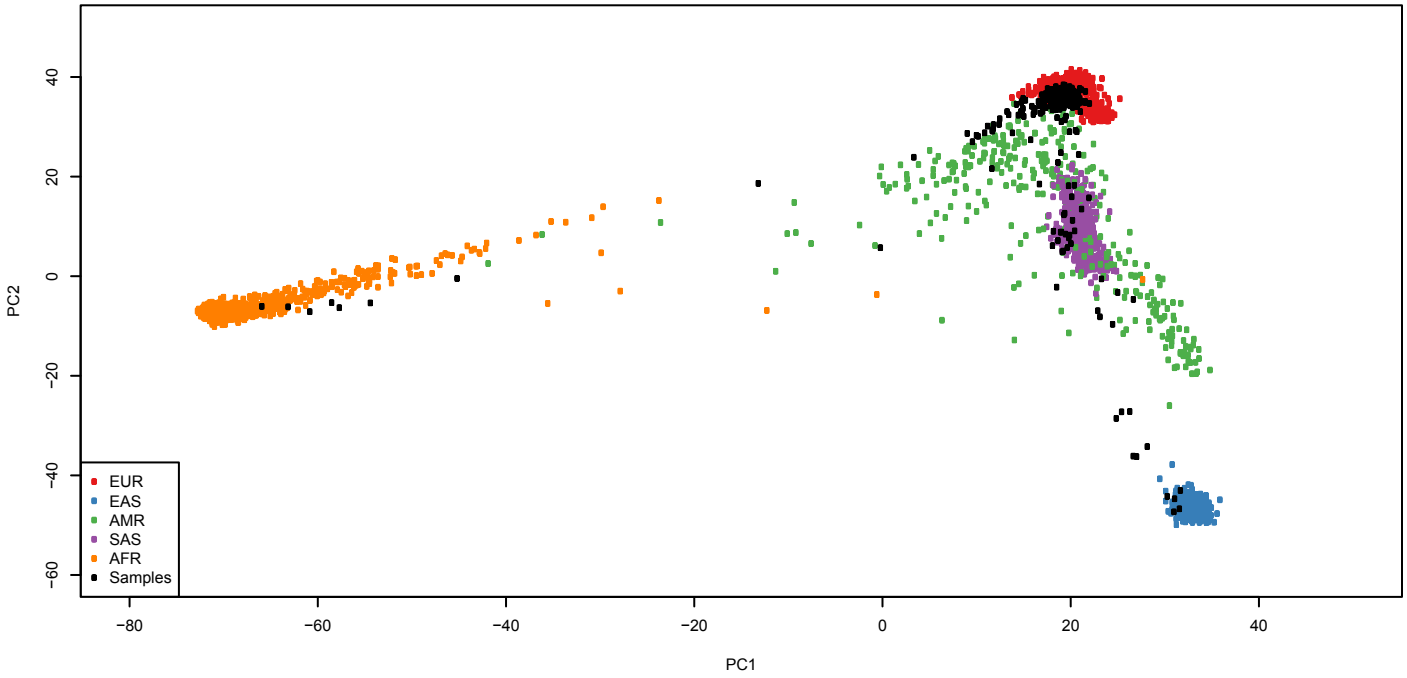


Figure S2. Evaluation of whole genome sequencing alignment and coverage. Performance metrics for whole genome sequencing data were computed across all samples with Picard Tools (http://broadinstitute.github.io/picard). Histograms show the distributions of (A) mean coverage; (B) standard deviation (SD) of coverage; (C) the fraction of bases that attained at least 20× sequence coverage; (D) the fraction of bases that attained at least 10× sequence coverage; (E) mean coverage across all protein-coding exons; (F) mean coverage across all protein-coding exons of hcCHD genes, across all samples in the cohort.

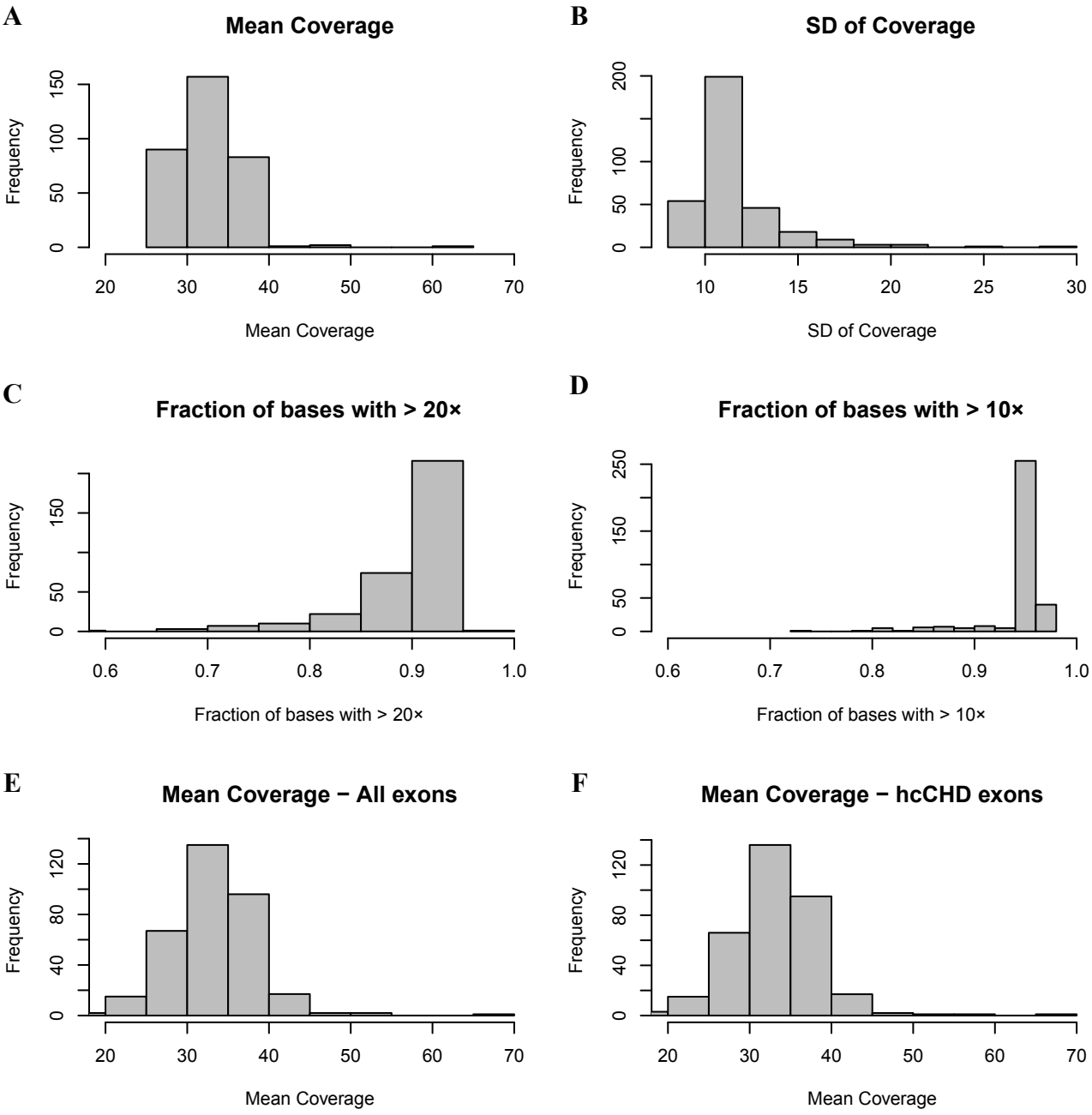


Figure S3. Pedigrees and genotypes of families with pathogenic and likely pathogenic variants.

Green areas indicate individuals, per represented family, of whom whole genome sequencing and analysis was performed. Squares indicate male persons, circles female persons, triangles first-trimester deaths, solid symbols affected persons (patients), slashes deceased persons, and double horizontal lines consanguineous marriages. + heterozygous genotype for the listed variant. ++homozygous genotype. [a] Gestational diabetes. ADHD: Attention deficit hyperactivity disorder; AI: aortic incompetence; AP: aorticopulmonary; AR: aortic regurgitation; ASD: atrial septal defect; AVA: aortic valve atresia; AVR: aortic valve regurgitation; AVSD: atrioventricular septal defect; BAV: bicuspid aortic valve; CoA: coarctation of the aorta; DORV: double outlet right ventricle; GORD: gastro-esophageal reflux disease; HLHS: hypoplastic left heart syndrome; HRH: hypoplastic right heart; MVI: mitral valve incompetence; MVR: mitral valve regurgitation; PA: pulmonary atresia; PAH: pulmonary arterial hypertension; PAPVR: partial anomalous pulmonary venous return; PDA: patent ductus arteriosus; PFO: patent foramen ovale; PS: pulmonary stenosis; PVR: pulmonary valve regurgitation; RAA: right aortic arch; RV: right ventricle; SAM: subaortic membrane; SVC: superior vena cava; TGA: transposition of the great arteries; TOF: Tetralogy of Fallot; TVS: tricuspid valve stenosis; VSD: ventricular septal defect.

Family 44

+ *HAAO* NM_012205.2: c.558G>A p.Trp186*

Comprehensive analysis | **ACMG-AMP:** Pathogenic (Ia): PVS1, PS3, PM2, PM3, PP3

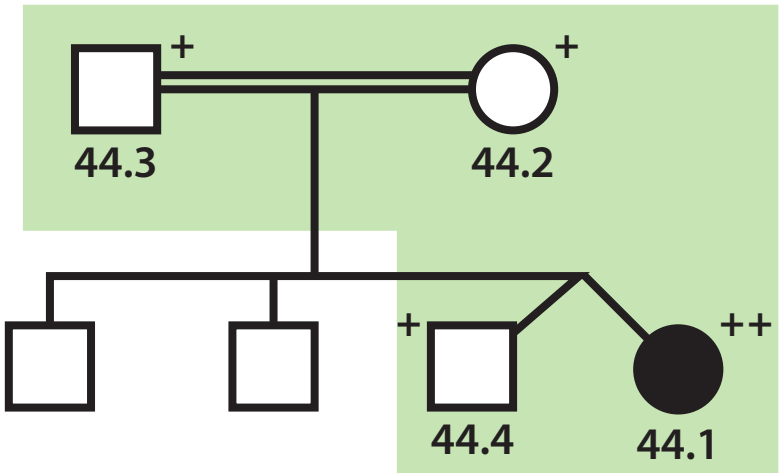
PVS1: Shi, *et al*.[261] showed homozygous loss-of-function (LOF) variants in *HAAO* cause Vertebral, cardiac, renal and limb defects syndrome 1 (VCRL1) (OMIM: 604521).

PS3: *In vivo* and *in vitro* functional assays show that the mutations abolish the enzymatic activity of HAAO.[261]

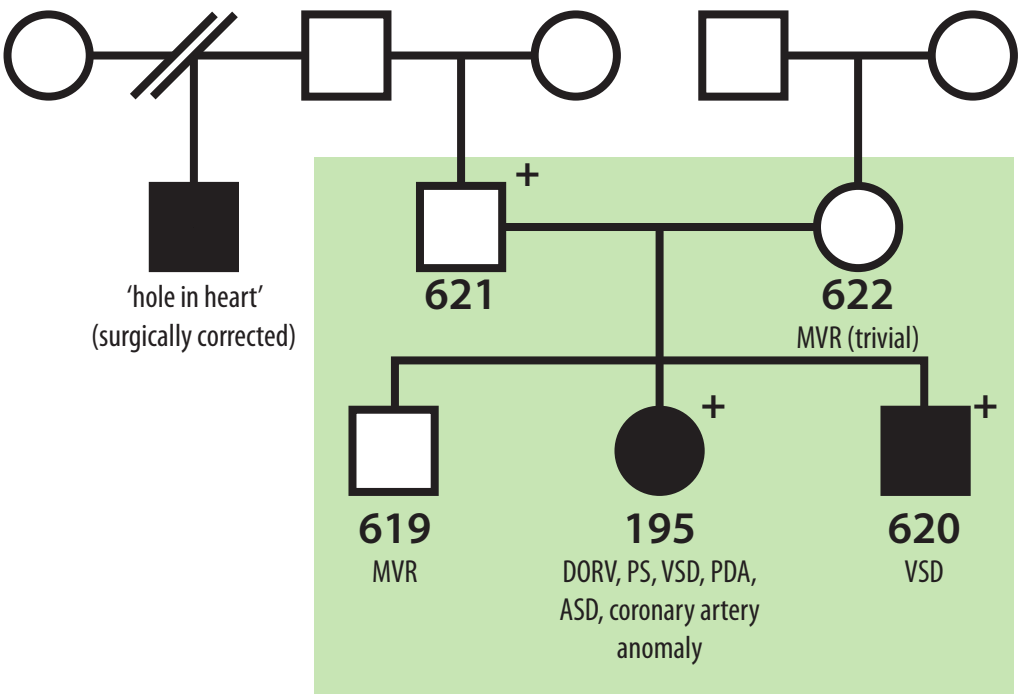
PM2: The variant is not present in population databases (ExAC, 1000 Genomes).

PM3: The proband is homozygous for the pathogenic variant.

PP3: The variant is predicted to be severely damaging due to truncation of the gene product leading to nonsense-mediated decay (NMD).



Family 195
+ *NODAL* NM_018055.4: c.123_142dup p.Tyr48Trpfs*5
hcCHD Gene Screen | **ACMG-AMP:** Pathogenic (Ia): PVS1, PM2, PP3, PP1-S, BS2
PVS1: Heterozygous LOF *NODAL* variants leading to CHD have been previously reported (See **Supplementary Table S2**) (OMIM: 601265).
PM2: The variant is not present in population databases (ExAC, 1000 Genomes).
PP3: The variant is predicted to be severely damaging due to truncation of the gene product leading to NMD. The pLI score of *NODAL* is 0.95.
PP1-S: Variant is also present in Family 835. It is found in 5 individuals, 4 of whom have manifested cardiac phenotypes with those consistent with what is previously reported for *NODAL* mutations.
BS2: Variant present in the asymptomatic father (Patient 621).



Family 575

+ *CHD7* NM_017780.3: c.2098A>G p.Asn700Asp (splicing)

hcCHD Gene Screen | **ACMG-AMP:** Pathogenic (Ic): PVS1, PM2, PP3, BS2

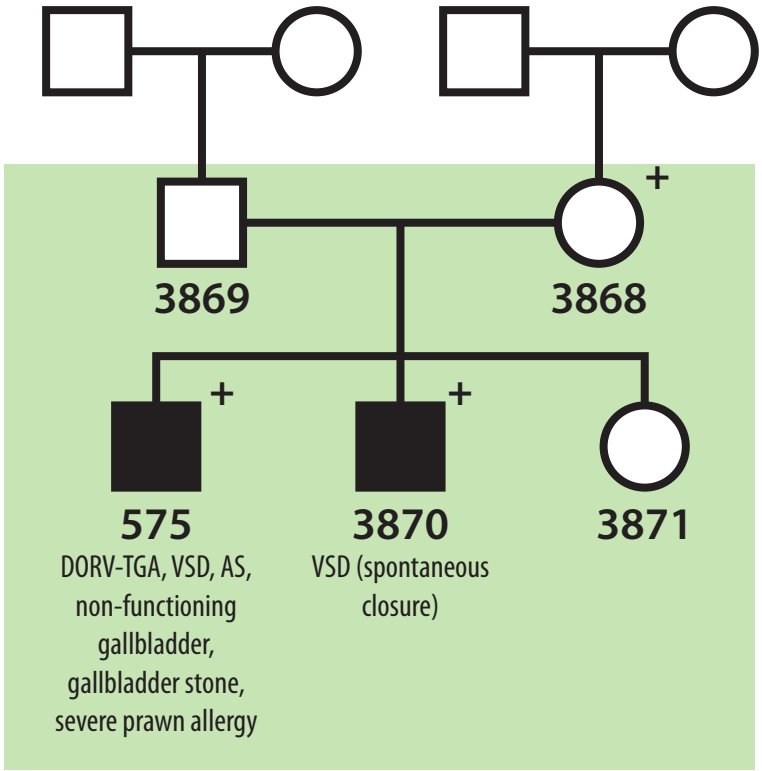
PVS1: Heterozygous LOF variants have previously been associated with cardiac defects (See **Supplementary Table S2**) (OMIM: 608892).

PM2: The variant is not present in population databases (ExAC, 1000 Genomes).

PP3: Variant predicted to lead to loss of splicing acceptor sequence motif on exon 4, thus causing aberrant splicing of the gene, loss of full/partial exons and loss of typical protein function. The pLI of *CHD7* is 1.00.

BS2: Variant inherited by two affected children (Patients 575 and 3870) from asymptomatic mother (Patient 3868).

NOTE: Pathogenic variants in *CHD7* are associated with CHARGE syndrome (OMIM: 214800) and hypogonadotropic hypogonadism (OMIM: 612370). Close monitoring of the patients may reveal further phenotypes associated with the gene-variant.



Family 835

+ *NODAL* NM_018055.4: c.123_142dup p.Tyr48Trpfs*5

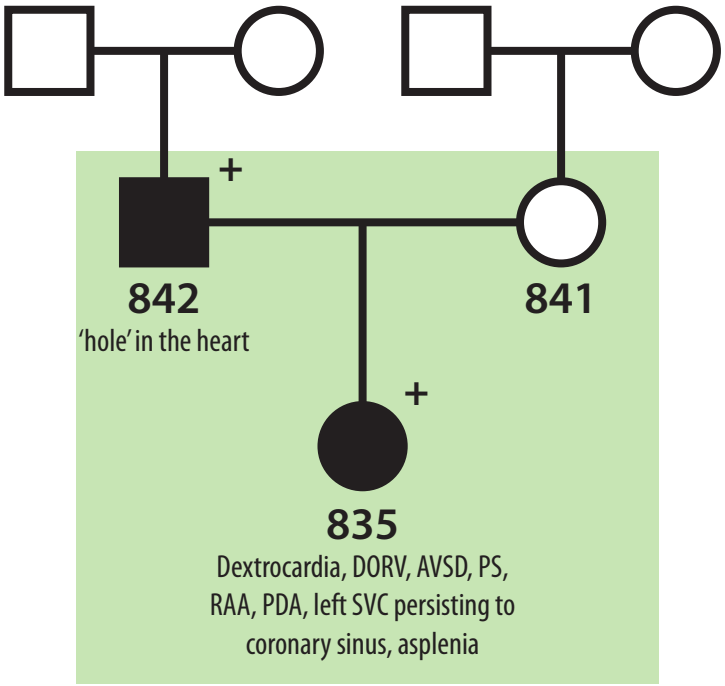
hcCHD Gene Screen | **ACMG-AMP:** Pathogenic (Ia): PVS1, PM2, PP3, PP1-S

PVS1: Heterozygous LOF *NODAL* variants leading to CHD have been previously reported (See **Supplementary Table S2**) (OMIM: 601265).

PM2: The variant is not present in population databases (ExAC, 1000 Genomes).

PP3: The variant is predicted to be severely damaging due to truncation of the gene product leading to NMD. The pLI score of *NODAL* is 0.95.

PP1-S: Variant is also present in Family 195. It is found in 5 individuals, 4 of whom have manifested cardiac phenotypes with those consistent with what is previously reported for *NODAL* mutations.



Family 939

+ *KMT2C* NM_070606.2: c.8390dup p.Glu2798Glyfs*11

Comprehensive analysis | **ACMG-AMP:** Likely pathogenic (II): PS2, PM2, PP3, BS4

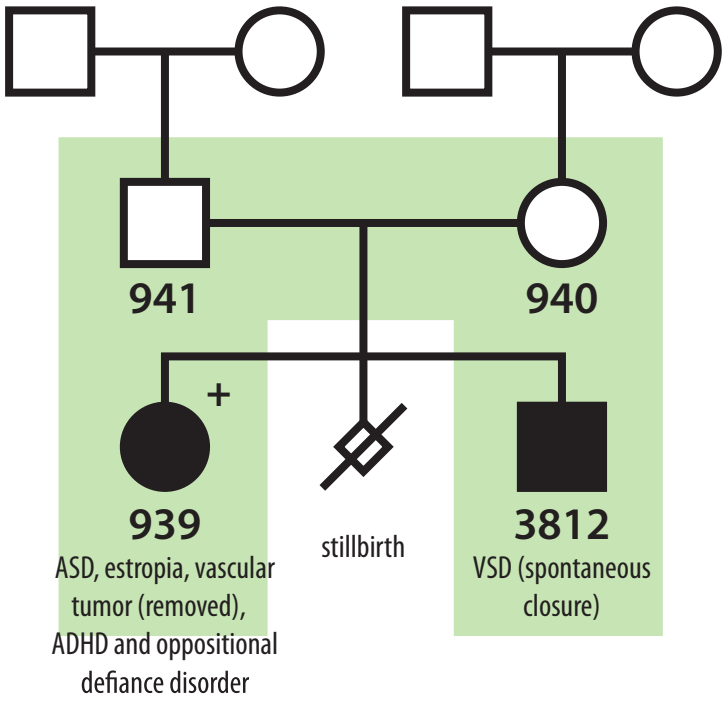
PS2: *De novo* frameshift variant in *KMT2C* present in the proband and not in the parents. Frameshift LOF variants in *KMT2C* have previously been reported to be disease causal (OMIM: 606833).[70,267,268]

PM2: The variant is not present in population databases (ExAC, 1000 Genomes).

PP3: The variant is predicted to be severely damaging due to truncation of the gene product leading to NMD. The pLI score of *KMT2C* is 1.00.

BS4: Variant is not present in the affected sibling (Patient 3812).

NOTE: KMT2C is a chromatin modifier involved in transcriptional regulation. Frameshift LOF variants in *KMT2C* have previously been reported to be disease-causal (OMIM: 606833).[70,267,268] The variant is *de novo* in the proband, who displays cardiac and extra-cardiac neurodevelopmental defects consistent with what has previously been observed for pathogenic *KMT2C* variants. The sibling, who has a CHD that did not require surgery and displays no other adverse phenotypes, does not carry the variant, suggesting variable disease etiologies within the family.



Family 1384

+ *MYH6* NM_002471.3: c.731G>A p.Arg244His

+ *MYH6* NM_002471.3: c.5794A>T p.Lys1932*

hcCHD Gene Screen | **ACMG-AMP:** Pathogenic (Id) (PVS1, PM3, PP2, PP3) and Likely Pathogenic (IV) (PM1, PM2, PM3, PP2, PP3)

PVS1: LOF variants in *MYH6* have previously been associated with cardiac defects (**Supplementary Table S2**) (OMIM: 160710).[268,284]

PM1: Missense variant falls within conserved myosin motor domain of MYH6.

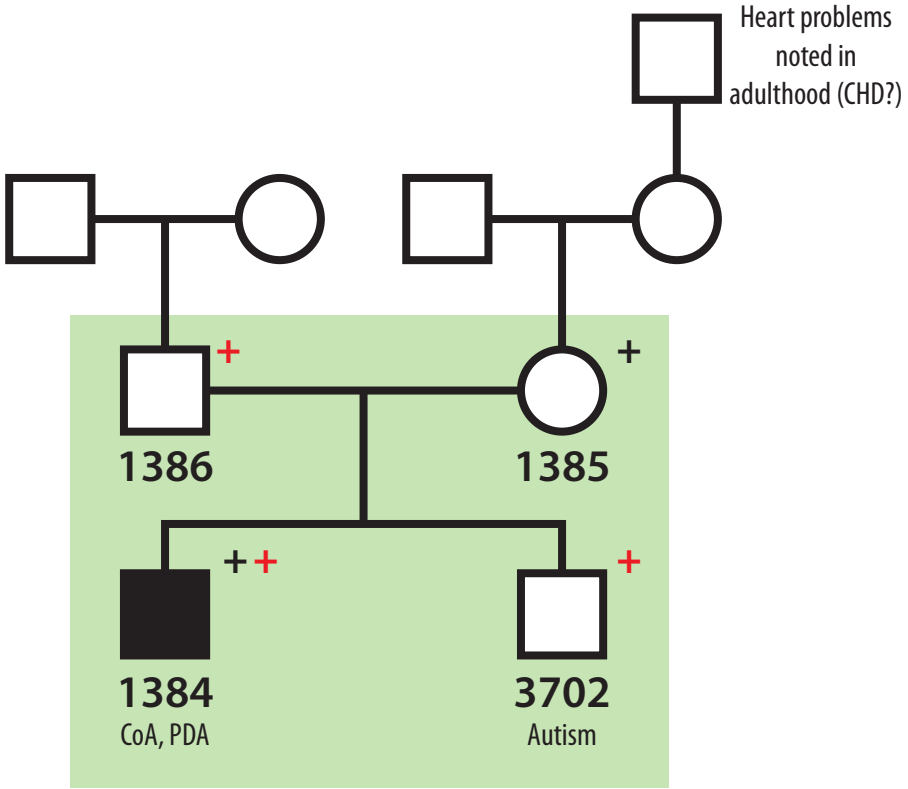
PM2: Both variants are found at very low frequencies in population databases (ExAC, 1000 Genomes).

PM3: The pathogenic/likely-pathogenic variants are inherited in a compound heterozygous manner.

PP2: *MYH6* is a gene intolerant to missense variation (RVIS score = 0.66%), where missense variants are a known mechanism of disease.

PP3: The stop-gain variant is predicted to truncate the protein as well as lead to loss of splicing donor. Missense variant where the highly conserved Arginine residue is replaced by a Histidine residue, is also predicted to be highly damaging for MYH6 protein structure/function.

NOTE: Although heterozygous variants in *MYH6* have previously been associated with cardiac defects, homozygous/compound heterozygous variants in *MYH6* causing congenital heart defects have also been observed.[268,284] Pathogenic *MYH6* variants are associated with cardiomyopathy (OMIM: 613252, 613251) and close monitoring of the siblings for manifestation of late-onset, gene-variant related phenotypes may be required.



Family 1449

+ *ACTC1* NM_005159.4: c.203C>T p.Thr68Ile

hcCHD Gene Screen | **ACMG-AMP:** Pathogenic (IIIc): PM1, PM2, PP1, PP2, PP3, PS1[b]

PM1: Variant is found at a junction of three actin monomers without tolerated variants in ExAC.

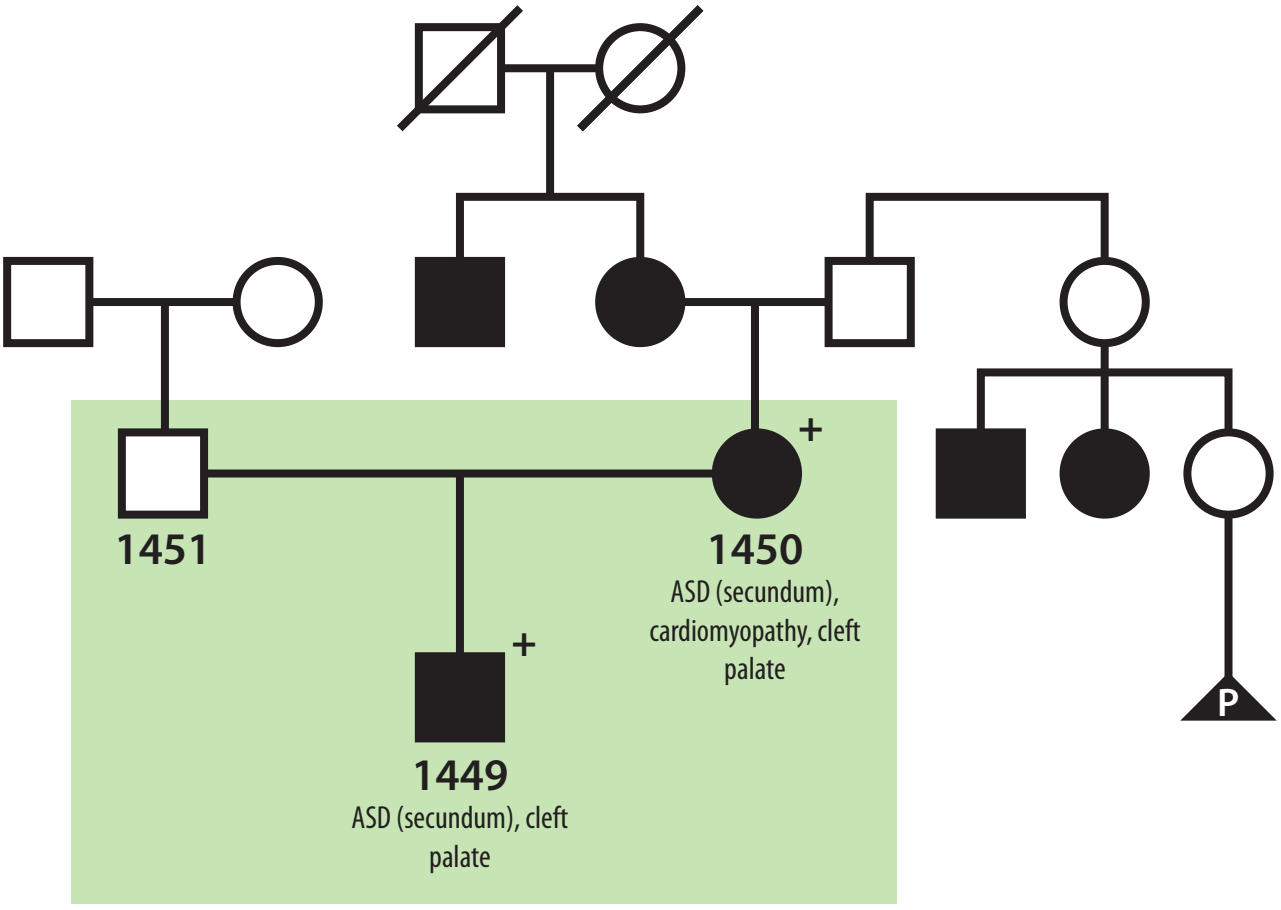
PM2: The variant is not present in population databases (ExAC, 1000 Genomes).

PP1: The variant is inherited by the proband from the affected mother.

PP2: *ACTC1* is relatively intolerant to missense variation (38.28%). Missense *ACTC1* variants are a known mechanism of disease (See **Supplementary Table S2**) (OMIM: 102540).

PP3: The variant is predicted to be damaging to ACTC1 structure/function by replacing the nucleophilic Threonine residue with a hydrophobic Isoleucine residue, thereby destabilizing the actin-myosin interface.

PS1[b]: A homologous Thr68Ile variant has previously been identified in alpha skeletal actin (*ACTA1*), which shares 99% amino acid identity with *ACTC1*, making this variant comparable to that observed here. The Thr68Ile variant-carrier exhibited a severe lethal hypotonia and minimal spontaneous movements of the legs and arms, dying of respiratory failure, indicative of protein dysfunction caused by this mutation.[285]

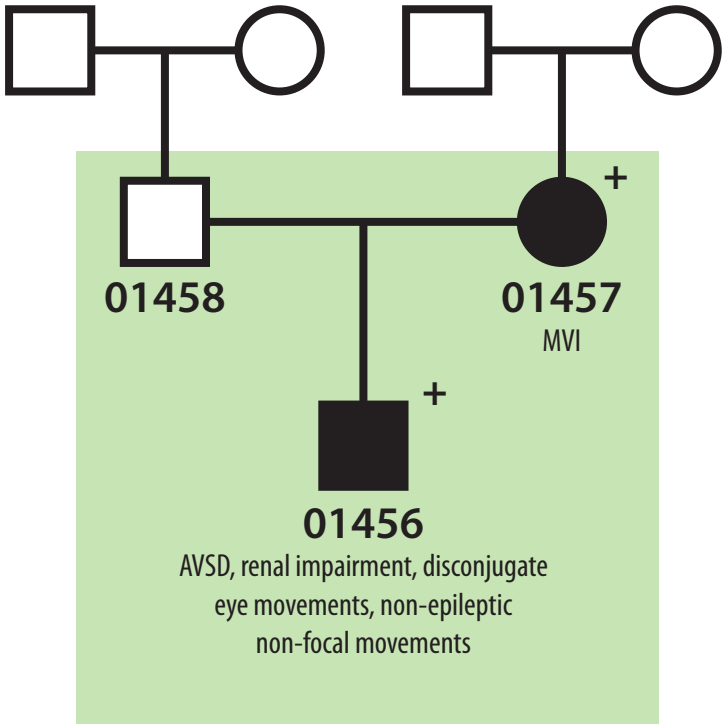


Family 1456

+ chr10q22.3-q23.2 deletion

Comprehensive analysis | **ACMG:** Pathogenic

The ~7.5 Mb deletion at 10q22.3-q23.2 removes 75 protein-coding and non-coding genes. Pathogenic deletions at this chromosomal location have previously been observed (ClinVar ID: 441795, 442036, 155111, 153276, 147367, 146022, 58757, 58755, 58754, 58751, 58748, 33096). The deleted region is flanked by low-copy repeats which mediate nonallelic homologous recombination.[286] 10q22.3-q23.2 syndrome is characterized by cognitive and behavioral abnormalities, and often accompanied by CHD (See **Supplementary Figure S5**) (OMIM: 612242).[286]



Family 1746

+ *HNRNPK* NM_002140.4: c.1259C>T p.Ser420Leu

Comprehensive analysis | **ACMG-AMP:** Likely pathogenic (II): PS2, PM1, PM2, PP3, BP1

PS2: *De novo* missense variant in *HNRNPK* found in the proband and absent in the parents.

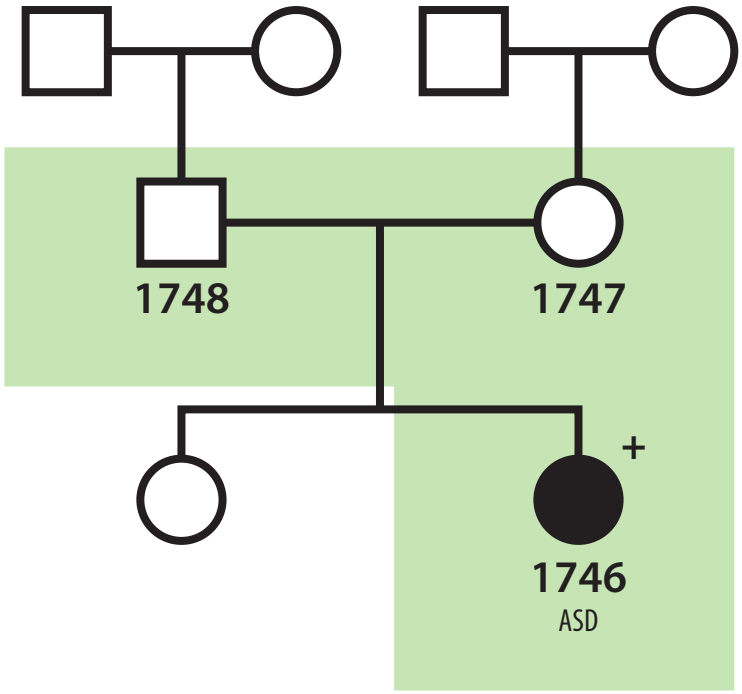
PM1: Variant falls within K-Homology (KH) RNA-binding domain of HNRNPK.

PM2: The variant is not present in population databases (ExAC, 1000 Genomes).

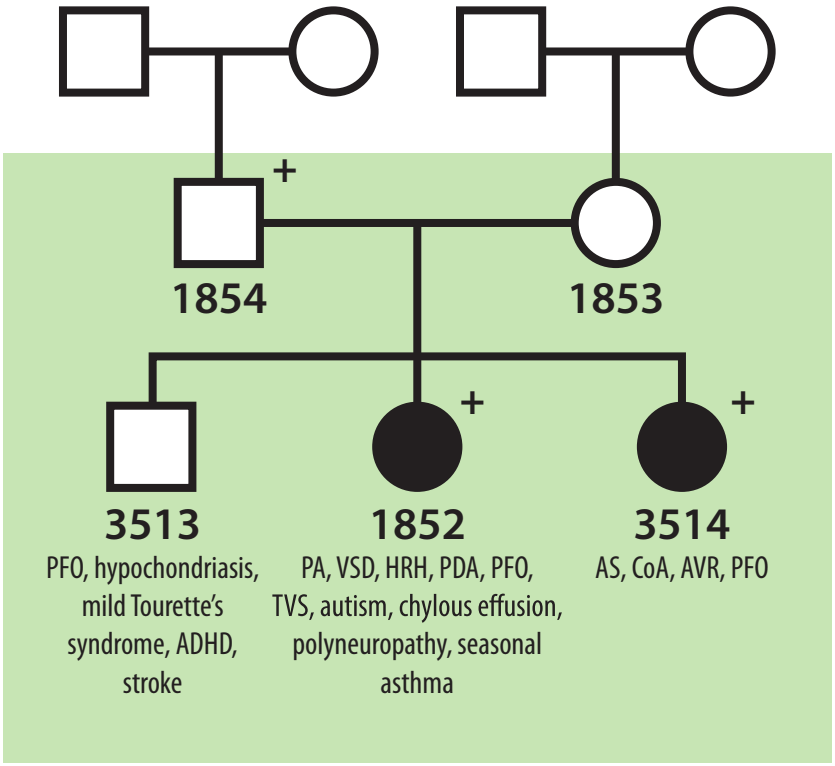
PP3: The variant is predicted to be damaging to protein structure/function due to replacement of nucleophilic Serine residue with hydrophobic Leucine residue. The substitution also leads to the loss of a phosphoserine at Ser420 in the KH domain.

BP1: Majority of disease-associated variants in *HNRNPK* are gene/protein truncating variants.

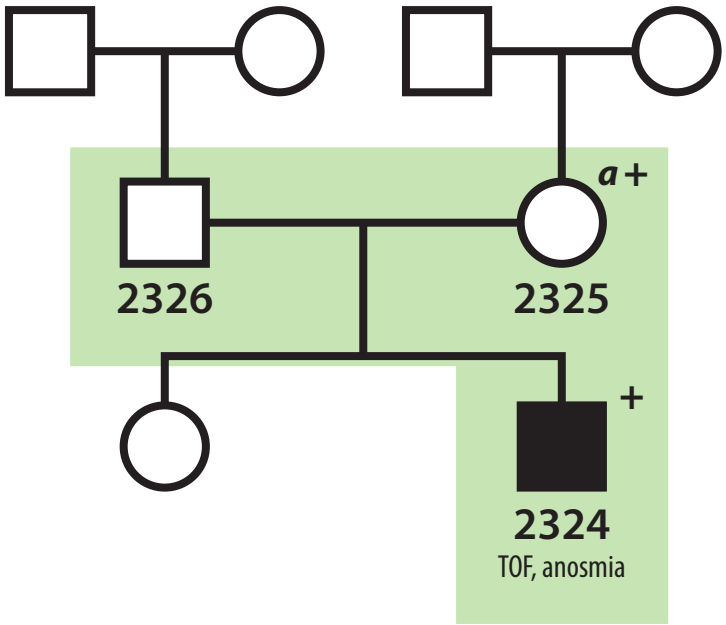
NOTE: HNRNPK is an RNA-splicing factor involved in transcription and translation regulation. *De novo* heterozygous LOF variants in *HNRNPK* are associated with Au-Kline syndrome, where septal defects are the common CHD phenotype (OMIM: 600712).[268,287] Although the proband currently does not exhibit ECA associated with Au-Kline syndrome, close monitoring may reveal further gene-variant associated phenotypes.



Family 1852
+ *NOTCH1* NM_017617.4: c.5865del p.Asn1955Lysfs*26
hcCHD Gene Screen | **ACMG-AMP:** Pathogenic (Id): PVS1, PM2, PP3, BS4
PVS1: Heterozygous LOF variants in *NOTCH1* leading to CHD have previously been reported (See **Supplementary Table S2**) (OMIM: 190198).
PM2: The variant is not present in population databases (ExAC, 1000 Genomes).
PP3: The variant is predicted to be severely damaging due to truncation of the gene product leading to NMD. The pLI score of *NOTCH1* is 1.00.
BS4: Variant is not present in sibling (Patient 3513) with PFO.



Family 2324
+ *SEMA3D* NM_152754.2: c.191C>A p.Ser64*
[a] Gestational diabetes
Comprehensive analysis | **ACMG-AMP:** Pathogenic (Ic): PVS1, PM2, PP3, BS2
PVS1: Heterozygous LOF variants in *SEMA3D* leading to CHD have previously been reported.[263,264,268]
PM2: The variant is not present in population databases (ExAC, 1000 Genomes).
PP3: The variant is predicted to be severely damaging due to truncation of the gene product leading to NMD.
BS2: The variant is present in the asymptomatic mother (Patient 2325).
NOTE: The mother suffered from gestational diabetes during pregnancy, which may have influenced variant penetrance in the proband.



Family 2632

+ *DCHS1* NM_003737.3: c.3326C>T p.Pro1109Leu

Comprehensive analysis | **ACMG-AMP:** Likely pathogenic (V): PM1, PM2, PP2, PP3, BS2

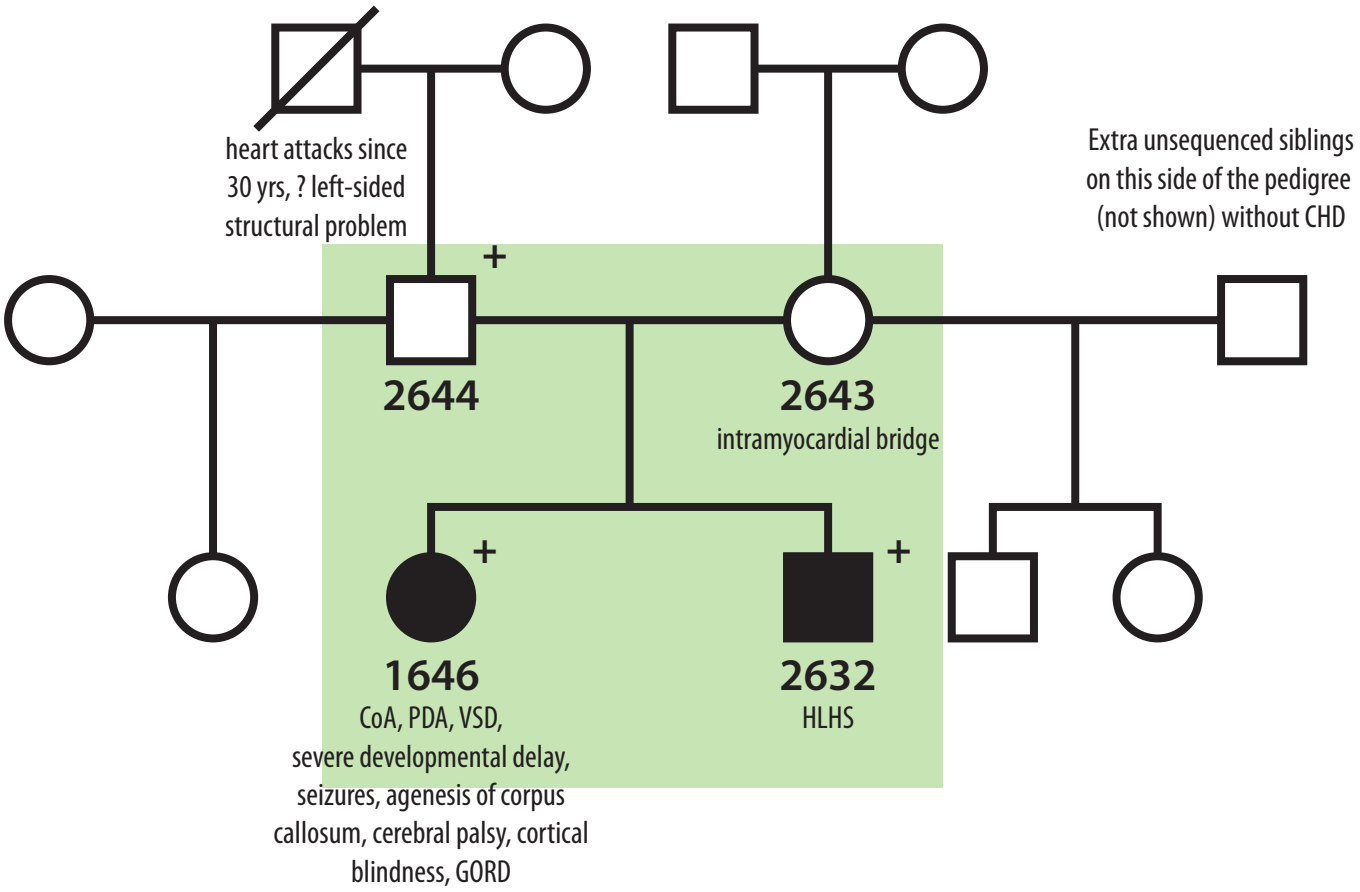
PM1: Variant falls within cadherin-10 domain of DCHS1, which binds calcium to facilitate formation of adherens junctions between cells.

PM2: The variant is not present in population databases (ExAC, 1000 Genomes).

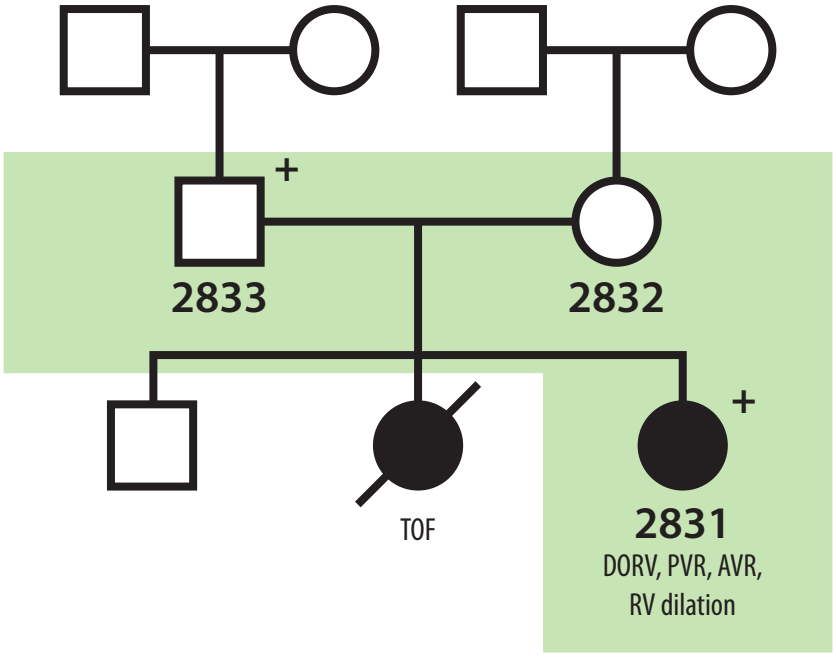
PP2: *DCHS1* is intolerant to missense variation (RVIS = 4.4%). Heterozygous missense variants in *DCHS1* have been associated with CHD.[270]

PP3: The variant is predicted to be damaging to protein structure/function due to replacement of cyclical Proline residue with a Leucine residue.

BS2: The *DCHS1* variant is inherited by the two affected siblings from the father diagnosed with intramyocardial bridge.



Family 2831
+ *NOTCH1* NM_017617.4: c.6105del p.Ala2036Profs*3
hcCHD Gene Screen | **ACMG-AMP:** Pathogenic (Ic): PVS1, PM2, PP3, BS2
PVS1: Heterozygous LOF variants in *NOTCH1* leading to CHD have previously been reported (See **Supplementary Table S2**) (OMIM: 190198).
PM2: The variant is not present in population databases (ExAC, 1000 Genomes).
PP3: The variant is predicted to be severely damaging due to truncation of the gene product leading to NMD. The pLI score of *NOTCH1* is 1.00.
BS2: The variant is inherited by the proband from the asymptomatic father.



Family 2985

+ *CFC1* NM_032545.3: c.522del p.Ala175Argfs*56

hcCHD Gene Screen | **ACMG-AMP:** Pathogenic (Ia): PVS1, PS1, PS3, PM2, PP1, PP3

PVS1: Heterozygous LOF variants in *CFC1* leading to CHD have previously been reported (See **Supplementary Table S2**) (OMIM: 605194).

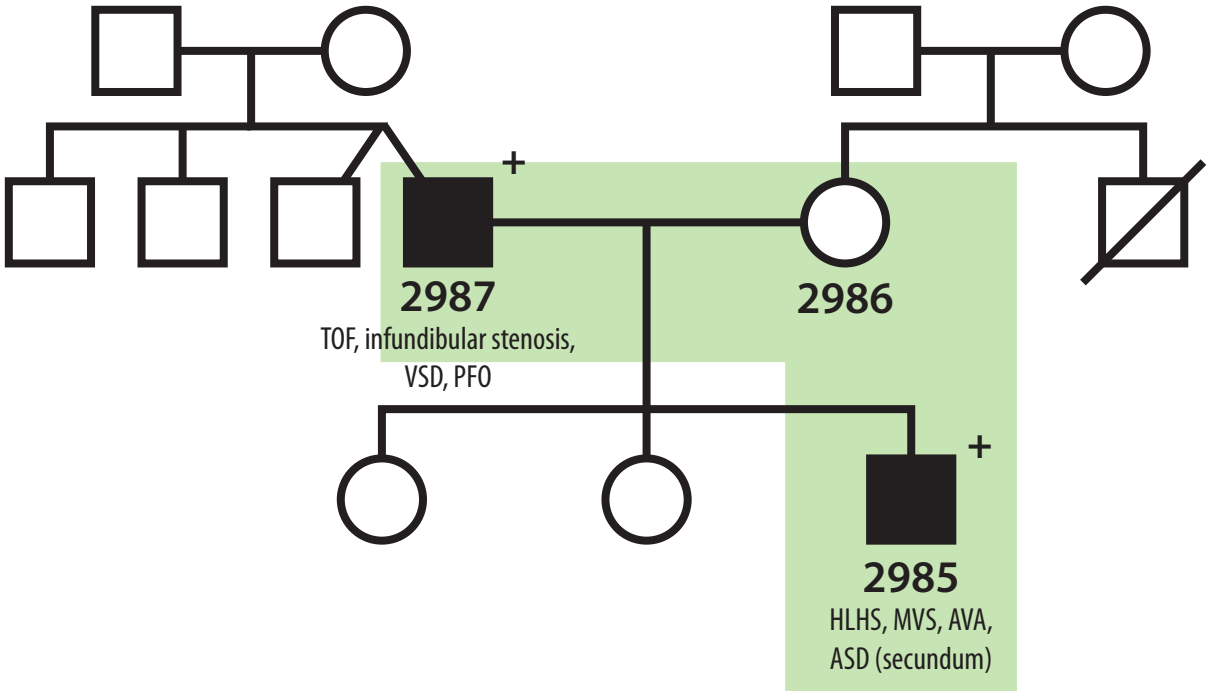
PS1: The variant has previously been reported to be pathogenic in patients with transposition of the great arteries, double outlet right ventricle, and Tetralogy of Fallot.[49,50,52]

PS3: *In vitro* studies by Bamford, *et al*.[49] showed inability of the mutant CFC1 receptor to localize to the cell membrane. *In vivo* studies, also conducted by Bamford, *et al*.,[49] in zebrafish showed that the mutant is unable to rescue a CFC1-deficient phenotype.

PM2: The variant is not present in population databases (ExAC, 1000 Genomes).

PP1: The variant is inherited by the proband from the affected father (Patient 2987).

PP3: This frameshift variant is predicted to be severely damaging due to the extension of the protein from residue 175 with 50 subsequently altered amino acids, normally essential for tethering the CFC1 protein to the cell membrane.[49] The pLI score of *CFC1* is 0.68.



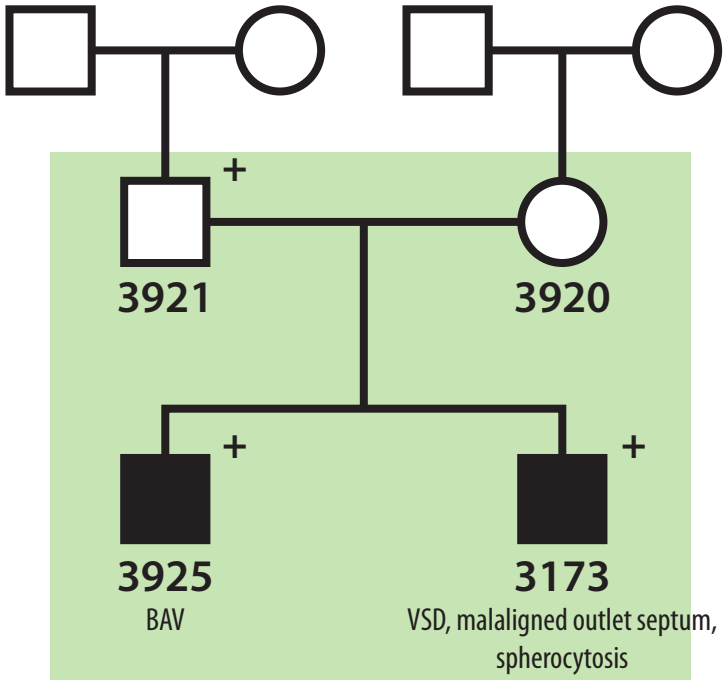
Family 3173

+ *NOTCH1* 22.5 kb deletion (chr9:136537696-136560250)

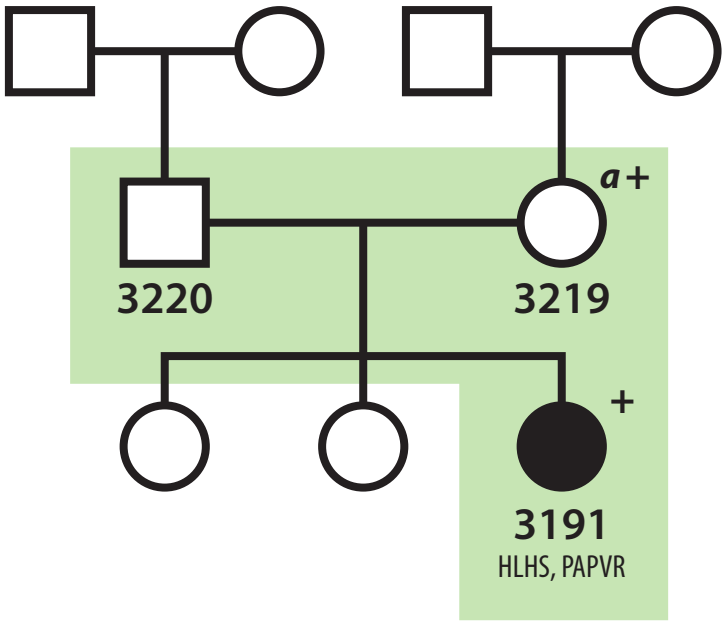
hcCHD Gene Screen | **ACMG:** Pathogenic: PVS1

PVS1: The deletion in *NOTCH1* removes exons 1 and 2. In a circumstance where transcriptional machinery skips to the next available start codon, it is at position 302 of the protein within EGF-like domain 8. The loss of the first 7 EGF-like domains of the extracellular domain of NOTCH1 would impair its function as a receptor and thus, this structural variant is most likely to lead to loss of function.

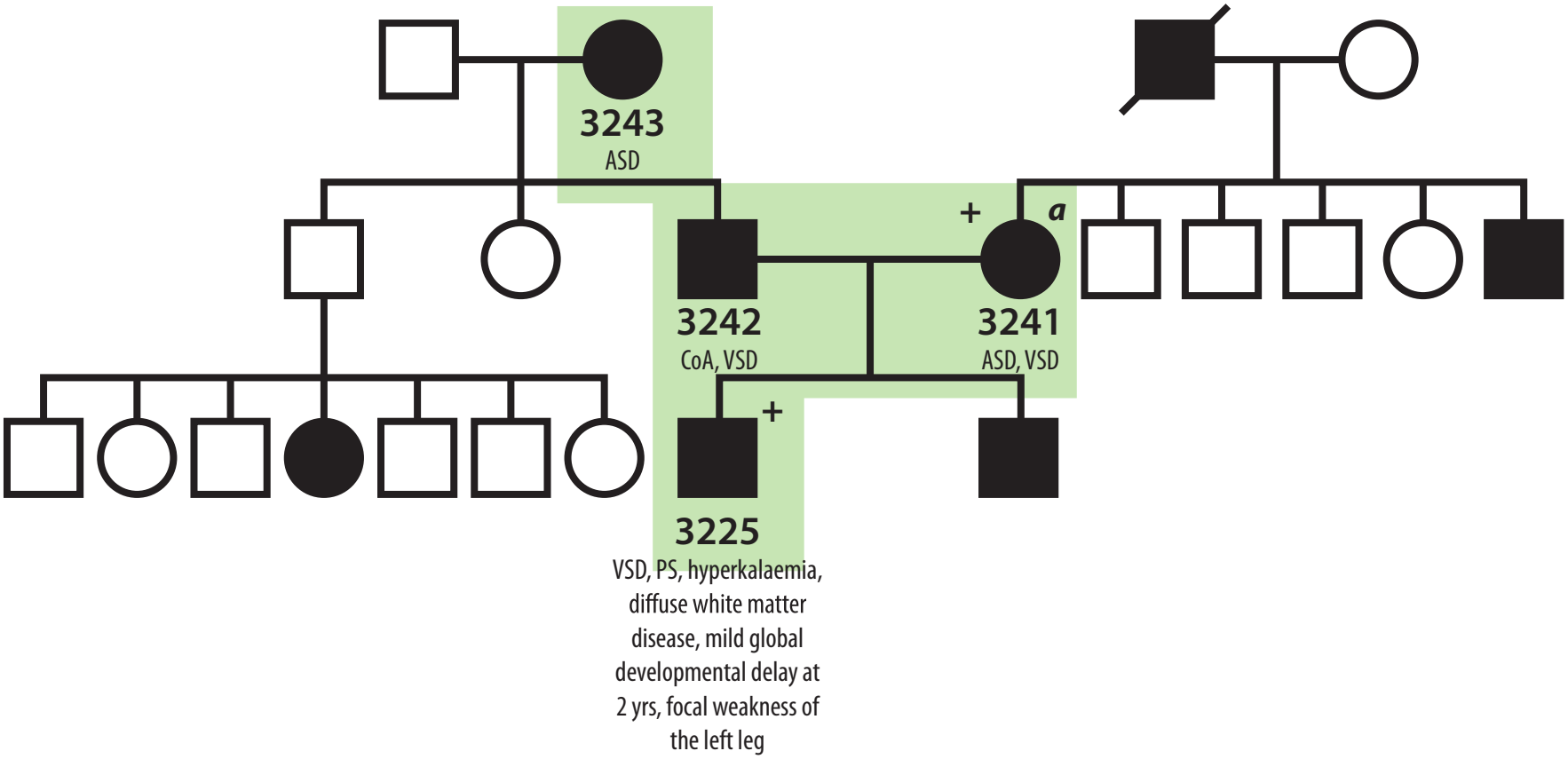
NOTE: The variant is inherited by the proband and affected sibling (Patient 3925) from the mosaic father (Patient 3921) (See **Supplementary Figure S4**).



Family 3191
+ *SMAD6* NM_005585.4: c.86del p.Gly29Alafs*35
[a] Gestational diabetes
hcCHD Gene Screen | **ACMG-AMP:** Pathogenic (Ic): PVS1, PM2, PP3, BS2
PVS1: Heterozygous LOF variants in *SMAD6* leading to CHD have previously been reported.[268]
PM2: The variant is not present in population databases (ExAC, 1000 Genomes).
PP3: The variant is predicted to be severely damaging due to truncation of the gene product leading to NMD.
BS2: The variant is inherited from asymptomatic mother (Patient 3219).
NOTE: The mother suffered from gestational diabetes during pregnancy, which may have influenced variant penetrance in the proband.



Family 3225


3243
ASD
+
a
3242
CoA, VSD
3241
ASD, VSD
+
3225
VSD, PS, hyperkalaemia, diffuse white matter disease, mild global developmental delay at 2 yrs, focal weakness of the left leg

+ *GATA4* NM_002052.4: c.959G>A p.Arg320Gln

[a] Gestational diabetes

hcCHD Gene Screen | **ACMG-AMP:** Pathogenic (IIIb): PM2, PP3, PP2, PM1, PP1, PS3

PS3: *In vitro* assays show that the mutation results in a speckled GATA4 protein aggregation in subnuclear compartments, incapable of transcriptional activity due to decreased DNA-binding capacity.[288]

PM1: Variant falls within the nuclear localization signal that coordinates the nuclear import of GATA4 protein.

PM2: The variant is not present in population databases (ExAC, 1000 Genomes).

PP1: Variant is inherited by the proband from the affected mother.

PP2: *GATA4* is relatively intolerant to missense variation. Missense *GATA4* variants are a known mechanism of disease (See **Supplementary Table S2**) (OMIM: 600576).

PP3: Variant is predicted to be damaging to protein structure/function due to replacement of the large, basic Arginine residue with a polar Glutamate residue, and thereby disturbing the conserved $RRX_{33}RXR$ nuclear localization motif.[288,289]

Family 3397

+ *TEK* NM_000459.4: c.2744G>A p.Arg915His

Comprehensive analysis | **ACMG-AMP:** Pathogenic (II): PS1, PS3, PM1, PM2, PP1, PP2, PP3

PS1: The same variant (Arg915His) has previously been published as pathogenic by Wouters, *et al*.[269]

PS3: *In vitro* studies conducted by Wouters, *et al*.,[269] showed ligand-independent hyperphosphorylation of TEK.

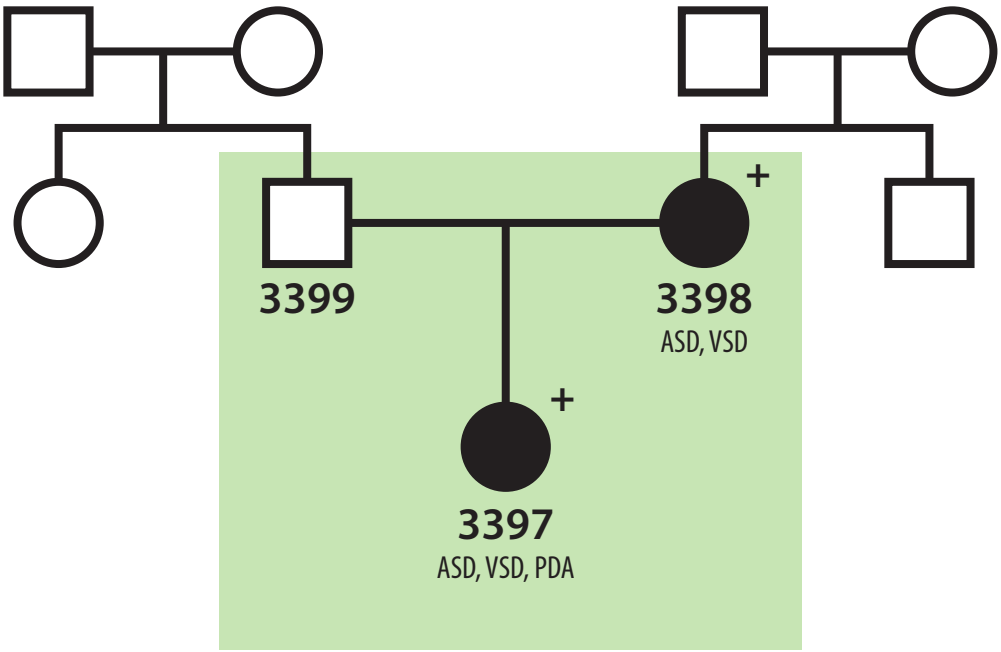
PM1: Variant is located within the tyrosine kinase domain of TEK, which dictates the kinase activity of the TEK receptor.

PM2: The variant is not present in population databases (ExAC, 1000 Genomes).

PP1: Variant is inherited by the proband from the affected mother (Patient 3398).

PP2: *TEK* is relatively intolerant to missense variation (RVIS = 30.9%). Heterozygous missense variants in *TEK* have been associated with CHD.[269]

PP3: Variant is predicted to be damaging to protein structure/function by replacing the large Arginine residue at position 915 with the smallest basic residue, Histidine.



Family 3595

+ *KMT2C* NM_170606.2: c.5636dup p.Gln1880Alafs*9

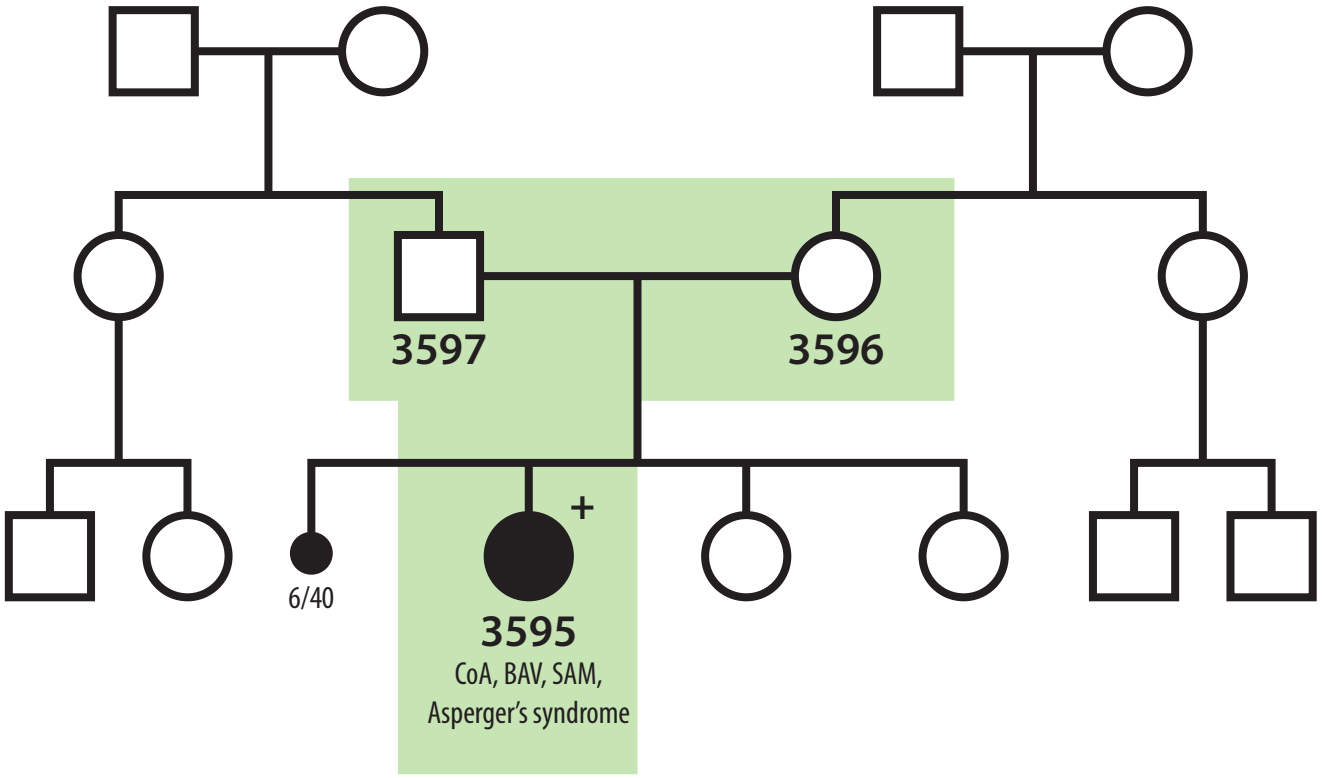
Comprehensive analysis | **ACMG-AMP:** Likely pathogenic (II): PM2, PP3, PS2

PS2: *De novo* frameshift variant in *KMT2C* present in the proband and not in the parents.

PM2: The variant is not present in population databases (ExAC, 1000 Genomes).

PP3: The variant is predicted to be severely damaging due to truncation of the gene product leading to NMD. The pLI score of *KMT2C* is 1.00.

NOTE: KMT2C is a chromatin modifier involved in transcriptional regulation. Frameshift LOF variants in *KMT2C* have previously been reported to be disease-causal (OMIM: 606833).[70,267,268] The variant is *de novo* in the proband, who displays cardiac and extra-cardiac neurodevelopmental defects consistent with what has been reported for *KMT2C* LOF variants.



Family 3642

+ *INVS* NM_014425.4: c.3182dup p.Asn1061Lysfs*20

hcCHD Gene Screen | **ACMG-AMP:** Pathogenic (Ic): PVS1, PM3, PP3, BS2

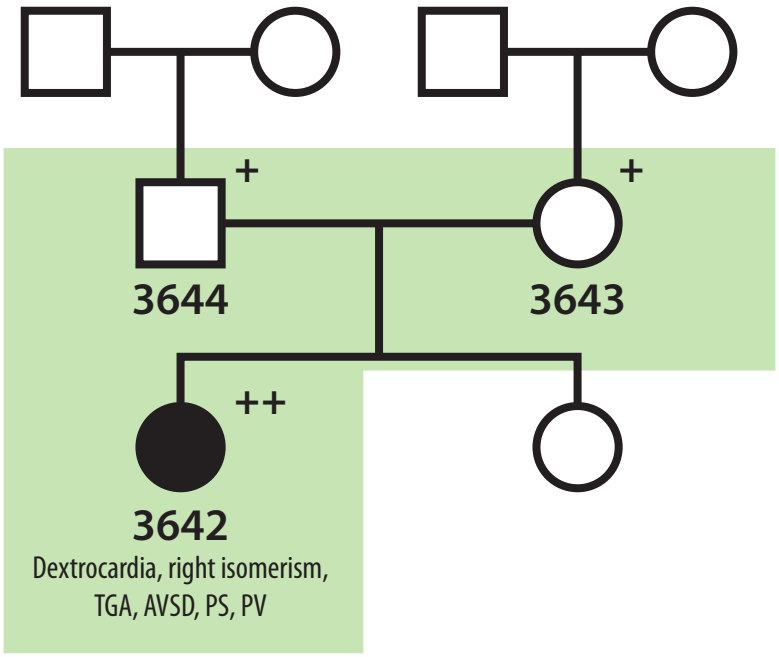
PVS1: Homozygous frameshift variants in *INVS* leading to CHD have previously been reported (see **Supplementary Table S2**).[290]

PM3: Variant is a homozygous frameshift that is present on both alleles.

PP3: Variant predicted to be damaging to protein function due to loss of stop-codon and elongation of the protein.

BS2: Variant present in an asymptomatic individual within our cohort (Family 150650086, mother).

NOTE: Pathogenic variants in *INVS* are associated with Nephronophthisis (OMIM: 602088). Close monitoring of the proband may reveal further phenotypes associated with the gene-variant.



Family 3648

+ *JAG1* NM_000214.2: c.2429C>T p.Pro810Leu

hcCHD Gene Screen | **ACMG-AMP:** Pathogenic (II): PS1, PS3, PM1, PP2, PP3, BS2

PS1: The variant has been previously published as pathogenic by Bauer, *et al*.[255]

PS3: *In vitro* assays by Bauer, *et al*.,[255] showed defective glycosylation of mutant JAG1, which led to abnormal trafficking and cellular localization. The mutant protein was also unable to activate NOTCH signaling.

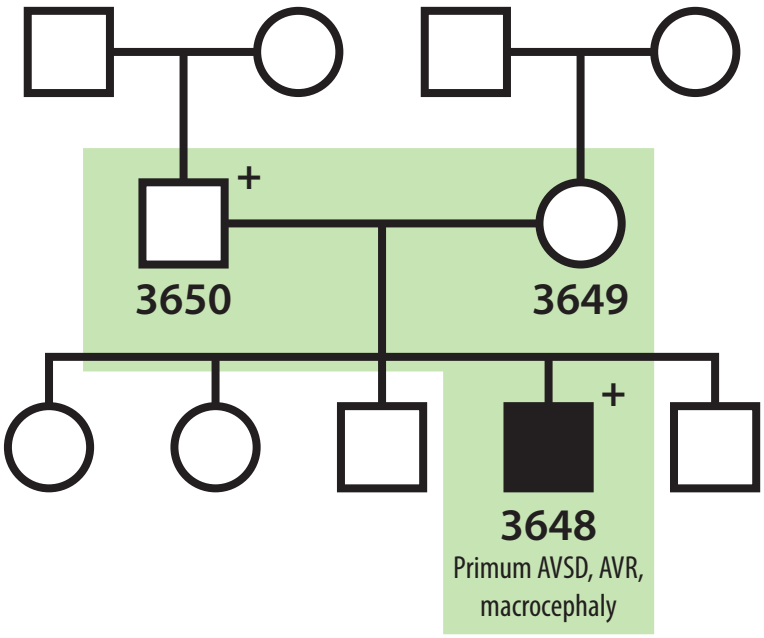
PM1: The variant occurs at a conserved Proline residue within the 15th EGF-like calcium binding domain.

PP2: *JAG1* is intolerant to missense variation (RVIS = 1.49%). Missense variants in *JAG1* have previously been associated with CHD (See **Supplementary Table S2**) (OMIM: 601920).

PP3: Variant predicted to be damaging to protein structure/function due to replacement of a conserved cyclical Proline residue with a Leucine residue.

BS2: The variant is inherited from the asymptomatic father (Patient 3650).

NOTE: Pathogenic variants in *JAG1* are associated with isolated CHD (OMIM: 187500) or Alagille syndrome (OMIM: 118450). Close monitoring of the proband, and the asymptomatic father who carries the variant, may reveal further gene-variant related phenotypes.



Family 3651

+ *KIAA0586* NM_014749.4: c.4268-1G>A

[a] Gestational diabetes

Comprehensive analysis | **ACMG-AMP:** Pathogenic (Ib): PVS1, PM2, PM3, PP3

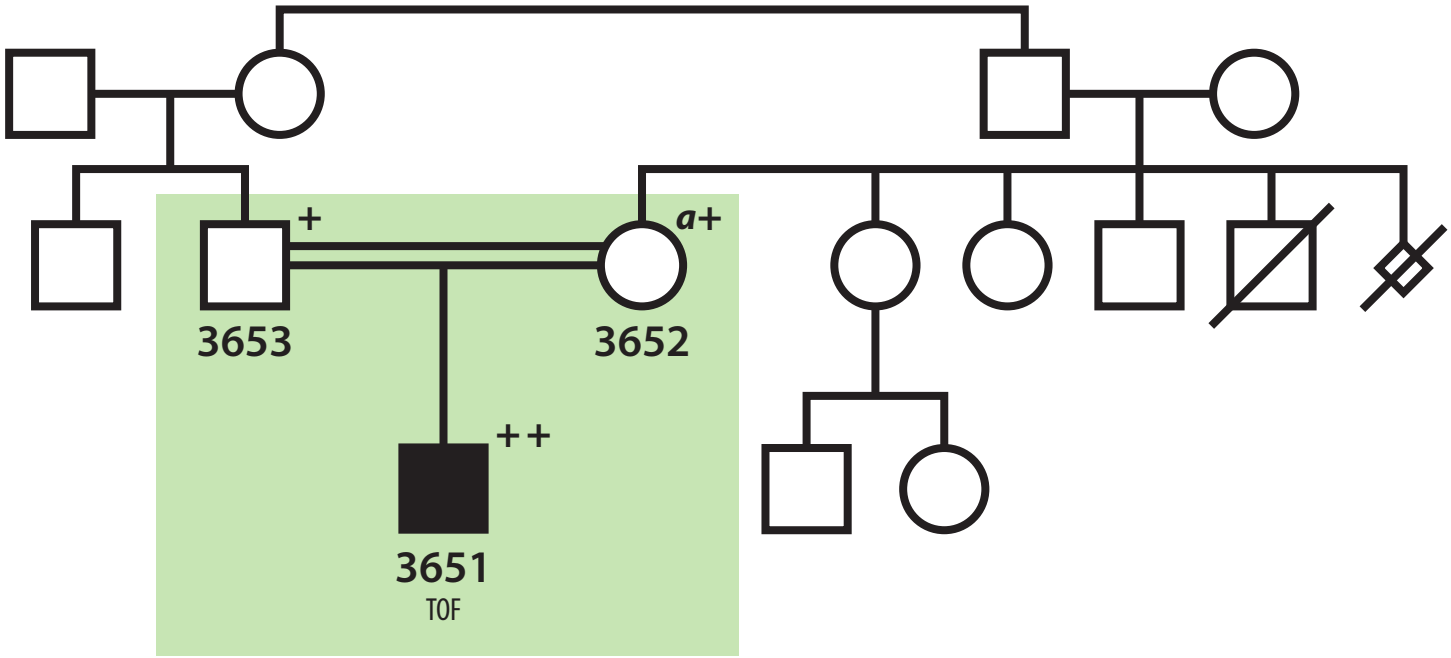
PVS1: Homozygous LOF variants in *KIAA0586* have previously been observed in patients with syndromic CHD.[291]

PM2: Variant is present at very low frequency in heterozygous individuals in population databases (ExAC, 1000 Genomes). There are no homozygote carriers of this variant in current databases.

PM3: Variant is a homozygous loss of splicing acceptor that is present on both alleles.

PP3: Variant is predicted to lead to loss of splicing acceptor sequence motif on exon 31, thus causing aberrant splicing of the gene, loss of full/partial exon and loss of typical protein function.

NOTE: Homozygous LOF variants in *KIAA0586* have previously been associated with Joubert syndrome (OMIM: 616490) or Short-rib thoracic dysplasia 14 with polydactyly (OMIM: 616546). The status of ECA manifestation in our patient is unknown, thus we are unable to determine the syndromic nature of the proband's CHD. The proband's mother (Patient 3652) suffered from gestational diabetes during pregnancy, which may have influenced variant penetrance in the proband.



Family 3695

+ *TLL1* NM_012464.4: c.2578A>G p.Thr860Ala

hcCHD Gene Screen | **ACMG-AMP:** Likely-pathogenic (V): PM1, PM2, PP1, PP2, PP3

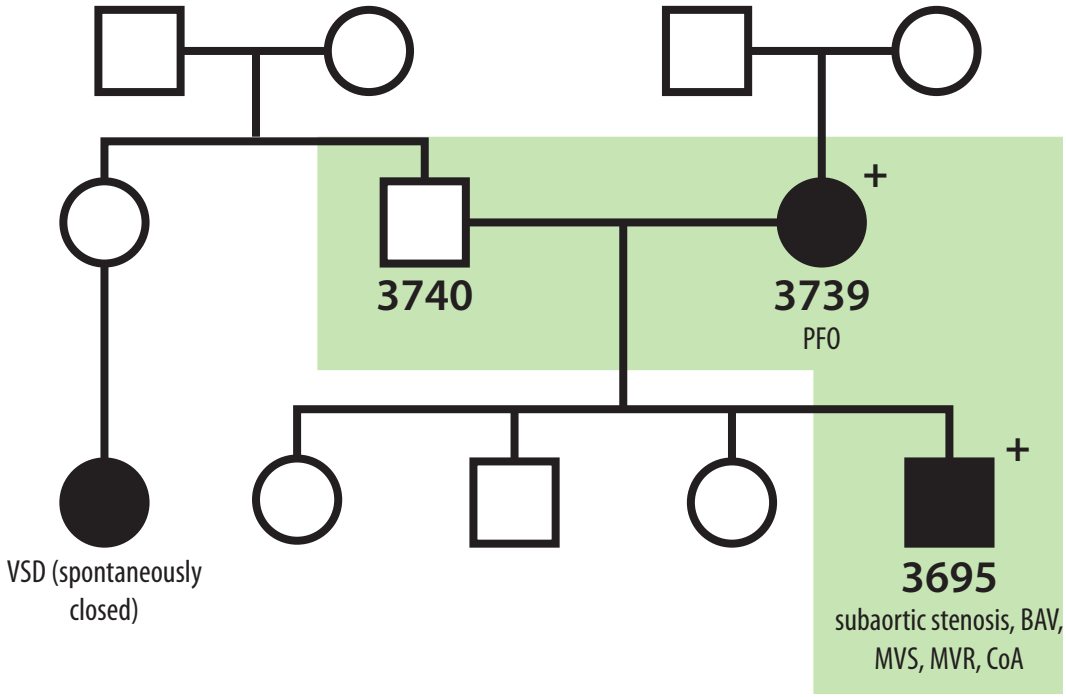
PM1: Variant falls within the fourth CUB domain, which plays a role in protein oligomerization and recognition of binding partners.

PM2: The variant is not present in population databases (ExAC, 1000 Genomes).

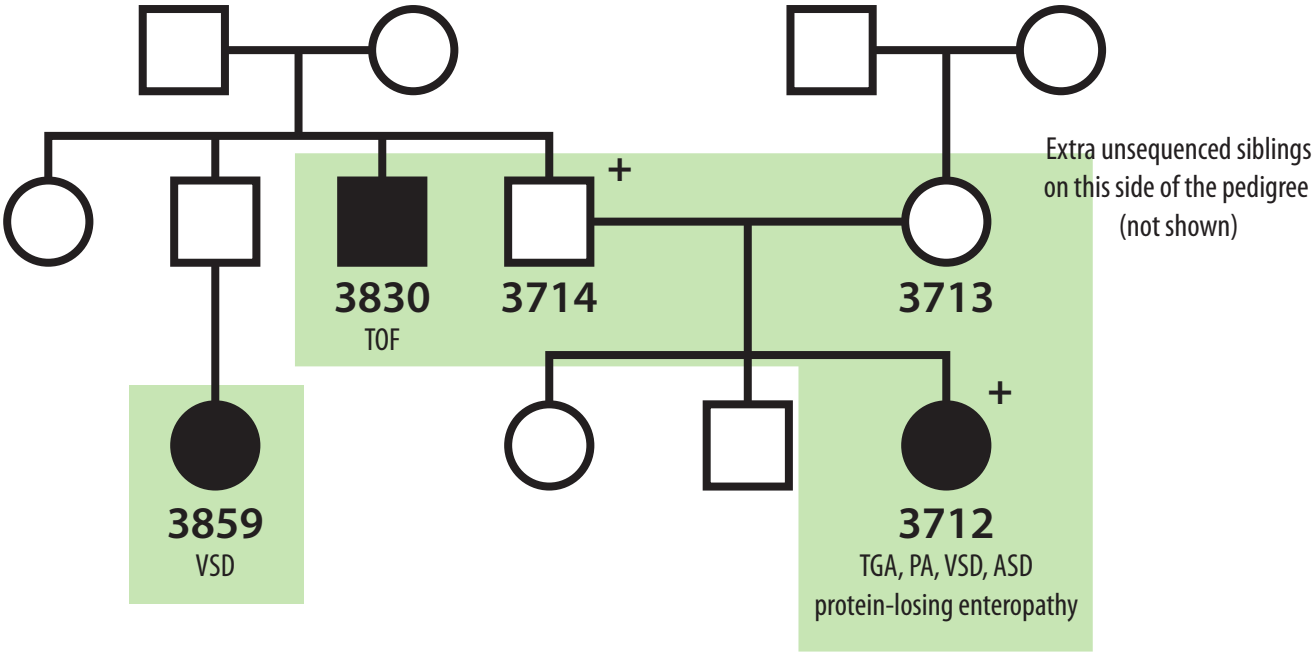
PP1: Variant is inherited by the proband from the affected mother (Patient 3739).

PP2: *TLL1* is intolerant to missense variation (RVIS = 9.72%). Missense variants in *TLL1* have previously been associated with CHD (See **Supplementary Table S2**) (OMIM: 606742).

PP3: Variant predicted to be damaging to protein structure/function due to loss of phosphorylated, polar Threonine residue to a non-polar Alanine residue.



Family 3712
+ *GATA6* NM_005257.5: c.1595_1596del p.Pro532Hisfs*100
hcCHD Gene Screen | **ACMG-AMP:** Pathogenic (Ic): PVS1, PM2, PP3, BS2
PVS1: Heterozygous frameshift variants in *GATA6* leading to CHD have previously been reported (See **Supplementary Table S2**) (OMIM: 601656).
PM2: The variant is not present in population databases (ExAC, 1000 Genomes).
PP3: The variant is predicted to be severely damaging due to change in amino acid sequence, loss of stop-codon, and elongation of the gene product.
BS2: The variant is inherited by the proband from the asymptomatic father (Patient 3714).



Family 3766

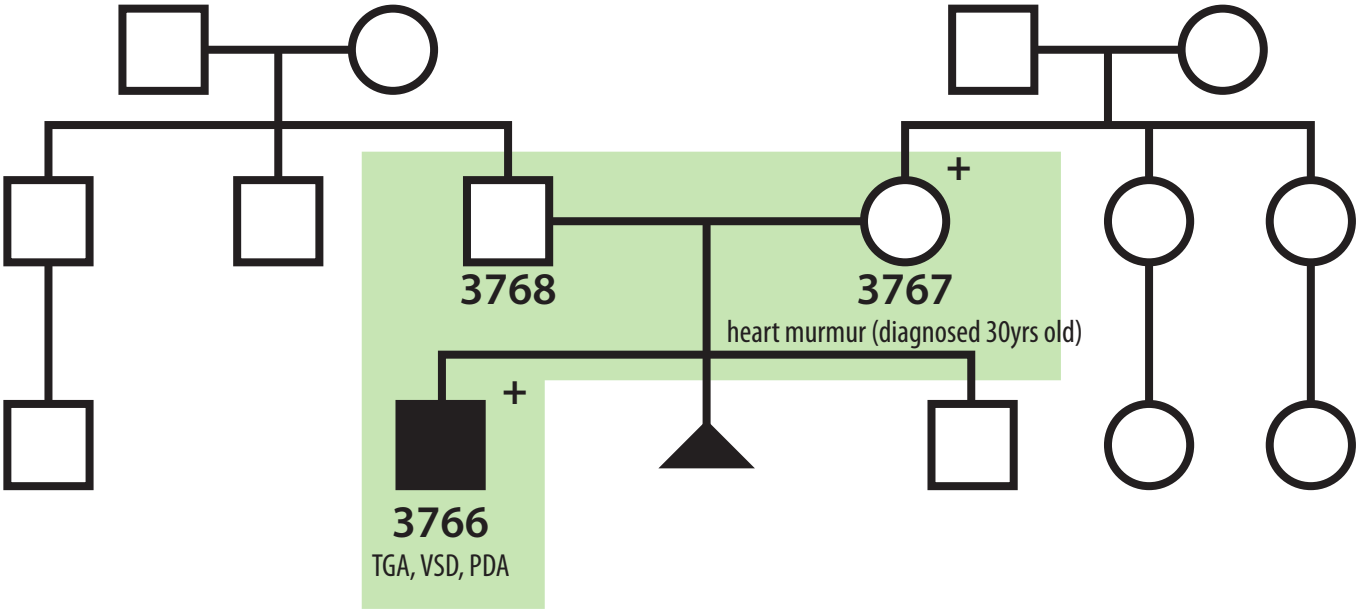
+ *NODAL* NM_018055.4: c.919C>T p.Arg307*

hcCHD Gene Screen | **ACMG-AMP:** Pathogenic (Ic): PVS1, PM2, PP3

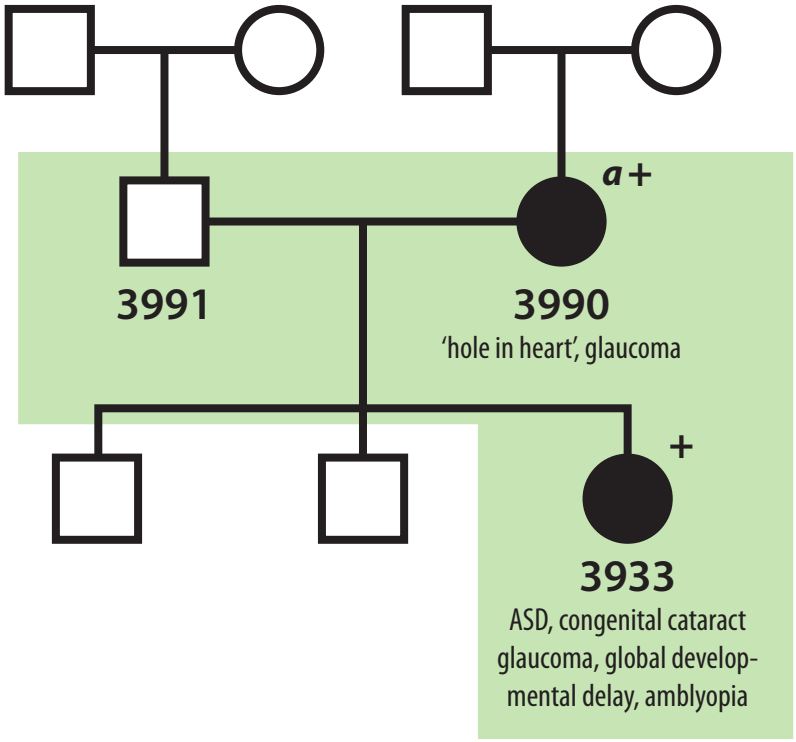
PVS1: Heterozygous LOF *NODAL* variants leading to CHD have been previously reported (See **Supplementary Table S2**) (OMIM: 601265).

PM2: The variant is not present in population databases (ExAC, 1000 Genomes).

PP3: The variant is predicted to be severely damaging due to truncation of the gene product leading to NMD. The pLI score of *NODAL* is 0.95.



Family 3933
+ *BCOR* NM_001123383.1: c. 2488_2489del p.Ser830Cysfs*6
[a] Gestational diabetes
hcCHD Gene Screen | **ACMG-AMP:** Pathogenic (Id): PVS1, PM2, PP1, PP3
PVS1: X-linked dominant LOF *BCOR* variants leading to syndromic CHD have been previously reported (see **Supplementary Table S2**) (OMIM: 300485).
PM2: The variant is not present in population databases (ExAC, 1000 Genomes).
PP1: Variant is inherited by the proband with CHD+ECA from the mother with CHD+ECA (Patient 3990).
PP3: The variant is predicted to be severely damaging due to truncation of the gene product leading to NMD. The pLI score of *BCOR* is 1.00.
NOTE: X-linked dominant LOF variants in *BCOR* cause syndromic CHD, Microphthalmia (OMIM: 300166). The CHD + ECA phenotypes of the proband and the affected mother are consistent with Microphthalmia.



Family 3953

+ *NF1* NM_000267.3: c.3560T>G p.Leu1187Arg

hcCHD Gene Screen | **ACMG-AMP:** Likely pathogenic (II): PS2, PM1, PM2, PP3, BP1

PS2: *De novo* missense variant in *NF1* found in the proband and absent in the parents.

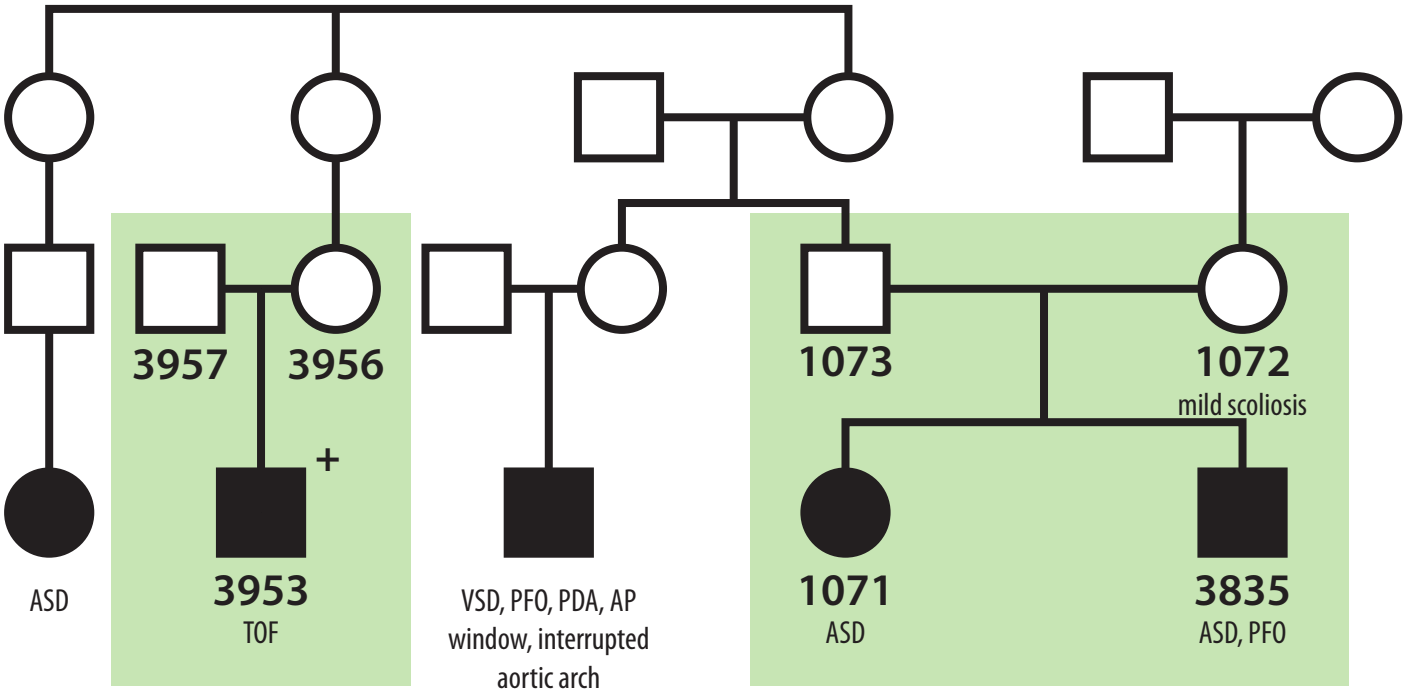
PM1: Pathogenic variants at amino acid positions 1182 (Val > Asp), 1188 (Gln > Pro) and 1189 (Gln*) have been reported to cause Neurofibromatosis (OMIM: 162200). Somatic variant at 1187 (Leu >Ile) has been reported in colorectal cancer (OMIM: 114500).

PM2: The variant is not present in population databases (ExAC, 1000 Genomes).

PP3: Variant is predicted to be damaging to NF1 protein structure/function due to replacement of conserved, small, non-polar Leucine residue to a large, basic Arginine residue.

BP1: Majority of reported pathogenic variants in *NF1* are gene/protein truncating variants.

NOTE: Pathogenic variants in *NF1* are associated with Neurofibromatosis (OMIM: 162200). Close monitoring of the proband may reveal further phenotypes associated with pathogenic gene-variant.



Family 150650086

+ *ACVR2B* NM_001106.3: c.1057G>T p.Gly353Trp

+ *DOCK6* NM_020812.3: c.3163G>A p.Val1055Met

[a] Gestational diabetes

hcCHD Gene Screen | **ACMG-AMP:** Likely pathogenic (V): PM1, PM2, PP2, PP3, BS2 and Likely pathogenic (V): PM2, PM3, PP2, PP3

PM1: Variant is found within the protein kinase domain of ACVR2B, which determines the catalytic function of the protein.

PM2: The variant is not present in population databases (ExAC, 1000 Genomes).

PP2: *ACVR2B* is intolerant to missense variation (RVIS = 12.88%). Missense variants in *ACVR2B* have previously been associated with CHD (See **Supplementary Table S2**) (OMIM: 602730).

PP3: Variant is predicted to be damaging to protein structure/function due to replacement of small Glycine residue with a large, aromatic Tryptophan residue.

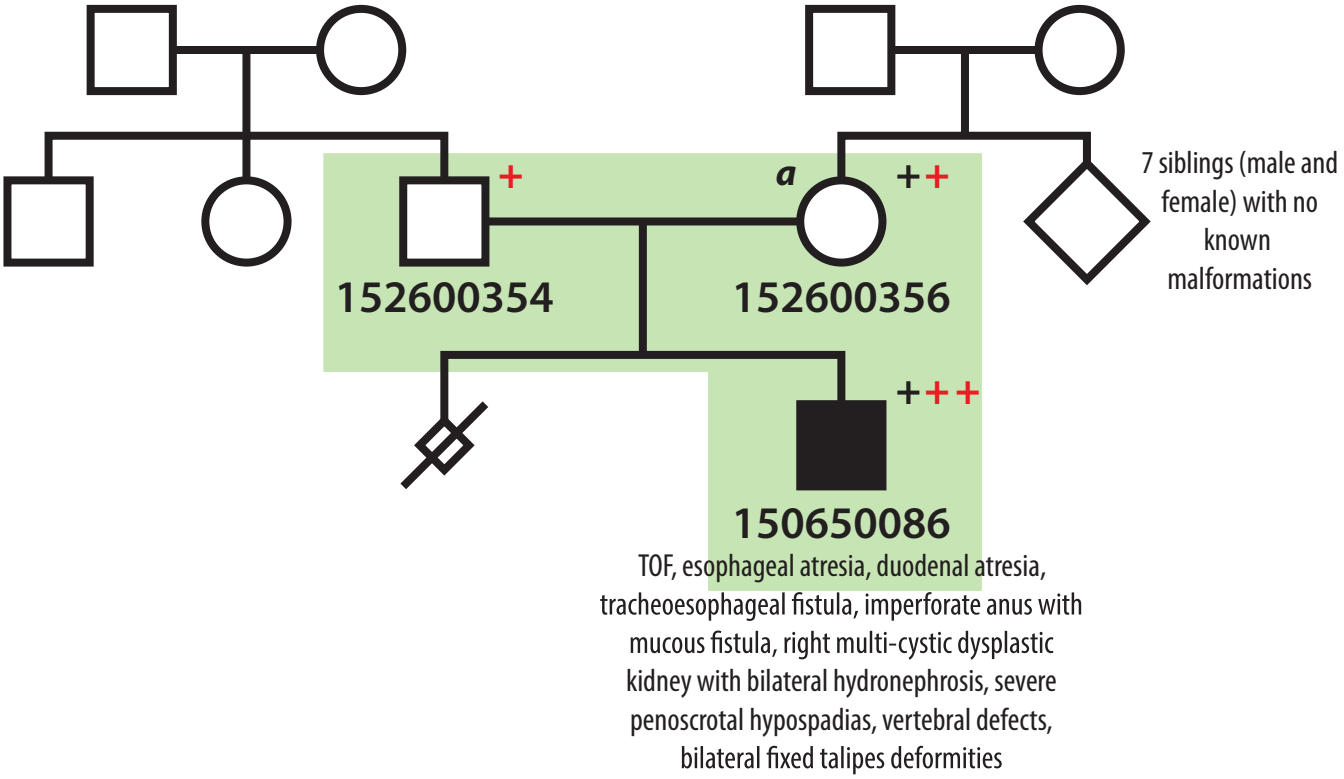
BS2: The variant is inherited by the proband from the asymptomatic mother (Patient 152600356).

PM2: Individuals heterozygous for the *DOCK6* variant are present in population databases (ExAC, 1000 Genomes), however there are no individuals homozygous for the variant.

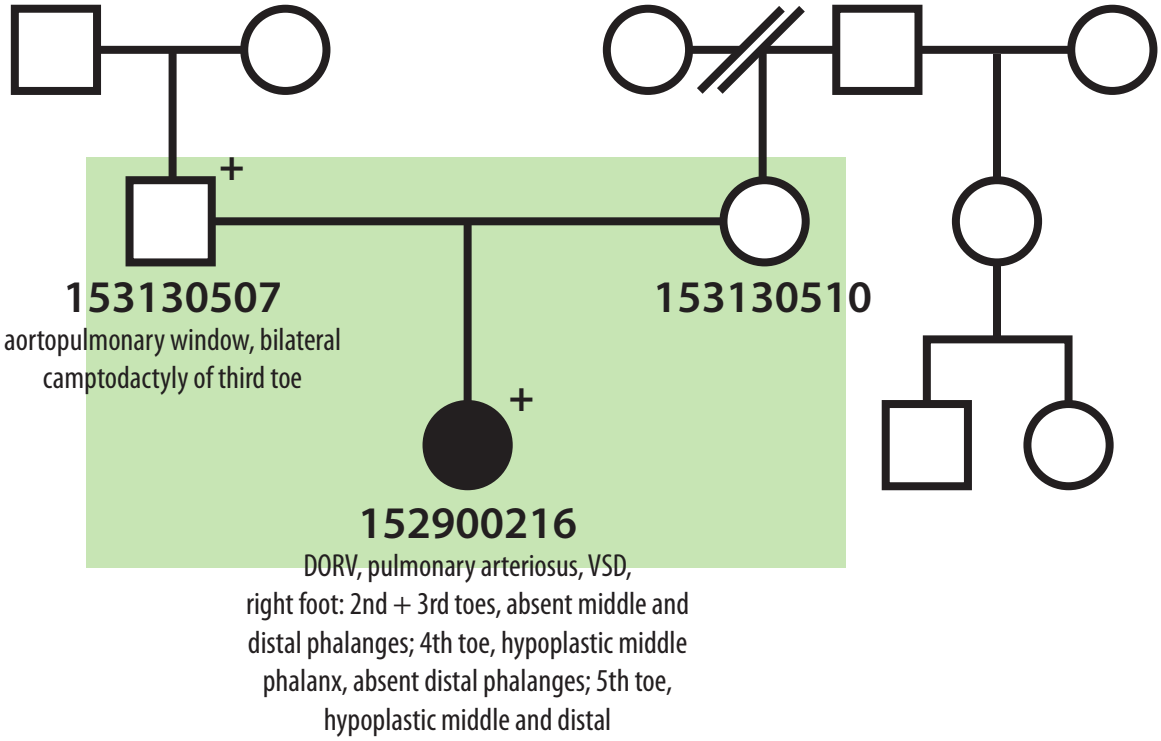
PM3: The proband is homozygous for the damaging variant.

PP2: *DOCK6* is intolerant to missense variation (RVIS = 9.42%). Homozygous missense *DOCK6* variants have been associated with syndromic CHD, Adams-Oliver syndrome 2 (See **Supplementary Table S2**) (OMIM: 614194).

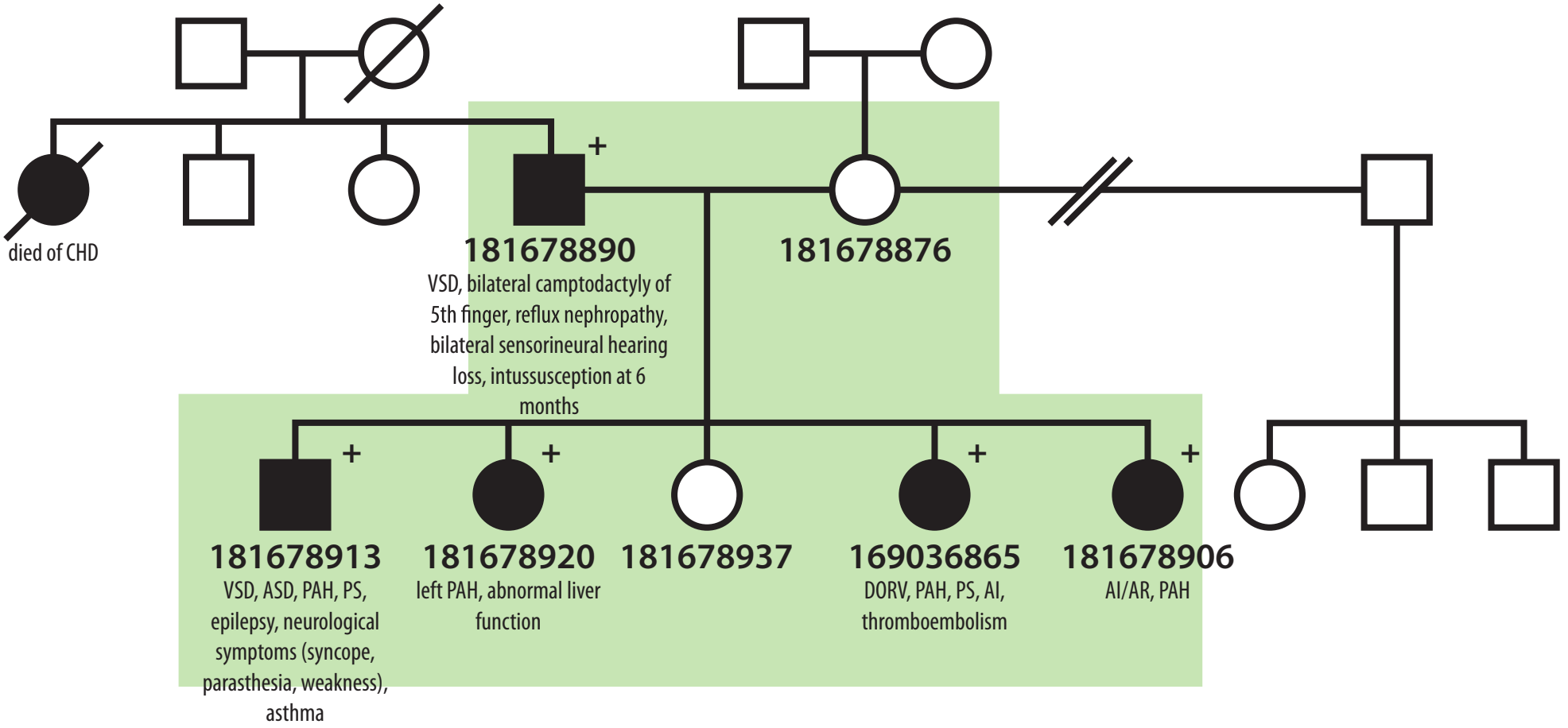
PP3: Variant is predicted to be damaging to protein structure/function due to replacement of a highly conserved Valine residue to a Methionine residue.



Family 152900216
+ *NOTCH1* NM_017617.4: c.4416C>G p.Cys1472Trp
hcCHD Gene Screen | **ACMG-AMP:** Likely pathogenic (V): PM1, PM2, PP1, PP2, PP3
PM1: Variant falls within Lin-12/Notch repeat (LNR) domain, which negatively regulates NOTCH receptor function.
PM2: The variant is not present in population databases (ExAC, 1000 Genomes).
PP1: Variant is inherited by the proband from the affected father (Patient 153130507).
PP2: *NOTCH1* is intolerant to missense variation (RVIS = 0.33%). Missense variants in *NOTCH1* have previously been associated with syndromic and isolated CHD (See **Supplementary Table S2**) (OMIM: 190198).
PP3: Variant is predicted to be damaging to NOTCH1 protein structure/function due to replacement of a crucial Cysteine residue that forms disulphide bonds with a large, aromatic Tryptophan residue.



Family 169036865

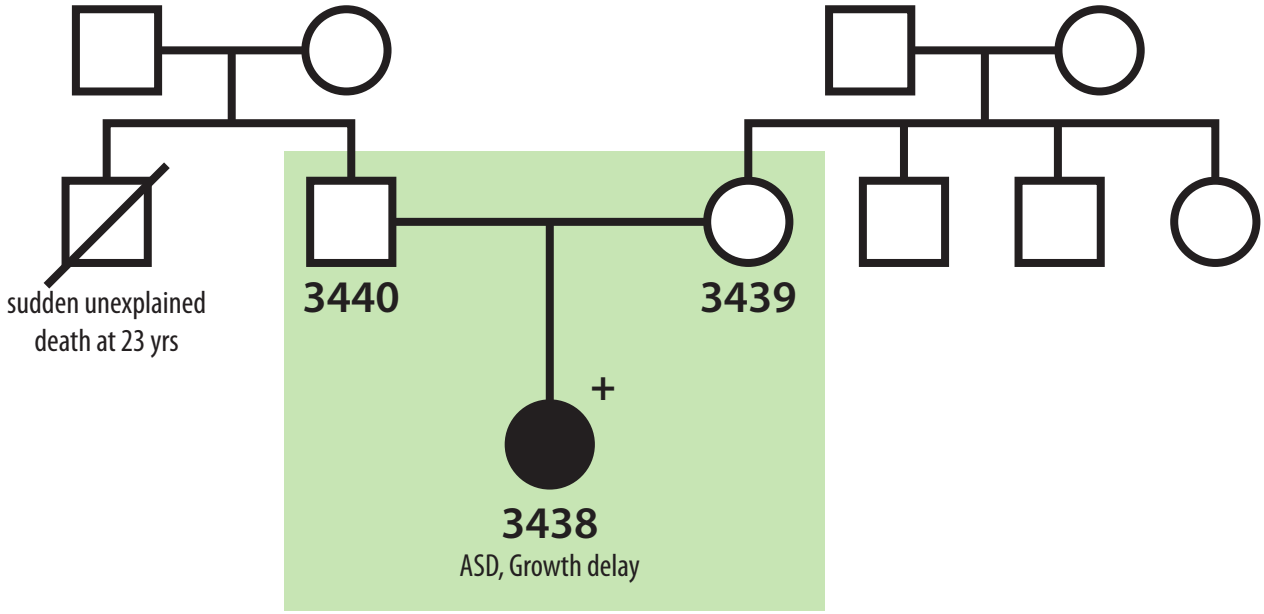


+ *JAG1* NM_000214.2: c.622G>C p.Gly208Arg
hcCHD Gene Screen | **ACMG-AMP:** Pathogenic (IIIb): PP1-S, PM1, PM2, PP3, PP4, BP1
PP1-S: Variant is inherited by the proband and siblings with CHD+ECA, from the father with CHD+ECA.
PM1: Variant is found within the Delta/Serrate/lag-2 (DSL) domain, which plays a role during ligand/receptor interactions. Variant is also immediately adjacent to the region of JAG1 that interacts with NOTCH1 (amino acids 199-207).
PM2: The variant is not present in population databases (ExAC, 1000 Genomes).
PP3: Variant is predicted to be damaging to JAG1 protein structure/function due to replacement of small Glycine residue with a large, basic Arginine residue.
PP4: The combination of CHD with the specific ECA phenotypes present in the affected individuals of the family is specific for Alagille syndrome caused by JAG1 mutations (OMIM: 601920).
BP1: Majority of reported pathogenic variants in *JAG1* are gene/protein truncating variants.
NOTE: The affected persons of the family display phenotypes associated with Alagille syndrome, caused by pathogenic *JAG1* variants. Close monitoring of the affected persons may reveal further phenotypes associated with the pathogenic gene-variant.

Family 3438

+ *HAND1* promoter (chr5:154478752 G>A)

Proband 3438 harbors a novel SNV ~450 bp upstream of the transcription start site of *HAND1*. This resides within the overlap of a CpG island, a DNase1 hypersensitivity site, and an H3K27me3 site, where transcription factor EZH2 is predicted to bind. As pathogenic variants in *HAND1* result in phenotypes similar to what we observe in our proband,[107-109] it is possible that this non-coding variant leads to aberrant transcriptional regulation, which contributes to the disease phenotype. However, further functional studies are required to prove this association.



Family 3400

+ *ACVR1* NM_001105.4: c.790+371G>C

Proband 3400 carried a unique intronic variant in *ACVR1* that overlaps a DNase1 hypersensitivity site. As pathogenic variants in *ACVR1* result in phenotypes similar to what we observe in our proband,[22,23] it is possible that this non-coding variant leads to aberrant transcriptional regulation, which contributes to the disease phenotype. However, further functional studies are required to prove this association.

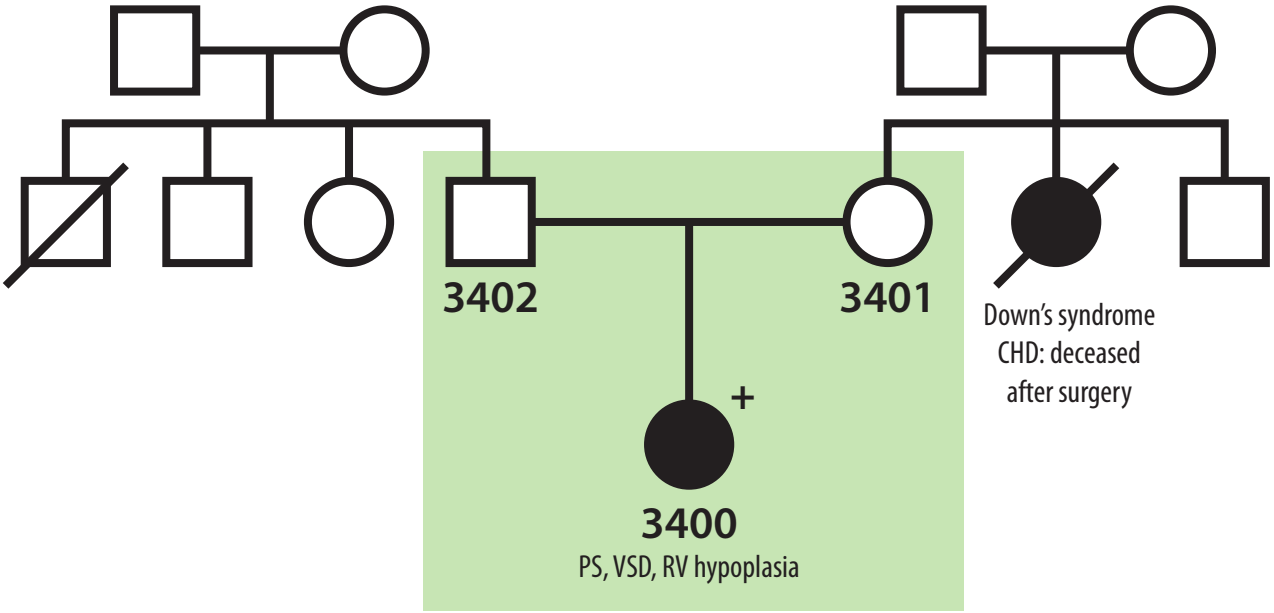
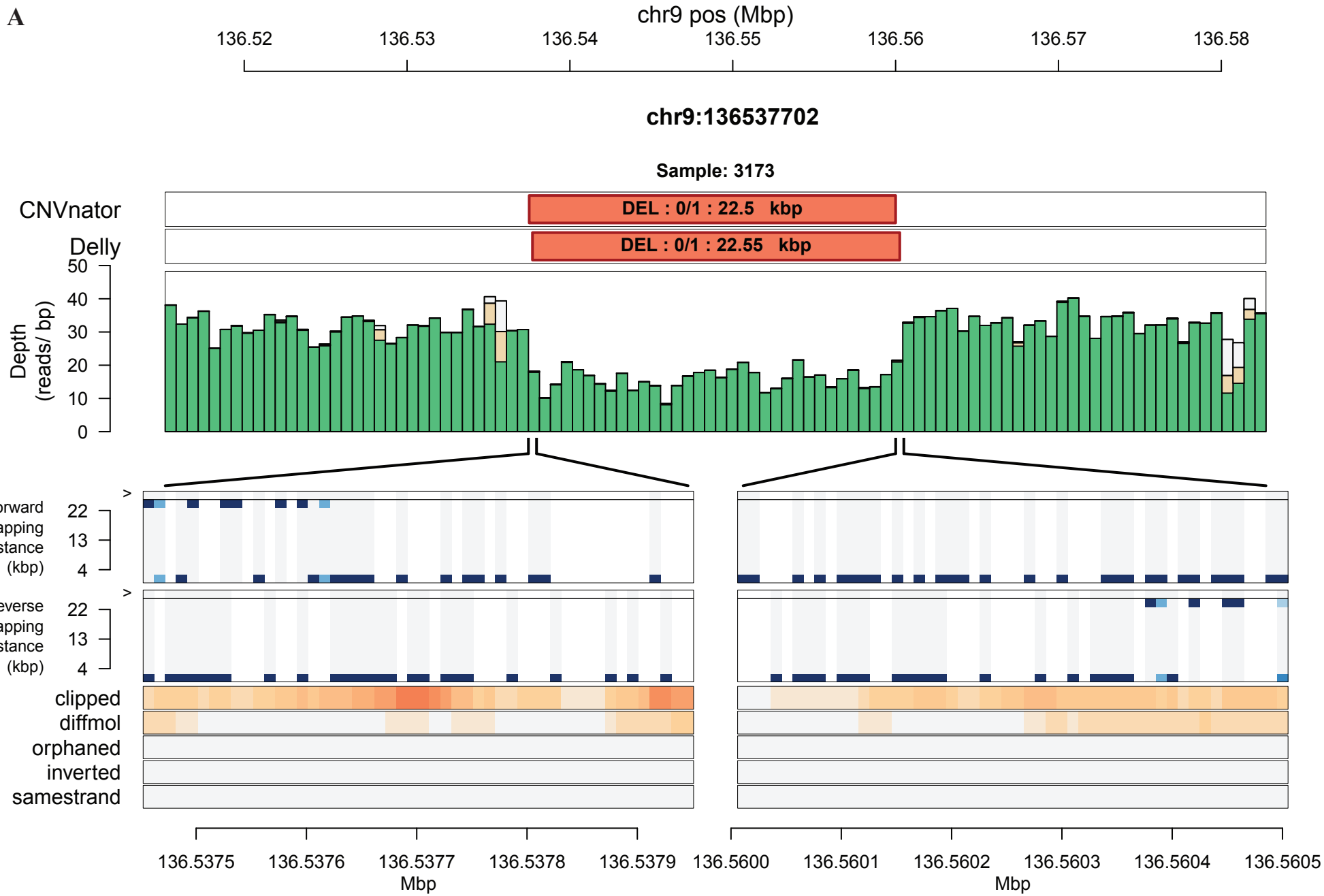


Figure S4. Pathogenic 22.55 kb *NOTCH1* deletion identified by the hcCHD gene screen. (A) SVPV output of pathogenic *NOTCH1* deletion in Family 3173, present in the proband (3173), affected sibling (3925), and the father (3921); (B) Sanger sequencing trace across the breakpoints of the 22.55 kb *NOTCH1* deletion in 3173 (proband), 3921 (father), and 3925 (affected sibling). Red and blue bars indicate sequencing 5' and 3' of the *NOTCH1* deletion breakpoints, respectively. (C) Agarose gel electrophoresis of amplified *NOTCH1* product across the *NOTCH1* deletion in Family 3173, confirming presence in 3173 and 3925, and potential mosaicism in 3921. Arrowhead indicates successful PCR product amplified across the *NOTCH1* deletion.


A
chr9 pos (Mbp)
136.52
136.53
136.54
136.55
136.56
136.57
136.58
chr9:136537702
Sample: 3173
CNVnator
DEL : 0/1 : 22.5 kbp
Delly
DEL : 0/1 : 22.55 kbp
Depth (reads/ bp)
50
40
30
20
10
0
forward mapping distance (kbp)
22
13
4
reverse mapping distance (kbp)
22
13
4
clipped
diffmol
orphaned
inverted
samestrand
136.5375
136.5376
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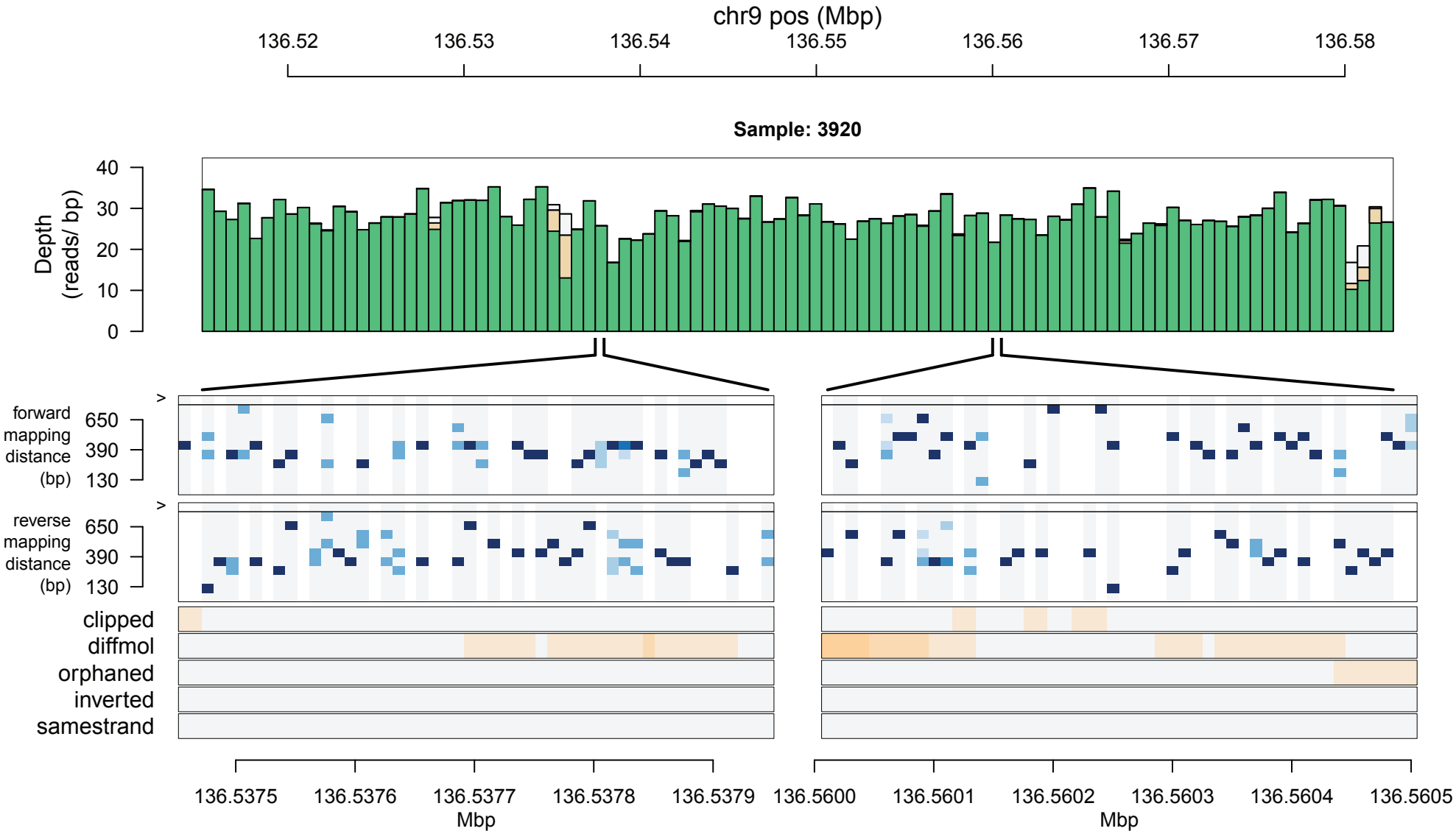
136.5602
136.5603
136.5604
136.5605
Mbp


chr9 pos (Mbp)
136.52
136.53
136.54
136.55
136.56
136.57
136.58
Sample: 3920
Depth (reads/ bp)
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30
20
10
0
forward mapping distance (bp)
650
390
130
reverse mapping distance (bp)
650
390
130
clipped
diffmol
orphaned
inverted
samestrand
136.5375
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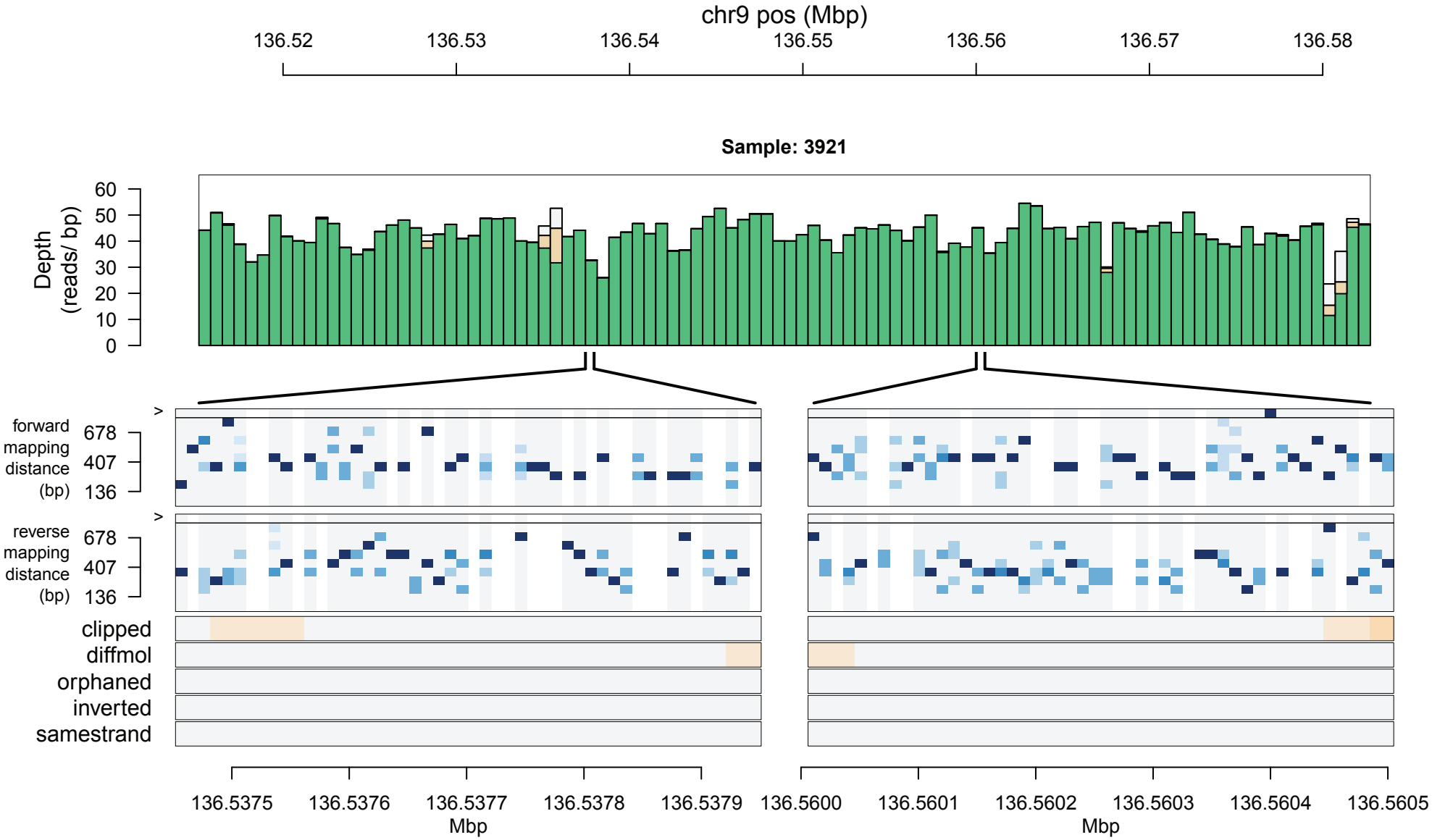
136.5603
136.5604
136.5605
Mbp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136.54
136.55
136.56
136.57
136.58
Sample: 3921
Depth (reads/ bp)
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40
30
20
10
0
forward mapping distance (bp)
678
407
136
reverse mapping distance (bp)
678
407
136
clipped
diffmol
orphaned
inverted
samestrand
136.5375
136.5376
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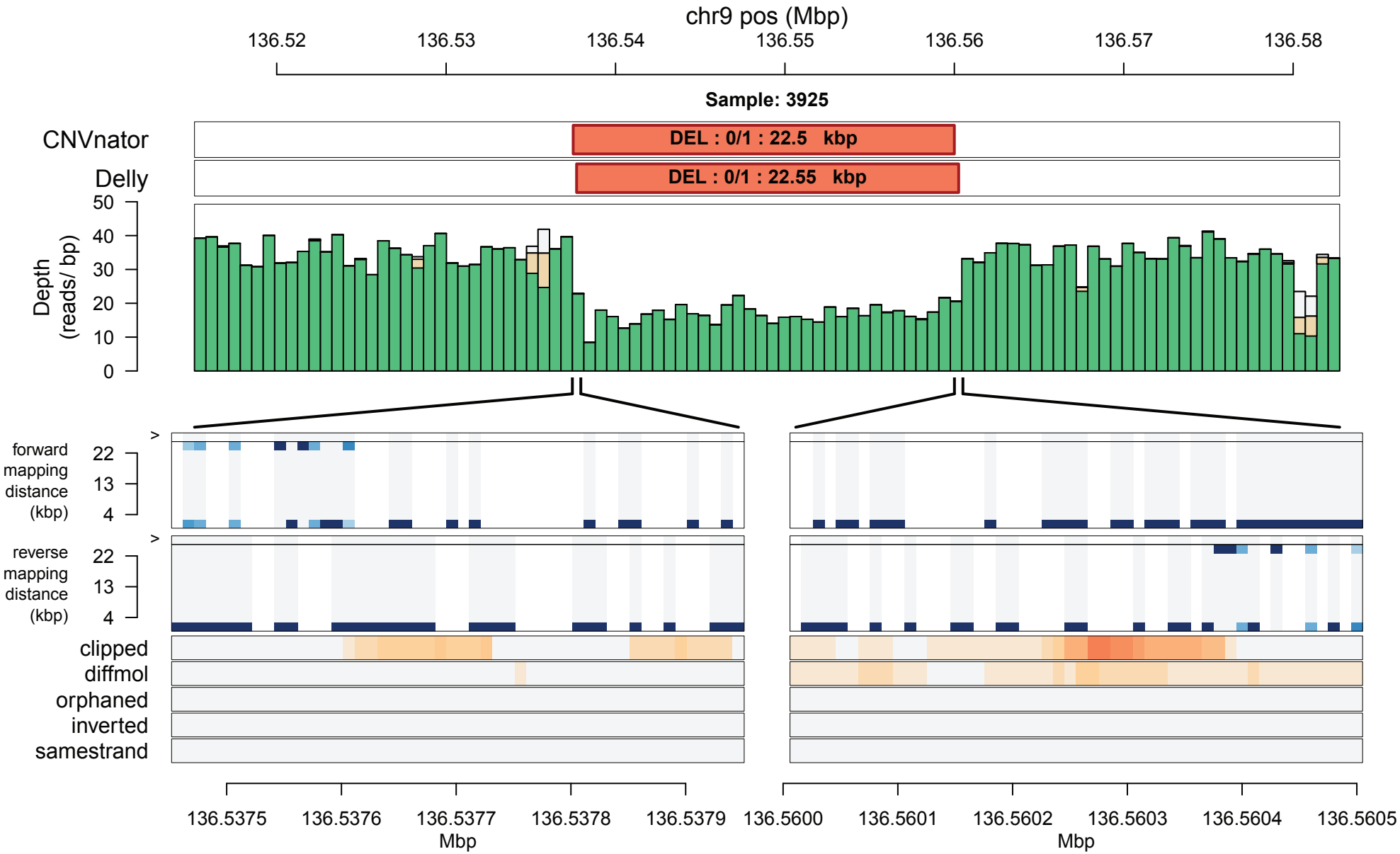
136.5602
136.5603
136.5604
136.5605
Mbp


chr9 pos (Mbp)
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136.53
136.54
136.55
136.56
136.57
136.58
Sample: 3925
CNVnator
DEL : 0/1 : 22.5 kbp
Delly
DEL : 0/1 : 22.55 kbp
Depth (reads/ bp)
50
40
30
20
10
0
forward mapping distance (kbp)
22
13
4
reverse mapping distance (kbp)
22
13
4
clipped
diffmol
orphaned
inverted
samestrand
136.5375
136.5376
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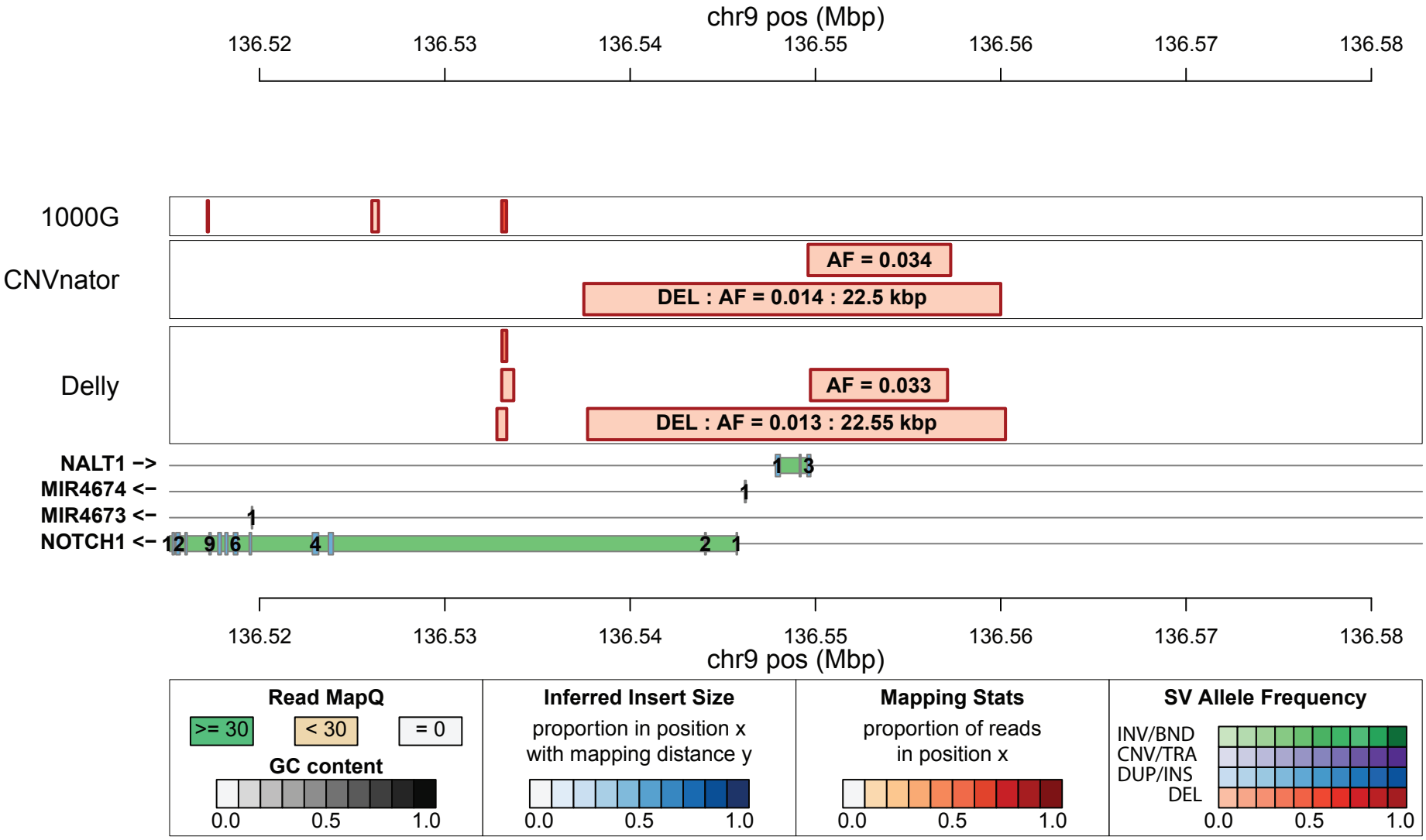
136.5603
136.5604
136.5605
Mbp



SVPV v1.01 Depth bin size: 676 Num depth bins: 100 Zoom bin size: 10 Num zoom bins: 50 Date: 2017−06−06

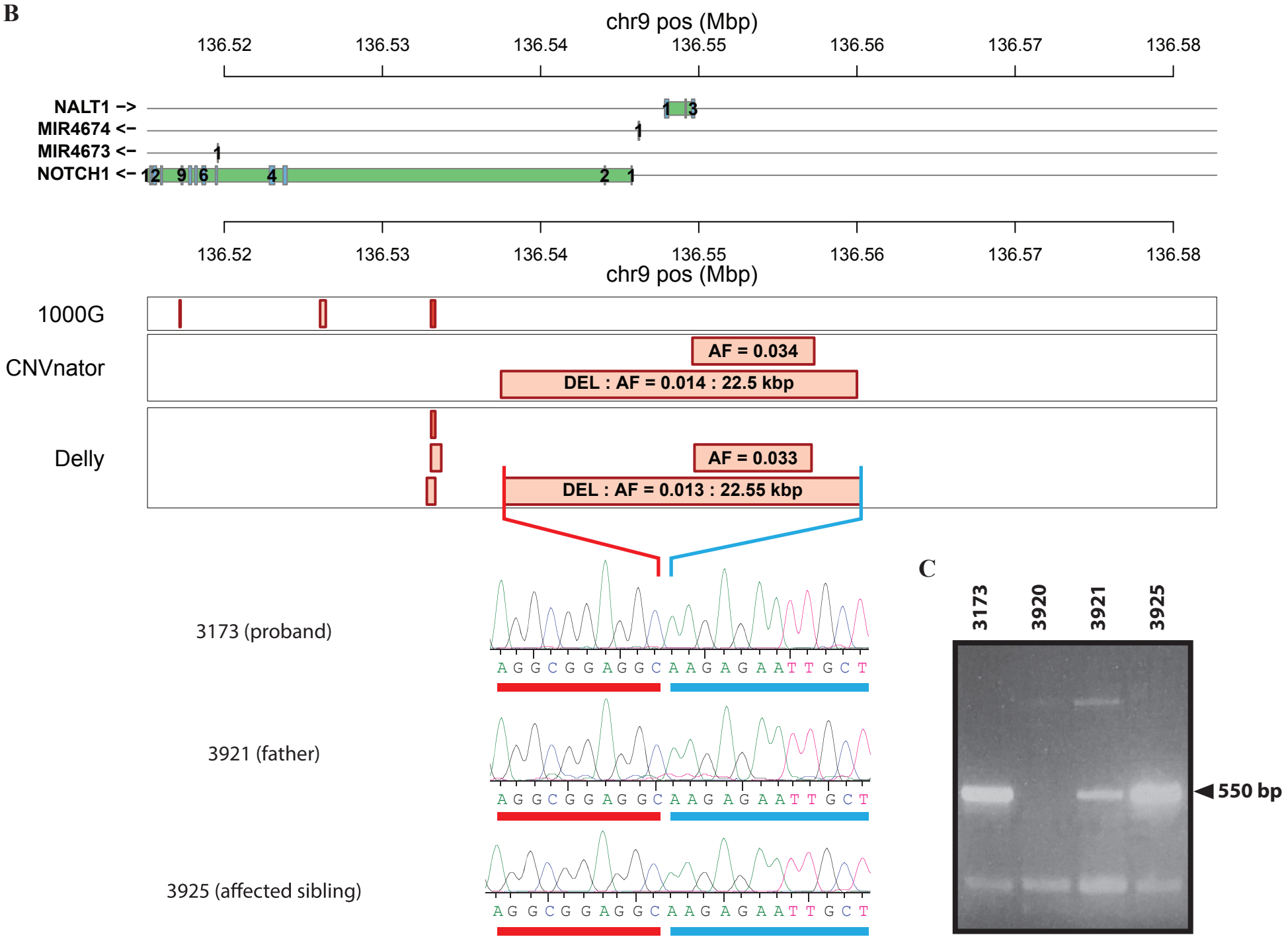

B
chr9 pos (Mbp)
136.52
136.53
136.54
136.55
136.56
136.57
136.58
NALT1 –>
MIR4674 <–
MIR4673 <–
NOTCH1 <–
1 3
1
1
12 9 6 4 2 1
1000G
CNVnator
AF = 0.034
DEL : AF = 0.014 : 22.5 kbp
Delly
AF = 0.033
DEL : AF = 0.013 : 22.55 kbp
3173 (proband)
3921 (father)
3925 (affected sibling)
A G G C G G A G G C A A G A G A A T T G C T
C
3173
3920
3921
3925
550 bp

Figure S5. Pathogenic ~7.5 Mb deletion in chr10 identified by comprehensive analysis. The large deletion, encompassing 75 unique genes, is inherited by the proband (1456) from the affected mother (1457).

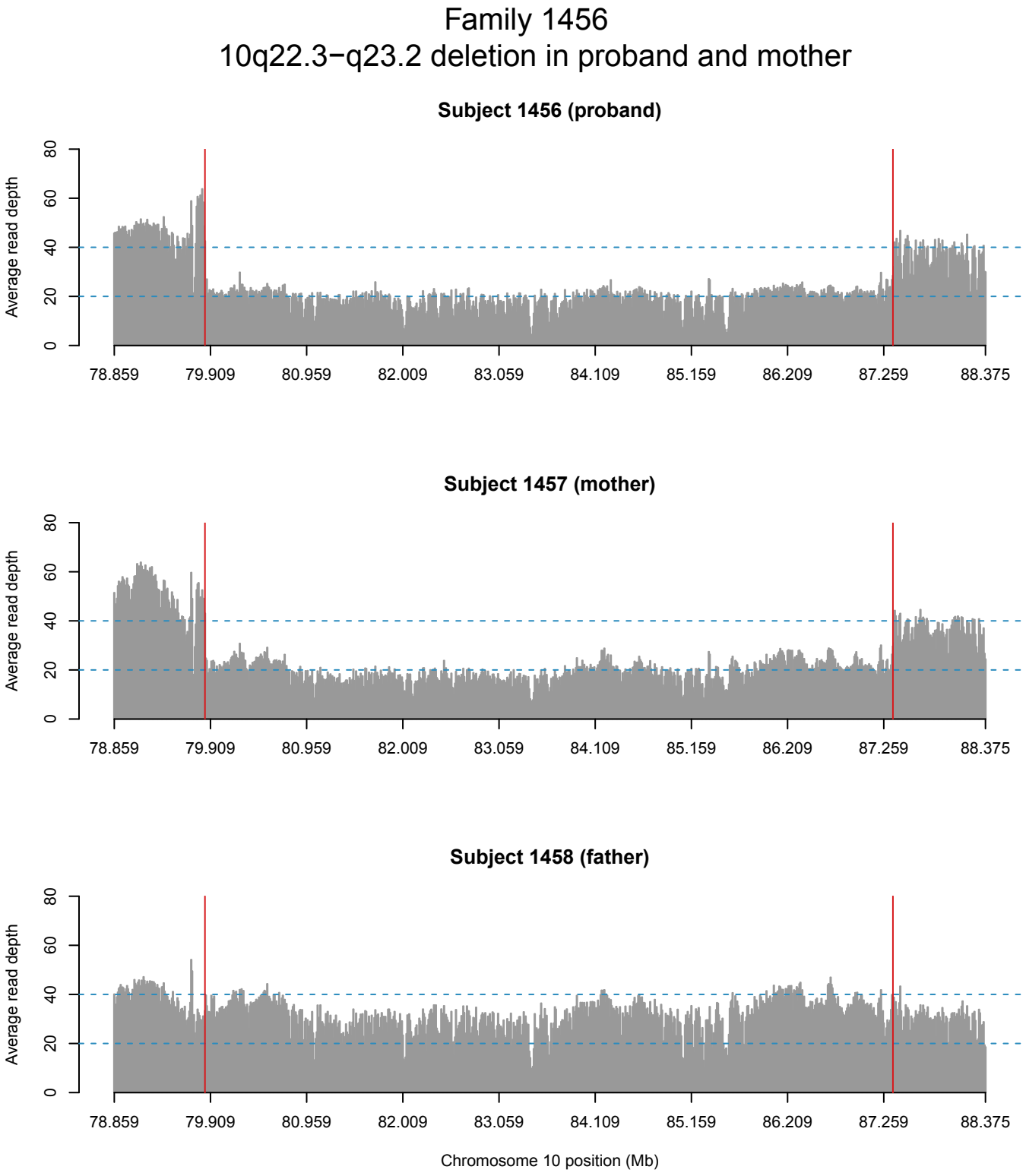
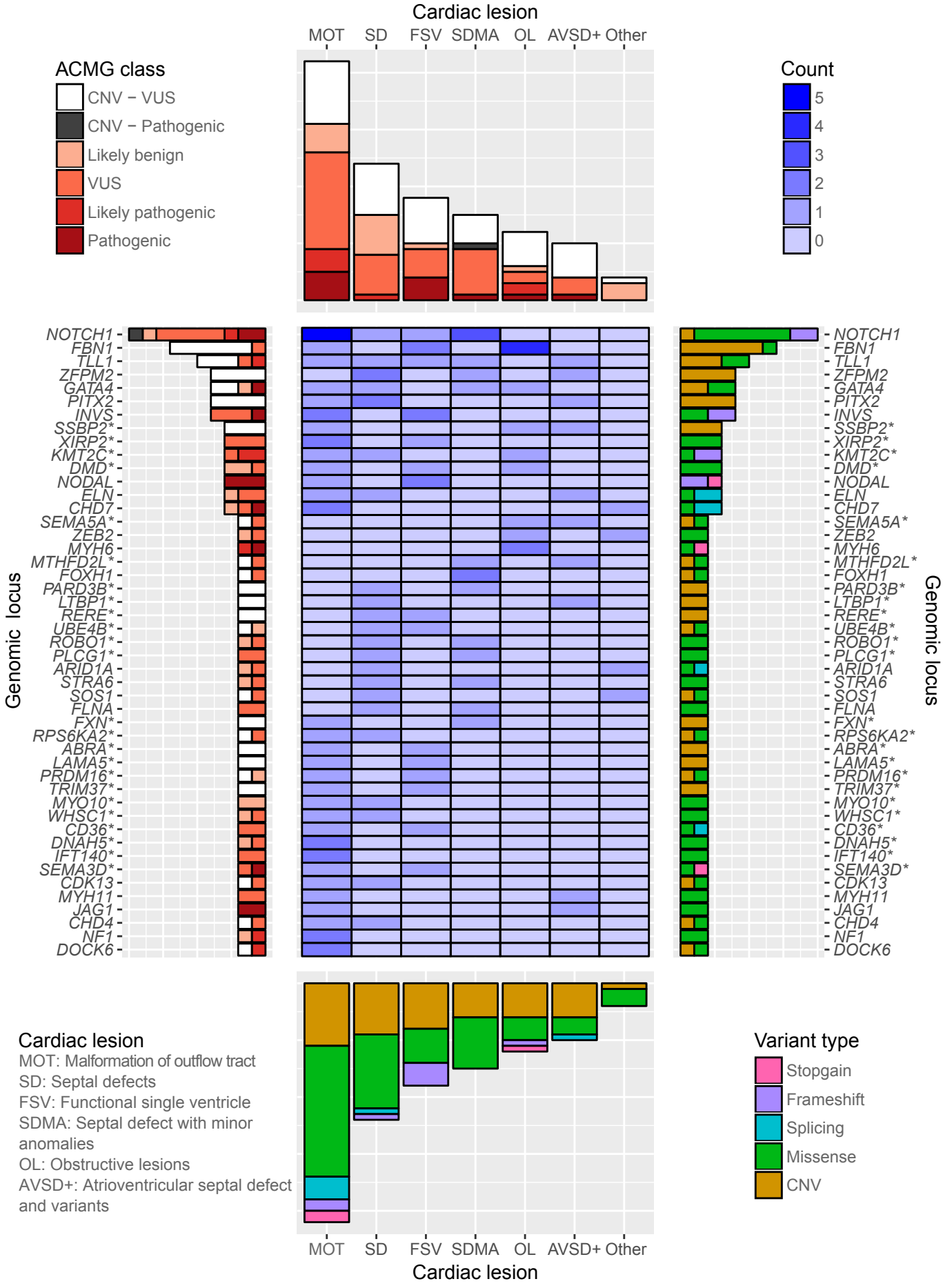


Figure S6. Visual representation of genomic loci with >1 variant identified by hcCHD screen and comprehensive analysis, showing frequency of rare variation per loci of interest and corresponding ACMG-AMP classification, with respect to cardiac lesion type. CNV: copy number variant; VUS: variant of uncertain significance. *Genes with variants identified by comprehensive analysis.



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