Supplementary Materials

De novo mutations in histone modifying genes in congenital heart disease

Congenital Heart Disease (CHD) Supplementary Materials Page #

Patient cohorts. Probands with or without parents were recruited from 9 centers in the United States and the United Kingdom into the Congenital Heart Disease Genetic Network Study of the Pediatric Cardiac Genomics Consortium (CHD Genes: NCT01196182)[7]. The protocol was approved by the Institutional Review Boards of Boston Children's Hospital, Brigham and Women's Hospital, Great Ormond St. Hospital, Children's Hospital of Los Angeles, Children's Hospital of Philadelphia, Columbia University Medical Center, Icahn School of Medicine and Mt. Sinai, Rochester School of Medicine and Dentistry, Steven and Alexandra Cohen Children's Medical Center of New York, and Yale School of Medicine. Written informed consent was obtained from each participating subject or their parent/guardian. Probands were selected for severe congenital heart disease (excluding isolated VSDs, ASDs, PDAs or PSs), availability of both parents, and absence of any CHD in first-degree relatives. Cardiac diagnoses were obtained from review of echocardiogram, catheterization and operative reports; extracardiac findings were extracted from medical records. Controls were from 264 previously studied quartets that included one offspring with autism, an unaffected sibling and unaffected parents, all recruited with written informed consent by the Simons Foundation Autism Research Initiative[28]. Parents and their unaffected sibling from this cohort were analyzed in the current study.

Exome sequencing. Trios were sequenced at the Yale Center for Genome Analysis following the same protocol. Genomic DNA from venous blood was captured with the NimbleGen v2.0 exome capture reagent (Roche) and sequenced (Illumina HiSeq 2000, 75 base paired-end reads). Reads were mapped to the reference genome using Eland. SNV and indel calls were assigned quality scores (QS) using SAMtools[8] and annotated for novelty using dbSNP, build 135, 1000 genomes, May 2011 release and the Yale Exome Database, for impact on encoded proteins, and conservation of variant position.

Identification and confirmation of *de novo* mutations. Heterozygous SNVs and indels in the proband that showed QS ≥ 60 and 600, respectively, and rare

non-reference calls in both parents were selected. Read plots of all putative indels were visually inspected in trio members to eliminate false calls. A Bayesian algorithm was used to assist *de novo* mutation calls. Elements included probability of the proband being heterozygous at the test position; probability that parents are homozygous for the reference allele, given frequency of reference and non-reference reads and probability of heterozygosity in offspring; probability that a variant is *de novo* given its population frequency. Resulting QSs scaled from 0 to 100. Their correlation with bona fide *de novo* mutations was determined by Sanger sequencing of PCR amplicons harboring 181 putative mutations distributed across the QS spectrum. Additionally, all six *de novo* indels with QS > 50 in the HHE gene set were tested and confirmed by Sanger sequencing.

RNA sequencing and analysis. Hearts from e14.5 mouse embryos (strain 129SvEv) were isolated, rinsed, and immersed in RNALater. Left and right atria, left ventricle (with interventricular septum, aortic and mitral valves), and right ventricle (with pulmonary and tricuspid valves) were dissected. Chamber-specific RNAs were extracted and pooled from 5 embryos, selected with oligo-dT, copied into double stranded DNA, and ligated to adaptors. 150-250 bp fragments were isolated after acrylamide gel electrophoresis, amplified and sequenced (Illumina HiSeq2000), with > 40 million paired-end 50 base reads per library as previously described[29]. Reads were aligned to the mouse genome (mm9)[30], and reads per gene per million mapped reads (rpm) was determined. The average of rpm of each gene from each chamber was used as the measure of heart expression. RNA from atria, ventricle and truncus/outflow tract at e9.5 was prepared, sequenced and analyzed by an analogous approach. RNA sequencing of control human adult tissues- lung, liver, heart and brain- from the Illumina Human Body Map (http://www.ebi.ac.uk/arrayexpress/browse.html?keywords=E-MTAB-513) was similarly performed and analyzed as reads per gene per million reads per kb of transcript.

Principal component analysis. The EIGENSTRAT program was used to compare SNP genotypes of probands and individuals of known ancestry in HapMap3 (http://hapmap.ncbi.nlm.nih.gov/). SNPs with MAF >5% without significant linkage disequilibrium with other SNPs were analyzed. The results of analysis correctly distinguished ancestry groups in HapMap3 samples; ancestries of CHD subjects were assigned accordingly.

Statistical analyses. The significance of mutation frequency differences between groups was tested with two-tailed binomial exact tests; two-tailed Fisher exact tests assessed differences in numbers of patients with one or more *de novo* mutations; tests among 3 groups was by Chi-square analysis. Gene expression at e14.5 of genes mutated in cases and controls was compared by Wilcoxon signed-rank test. Correlation of mutation rate and parental age was tested by Pearson's correlation. The expected number of genes with more than one *de novo* mutation was determined by Monte Carlo simulation (10^8 iterations) specifying the total number of protein-altering mutations and 21,000 genes of observed coding length. Analogous approaches were used to determine probabilities of any gene having $\geq$ 2 damaging mutations, $\geq$ 1 damaging and $\geq$ 1 mutation at a conserved position, and $\geq$ 13 genes mutated in both CHD and autism. The fit to the Poisson distribution of the observed numbers of *de novo* mutations per subject was assessed by Chi-square test.

Overrepresentation of *de novo* mutations in the H3K4me pathway and the presence of significant enrichment of other gene pathways was tested via Gene Ontology (GO) analysis, using a modified Fisher's exact test with Bonferroni correction as implemented in DAVID (http://david.abcc.ncifcrf.gov/). Input was all genes with protein-altering *de novo* mutations in CHD or control subjects, and all genes sequenced. The H3K4me gene set was: *CHD8, MLL3, SETD7, WHSC1L1, CDC73, WHSC1, SETD1A, MLL2, KDM5A, MLL4, MLL5, UBE2B, ASH1L, SETD1B, MLL, LEO1, PAF1, KDM5C, CTR9, PRDM9, MEN1, CHD7, RNF20, KDM1A, RNF40, SMYD3, KDM6A, KDM5B, USP44, WDR5*. The

expected number of mutations in the H3K4me set was calculated from the fraction of the exome coding region attributable to this gene set and the total number of *de novo* mutations.

Estimating number of genes in which *de novo* mutations contribute to CHD. We addressed this question using the 'unseen species problem[9]. We infer that the number of probands with non-synonymous mutations in the HHE set (81) minus the expected number (44; calculated from the number observed in controls), represents the number of subjects in whom *de novo* mutations confer CHD risk (37; 10.0% of probands). The number of genes with > 1 protein-altering *de novo* mutation (six) minus the most likely number expected by chance (three) represents risk-associated genes with more than 1 mutation (three). The number of risk-associated genes (C) is estimated as follows:

$C = c/u + g^2 \times d \times (1-u)/u$

c = number of observed risk-associated genes (34)

c_1 = number of genes mutated once (31)

d = total number of risk-associated mutations (37)

g = variation in effect size of individual *de novo* mutations (assumed to be 1, which minimizes underestimation of set size)

$u = 1 - c_1/d$ (probability that newly added mutation hits a previously mutated gene)

C = 401

From 95% confidence intervals of the number of risk-associated events, the 95% confidence interval for number of risk genes is calculated as 197-837.

Table S1. Description of cohorts subjected to sequencing

Demographics	Cases (362)[†]	Controls (264)
Male: Female	220:142	163:161
Age at enrollment *(mean ± SD)*	7.8 ± 9.6	13.7 ± 4.6
Maternal age at birth of proband *(mean ± SD)*	31.2 ± 5.5	29.9±4.8
Paternal age at birth of proband *(mean ± SD)*	31.9 ± 6.1	32.5±6
African-American	32 (8.8%)	9 (3.8%)
European	305 (84.3%)	187 (79.2%)
Asian	31 (8.6%)	13 (5.5%)
Native American	4 (1.1%)	0 (0%)
Hispanic	50 (13.8%)	27 (11.5%)
Cardiac Lesions in Probands[§]		
Conotruncal Defects (CTD): 153 probands		
TOF	63 (17%)	
TOF/PA	9 (2.5%)	
TOF/PA-MAPCA	12 (3%)	
D-TGA	47 (13%)	
DORV	18 (5%)	
TA	7 (2%)	
AoAA	3 (1%)	
Left Ventricular Obstruction (LVO): 132 probands		
HLHS	60 (17%)	
CoA	36 (10%)	
BAV	40 (11%)	
AS (not BAV)	13 (4%)	
MS	1 (0.3%)	
Heterotaxy (HTX): 70 probands		
RAI	8 (2%)	
LAI	16 (4%)	
DEX	19 (5%)	
CAVC	26 (7%)	
DORV	17 (5%)	
L-Loop	12 (3%)	
L-TGA	18 (5%)	
Other Cardiac Diagnosis: 6 probands		
TAPVR	1 (0.3%)	
CAVC	1 (0.3%)	
DILV	3 (1%)	
other	1 (0.3%)	
Extracardiac abnormalities	81 probands (22%)	

[†]Self-reported ethinicity: 15 probands self-idenitfied as multi-ethnitic, hence ethnicities total >100%

[§]Numerous probands have more than one cardiac feature; for this reason, sum of sub-phenotypic classification does not equal 100%.

Abbreviations: TOF-tetralogy of Fallot; TOF/PA-tetralogy of Fallot with pulmonary atresia; TOF/PA-MAPCA-tetralogy of Fallot with pulmonary atresia and multiple aortico-pulmonary collaterals; D-TGA-D-transposition of the great arteries; DORV-double outlet right ventricle; AoAA-aortic arch anomaly; TA-truncus arteriosus; HLHS-hypoplastic left heart syndrome; CoA-coarctation of the aorta; BAV-bicuspid aortic valve; AS-aortic stenosis; MS-mitral stenosis; RAI-right atrial isomerism; LAI-left atrial isomerism; Dex-dextrocardia; CAVC-complete atrioventricular canal; L-Loop-L-looped ventricles; L-TGA-L-Transposition of the great arteries; TAPVR-total anomalous pulmonary venous return; DILV-double-inlet left ventricle.

Table S2: Sequencing QA/QC for CHD and control cohorts

Category	Cases (1086 samples)	Controls (792 samples)
Read length (bp)	74	74
# of reads per sample (M)	102.6 ± 28	120 ± 45.5
Median coverage at each targeted base (X)	91.2 ± 23.9	99.3 ± 36.6
Mean coverage at each targeted base (X)	107 ± 27.7	117.1 ± 42
% of all bases that map to human genome	91.3% ± 0.9	91.3% ± 2
% of all bases that map to target	69.1% ± 4.8	67.4% ± 9.3
% of targeted bases read at least 8x	96.0% ± 1	95.6% ± 1.6
% of targeted bases read at least 20x	91.4% ± 3.4	90.5% ± 6.8
Mean error rate	0.5% ± 0.1	0.6% ± 0.2
% PCR duplicates	5.53% ± 2.39	5.39% ± 2.79

Table S3. Relationship of *de novo* Bayesian quality score vs. confirmation by Sanger sequencing

Bayesian Quality Score	# of putative *de novo* mutations	# of attempted confirmations	# validated by Sanger sequencing	% of all *de novo* mutations
≥50	324	88	88 (100%)	88.5%
40-50	9	9	7 (77%)	1.9%
30-40	11	11	8 (72%)	2.2%
20-30	34	31	13 (42)	3.9%
10-20	64	15	3 (20%)	3.5%
5-10	77	5	0 (0%	0%

Table S4. All *de novo* mutations with Bayesian QS≥50

											Proband			Father		Mother		
ID	Primary Cardiac Classification	Gene	Accession IDs	Mutation	Amino Acid Change	dbSNP	Chr	Position (hg19)	Base change	Mean Heart Expression	Variant Quality Score	Reference Coverage	Nonreference Coverage	Reference Coverage	Nonreference Coverage	Reference Coverage	Nonreference Coverage	Bayesian Quality Score
Damaging mutations in HHE genes in CHD Cases																		
1-00534	CTD	CHD7	NM_017780	Nonsense	Q1599X	Novel	8	61754556	C>T	124.85	228	53	68	128	0	121	0	100
1-02279	LVOTO	AHNAK	NM_024060	Nonsense	G254X	Novel	11	62301129	C>A	175.75	228	27	23	88	0	96	0	100
1-02144	CTD	LIG1	NM_000234	Nonsense	Y765X	Novel	19	48624517	G>T	118.41	228	33	27	34	0	58	0	100
1-01360	LVOTO	NCKAP1	NM_013436	Nonsense	E1057X	Novel	2	183792856	C>A	103.97	228	70	76	175	0	128	4	100
1-01965	LVOTO	KDM5B	NM_006618	Splice	1 bp beyond exon 12	Novel	1	202722032	C>T	68.06	228	47	39	112	0	76	0	100
1-00075	HTX	RNF20	NM_019592	Nonsense	Q83X	Novel	9	104302602	C>T	58.23	228	66	47	129	0	114	0	100
1-01028	CTD	GTPBP4	NM_012341	Nonsense	K332X	Novel	10	1051839	A>T	67.83	228	132	138	235	0	263	0	100
1-00596	LVOTO	MLL2	NM_003482	Frameshift	S1722	Novel	12	49438005	-A	216.12	*Indel-Pass*	126	39	125	0	120	0	100
1-00455	HTX	NAA15	NM_057175	Frameshift	D335	Novel	4	140272757	-AAAG	213.74	*Indel-Pass*	68	17	60	0	72	0	100
1-00577	LVOTO	OS9	NM_001017957	Frameshift	T158	Novel	12	58089814	-A	67.99	*Indel-Pass*	78	39	80	0	84	0	100
1-02227	LVOTO	FTSJ3	NM_017647	Frameshift	786/847	Novel	17	61897350	-GA/+C	59.29	*Indel-Pass*	62	69	100	0	87	0	100
1-00577	LVOTO	CUL3	NM_001257197	Frameshift	I144	Novel	2	225379434	-TAAT	57.06	*Indel-Pass*	163	49	170	0	190	0	100
1-00448	CTD	NF1	NM_001128147	Splice	4 bp beyond exon 6	Novel	17	29508509	-A	54.87	*Indel-Pass*	139	48	110	0	145	0	100
1-00141	CTD	NAA15	NM_057175	Nonsense	S761X	Novel	4	140306112	C>A	213.74	196	16	12	30	0	41	0	92
1-01907	CTD	SERPINH1	NM_001207014	Nonsense	R415X	Novel	11	75283114	C>T	847.33	137	5	15	32	0	21	0	55
Mutations at conserved position in HHE genes in CHD Cases																		
1-00148	LVOTO	LAMC1	NM_002293	Missense	G170E	Novel	1	183072553	G>A	510.65	169	22	15	54	0	63	0	100
1-00522	LVOTO	TLN1	NM_006289	Missense	L684V	Novel	9	35717729	G>C	321.59	228	53	49	96	0	132	0	100
1-01664	HTX	OBSCN	NM_001098623	Missense	F5295S	Novel	1	228521311	T>C	298.14	228	43	52	125	1	96	0	100
1-00750	LVOTO	HUWE1	NM_031407	Missense	R3219C	Novel	X	53576300	G>A	259.79	228	38	56	48	0	146	0	100
1-00522	LVOTO	LAMA5	NM_005560	Missense	C1625Y	Novel	20	60902649	C>T	208.82	228	39	39	73	0	96	0	100
1-01637	CTD	KIAA0664	NM_015229	Missense	H823Y	Novel	17	2598535	G>A	182.48	228	70	61	186	0	135	0	100
1-01907	CTD	UBE2B	NM_003337	Missense	R8T	Novel	5	133707309	G>C	146.23	228	26	21	101	0	63	0	100
1-00808	CTD	RAVER1	NM_133452	Missense	H93R	Novel	19	10441195	T>C	130.73	228	124	82	125	0	132	0	100
1-00479	LVOTO	GANAB	NM_198334	Missense	N171S	Novel	11	62402341	T>C	129.52	228	81	83	123	0	69	0	100
1-02394	LVOTO	DST	NM_015548	Missense	K2653I	Novel	6	56417763	T>A	121.78	189	46	67	104	0	58	1	100
1-02189	LVOTO	EIF3H	NM_003756	Missense	H109R	Novel	8	117671183	T>C	118.19	228	66	60	116	0	147	0	100
1-00161	HTX	SBNO1	NM_001167856	Missense	T1339M	Novel	12	123782548	G>A	108.82	228	84	114	239	0	240	0	100
1-00753	LVOTO	FYCO1	NM_024513	Missense	E1286K	Novel	3	45996829	C>T	96.55	228	78	80	124	0	60	0	100
1-02153	CTD	RNF44	NM_014901	Missense	R421Q	Novel	5	175956066	C>T	88.67	228	15	17	43	0	54	0	100
1-00197	LVOTO	BCL9	NM_004326	Missense	M1395K	Novel	1	147096663	T>A	87.75	228	12	16	47	0	71	0	100
1-00325	LVOTO	TSHZ1	NM_005786	Missense	Q288R	Novel	18	72998360	A>G	82.03	228	30	24	65	0	49	0	100
1-01026	LVOTO	RUFY2	NM_017987	Missense	P621L	Novel	10	70105589	G>A	77.03	228	90	105	216	0	221	0	100
1-00541	HTX	EFHD2	NM_024329	Missense	A230V	Novel	1	15755186	C>T	76.90	228	73	86	143	0	99	0	100
1-00230	LVOTO	KDM5A	NM_001042603	Missense	R1508W	Novel	12	402269	G>A	69.89	228	53	36	90	0	108	0	100
1-02923	LVOTO	PHIP	NM_017934	Missense	S674C	Novel	6	79707311	G>C	67.31	193	34	30	91	0	106	0	100
1-02264	LVOTO	C11orf9	NM_001127392	Missense	F387S	Novel	11	61541483	T>C	66.17	228	22	18	48	1	87	0	100
1-01783	LVOTO	FADS3	NM_021727	Missense	G412S	Novel	11	61643375	C>T	65.71	228	22	19	45	0	84	0	100
1-01138	LVOTO	USP34	NM_014709	Missense	L432P	Novel	2	61577785	A>G	64.48	228	56	69	154	0	115	1	100
1-02133	CTD	CPSF1	NM_013291	Missense	N29K	Novel	8	145634456	G>C	63.04	228	41	29	80	0	82	0	100
1-02437	HTX	LZTR1	NM_006767	Missense	G248R	Novel	22	21344765	G>A	60.72	228	35	27	107	0	99	0	100
1-01365	CTD	GTPBP1	NM_004286	Missense	E291K	Novel	22	39117783	G>A	56.59	228	45	55	129	0	150	0	100
1-00934	LVOTO	FREM2	NM_207361	Missense	D2206N	Novel	13	39425119	G>A	51.87	228	68	69	132	1	157	0	100
1-01341	CTD	KIAA0196	NM_014846	Missense	V167D	Novel	8	126093921	A>T	48.07	228	95	72	138	0	134	0	100
1-00587	LVOTO	SMAD4	NM_005359	Missense	I500V	Novel	18	48604676	A>G	45.78	228	47	56	82	0	139	0	100
1-00491	LVOTO	KPNA1	NM_002264	Missense	P350S	Novel	3	122156091	G>A	45.35	228	101	110	199	0	144	0	100
1-00116	CTD	NUB1	NM_016118	Missense	D310H	Novel	7	151064080	G>C	45.07	228	95	86	149	0	176	0	100
1-01036	CTD	BCL2L11	NM_001204113	Missense	P59S	Novel	2	111881677	C>T	41.14	228	51	48	104	0	119	0	100
1-03300	LVOTO	DHX38	NM_014003	Missense	G332D	Novel	16	72133665	G>A	40.76	228	34	28	84	0	103	0	100
1-02093	CTD	LOXL2	NM_002318	Missense	R327Q	Novel	8	23186065	C>T	110.95	190	27	17	27	0	25	0	91
1-01828	CTD	DAPK3	NM_001348	Missense	P193L	Novel	19	3963893	G>A	54.61	228	34	38	50	0	63	0	90
1-01984	LVOTO	PCDHGA2	NM_018915	Missense	L172F	Novel	5	140719052	C>T	182.84	228	28	29	33	0	43	0	89
1-03151	LVOTO	SUPT5H	NM_001130825	Missense	E451D	Novel	19	39960029	G>C	132.72	228	22	23	33	0	25	0	72
1-02788	CTD	MINK1	NM_153827	Missense	R299C	Novel	17	4789867	C>T	77.77	150	16	16	23	0	27	0	66
1-01696	CTD	GLT25D1	NM_024656	Missense	R471W	Novel	19	17691524	C>T	73.64	228	35	33	47	0	57	0	55
Mutations at nonconserved position in HHE genes in CHD Cases																		
1-00638	CTD	FBN2	NM_001999	Missense	D2191N	Novel	5	127624885	C>T	263.94	228	127	111	325	0	312	0	100
1-00258	CTD	PFKM	NM_001166686	Missense	A522G	Novel	12	48535104	C>G	218.03	228	88	81	208	0	151	0	100
1-01817	CTD	MAPK8IP3	NM_001040439	Missense	P852R	Novel	16	1816090	C>G	134.18	228	16	16	51	0	45	0	100
1-01432	CTD	LAMB2	NM_002292	Missense	R1661W	Novel	3	49159236	G>A	129.86	176	26	11	50	0	43	0	100
1-01997	CTD	PRPF4B	NM_003913	Missense	E14Q	Novel	6	4021699	G>C	129.14	228	26	21	58	0	71	0	100
1-00222	LVOTO	NUCB1	NM_006184	Missense	R189C	Novel	19	49416352	C>T	123.31	228	115	70	258	0	164	0	100
1-00381	LVOTO	STAB1	NM_015136	Missense	A1102V	Novel	3	52547767	C>T	122.21	228	71	47	89	0	128	0	100
1-02121	CTD	DST	NM_015548	Missense	G2936D	Novel	6	56401671	C>T	121.78	228	104	64	229	0	180	0	100
1-01913	Other	RAB10	NM_016131	Missense	N112S	Novel	2	26350020	A>G	118.86	225	23	21	59	0	56	0	100
1-01933	LVOTO	CPD	NM_001304	Missense	P425R	Novel	17	28748818	C>G	98.86	228	108	69	169	2	142	0	100
1-02141	CTD	LRPPRC	NM_133259	Missense	D486N	Novel	2	44190759	C>T	93.03	228	100	98	278	0	169	0	100
1-00186	CTD	DSG2	NM_001943	Missense	L499Q	Novel	18	29116237	T>A	89.11	228	79	69	157	0	90	0	100
1-01788	LVOTO	MYEF2	NM_016132	Missense	I264V	Novel	15	48451047	T>C	69.68	228	87	106	191	0	201	0	100
1-00305	CTD	GRIP2	NM_001080423	Missense	T954M	Novel	3	14547126	G>A	51.51	228	38	24	70	0	53	0	100
1-02888	LVOTO	AP3B1	NM_003664	Missense	E771K	Novel	5	77406117	C>T	63.56	228	81	74	108	0	116	0	100
1-00323	HTX	NUP62	NM_012346	Missense	Q70R	Novel	19	50412856	T>C	62.55	228	55	64	99	0	93	0	100
1-00465	LVOTO	TOMM40L	NM_032174	Missense	S171I	Novel	1	161198003	G>T	62.29	228	69	67	96	0	73	0	100
1-00174	CTD	ZNF326	NM_182976	Missense	E338A	Novel	1	90482962	A>C	54.95	228	137	100	125	0	110	0	100
1-00934	LVOTO	TRIM41	NM_201627	Missense	P167S	Novel	5	180651498	C>T	53.73	228	28	20	56	0	62	0	100
1-00824	HTX	MAP2K7	NM_145185	Missense	V409I	Novel	19	7977281	G>A	52.58	228	26	25	41	0	43	0	100
1-01151	CTD	SUV420H1	NM_017635	Missense	R143C	Novel	11	67942601	G>A	44.49	228	110	105	295	0	411	0	100
1-02254	LVOTO	ELMO2	NM_133171	Missense	N332S	Novel	20	45003945	T>C	44.31	228	87	71	201	0	111	0	100
1-00344	CTD	SERINC4	NM_001033517	Missense	R65H	Novel	15	44089057	C>T	43.79	228	68	73	112	0	126	0	100
1-01941	CTD	NOP2	NM_006170	Missense	I351V	Novel	12	6671053	T>C	42.04	228	55	44	129	0	210	0	100
1-03190	CTD	OBSCN	NM_001098623	Missense	T4421M	Novel	1	228503797	C>T	298.14	228	15	30	21	0	45	0	87
1-00373	LVOTO	DGCR2	NM_005137	Missense	A64T	Novel	22	19076893	C>T	77.33	228	22	17	28	0	47	0	72
1-01505	LVOTO	TTN	NM_001256850	Missense	T4852N	Novel	2	179598610	G>T	2093.26	154	12	10	35	0	23	0	71
Silent mutations in HHE genes in CHD cases																		
1-03234	HTX	NDUFA13	NM_015965	Silent	I50I	Novel	19	19637046	A>C	428.99	228	23	27	84	0	139	0	100
1-00650	HTX	RYR2	NM_001035	Silent	T1464T	Novel	1	237756892	A>C	343.20	228	46	29	105	0	90	0	100
1-01816	LVOTO	ARHGDIA	NM_001185078	Silent	T132T	Novel	17	79827068	C>T	289.36	228	32	34	77	0	46	0	100
1-01637	CTD	PSMB1	NM_002793	Silent	T5T	Novel	6	170862316	T>C	243.58	194	23	11	94	0	66	0	100
1-01696	CTD	PKP4	NM_003628	Silent	S812S	Novel	2	159519816	G>A	173.79	228	67	68	97	0	119	0	100
M003-10	HTX	SARS	NM_006513	Silent	P384P	Novel	1	109779065	G>A	137.97	228	57	72	153	0	132	0	100
1-01505	LVOTO	AKAP8	NM_005858	Silent	Y53Y	Novel	19	15484809	G>A	121.48	228	57	31	98	0	75	0	100
1-00465	LVOTO	CAND1	NM_018448	Silent	L180L	Novel	12	67691235	A>G	101.74	228	129	108	122	0	118	0	100
1-00425	LVOTO	ANLN	NM_018685	Silent	T232T	Novel	7	36445998	C>T	97.65	228	65	61	198	0	152	0	100
M004-11	HTX	TAX1BP1	NM_001206901	Silent	G325G	Novel	7	27833977	G>A	93.81	228	229	218	322	0	494	0	100

1-00344	CTD	UBR5	NM_015902	Silent	L291L	Novel	8	103357639	G>A	92.92	228	145	55	220	0	149	0	100
1-01816	LVOTO	SPARCL1	NM_001128310	Silent	Q448Q	Novel	4	88411978	T>C	83.47	228	90	85	210	0	153	0	100
1-00738	CTD	WWC2	NM_024949	Silent	T157T	Novel	4	184130127	T>C	63.65	228	40	46	111	0	149	0	100
1-00534	CTD	FAM111A	NM_001142521	Silent	E219E	Novel	11	58919798	A>G	54.71	228	46	52	131	0	115	0	100
1-02708	LVOTO	FOXM1	NM_021953	Silent	P451P	Novel	12	2968698	A>G	52.16	228	74	66	124	0	160	0	100
1-01365	CTD	C20orf152	NM_080834	Silent	G297G	Novel	20	34582995	G>T	45.23	228	110	91	226	0	207	2	100
1-02093	CTD	LIFR	NM_002310	Silent	Q527Q	Novel	5	38502758	T>C	42.39	228	69	62	142	0	121	0	100
1-00305	CTD	SHANK3	NM_001080420	Silent	C290C	Novel	22	51117841	T>C	65.68	111	28	9	55	1	38	1	91
1-01341	CTD	NISCH	NM_007184	Silent	H989H	Novel	3	52522475	C>T	490.14	228	15	14	24	0	34	0	77
1-02786	OTHER	CCNL1	NM_020307	Silent	A19A	Novel	3	156877827	G>A	40.50	199	13	14	32	0	28	0	76
1-01933	LVOTO	FBLN2	NM_001998	Silent	P312P	Novel	3	13612791	A>C	46.48	130	20	10	18	0	18	0	55
Damaging mutations in LHE genes in CHD Cases																		
1-00604	CTD	SLC5A2	NM_003041	Nonsense	W172X	Novel	16	31497537	G>A	3.43	228	20	23	100	0	78	0	100
1-02282	LVOTO	DNAH10	NM_207437	Nonsense	W1406X	Novel	12	124317687	G>A	0.29	228	27	31	65	0	65	0	100
1-00323	HTX	CATSPERG	NM_021185	Nonsense	Y752X	Novel	19	38853114	C>G	0.24	228	48	26	47	0	43	0	100
1-02020	HTX	SMAD2	NM_001135937	Splice	1 bp beyond exon 6	Novel	18	45374845	C>T	38.29	228	38	35	114	2	96	0	100
1-01451	HTX	MED20	NM_004275	Splice	2 bp beyond exon 2	Novel	6	41884521	A>G	25.19	168	118	33	153	0	158	0	100
1-03300	LVOTO	NTM	NM_001048209	Frameshift	204/344	Novel	11	132177668	-A	14.02	*Indel-Pass*	25	70	95	0	80	0	100
1-00185	LVOTO	NAT8L	NM_178557	Frameshift	S217	Novel	4	2065595	+A	9.34	*Indel-Pass*	47	20	35	0	37	0	100
1-00141	CTD	GREB1L	NM_001142966	Nonsense	W1373X	Novel	18	19085419	G>A	3.96	228	94	87	159	0	151	0	100
1-00393	CTD	PLCZ1	NM_033123	Frameshift	Y603	Novel	12	18836193	+AAAC	0.00	*Indel-Pass*	321	91	250	0	190	0	100
1-01664	HTX	IQCB1	NM_001023570	Nonsense	Q51X	Novel	3	121547429	G>A	9.73	163	35	14	49	0	14	0	57
Mutations at conserved position in LHE genes in CHD Cases																		
1-00381	LVOTO	COL4A3BP	NM_031361	Missense	G131D	Novel	5	74722260	C>T	39.13	228	76	57	122	0	111	0	100
1-00788	CTD	MARCH5	NM_017824	Missense	E28K	Novel	10	94070938	G>A	38.87	228	67	47	143	0	162	0	100
1-02621	HTX	SMAD2	NM_001135937	Missense	W244C	Novel	18	45375021	C>G	38.29	228	45	39	134	0	42	0	100
1-01053	CTD	FAM135A	NM_001105531	Missense	R1138H	Novel	6	71245998	G>A	37.73	228	156	113	205	0	190	0	100
1-00753	LVOTO	KIAA1737	NM_033426	Missense	P132L	Novel	14	77579856	C>T	36.17	228	65	58	91	0	36	0	100
1-00373	LVOTO	PAPSS1	NM_005443	Missense	T399S	Novel	4	108574688	G>C	33.63	228	89	78	178	0	181	0	100
1-01943	CTD	PES1	NM_014303	Missense	P409T	Novel	22	30975867	G>T	33.10	228	12	22	80	0	88	0	100
1-01995	CTD	TM2D2	NM_031940	Missense	T74A	Novel	8	38851146	T>C	27.69	228	40	23	81	0	76	0	100
1-01412	LVOTO	ODZ4	NM_001098816	Missense	R1444K	Novel	11	78413327	C>T	22.75	228	60	61	95	0	115	2	100
1-02955	CTD	MAK16	NM_032509	Missense	R122L	Novel	8	33346630	G>T	21.55	228	101	83	265	1	365	0	100
1-00998	CTD	INTS6	NM_012141	Missense	T86R	Novel	13	52025243	G>C	20.84	228	109	83	251	0	246	0	100
1-00750	LVOTO	SSH2	NM_033389	Missense	V108L	Novel	17	28011657	C>A	20.74	228	70	64	149	0	189	0	100
1-02955	CTD	XRCC5	NM_021141	Missense	K238Q	Novel	2	216990668	A>C	20.47	228	58	52	166	0	226	0	100
1-01411	LVOTO	EPDR1	NM_001242946	Missense	T209M	Novel	7	37989949	C>T	15.67	228	57	59	78	0	76	0	100
1-01119	CTD	NAA16	NM_024561	Missense	R70C	Novel	13	41893010	C>T	12.47	228	142	121	234	0	219	0	100
1-01312	CTD	SESTD1	NM_178123	Missense	R24Q	Novel	2	180047900	C>T	11.25	228	77	101	191	0	195	0	100
1-02461	CTD	DTNA	NM_032975	Missense	P295S	Novel	18	32400761	C>T	10.09	228	56	49	97	0	133	0	100
1-01175	HTX	ITGA7	NM_001144997	Missense	R279W	Novel	12	56092245	G>A	7.79	228	53	40	91	0	94	0	100
1-00096	CTD	PIK3CD	NM_005026	Missense	L347V	Novel	1	9778770	C>G	4.38	210	40	19	107	0	117	0	100
1-02364	CTD	NR6A1	NM_033334	Missense	C120R	Novel	9	127316634	A>G	3.40	228	44	62	145	0	198	0	100
1-02126	CTD	BICD1	NM_001714	Missense	D760E	Novel	12	32490460	T>A	3.39	228	22	29	70	0	76	0	100
1-03171	CTD	ALPL	NM_001177520	Missense	A102T	Novel	1	21890596	G>A	3.10	215	34	19	40	0	87	0	100
1-02107	LVOTO	RDH5	NM_002905	Missense	R280S	Novel	12	56118210	C>A	2.42	228	21	28	59	0	62	0	100
1-00734	LVOTO	MYO16	NM_015011	Missense	R1164H	Novel	13	109777481	G>A	1.45	228	28	24	68	0	59	0	100
1-02956	HTX	HYDIN	NM_032821	Missense	I2216N	Novel	16	70977734	A>T	0.98	188	54	19	66	0	51	0	100
1-00938	LVOTO	FGFR4	NM_002011	Missense	D297N	Novel	5	176519483	G>A	0.55	228	34	18	42	0	86	0	100
1-01179	CTD	TDRD12	NM_001110822	Missense	A155E	Novel	19	33239405	C>A	0.20	228	60	58	92	0	171	0	100
1-00323	HTX	IL2RB	NM_000878	Missense	R170Q	Novel	22	37533655	C>T	0.16	228	25	34	35	0	35	0	100
1-02527	CTD	GRM8	NM_001127323	Missense	N778S	Novel	7	126173103	T>C	0.12	228	65	68	269	0	150	0	100
1-01179	CTD	C11orf41	NM_012194	Missense	S303C	Novel	11	33564908	C>G	0.02	228	77	75	138	0	207	0	100
1-02144	CTD	KNDC1	NM_152643	Missense	T81M	Novel	10	134981024	C>T	0.02	228	18	16	30	0	44	0	100
1-02023	LVOTO	SIGLEC5	NM_003830	Missense	C269Y	Novel	19	52131278	C>T	0.02	228	15	24	84	0	67	0	100
1-01536	CTD	C1orf94	NM_032884	Missense	R222Q	Novel	1	34666598	G>A	0.00	228	36	32	121	0	58	0	100
1-00619	LVOTO	KLF2	NM_016270	Missense	C334Y	Novel	19	16437775	G>A	21.00	104	17	8	29	0	47	0	86
1-02515	HTX	KCNH6	NM_030779	Missense	T274M	Novel	17	61611392	C>T	0.38	228	28	25	47	0	37	0	83
1-03173	CTD	IGFN1	NM_001164586	Missense	I1864M	Novel	1	201179613	A>G	0.06	109	40	17	26	1	24	0	82
1-00281	HTX	SPATA2	NM_006038	Missense	D203V	Novel	20	48523111	T>A	23.64	194	21	14	27	0	29	0	64
Mutations at nonconserved position in LHE genes in CHD Cases																		
1-00853	CTD	WDR5	NM_017588	Missense	K7Q	Novel	9	137005018	A>C	39.47	228	35	25	75	0	62	0	100
1-01538	HTX	MPI	NM_002435	Missense	A38V	Novel	15	75182964	C>T	39.04	228	36	39	99	0	84	0	100
1-02013	LVOTO	TFIP11	NM_012143	Missense	M432T	Novel	22	26895104	A>G	36.10	228	51	45	90	0	101	0	100
1-02772	LVOTO	TARS2	NM_025150	Missense	P155R	Novel	1	150463153	C>G	35.84	228	51	37	148	0	130	0	100
1-02121	CTD	KRBA1	NM_032534	Missense	R351G	Novel	7	149421865	A>G	32.33	201	21	14	70	0	51	0	100
1-00802	LVOTO	PTCH1	NM_001083607	Missense	R831Q	Novel	9	98220518	C>T	32.19	228	21	28	34	0	51	0	100
1-02772	LVOTO	TBC1D4	NM_014832	Missense	P634R	Novel	13	75900465	G>C	29.44	228	131	85	273	0	239	0	100
1-00980	CTD	NCAPD3	NM_015261	Missense	A1041V	Novel	11	134038929	G>A	29.32	228	62	38	93	0	101	0	100
1-01048	LVOTO	SDK1	NM_152744	Missense	V625M	Novel	7	4014056	G>A	29.25	228	93	104	155	0	138	1	100
1-02458	other	SOS1	NM_005633	Missense	T266K	Novel	2	39278352	G>T	28.25	228	97	70	228	0	125	0	100
1-00982	HTX	CREB5	NM_182899	Missense	T236M	Novel	7	28843919	C>T	27.24	228	125	125	193	1	111	0	100
1-00482	HTX	NFATC2	NM_012340	Missense	D584A	Novel	20	50071183	T>G	26.75	228	97	86	210	0	205	0	100
1-02254	LVOTO	ZNFX1	NM_021035	Missense	Y1789C	Novel	20	47864195	T>C	25.62	228	46	70	143	0	70	0	100
1-01795	CTD	DDX10	NM_004398	Missense	V427L	Novel	11	108577521	G>T	25.53	228	128	92	188	1	295	0	100
1-01106	HTX	TMEM63A	NM_014698	Missense	I413V	Novel	1	226047036	T>C	25.32	228	73	62	155	0	216	0	100
1-00140	CTD	KIAA1468	NM_020854	Missense	S416P	Novel	18	59895629	T>C	23.72	228	55	73	129	0	148	0	100
1-00347	LVOTO	C9orf64	NM_032307	Missense	L144F	Novel	9	86570461	T>A	23.30	228	45	36	105	0	95	0	100
1-00448	CTD	ITPR3	NM_002224	Missense	R1027H	Novel	6	33642006	G>A	23.06	228	60	64	191	0	232	0	100
1-01457	HTX	VPS13C	NM_017684	Missense	T423A	Novel	15	62283959	T>C	22.39	228	121	79	229	0	228	0	100
1-00312	LVOTO	FAM76A	NM_001143915	Missense	I228V	Novel	1	28075646	A>G	21.69	228	54	43	143	0	123	0	100
1-01360	LVOTO	NEURL2	NM_080749	Missense	S92T	Novel	20	44519356	C>G	21.38	228	23	21	47	0	36	0	100
1-02020	HTX	WIBG	NM_032345	Missense	G203V	Novel	12	56295663	C>A	20.50	228	86	78	191	0	169	0	100
1-01621	HTX	TWF2	NM_007284	Missense	E185Q	Novel	3	52264942	C>G	20.49	228	72	68	184	0	311	0	100
1-00230	LVOTO	BACH2	NM_021813	Missense	T803A	Novel	6	90642246	T>C	20.11	228	60	33	107	0	78	1	100
1-00111	LVOTO	PPWD1	NM_015342	Missense	I190V	Novel	5	64867712	A>G	19.27	228	47	51	55	0	103	1	100
1-02265	LVOTO	PKN3	NM_013355	Missense	R255Q	Novel	9	131469613	G>A	19.26	228	25	21	53	0	62	0	100
1-00249	LVOTO	SYNM	NM_015286	Missense	K523R	Novel	15	99670134	A>G	19.13	228	33	32	81	0	79	0	100
1-00230	LVOTO	MKRN2	NM_014160	Missense	R50W	Novel	3	12610495	C>T	18.87	228	62	59	110	0	115	0	100
1-01538	HTX	MKRN2	NM_014160	Missense	A251V	Novel	3	12616400	C>T	18.87	228	35	38	100	0	81	0	100
1-00293	LVOTO	HIVEP2	NM_006734	Missense	P123L	Novel	6	143095508	G>A	18.54	228	63	62	141	0	109	0	100
1-01106	HTX	GPRC5B	NM_016235	Missense	I205T	Novel	16	19883554	A>G	18.06	228	29	23	54	0	84	0	100
1-02952	LVOTO	PITX2	NM_000325	Missense	A47V	Novel	4	111553543	G>A	17.99	228	77	52	156	0	101	0	100
1-01836	CTD	KIAA2018	NM_001009899	Missense	S1168L	Novel	3	113377026	G>A	17.96	228	103	35	215	1	165	0	100
1-01019	HTX	PCDH1	NM_032420	Missense	K1203R	Novel	5	141233713	T>C	17.66	228	88	90	194	0	172	0	100
M007-20	HTX	SBNO2	NM_001100122	Missense	V78M	Novel	19	1147355	C>T	16.34	228	84	40	115	0	68	0	100
1-03171	CTD	EEPD1	NM_030636	Missense	E414D	Novel	7	36327313	G>T	16.10	228	34	38	47	0	84	0	100
1-01190	HTX	ATM	NM_000051	Missense	S821N	Novel	11	108129798	G>A	15.91	228	116	96	313	0	111	1	100
1-00096	CTD	LPHN3	NM_015236	Missense	K1406R	Novel	4	62936433	A>G	15.44	228	50	42	150	0	154	0	100
M007-20	HTX	MASTL	NM_032844	Missense	D537N	Novel	10	27459497	G>A	12.82	228	162	143	276	0	168	0	100
1-02212	CTD	ZNF536	NM_014717	Missense	E727K	Novel	19	31025762	G>A	11.70	129	24	10	53	0	39	0	100

1-00878	HTX	CRB2	NM_173689	Missense	R1189Q	Novel	9	126137555	G>A	11.59	228	45	36	153	0	99	0	100
1-01184	HTX	APLP1	NM_005166	Missense	R330C	Novel	19	36364547	C>T	11.43	228	66	52	86	0	208	0	100
1-01327	CTD	KLHDC4	NM_001184854	Missense	V443I	Novel	16	87742022	C>T	10.94	228	36	34	81	0	69	0	100
1-00878	HTX	CCDC57	NM_198082	Missense	A77T	Novel	17	80159592	C>T	10.18	228	18	23	58	0	48	0	100
1-00818	LVOTO	C16orf48	NM_032140	Missense	A192T	Novel	16	67697845	C>T	9.31	228	39	27	100	0	124	0	100
1-00820	LVOTO	PABPC4L	NM_001114734	Missense	K224Q	Novel	4	135121679	T>G	9.05	228	53	46	78	0	96	0	100
1-02070	CTD	HAUS3	NM_024511	Missense	F572L	Novel	4	2233750	G>T	8.44	228	27	17	72	0	90	0	100
1-00235	CTD	ZC3HAV1	NM_020119	Missense	A371T	Novel	7	138764576	C>T	8.33	228	91	79	151	0	174	0	100
1-00325	LVOTO	LYST	NM_000081	Missense	N3017T	Novel	1	235892952	T>G	6.80	228	51	24	118	0	89	0	100
1-00151	CTD	FAN1	NM_001146095	Missense	T905M	Novel	15	31221527	C>T	6.18	228	61	56	83	0	75	0	100
1-01994	LVOTO	DFFB	NM_004402	Missense	A91S	Novel	1	3782405	G>T	5.85	218	25	17	50	0	30	0	100
1-00312	LVOTO	USH1C	NM_005709	Missense	R875K	Novel	11	17517147	C>T	5.66	228	57	59	127	0	62	0	100
1-02070	CTD	NCKAP5	NM_207481	Missense	T1202I	Novel	2	133540779	G>A	5.65	228	75	62	136	0	135	0	100
1-00305	CTD	CHIC1	NM_001039840	Missense	R129H	Novel	X	72804287	G>A	5.33	228	132	57	257	0	377	0	100
1-02189	LVOTO	DDO	NM_004032	Missense	A107V	Novel	6	110729582	G>A	4.81	228	47	58	108	0	153	0	100
1-02598	HTX	LRP2	NM_004525	Missense	E4372K	Novel	2	169997050	C>T	4.53	228	19	21	53	0	64	0	100
1-00392	HTX	CYP2J2	NM_000775	Missense	S154N	Novel	1	60377896	C>T	4.43	228	114	95	215	1	167	1	100
1-01984	LVOTO	FAM129C	NM_001098524	Missense	R650W	Novel	19	17662699	C>T	3.12	228	78	80	105	0	135	0	100
1-01536	CTD	ALS2CL	NM_001190707	Missense	R129W	Novel	3	46716141	G>A	2.72	228	23	22	161	0	100	0	100
1-00186	CTD	INPP4B	NM_003866	Missense	D23N	Novel	4	143352346	C>T	2.48	228	143	126	354	0	211	0	100
1-02121	CTD	CDYL2	NM_152342	Missense	S267I	Novel	16	80666950	C>A	1.99	228	40	40	144	0	71	0	100
1-01866	CTD	FAM65C	NM_080829	Missense	G488S	Novel	20	49218794	C>T	1.85	228	35	32	111	0	92	1	100
1-02123	CTD	UNC13C	NM_001080534	Missense	R1182Q	Novel	15	54556462	G>A	1.77	228	64	54	128	0	101	0	100
1-01788	LVOTO	LMBRD2	NM_001007527	Missense	I329L	Novel	5	36122517	T>G	1.61	228	25	29	57	0	43	0	100
1-01474	CTD	MYOG	NM_002479	Missense	T189M	Novel	1	203053147	G>A	1.51	228	65	67	183	0	187	0	100
1-02020	HTX	SLC38A3	NM_006841	Missense	T174I	Novel	3	50253226	C>T	1.34	228	24	18	50	0	45	0	100
M006-17	HTX	AIPL1	NM_014336	Missense	E195K	Novel	17	6330260	C>T	1.29	228	63	50	126	0	111	0	100
1-01913	Other	FAM171B	NM_177454	Missense	T234S	Novel	2	187611953	A>T	1.24	150	84	29	114	0	103	0	100
1-01696	CTD	ITGA4	NM_000885	Missense	R261W	Novel	2	182346351	C>T	1.08	228	90	104	176	0	193	0	100
1-02290	LVOTO	CNTF	NM_000614	Missense	T111N	Novel	11	58391724	C>A	0.92	228	14	18	55	0	42	0	100
1-00305	CTD	KCNJ15	NM_002243	Missense	T77I	Novel	21	39671413	C>T	0.85	228	30	30	66	0	39	0	100
1-00689	CTD	KRT1	NM_006121	Missense	G137R	Novel	12	53073724	C>G	0.35	228	74	65	48	0	153	0	100
1-01970	CTD	ANKS1B	NM_152788	Missense	A67V	Novel	12	100219102	G>A	0.33	228	46	34	98	0	98	0	100
1-02411	CTD	CDH23	NM_052836	Missense	R1136C	Novel	10	73483838	C>T	0.30	228	43	45	158	0	199	0	100
1-00894	CTD	CYP11A1	NM_001099773	Missense	E157K	Novel	15	74637541	C>T	0.16	228	35	36	96	1	92	0	100
1-01847	LVOTO	RAB11FIP4	NM_032932	Missense	E138K	Novel	17	29844744	G>A	0.14	228	26	25	42	0	52	0	100
1-01371	CTD	DNAH9	NM_001372	Missense	R668W	Novel	17	11550420	C>T	0.12	228	101	108	222	0	198	0	100
1-00571	LVOTO	HEPACAM	NM_152722	Missense	D213N	Novel	11	124793697	C>T	0.12	228	60	59	131	0	115	0	100
1-01260	LVOTO	USP44	NM_032147	Missense	E71D	115488	12	95927820	C>A	0.10	174	89	23	110	0	127	0	100
1-00370	CTD	IL12RB1	NM_005535	Missense	P342Q	Novel	19	18180520	G>T	0.08	228	69	47	80	0	148	0	100
1-02504	CTD	C12orf56	NM_001099676	Missense	T263R	Novel	12	64678486	G>C	0.06	228	157	117	181	0	356	0	100
1-00508	LVOTO	CYP26C1	NM_183374	Missense	D268N	Novel	10	94824234	G>A	0.06	228	23	25	82	0	111	0	100
1-00728	CTD	FABP2	NM_000134	Missense	R11Q	Novel	4	120243226	C>T	0.06	228	89	74	193	0	199	1	100
1-01866	CTD	ABCA13	NM_152701	Missense	E574Q	Novel	7	48287896	G>C	0.04	228	36	21	91	0	173	0	100
1-00788	CTD	ALS2CR11	NM_001168221	Missense	I935V	Novel	2	202358261	T>C	0.04	172	12	8	53	0	36	0	100
1-00522	LVOTO	GPR1	NM_005279	Missense	A293S	Novel	2	207041095	C>A	0.02	228	28	37	59	0	82	0	100
1-00625	LVOTO	BTN3A3	NM_197974	Missense	V367M	Novel	6	26452130	G>A	0.00	228	160	95	283	0	284	0	100
1-00392	HTX	CCDC129	NM_001257967	Missense	A155G	Novel	7	31614222	C>G	0.00	228	49	68	108	0	81	0	100
1-01941	CTD	DEFB128	NM_001037732	Missense	G45E	Novel	20	168675	C>T	0.00	228	47	31	144	0	207	0	100
1-01474	CTD	DMBX1	NM_172225	Missense	E140Q	Novel	1	46976691	G>C	0.00	222	23	14	39	0	39	1	100
1-00305	CTD	DNAJC5B	NM_033105	Missense	E22K	Novel	8	66963846	G>A	0.00	228	77	83	176	0	129	0	100
1-01119	CTD	DSC1	NM_024421	Missense	V550D	Novel	18	28719725	A>T	0.00	228	33	44	60	0	65	0	100
1-02696	CTD	KCNH5	NM_172375	Missense	N817S	Novel	14	63174743	T>C	0.00	228	104	83	121	0	122	0	100
1-01440	CTD	NRG3	NM_001010848	Missense	V616F	Novel	10	84745116	G>T	0.00	228	52	40	89	0	86	0	100
1-01341	CTD	UMODL1	NM_001199527	Missense	V1090M	Novel	21	43543213	G>A	0.00	228	23	20	39	0	34	0	100
1-02408	HTX	UMODL1	NM_001199527	Missense	K1251E	Novel	21	43547834	A>G	0.00	228	57	60	108	0	165	0	100
1-02290	LVOTO	MYBPC2	NM_004533	Missense	S607I	Novel	19	50957347	G>T	4.40	188	26	13	51	0	35	0	96
1-02956	HTX	ASIC4	NM_182847	Missense	R593W	Novel	2	220402405	C>T	0.35	164	16	9	73	0	31	0	95
1-02458	other	PDCD1LG2	NM_025239	Missense	S36N	Novel	9	5534796	G>A	0.00	228	18	17	58	0	23	0	87
1-01951	CTD	ABCB6	NM_005689	Missense	A176G	Novel	2	220082869	G>C	12.02	209	13	13	28	0	27	0	77
1-02769	HTX	MAP3K1	NM_005921	Missense	T985P	Novel	5	56177980	A>C	32.48	132	63	32	20	0	97	0	66
1-02708	LVOTO	ANGPT4	NM_015985	Missense	A232T	Novel	20	865862	C>T	0.04	102	26	9	30	0	32	0	63
1-01190	HTX	AFTPH	NM_017657	Missense	R262L	Novel	2	64779393	G>T	15.01	201	21	12	38	0	20	0	55
Silent mutations in LHE genes in CHD cases																		
1-00079	LVOTO	RBM27	NM_018989	Silent	D775D	Novel	5	145643188	T>C	39.43	228	98	86	159	0	126	0	100
1-00448	CTD	ATCAY	NM_033064	Silent	N129N	Novel	19	3907760	C>T	36.44	228	23	23	69	0	56	0	100
1-01360	LVOTO	CLPB	NM_030813	Silent	V615V	Novel	11	72005096	C>T	31.25	228	46	57	109	0	94	0	100
1-02189	LVOTO	ZDHHC16	NM_032327	Silent	L196L	Novel	10	99213570	C>T	30.84	224	10	11	43	0	40	0	100
1-00650	HTX	ADCY4	NM_001198592	Silent	N844N	Novel	14	24791326	G>A	29.08	228	47	40	119	0	99	0	100
1-02202	CTD	SNX13	NM_015132	Silent	N284N	Novel	7	17890573	G>A	28.19	210	61	51	123	0	129	0	100
1-02573	LVOTO	TTLL5	NM_015072	Silent	T1078T	Novel	14	76286412	C>T	27.45	228	35	50	112	0	113	0	100
1-00739	CTD	MOV10L1	NM_018995	Silent	T651T	Novel	22	50573002	G>A	26.76	228	100	76	192	0	198	0	100
1-01411	LVOTO	MYO6	NM_004999	Silent	C691C	Novel	6	76583013	T>C	23.04	228	106	86	196	0	131	0	100
1-02765	CTD	CSF1	NM_172212	Silent	A27A	Novel	1	110456922	G>A	21.43	228	71	83	191	0	190	0	100
1-01042	HTX	EPB41L4A	NM_022140	Silent	D545D	Novel	5	111506677	A>G	21.14	228	175	159	397	0	210	0	100
1-02050	HTX	SLC1A5	NM_005628	Silent	P120P	Novel	19	47282024	G>A	19.32	228	34	33	211	0	122	0	100
1-01327	CTD	PRKAR2B	NM_002736	Silent	D377D	Novel	7	106799901	T>C	18.50	228	49	38	117	0	104	0	100
1-02282	LVOTO	ITPA	NM_181493	Silent	P84P	Novel	20	3194693	C>T	15.15	228	61	35	88	0	99	0	100
1-00436	LVOTO	PDPN	NM_006474	Silent	I225I	Novel	1	13940871	C>T	14.35	228	139	120	258	1	319	0	100
1-02212	CTD	ZFP62	NM_152283	Silent	K478K	Novel	5	180277061	C>T	12.91	228	54	73	135	0	81	0	100
M006-17	HTX	VPS33B	NM_018668	Silent	V512V	Novel	15	91543765	G>C	9.41	228	188	193	423	1	308	0	100
1-01019	HTX	DYNC2H1	NM_001080463	Silent	E175E	Novel	11	102985928	G>A	7.91	228	63	62	127	0	120	0	100
1-03151	LVOTO	DENND2C	NM_198459	Silent	T287T	Novel	1	115166210	C>T	6.02	228	155	117	205	0	226	0	100
1-00756	LVOTO	MFSD8	NM_152778	Silent	G335G	Novel	4	128843112	G>A	5.60	228	46	30	93	1	79	0	100
1-00934	LVOTO	AHR	NM_001621	Silent	R430R	Novel	7	17378739	G>A	5.34	228	54	53	121	0	142	0	100
1-00571	LVOTO	NFE2L3	NM_004289	Silent	V215V	Novel	7	26217637	G>A	2.59	228	23	31	71	0	77	0	100
1-00465	LVOTO	AFAP1L2	NM_001001936	Silent	A553A	Novel	10	116060333	T>C	1.72	228	75	75	108	0	91	1	100
1-02121	CTD	CSF2RB	NM_000395	Silent	I539I	Novel	22	37333467	A>C	1.49	228	19	23	79	0	54	0	100
1-00525	HTX	PRDM1	NM_001198	Silent	R442R	Novel	6	106553361	G>A	1.21	228	22	38	71	0	49	0	100
1-00739	CTD	HIST1H2BB	NM_021062	Silent	A10A	Novel	6	26043856	G>A	0.51	228	79	79	111	0	133	0	100
1-00230	LVOTO	SPATA9	NM_031952	Silent	Q233Q	Novel	5	94994393	C>T	0.45	228	58	56	153	0	125	0	100
1-00159	HTX	PRKG2	NM_006259	Silent	I225I	Novel	4	82092912	G>A	0.42	228	99	74	160	0	133	0	100
1-01184	HTX	TMEM125	NM_144626	Silent	L77L	Novel	1	43738622	C>T	0.41	228	28	31	49	0	123	0	100
1-01151	CTD	KCNU1	NM_001031836	Silent	I644I	Novel	8	36721962	C>T	0.25	228	59	33	161	0	214	0	100
1-00249	LVOTO	ASPHD1	NM_181718	Silent	P357P	Novel	16	29917116	C>G	0.17	228	58	47	114	0	111	0	100
1-00587	LVOTO	C9orf11	NM_020641	Silent	P129P	Novel	9	27291051	T>C	0.08	228	145	86	131	0	211	0	100
1-01385	LVOTO	FUT9	NM_006581	Silent	N52N	Novel	6	96651187	C>T	0.06	228	36	39	94	0	89	1	100
1-00565	CTD	GPR151	NM_194251	Silent	A24A	Novel	5	145895605	G>A	0.04	228	30	18	47	0	37	0	100
1-00977	CTD	CES4A	NM_173815	Silent	N274N	Novel	16	67037185	C>T	0.02	228	52	62	101	0	109	0	100
1-01401	LVOTO	AFM	NM_001133	Silent	P523P	Novel	4	74365867	T>C	0.00	228	78	72	197	1	242	0	100
M004-11	HTX	ASCL3	NM_020646	Silent	E100E	Novel	11	8959409	C>T	0	228	54	59	72	0	134	0	100

1-02736	LVOTO	DNAH5	NM_001369	Silent	A3456A	Novel	5	13759006	C>T	0.00	228	65	75	135	0	165	0	100
1-02313	CTD	FAM135B	NM_015912	Silent	I408I	Novel	8	139180172	G>A	0	228	43	42	117	0	92	1	100
1-00771	CTD	IGSF21	NM_032880	Silent	N368N	Novel	1	18703296	C>T	0	228	30	29	58	0	58	0	100
1-00243	LVOTO	RXFP3	NM_016568	Silent	L119L	Novel	5	33937202	C>T	0.00	228	68	47	110	0	106	0	100
1-00305	CTD	NPDC1	NM_015392	Silent	P43P	Novel	9	139937509	G>A	13.03	228	11	21	40	0	30	0	89
1-01816	LVOTO	RALGDS	NM_006266	Silent	A344A	Novel	9	135983375	C>T	23.66	144	12	9	34	0	25	0	84
1-00660	CTD	PDZD3	NM_001168468	Silent	E352E	Novel	11	119059387	G>A	0.04	224	9	16	29	0	30	0	71
Mutations in genes without identified mouse orthologs in CHD cases																		
1-00243	LVOTO	LIPJ	NM_001010939	Nonsense	L348X	Novel	10	90366606	T>G	-	228	17	18	49	0	39	0	100
1-00154	LVOTO	CALML6	NM_138705	Missense	Q10L	Novel	1	1847125	A>T	-	228	62	49	93	0	143	0	100
1-02888	LVOTO	KIAA0090	NM_015047	Missense	R881C	Novel	1	19547289	G>A	-	228	71	55	125	0	90	0	100
1-00894	CTD	ZNF576	NM_024327	Missense	S23N	Novel	19	44101328	G>A	-	228	85	95	169	1	228	1	100
1-02338	CTD	ZNF79	NM_007135	Missense	G385V	Novel	9	130207133	G>T	-	228	32	35	76	0	85	0	100
1-01401	LVOTO	ABCA10	NM_080282	Missense	I453L	Novel	17	67190119	T>G	-	137	40	41	107	0	61	2	100
1-00826	LVOTO	ADH1B	NM_000668	Missense	V77A	Novel	4	100239232	A>G	-	228	182	75	237	0	241	0	100
1-02290	LVOTO	C15orf42	NM_152259	Missense	R301Q	Novel	15	90126164	G>A	-	228	59	87	135	0	101	0	100
1-00303	CTD	C7orf33	NM_145304	Missense	H67P	Novel	7	148288217	A>C	-	228	27	29	61	0	43	0	100
1-00198	CTD	COPG1	NM_016128	Missense	I425V	Novel	3	128984440	A>G	-	228	70	74	166	0	172	0	100
1-00847	HTX	DCHS2	NM_017639	Missense	S2818Y	Novel	4	155155986	G>T	-	228	34	27	56	0	58	0	100
1-00148	LVOTO	LPA	NM_005577	Missense	G1210S	Novel	6	161014991	C>T	-	228	17	17	162	0	177	0	100
1-02652	CTD	TARBP1	NM_005646	Missense	V1306I	Novel	1	234541722	C>T	-	208	66	20	70	0	89	0	100
1-00522	LVOTO	ZNF221	NM_013359	Missense	Q414H	Novel	19	44470896	A>T	-	145	42	11	54	0	48	0	100
1-00392	HTX	ZNF268	NM_001165887	Missense	W92R	Novel	12	133768114	T>C	-	228	111	91	212	0	175	0	100
1-01327	CTD	ZNF34	NM_030580	Missense	R91Q	Novel	8	146002914	C>T	-	228	38	39	93	0	79	0	100
1-01265	CTD	ZNF544	NM_014480	Missense	G54E	Novel	19	58758077	G>A	-	228	77	58	101	0	117	0	100
1-01520	LVOTO	ZNF675	NM_138330	Missense	Y250D	Novel	19	23836987	A>C	-	228	25	27	59	2	46	0	100
1-02041	LVOTO	CEP85	NM_022778	Silent	T648T	Novel	1	26603067	A>G	-	228	16	19	34	0	48	0	100
1-00101	HTX	DZANK1	NM_001099407	Silent	C563C	Novel	20	18374917	G>A	-	228	21	17	42	0	39	0	100
1-01653	HTX	TARBP1	NM_005646	Silent	L1604L	Novel	1	234527377	C>A	-	228	50	41	94	0	65	0	100
1-02643	LVOTO	ZNF880	NM_001145434	Silent	N140N	Novel	19	52887253	T>C	-	103	14	51	89	4	81	4	100
1-00392	HTX	DNAH17	NM_173628	Silent	R989R	.617387	17	76528711	C>T	-	228	20	27	47	0	40	0	69
Damaging mutations in HHE genes in Controls																		
14084	Control	ITM2C	NM_030926	Frameshift	F93	None	2	231448595	-C	170.37	*Indel-Pass*	41	49	High	0	High	0	100
11382	Control	KANK1	NM_001256876	Nonsense	E309X	Novel	9	702165	G>T	73.40	228	51	38	140	0	110	0	100
Mutations at conserved position in HHE genes in Controls																		
13781	Control	ACTN1	NM_001130005	Missense	I835N	Novel	14	68413634	A>T	139.44	228	82	86	119	0	132	0	100
14249	Control	COPG2	NM_012133	Missense	L45F	Novel	7	130002265	C>G	116.90	228	143	105	275	0	220	0	100
12197	Control	GART	NM_001136006	Missense	N622S	Novel	21	33811623	T>C	70.62	228	130	134	296	0	226	0	100
13271	Control	GLRX3	NM_001199868	Missense	P145A	Novel	10	131849206	C>G	42.58	228	60	55	85	0	89	0	100
11089	Control	HEATR5B	NM_019024	Missense	R176C	Novel	2	37156203	G>A	40.05	228	71	92	233	0	221	0	100
11219	Control	LAMA4	NM_001105206	Missense	N176I	Novel	6	112619722	T>A	223.89	228	67	42	51	0	73	0	100
14260	Control	LSMD1	NM_032356	Missense	M151I	Novel	17	7700843	C>T	88.31	228	44	30	106	1	70	0	100
11085	Control	POGK	NM_017542	Missense	E163K	Novel	1	165084927	G>A	40.69	228	71	94	117	0	134	0	100
11999	Control	POGZ	NM_207171	Missense	R321Q	Novel	1	149664119	C>T	59.41	228	83	47	119	0	139	0	100
11881	Control	PRCP	NM_005040	Missense	T124M	Novel	11	82248668	G>A	44.87	228	49	57	96	0	120	0	100
13747	Control	SETD5	NM_001080517	Missense	K170R	Novel	3	9452532	A>G	158.05	228	94	89	193	0	170	0	100
14116	Control	SUPT16H	NM_007192	Missense	R278C	Novel	14	20906391	G>A	96.90	228	68	66	102	0	105	0	100
13614	Control	ZCCHC24	NM_153367	Missense	R239C	Novel	10	80816118	G>A	77.79	221	11	22	70	0	54	0	65
Mutations at nonconserved position in HHE genes in Controls																		
13855	Control	ACO2	NM_001098	Missense	P745H	Novel	22	40254454	C>A	409.24	228	103	99	233	0	271	0	100
14259	Control	ASNSD1	NM_019048	Missense	R341C	Novel	2	190240124	C>T	62.44	228	67	58	121	0	141	0	100
11700	Control	BECN1	NM_003766	Missense	C159R	Novel	17	38224101	A>G	71.54	228	51	48	74	0	77	0	100
13204	Control	C1orf131	NM_152379	Missense	K254R	Novel	1	229426769	T>C	80.15	228	73	66	148	0	149	0	100
11667	Control	CDH3	NM_001793	Missense	V698F	Novel	16	67286748	G>T	50.78	228	39	35	48	0	56	0	100
11356	Control	DHX30	NM_138615	Missense	E439K	Novel	3	47862998	G>A	64.41	228	49	43	65	0	61	0	100
11028	Control	IPO4	NM_024658	Missense	A188T	Novel	14	23726472	C>T	53.82	228	35	29	106	0	83	0	100
12299	Control	KLHL21	NM_014851	Missense	R538H	Novel	1	6576193	C>T	63.48	228	39	42	65	0	56	0	100
11045	Control	LRP1	NM_002332	Missense	R2244W	Novel	12	55865847	C>T	118.73	228	52	50	80	0	58	0	100
11881	Control	MMP15	NM_002428	Missense	E360K	Novel	16	56633189	G>A	260.55	228	42	49	98	0	71	0	100
11740	Control	N4BP1	NM_153029	Missense	Q361K	Novel	16	47152974	G>T	45.41	228	25	14	35	0	40	0	100
14072	Control	PAPOLA	NM_001252006	Missense	P624L	Novel	14	96092370	C>T	233.58	228	80	54	126	0	120	0	100
12162	Control	PKP4	NM_003628	Missense	V358L	Novel	2	159190104	G>C	173.79	228	46	37	69	0	73	0	100
11247	Control	SLC8A1	NM_001112801	Missense	M634T	Novel	2	40259045	A>G	923.03	228	122	124	151	0	203	0	100
11014	Control	TXNL1	NM_004786	Missense	R234H	Novel	18	52432687	C>T	67.99	228	143	143	276	0	185	0	100
13876	Control	UTRN	NM_007124	Missense	K987E	Novel	6	144825588	A>G	124.53	111	18	7	206	0	166	0	100
12235	Control	YLPM1	NM_019589	Missense	E2022K	Novel	14	74357546	G>A	84.43	228	40	38	72	0	97	0	100
Silent mutations in HHE genes in Controls																		
11519	Control	BAHCC1	NM_001080519	Silent	D1994D	Novel	17	77042272	C>T	51.91	125	14	7	32	0	36	0	100
11077	Control	CCNG1	NM_199246	Silent	I212I	Novel	5	162801667	C>A	119.21	228	136	129	151	0	291	0	100
13073	Control	DDR1	NM_001954	Silent	D717D	Novel	6	30974781	C>T	220.24	228	38	23	60	0	44	0	100
14058	Control	GSPT1	NM_001130007	Silent	A322A	Novel	16	11888278	G>A	98.71	228	90	95	198	0	205	0	100
11382	Control	KANK1	NM_001256876	Silent	R829R	Novel	9	703253	T>C	73.40	228	17	22	90	0	30	0	100
14046	Control	KIAA0232	NM_014743	Silent	P24P	Novel	4	6877153	T>C	51.78	228	88	81	192	0	152	0	100
12853	Control	LAMA4	NM_001105206	Silent	H1680H	Novel	6	112543810	G>A	223.89	228	167	152	359	0	333	0	100
11263	Control	MAPK8IP3	NM_001040439	Silent	L997L	Novel	16	1756795	C>T	134.18	198	23	18	111	0	130	0	100
11334	Control	MLL5	NM_182931	Silent	G1097G	Novel	7	104535431	A>C	147.53	228	78	55	127	0	145	0	100
11835	Control	NFE2L1	NM_003204	Silent	T61T	Novel	17	43483662	C>A	234.68	228	57	49	69	0	113	1	100
13093	Control	PLXNA1	NM_032242	Silent	G750G	Novel	3	128213628	C>A	73.60	228	28	47	103	0	64	0	100
14256	Control	PPP1R12B	NM_001167857	Silent	P48P	Novel	1	200798553	T>G	61.93	166	47	16	79	0	53	0	100
11014	Control	QKI	NM_206855	Silent	T7T	Novel	6	163756236	G>C	228.31	228	51	40	65	0	54	0	100
11676	Control	RRBP1	NM_004587	Silent	R395R	Novel	20	17562231	C>G	529.18	228	83	77	125	0	92	0	100
11561	Control	SBNO1	NM_001167856	Silent	L664L	Novel	12	122374313	G>T	108.82	228	22	23	206	0	101	0	100
13912	Control	SNRNP200	NM_014014	Silent	T389T	Novel	2	96327138	T>C	101.27	228	102	117	167	0	221	0	100
11654	Control	TBC1D1	NM_001253912	Silent	C454C	Novel	4	37713663	T>C	48.09	228	34	43	66	1	40	0	100
13855	Control	THOC2	NM_001081550	Silent	L353L	Novel	X	122628769	T>C	79.41	228	93	114	105	0	190	0	100
11651	Control	TSC22D3	NM_198057	Silent	P76P	Novel	X	106905078	C>A	78.09	228	140	146	75	0	283	0	100
11180	Control	WDR6	NM_018031	Silent	R919R	Novel	3	49026820	G>A	111.93	228	30	21	44	0	37	0	100
12297	Control	ZC3H7B	NM_017590	Silent	P296P	Novel	22	40065837	C>T	143.86	228	42	69	123	0	148	0	100
Damaging mutations in LHE genes in Controls																		
12228	Control	ACPP	NM_001099	Splice	1 bp up of exon 3	Novel	3	133533180	G>A	0.12	154	10	9	23	0	28	0	68
13795	Control	ADARB1	NM_001112	Frameshift	2 bp up of exon 13	Novel	21	45420294	+T	30.40	*Indel-Pass*	1420	94	High	0	High	0	100
14046	Control	CDK14	NM_012395	Frameshift	P207	None	7	90366577	+G	22.63	*Indel-Pass*	46	101	High	0	High	0	100
13720	Control	FAM46C	NM_017709	Nonsense	Q227X	Novel	1	117967692	C>T	15.81	173	19	10	35	0	26	0	99
14147	Control	FAM82A1	NM_001170791	Splice	1 bp up of exon 9	Novel	2	38084782	G>C	7.45	228	91	112	256	0	217	0	100
12299	Control	GBA2	NM_020944	Nonsense	R574X	Novel	9	35729074	G>A	33.08	228	69	69	160	0	123	0	100
12340	Control	MRPL48	NM_016055	Nonsense	R201X	Novel	11	73253025	C>T	22.01	228	66	71	74	0	100	0	100
14054	Control	NSL1	NM_015471	Frameshift	E81	None	1	211027596	-AA	15.84	*Indel-Pass*	64	110	High	0	High	0	100
13799	Control	PCOLCE2	NM_013363	Frameshift	G39	None	3	144089276	-C	0.14	*Indel-Pass*	98	128	High	0	High	0	100
11028	Control	PDZD7	NM_001195263	Frameshift	V346	Novel	10	102770344	-G	4.72	*Indel-Pass*	1027	95	High	0	High	0	100
14239	Control	RPRD1B	NM_021215	Frameshift	S90	None	20	36102367	+C	28.67	*Indel-Pass*	73	331	High	0	High	0	100
11766	Control	SYTL3	NM_001242395	Nonsense	K127X	Novel	6	159023994	A>T	0.09	228	23	35	77	0	66	0	100

12650	Control	TELO2	NM_016111	Frameshift	L140	Novel	16	1492085	-AG	18.71	*Indel-Pass*	2812	90	High	0	High	0	100
11296	Control	WRNIP1	NM_020135	Frameshift	T2398	Novel	6	2785347	+A	26.53	*Indel-Pass*	1877	103	High	0	High	0	100
13842	Control	ZNF407	NM_017757	Splice	1 bp up of exon 7	Novel	18	70761457	G>A	10.74	228	56	42	141	0	117	0	100
Mutations at conserved position in LHE genes in Controls																		
12320	Control	ABHD5	NM_016006	Missense	G305V	Novel	3	43734307	G>T	16.56	228	99	90	261	1	235	0	100
11501	Control	ANPEP	NM_001150	Missense	R855W	Novel	15	88135294	G>A	24.25	228	231	260	311	0	422	0	100
13023	Control	ATG14	NM_014924	Missense	P441R	Novel	14	54906247	G>C	9.63	228	31	29	54	0	66	0	100
11412	Control	C16orf62	NM_020314	Missense	E971K	Novel	16	19610292	G>A	37.68	228	37	45	130	0	96	0	100
13973	Control	CABP5	NM_019855	Missense	T59M	Novel	19	53235736	G>A	0.02	228	113	117	231	0	195	1	100
12834	Control	CADPS	NM_003716	Missense	N1022S	Novel	3	62442546	T>C	0.68	228	103	69	274	0	268	0	100
12020	Control	CCDC129	NM_001257967	Missense	R103T	Novel	7	31575948	G>C	0.00	228	476	391	498	0	702	0	100
12317	Control	CHST2	NM_004267	Missense	S177W	Novel	3	144322878	C>G	13.04	228	14	26	47	0	40	0	100
11397	Control	CNTN1	NM_001256063	Missense	D771V	Novel	12	39696878	A>T	1.77	228	108	64	236	0	222	1	100
13739	Control	COL11A1	NM_001854	Missense	R1568I	Novel	1	103125142	C>A	13.65	156	161	42	137	0	161	0	100
11356	Control	DLGAP3	NM_001080418	Missense	R428C	Novel	1	35138287	G>A	1.30	228	130	123	171	0	139	0	100
13748	Control	EPHB1	NM_004441	Missense	G783R	Novel	3	136442680	G>A	3.30	228	30	24	60	0	41	0	99
13660	Control	ESRRB	NM_004452	Missense	R152C	Novel	14	75998697	C>T	16.13	150	23	9	32	0	30	0	100
12297	Control	GNA14	NM_004297	Missense	P294L	Novel	9	79228902	G>A	0.42	228	68	79	186	0	165	0	100
11304	Control	KLC2	NM_001134775	Missense	L201P	Novel	11	65786933	T>C	35.87	228	28	36	84	1	84	1	100
11075	Control	MED12L	NM_053002	Missense	N1061H	Novel	3	152566428	A>C	18.13	228	174	219	115	0	415	1	100
13737	Control	PAFAH2	NM_000437	Missense	L361V	Novel	1	26171639	G>C	11.57	228	35	54	61	0	81	0	100
11008	Control	PITPNM3	NM_001165966	Missense	R601Q	Novel	17	6308796	C>T	3.31	186	19	14	62	0	51	0	100
12219	Control	RALGAPA2	NM_020343	Missense	T1236I	Novel	20	20453529	G>A	27.17	228	80	67	141	0	139	0	100
11336	Control	RGS7	NM_002924	Missense	N20K	Novel	1	239585640	G>T	0.08	228	90	78	125	2	207	0	100
13912	Control	RIT2	NM_002930	Missense	R85W	Novel	18	38757708	G>A	0.00	228	46	38	69	0	59	0	100
14084	Control	SASH1	NM_015278	Missense	R1158C	Novel	6	148908967	C>T	1.92	228	39	43	71	0	40	0	100
14075	Control	SLC4A10	NM_001178016	Missense	V282A	Novel	2	162437127	T>C	0.12	228	62	64	187	0	101	0	100
11052	Control	SLC5A9	NM_001011547	Missense	G311R	Novel	1	48470369	G>A	0.00	228	79	97	205	1	110	0	100
12804	Control	SLC6A17	NM_001010898	Missense	V435M	Novel	1	110538727	G>A	0.96	228	26	42	83	0	83	0	100
13799	Control	SNX29	NM_032167	Missense	R608C	Novel	16	12357560	C>T	2.35	228	46	74	77	0	90	0	100
13614	Control	SPTLC2	NM_004863	Missense	G143A	Novel	14	77115105	C>G	29.95	228	66	51	141	0	121	0	100
12403	Control	SRPK1	NM_003137	Missense	K267N	Novel	6	35946226	C>A	31.59	228	83	53	224	0	225	0	100
13744	Control	TMC5	NM_001105248	Missense	G847R	Novel	16	19406115	G>A	0.08	228	60	53	79	0	88	0	100
13300	Control	TNFRSF19	NM_148957	Missense	G55V	Novel	13	23065578	G>T	23.96	228	116	108	202	0	196	0	100
11382	Control	WDR65	NM_001167965	Missense	G513R	Novel	1	43437756	G>A	1.50	228	109	121	395	0	221	0	100
13660	Control	ZNF423	NM_015069	Missense	F76V	Novel	16	48322234	A>C	8.50	228	122	109	311	1	275	0	100
11897	Control	ZNF423	NM_015069	Missense	M1009I	Novel	16	48227537	C>T	8.50	228	42	43	104	0	93	0	100
Mutations at nonconserved position in LHE genes in Controls																		
13093	Control	ABCA4	NM_000350	Missense	P1464A	Novel	1	94267738	G>C	5.90	228	60	45	116	0	109	0	100
13023	Control	AGTPBP1	NM_015239	Missense	Q352E	Novel	9	87451067	G>C	17.14	228	84	61	150	0	178	0	100
13825	Control	ANKRD44	NM_001195144	Missense	N784S	Novel	2	197574806	T>C	12.68	187	9	24	65	0	50	0	100
13714	Control	ANO10	NM_001204834	Missense	G378E	Novel	3	43577726	C>T	32.67	228	51	35	124	0	124	0	100
13730	Control	ARHGEF10L	NM_018125	Missense	V510M	Novel	1	17826529	G>A	17.02	228	20	16	43	0	28	0	100
13271	Control	ATF7IP2	NM_001256160	Missense	I58K	Novel	16	10432151	T>A	0.55	187	24	26	32	0	31	0	100
11075	Control	C4orf40	NM_214711	Missense	P110L	Novel	4	71058887	C>T	0.00	228	162	147	104	0	307	1	100
13997	Control	CACNA1D	NM_001128840	Missense	R1955Q	Novel	3	53819109	G>A	10.99	228	10	40	56	0	44	0	100
13799	Control	CATSPERD	NM_152784	Missense	G148R	Novel	19	5688199	G>A	0.67	228	266	241	405	0	452	0	100
14058	Control	CCR9	NM_006641	Missense	V291F	Novel	3	45918155	G>T	0.06	228	68	70	145	0	161	0	100
11888	Control	CD19	NM_001770	Missense	M476T	Novel	16	28856500	T>C	0.00	228	60	56	110	0	120	0	100
14090	Control	COBLL1	NM_014900	Missense	D1164N	Novel	2	165250713	C>T	24.42	228	74	70	168	0	120	0	100
14055	Control	CSRNP2	NM_030809	Missense	Q382E	Novel	12	49744284	G>C	16.80	228	25	30	65	0	49	0	63
11411	Control	DCLRE1B	NM_022836	Missense	R395W	Novel	1	114255920	C>T	12.27	200	10	13	38	0	30	0	100
12383	Control	DDC	NM_000790	Missense	A45T	Novel	7	50579145	C>T	37.60	228	49	47	161	0	151	1	100
12275	Control	DGKD	NM_152879	Missense	R517Q	Novel	2	234021696	G>A	39.32	228	48	29	55	0	76	0	100
12060	Control	DNMBP	NM_015221	Missense	A138T	Novel	10	101706809	C>T	23.59	228	17	19	73	0	29	0	98
11075	Control	DOLK	NM_014908	Missense	C45R	Novel	9	130749271	A>G	16.05	228	28	54	25	0	64	0	100
13821	Control	DPEP2	NM_022355	Missense	R434H	Novel	16	66579070	C>T	0.12	228	102	74	75	0	138	0	100
11456	Control	EPG5	NM_020964	Missense	G1591S	Novel	18	41733405	C>T	9.13	228	66	58	117	0	109	0	100
12507	Control	ESF1	NM_016649	Missense	R799Q	Novel	20	13643681	C>T	35.20	228	46	37	127	0	110	0	100
14116	Control	EXOC2	NM_018303	Missense	Q879E	Novel	6	434025	G>C	26.73	228	60	82	100	0	103	0	100
11676	Control	GLTSCR1	NM_015711	Missense	A982T	Novel	19	52890017	G>A	28.30	136	15	24	44	0	30	0	100
13614	Control	GRAMD1C	NM_017577	Missense	R442Q	Novel	3	115135163	G>A	5.05	228	65	56	185	0	150	0	100
13322	Control	HPS6	NM_024747	Missense	R693C	Novel	10	103817298	C>T	5.36	165	18	16	36	0	30	0	100
11429	Control	IL6R	NM_181359	Missense	R232C	Novel	1	152674143	C>T	1.41	228	37	30	68	0	46	0	100
11014	Control	IRAK2	NM_001570	Missense	S441C	Novel	3	10251192	C>G	6.97	228	74	45	135	0	82	0	100
13543	Control	KCTD14	NM_023930	Missense	V168M	Novel	11	77405553	C>T	0.49	228	36	26	45	0	72	0	95
11057	Control	MIB2	NM_080875	Missense	R96Q	Novel	1	1548637	G>A	24.72	228	21	21	34	0	30	0	100
11881	Control	MYCBPAP	NM_032133	Missense	E663K	Novel	17	45958316	G>A	5.42	228	32	32	78	0	92	0	100
14116	Control	NAALAD2	NM_005467	Missense	R712C	Novel	11	89564474	C>T	4.05	228	76	73	108	0	166	0	100
12622	Control	NDC80	NM_006101	Missense	K288E	Novel	18	2579301	A>G	21.66	228	30	39	56	0	45	0	100
13777	Control	NLRP5	NM_153447	Missense	K697T	Novel	19	61231501	A>C	0.19	116	21	7	61	0	35	1	93
12303	Control	NMBR	NM_002511	Missense	V360A	Novel	6	142438572	A>G	0.10	228	98	71	116	1	176	0	100
12853	Control	NSUN6	NM_182543	Missense	L191R	Novel	10	18943398	A>C	3.92	228	47	50	89	0	73	1	100
13964	Control	NTRK2	NM_006180	Missense	V220A	Novel	9	86528383	T>C	0.37	228	183	160	390	0	420	0	100
11296	Control	NUP155	NM_153485	Missense	I597T	Novel	5	37365072	A>G	34.78	228	112	93	226	0	204	1	100
13748	Control	PAQR3	NM_001040202	Missense	T196M	Novel	4	80066814	G>A	4.70	228	91	90	229	0	143	0	65
13855	Control	PENK	NM_006211	Missense	Q78K	Novel	8	57516957	G>T	0.32	228	34	23	61	0	63	0	100
14167	Control	PPARD	NM_006238	Missense	E24K	Novel	6	35486912	G>A	26.87	228	40	37	110	0	115	0	100
11888	Control	PRIC285	NM_033405	Missense	S182T	Novel	20	61668903	C>G	9.80	228	17	18	46	0	34	0	100
11089	Control	PRKAR1B	NM_001164761	Missense	V177I	767141[illegible]	7	612376	C>T	8.93	228	186	181	475	0	467	0	100
11502	Control	PRR16	NM_016644	Missense	Q75P	Novel	5	120049681	A>C	0.94	228	81	62	118	0	137	0	100
11397	Control	RAB3GAP2	NM_012414	Missense	V1124L	Novel	1	218397420	C>G	34.57	228	48	56	122	0	126	0	100
13236	Control	RHBDF2	NM_024599	Missense	I483M	Novel	17	71982152	G>C	13.47	228	27	39	66	1	74	0	65
11835	Control	SGTB	NM_019072	Missense	R292W	Novel	5	65001870	G>A	3.01	228	110	111	226	0	189	0	100
12154	Control	SH3RF3	NM_001099289	Missense	D738N	Novel	2	109616423	G>A	1.45	228	63	54	93	0	58	0	100
13777	Control	SHC2	NM_012435	Missense	P394L	Novel	19	376225	G>A	1.73	189	13	12	32	0	20	0	82
14084	Control	SKOR1	NM_001031807	Missense	S61R	Novel	15	65905430	C>A	0.02	228	13	25	26	0	27	0	100
12987	Control	SLC12A2	NM_001046	Missense	I324L	Novel	5	127478194	A>C	18.55	228	26	36	275	0	274	0	100
14084	Control	SLC16A13	NM_201566	Missense	S138Y	Novel	17	6882264	C>A	4.51	228	59	58	108	0	71	0	100
13805	Control	SLC30A6	NM_001193515	Missense	P351R	Novel	2	32299039	C>G	18.08	228	59	65	102	0	131	0	100
13852	Control	SNF8	NM_007241	Missense	G159S	Novel	17	44365655	C>T	31.42	228	195	155	574	0	393	0	100
13842	Control	SPATA20	NM_022827	Missense	R308Q	Novel	17	45982405	G>A	0.30	228	38	37	117	0	77	0	100
13621	Control	SPICE1	NM_144718	Missense	R354H	Novel	3	114669770	C>T	20.58	228	58	50	82	0	80	0	100
14260	Control	SPTBN2	NM_006946	Missense	Q935R	Novel	11	66225643	T>C	3.63	228	103	47	157	0	131	0	100
14260	Control	SPTBN2	NM_006946	Missense	E934D	Novel	11	66225645	C>G	3.63	228	76	75	165	0	134	0	100
11356	Control	STEAP3	NM_018234	Missense	A22S	Novel	2	119719606	G>T	22.15	228	35	23	48	0	35	0	100
13795	Control	STX3	NM_001178040	Missense	K199E	Novel	11	59317483	A>G	7.27	228	72	66	140	0	121	0	100
14058	Control	TBCK	NM_001163435	Missense	H588Y	Novel	4	107370981	G>A	5.72	228	84	74	196	0	180	0	100
11888	Control	TMEM39A	NM_018266	Missense	N176S	Novel	3	120648603	T>C	27.83	228	59	64	126	0	97	0	100
12834	Control	TRAM1L1	NM_152402	Missense	Y214F	Novel	4	118225357	T>A	3.00	228	43	42	122	0	136	0	100
12275	Control	TSSC4	NM_005706	Missense	R124W	Novel	11	2380809	C>T	26.44	228	11	20	26	0	18	0	84

12020	Control	TXNDC16	NM_001160047	Missense	T439I	Novel	14	52007145	G>A	19.76	228	221	170	232	1	306	0	100
13814	Control	VPS11	NM_021729	Missense	T554P	Novel	11	118453886	A>C	30.00	228	40	36	99	0	106	0	100
14110	Control	VPS53	NM_001128159	Missense	A164V	Novel	17	503398	G>A	23.55	228	84	74	206	0	245	1	100
11651	Control	VWDE	NM_001135924	Missense	A1310V	Novel	7	12350578	G>A	0.00	153	20	11	87	0	27	0	100
11114	Control	ZDHHC16	NM_032327	Missense	V75M	Novel	10	99201645	G>A	30.84	186	40	29	41	0	46	0	100
13965	Control	ZNF174	NM_001032292	Missense	S128F	Novel	16	3392388	C>T	1.59	177	25	12	32	0	26	0	94
11474	Control	ZW10	NM_004724	Missense	R83W	Novel	11	113136844	G>A	20.92	228	86	62	54	0	70	0	100
Silent mutations in LHE genes in Controls																		
13621	Control	ABCC6	NM_001171	Silent	H786H	Novel	16	16180213	G>A	0.02	228	11	19	26	0	19	0	57
14084	Control	ACSF2	NM_025149	Silent	F588F	Novel	17	45906594	T>C	13.15	228	110	83	198	0	145	0	100
11052	Control	ADAMTSL1	NM_052866	Silent	A1539A	Novel	9	18879720	G>A	13.25	228	41	29	102	0	35	0	100
13966	Control	APOL6	NM_030641	Silent	P37P	Novel	22	34384668	C>T	0.51	228	13	22	37	0	46	0	100
12375	Control	ATP1B4	NM_012069	Silent	D314D	Novel	X	119397397	C>T	0.17	228	78	80	66	0	162	0	100
13973	Control	BRCA1	NM_007297	Silent	E358E	Novel	17	38482129	T>C	14.78	228	111	103	248	0	235	1	100
13864	Control	CCDC116	NM_152612	Silent	G145G	Novel	22	20318673	A>C	0.45	228	42	53	53	0	50	0	100
11336	Control	CCDC99	NM_017785	Silent	V356V	Novel	5	168958093	C>T	18.68	228	119	87	152	0	270	0	100
13271	Control	CENPW	NM_001012507	Silent	T5T	Novel	6	126703127	C>T	28.55	228	44	30	61	0	57	0	100
11622	Control	CGN	NM_020770	Silent	L452L	Novel	1	149763413	G>A	6.26	228	125	124	179	0	224	0	100
11519	Control	CLEC3A	NM_005752	Silent	V92V	Novel	16	76622077	C>G	0.02	228	51	32	136	0	132	0	100
13814	Control	DIAPH3	NM_001042517	Silent	G40G	Novel	13	59635782	G>A	28.38	127	8	12	32	0	31	0	87
12056	Control	DRD1	NM_000794	Silent	H237H	Novel	5	174801998	G>A	0.04	228	64	64	67	0	76	0	100
12492	Control	ESYT1	NM_001184796	Silent	P568P	Novel	12	54816866	A>G	35.27	228	13	15	52	0	46	0	100
12492	Control	FAM149B1	NM_173348	Silent	D217D	Novel	10	74638491	C>T	18.66	228	17	37	91	1	104	1	100
11892	Control	FAM184A	NM_001100411	Silent	S288S	Novel	6	119382950	C>T	4.96	228	42	53	61	1	54	0	100
11028	Control	FOXD1	NM_004472	Silent	E33E	Novel	5	72779845	C>T	0.08	219	51	27	103	0	98	0	100
12492	Control	GPR133	NM_198827	Silent	T106T	Novel	12	130032389	G>A	4.29	228	16	19	44	1	47	0	100
14123	Control	GPR77	NM_018485	Silent	Y190Y	Novel	19	52536466	C>T	0.23	228	32	46	96	0	77	3	100
13300	Control	HMGCLL1	NM_001042406	Silent	P227P	Novel	6	55472008	C>A	1.15	228	145	117	236	0	229	0	100
13355	Control	IBTK	NM_015525	Silent	S29S	Novel	6	83006836	G>A	36.04	228	50	38	72	0	68	0	100
12299	Control	IFT57	NM_018010	Silent	L241L	Novel	3	109393114	G>A	18.81	228	154	159	427	0	340	1	100
14076	Control	ITGAM	NM_000632	Silent	S890S	Novel	16	31245719	C>T	10.59	228	104	110	159	0	193	0	100
11797	Control	KIF14	NM_014875	Silent	Y498Y	Novel	1	198844641	A>G	14.13	228	21	20	38	0	52	0	100
13820	Control	KRT12	NM_000223	Silent	A230A	Novel	17	36274701	G>A	0.02	228	63	85	162	1	112	0	100
13966	Control	LCT	NM_002299	Silent	V943V	Novel	2	136283558	C>T	0.04	226	29	18	39	0	49	0	94
13773	Control	LILRB4	NM_006847	Silent	P354P	Novel	19	59870921	C>G	0.63	136	24	10	136	0	162	7	65
11391	Control	PTH1R	NM_000316	Silent	C568C	Novel	3	46920072	C>T	25.38	228	34	35	69	0	92	1	100
12197	Control	PTRH2	NM_016077	Silent	S62S	Novel	17	55129936	G>A	39.92	228	125	108	191	0	178	0	100
13382	Control	RGS7	NM_002924	Silent	V471V	Novel	1	239031378	C>T	0.08	228	61	46	156	0	109	1	100
11897	Control	SBNO2	NM_001100122	Silent	T168T	Novel	19	1073998	T>C	16.34	228	112	113	358	0	280	0	100
11892	Control	SERPINA5	NM_000624	Silent	T264T	Novel	14	94126303	G>A	0.00	228	52	35	109	0	123	0	100
13912	Control	SLC24A1	NM_004727	Silent	N303N	Novel	15	63704380	C>T	0.12	228	24	28	36	0	42	0	100
12524	Control	SMPDL3B	NM_014474	Silent	P239P	Novel	1	28154808	G>A	4.30	223	23	45	41	0	43	0	100
13621	Control	TDRD5	NM_001199091	Silent	P528P	Novel	1	177875660	G>A	0.16	228	47	46	58	0	41	0	100
12289	Control	TLR9	NM_017442	Silent	S6S	5617227	3	52233354	G>A	0.25	199	24	14	33	0	36	0	68
11716	Control	TMEM110	NM_198563	Silent	D269D	Novel	3	52849628	G>A	9.70	161	43	23	51	0	52	0	100
11258	Control	TMEM63A	NM_014698	Silent	A666A	Novel	1	224104309	G>A	25.32	228	22	29	98	0	110	0	100
11597	Control	TTC21B	NM_024753	Silent	P282P	Novel	2	166496562	T>A	19.00	228	191	93	300	0	284	0	100
Mutations in genes without identified mouse orthologs in Controls																		
13799	Control	C7orf58	NM_024913	Missense	H122R	Novel	7	120443070	A>G	-	228	83	79	127	0	147	0	100
11114	Control	DZANK1	NM_001099407	Missense	R225C	Novel	20	18372065	G>A	-	228	20	21	44	0	40	0	100
13095	Control	MOB3C	NM_145279	Missense	I173F	Novel	1	46848365	T>A	-	228	146	150	160	0	176	0	100
11622	Control	ZNF780B	NM_001005851	Missense	D78N	Novel	19	45245122	C>T	-	228	207	174	295	0	447	0	100
11654	Control	COL21A1	NM_030820	Missense	A747T	Novel	6	56033761	C>T	-	228	29	19	31	0	24	0	95
14259	Control	MIS18BP1	NM_018353	Missense	M696V	Novel	14	44763454	T>C	-	171	22	17	33	0	39	0	92
12020	Control	OR2M2	NM_001004688	Missense	Y319N	Novel	1	246410865	T>A	-	228	258	302	361	0	425	0	100
11352	Control	PRAMEF10	NM_001039361	Missense	R48H	Novel	1	12878123	C>T	-	102	4	4	34	0	119	0	52
12345	Control	ZNF320	NM_207333	Missense	C191Y	Novel	19	58076619	C>T	-	228	79	63	184	0	167	0	100
13867	Control	ZNF44	NM_001164276	Missense	A449T	Novel	19	12244869	C>T	-	228	31	38	106	0	104	1	100
13867	Control	IL29	NM_172140	Silent	T193T	Novel	19	44480972	G>A	-	228	105	88	202	0	244	0	100
13795	Control	MOGAT3	NM_178176	Silent	L7L	Novel	7	100630835	C>T	-	228	29	31	61	0	67	0	100
14147	Control	ZNF554	NM_001102651	Silent	E437E	Novel	19	2785544	A>G	-	228	46	38	134	0	109	0	100

Table S5. *De novo* mutations (damaging and missense at conserved positions) in CHD are enriched in genes that are more highly expressed in the developing heart at e14.5

			Total # *de novo* mutations		*De novo* mutations/subject		Odds Ratios Cases: Cont (95% CI) †	P-value ††
			CHD	Controls	CHD	Controls		
% of all genes	≥ *n* reads per million @ e14.5	# genes	362 trios	264 trios	362 trios	264 trios		
100%	0	16,676	101	63	0.279	0.239	1.48 (0.9-2.4)	0.34
50%	13	8,338	73	36	0.202	0.136	1.97 (1.1-3.6)	0.06
45%	17	7,504	71	30	0.196	0.114	2.16 (1.1-4.1)	0.01
40%	21	6,670	67	28	0.185	0.106	2.03 (1.0-4.0)	0.01
35%	26	5,837	63	24	0.174	0.091	2.35 (1.2-4.8)	0.006
30%	32	5,003	61	18	0.169	0.068	3.54 (1.6-7.7)	0.0004
25%	40	4,169	54	15	0.149	0.057	3.60 (1.6-8.3)	0.0005
20%	50	3,335	48	11	0.133	0.042	5.13 (2.0-12.9)	0.0002
15%	66	2,501	37	10	0.102	0.038	4.84 (1.8-13.2)	0.004
10%	93	1,668	24	6	0.066	0.023	4.73 (1.4-15.7)	0.02
5%	161	834	12	2	0.033	0.008	5.00 (0.7-33.8)	0.05

† The odds ratio is the ratio of protein-altering to silent variants in cases divided by the corresponding ratio in controls

†† *P*-values compare the number of variants in each category between cases and controls using a two-tailed binomial exact test

Genes were ranked for level of expression in heart at e14.5, and partitioned at successive percentiles (e.g. '25%' denotes the genes in the top quartile of expression). Genes in resulting groups were analyzed for burden of damaging or missense mutations at conserved positions in CHD cases and controls.

Table S6. Increased frequency of *de novo* mutations (damaging and missense at conserved positions) in CHD cases and controls stratified for gene expression in developing heart at e9.5

Cases vs. controls, High heart expressed genes at e9.5 (top 25%)	Total # of *de novo* mutations		*De novo* mutations/subject		Odds Ratio Cases:Cont (95% CI) †	P-value††
	CHD 362 trios	Controls 264 trios	CHD 362 trios	Controls 264 trios		
Silent	18	23	0.050	0.087	1.00 (0.42 - 2.39)	0.08
Nonconserved Missense	29	21	0.080	0.080	1.76 (0.77 - 4.06)	1.00
Silent and Protein Changing	93	58	0.257	0.220	2.05 (1.02 - 4.12)	0.37
All Protein Changing	75	35	0.207	0.133	2.74 (1.31 - 5.71)	0.03
Conserved Missense	32	12	0.088	0.045	3.41 (1.38 - 8.43)	0.05
Conserved and Damaging Protein Altering	46	14	0.127	0.053	4.20 (1.78 - 9.91)	0.004
Damaging	14	2	0.039	0.008	8.94 (1.80 - 44.52)	0.02

†The odds ratio is the ratio of protein-altering to silent variants in cases divided by the corresponding ratio in controls

††*P*-values compare the number of variants in each category between cases and controls using a two-tailed binomial exact test

Table S7. Comparisons of *de novo* mutation frequencies using RNA expression data at e14.5

a. Cases vs. controls, Low heart expressed genes (bottom 75%)	Total # of *de novo* mutations		*De novo* mutations/subject		Odds Ratio Cases:Cont (95% CI) †	P-value ††
	CHD 362 trios	Controls 264 trios	CHD 362 trios	Controls 264 trios		
Silent	44	39	0.12	0.15	1.00 (0.54-1.84)	0.38
Nonconserved Missense	103	70	0.28	0.27	1.30 (0.77-2.21)	0.70
Silent and Protein Changing	194	157	0.54	0.59	1.10 (0.68-1.77)	0.33
All Protein Changing	150	118	0.41	0.45	1.13 (0.69-1.85)	0.54
Conserved Missense	37	33	0.10	0.13	0.99 (0.53-1.88)	0.40
Conserved and Damaging Protein Altering	47	48	0.13	0.18	0.87 (0.48-1.56)	0.12
Damaging	10	15	0.03	0.06	0.59 (0.24-1.47)	0.10
b. High vs. Low heart-expressed genes in CHD cases	Total # of *de novo* mutations		*De novo* mutations/subject		Odds Ratio High:Low (95% CI) †	P-value ††
	High heart 4,169 genes	Low heart 12,507 genes	High heart 4,169 genes	Low heart 12,507 genes		
Silent	21	44	0.06	0.12	1.00 (0.48-2.09)	0.59
Nonconserved Missense	27	103	0.07	0.28	0.55 (0.28-1.07)	0.04
Silent and Protein Changing	102	194	0.28	0.54	1.10 (0.62-1.95)	0.05
All Protein Changing	81	150	0.22	0.41	1.13 (0.63-2.03)	0.05
Conserved Missense	39	37	0.11	0.1	2.21 (1.11-4.39)	6.40E-05
Conserved and Damaging Protein Altering	55	47	0.15	0.13	2.45 (1.28-4.69)	1.63E-07
Damaging	15	10	0.04	0.03	3.14 (1.21-8.16)	0.001
c. High vs. Low heart-expressed genes in Controls	Total # of *de novo* mutations		*De novo* mutations/subject		Odds Ratio High:Low (95% CI) †	P-value ††
	High heart 4,169 genes	Low heart 12,507 genes	High heart 4,169 genes	Low heart 12,507 genes		
Silent	21	39	0.08	0.15	1.00 (0.47-2.12)	0.32
Nonconserved Missense	17	70	0.06	0.27	0.45 (0.21-0.95)	0.06
Silent and Protein Changing	53	157	0.20	0.59	0.63 (0.34-1.16)	0.25
All Protein Changing	32	118	0.12	0.45	0.50 (0.26-0.97)	0.04
Conserved Missense	13	33	0.05	0.13	0.73 (0.32-1.68)	1.00
Conserved and Damaging Protein Altering	15	48	0.06	0.18	0.58 (0.26-1.27)	0.41
Damaging	2	15	0.01	0.06	0.25 (0.05-1.19)	0.18

† The odds ratio is the ratio of protein-altering to silent variants in high-heart genes divided by the corresponding ratio in low-heart genes for cases (a) or controls (b)

†† *P*-values compare the number of bases in each category between high-heart expressed genes and low-heart expressed genes using a two-tailed binomial exact test

Table S8. *De novo* mutations in CHD probands and controls stratified for gene expression in developing heart at e14.5; categorical analysis of the presence or absence of any *de novo* mutation in probands

a. Cases vs. controls, High heart expressed genes (top 25%)	Total # of subjects with ≥1 *de novo* mutations		Fraction of subjects with ≥1 *de novo* mutation		Odds Ratio Cases:Cont (95% CI)	P-value
	CHD 362 trios	Controls 264 trios	CHD 362 trios	Contr ls 264 trios		
Silent	20	21	0.052	0.080	0.68 (0.34-1.35)	0.25
Nonconserved Missense	27	17	0.075	0.064	1.17 (0.6-2.34)	0.75
Silent and Protein Changing	86	49	0.238	0.186	1.37 (0.91-2.07)	0.14
All Protein Changing	77	31	0.213	0.117	2.03 (1.27-3.3)	0.002
Conserved Missense	38	13	0.105	0.049	2.26 (1.15-4.73)	0.01
Conserved and Damaging Protein Altering	51	15	0.141	0.057	2.72 (1.46-5.33)	0.0006
Damaging	14	2	0.039	0.008	5.26 (1.19-48.08)	0.02
b. Cases vs. controls, Low heart expressed genes (bottom 75%)	Total # of subjects with ≥1 *de novo* mutations		Fraction of subjects with ≥1 *de novo* mutation		Odds Ratio Cases:Cont (95% CI)	P-value
	CHD 362 trios	Controls 264 trios	CHD 362 trio	Controls 264 trios		
Silent	43	34	0.119	0.129	0.91 (0.55-1.52)	0.71
Nonconserved Missense	85	61	0.235	0.231	1.02 (0.69-1.52)	0.92
Silent and Protein Changing	150	115	0.414	0.436	0.92 (0.66-1.28)	0.62
All Protein Changing	122	94	0.337	0.356	0.92 (0.65-1.3)	0.67
Conserved Missense	35	32	0.097	0.121	0.78 (0.45-1.34)	0.36
Conserved and Damaging Protein Altering	44	46	0.122	0.174	0.66 (0.41-1.05)	0.07
Damaging	10	15	0.028	0.057	0.47 (0.19-1.14)	0.10
c. High vs. Low heart-expressed genes, CHD cases	Total # of subjects with ≥1 *de novo* mutations		Fraction of subjects with ≥1 *de novo* mutation		Odds Ratio High:Low (95% CI)	P-value
	High heart 4,169 genes	Low heart 12,507 genes	High hear 4,169 gene	Low heart 12,507 genes		
Silent	20	43	0.052	0.163	1.13 (0.63-1.97)	0.68
Nonconserved Missense	27	85	0.075	0.322	0.77 (0.48-1.21)	0.30
Silent and Protein Changing	86	150	0.238	0.568	1.40 (1.06-1.83)	0.02
All Protein Changing	77	122	0.213	0.462	1.54 (1.14-2.06)	0.004
Conserved Missense	38	35	0.105	0.133	2.65 (1.63-4.31)	4.51×10^{-5}
Conserved and Damaging Protein Altering	51	44	0.141	0.167	2.83 (1.85-4.33)	6.33×10^{-7}
Damaging	14	10	0.039	0.038	3.41 (1.41-8.59)	0.003
d. High vs. Low heart-expressed genes, Controls	Total # of subjects with ≥1 *de novo* mutations		Fraction of subjects with ≥1 *de novo* mutation		Odds Ratio High:Low (95% CI)	P-value
	High heart 4,169 genes	Low heart 12,507 genes	High heart 4,169 genes	Low heart 12,507 genes		
Silent	21	34	0.058	0.129	1.51 (0.83-2.67)	0.14
Nonconserved Missense	17	61	0.047	0.231	0.68 (0.37-1.18)	0.17
Silent and Protein Changing	49	115	0.135	0.436	1.04 (0.73-1.46)	0.86
All Protein Changing	31	94	0.086	0.356	0.80 (0.52-1.22)	0.33
Conserved Missense	13	32	0.036	0.121	0.99 (0.48-1.94)	1.00
Conserved and Damaging Protein Altering	15	46	0.041	0.174	0.79 (0.41-1.45)	0.48
Damaging	2	15	0.006	0.057	0.33 (0.04-1.4)	0.18

The odds ratio is calculated from the ratio of cases with and without *de novo* mutations in each category in cases divided by the corresponding ratio in controls. The *P*-values compares the number of subjects with and without variants in a specific category between cases and controls using a two-tailed Fisher exact test

Table S9. Odds ratios in different disease classes

a. Cases vs. controls, High heart expressed genes (top 25%)	Total # of *de novo* mutations				*De novo* mutations/subject				Odds Ratio (95% CI) †		
	CTD	LVO	HTX	Controls	CTD	LVO	HTX	Controls	CTD	LVO	HTX
	154 trios	132 trios	70 trios	264 trios	154 trios	132 trios	70 trios	264 trios			
Silent	9	7	3	21	0.06	0.05	0.04	0.08	N/A	N/A	N/A
Nonconserved Missense	14	10	2	17	0.08	0.08	0.03	0.06	1.92 (0.67 - 5.51)	1.76 (0.55 - 5.6)	0.82 (0.12 - 5.5)
Silent and All Protein Changing	42	46	11	53	0.27	0.35	0.16	0.20	N/A	N/A	N/A
All Protein Changing	33	39	8	32	0.21	0.30	0.11	0.12	2.41 (0.96 - 6.04)	3.66 (1.38 - 9.7)	1.75 (0.4 - 7.36)
Conserved Missense	13	22	4	13	0.08	0.17	0.06	0.05	2.33 (0.78 - 6.98)	5.08 (1.7 - 15.2)	2.15 (0.4 - 11.2)
Conserved and Damaging Protein Altering	20	29	6	15	0.13	0.22	0.09	0.06	3.11 (1.11 - 8.7)	5.80 (2 - 16.7)	2.80 (0.6 - 13)
Damaging	6	7	2	2	0.05	0.05	0.03	0.01	7.00 (1.2 - 41.54)	10.50 (1.8 - 62.8)	7.00 (0.7 - 70.1)

b. Cases vs. controls, Low heart expressed genes (bottom 75%)	Total # of *de novo* mutations				*De novo* mutations/subject				Odds Ratio (95% CI) †		
	CTD	LVO	HTX	Controls	CTD	LVO	HTX	Controls	CTD	LVO	HTX
	154 trios	132 trios	70 trios	264 trios	154 trios	132 trios	70 trios	264 trios			
Silent	15	20	9	39	0.10	0.15	0.13	0.15	N/A	N/A	N/A
Nonconserved Missense	42	33	25	70	0.27	0.25	0.36	0.27	1.56 (0.8 - 3.2)	0.92 (0.47 - 1.8)	1.55 (0.66 - 3.65)
Silent and All Protein Changing	80	67	44	157	0.52	0.51	0.63	0.59	N/A	N/A	N/A
All Protein Changing	65	47	35	118	0.42	0.36	0.50	0.45	1.43 (0.7 - 2.79)	0.78 (0.4 - 1.47)	1.29 (0.57 - 2.91)
Conserved Missense	20	11	6	33	0.13	0.08	0.09	0.13	1.58 (0.7 - 3.56)	0.65 (0.27 - 1.6)	0.79 (0.25 - 2.44)
Conserved and Damaging Protein Altering	23	14	10	48	0.15	0.11	0.14	0.18	1.25 (0.57 - 2.7)	0.57 (0.3 - 1.27)	0.90 (0.33 - 2.44)
Damaging	3	3	4	15	0.02	0.02	0.06	0.06	0.52 (0.1 - 2.06)	0.39 (0.1 - 1.51)	1.16 (0.31 - 4.32)

† The odds ratio is the ratio of protein-altering to silent variants in each disease class divided by the corresponding ratio in controls

Abbreviations: *CTD: Conotruncal defects, LVO: Left ventricular obstruction, HTX: Heterotaxy*

Table S10. Chromatin modifying and other genes of interest with *de novo* mutations in CHD probands

ID	Gene	Heart Exp[†]	Mutation	Primary Classification: Specific Cardiovascular Diagnoses[§]	Extracardiac Structural Anomalies	Neuro-Develop-mental	Somatic Growth	
							Ht(%)	Wt(%)
1-00596	*MLL2*	216	p.Ser1722 Argfs*9	LVO: Mitral atresia, HLHS, aortic atresia, dbl AA	Epicanthal folds, telecanthus, large low-set ears, excess nuchal skin, high arched palate, wide-spaced nipples, undescended testes, club foot, hyperpigmented lesions,	motor delay, hypotonia	50	<5
1-00853	*WDR5*	39	p.Lys7Gln	CTD: TOF, right aortic arch, aberrant LSA, coronary abnormality	No	abnormal	90	90
1-00534	*CHD7*	125	p.Gln1599*	CTD: TOF-PA	Cleft lip, cleft palate, inguinal hernia, micropenis, sensorineural hearing loss	abnormal	<5	<5
1-00230	*KDM5A*	70	p.Arg1508Trp	LVO: LSVC, Primum ASD, cleft MV, sub-AS, BAV	No	normal	<5	<5
1-01965	*KDM5B*	68	p.IVS12+1 G>A	LVO: Coarctation	No	n/a	<5	<5
1-01907	*UBE2B*	146	p.Arg8Thr	CTD: TOF	No	normal	50	10
1-00075	*RNF20*	58	p.Gln83*	HTX: Dextrocardia, RAI, TAPVR, L-ventricular loop, CAVC unbalanced-right dominant, PA	Low-set ears,excess nuchal skin, hydronephrosis, wide-spaced 2nd toe, Asplenia, primary cilia dyskinesia	abnormal	<5	<5
1-01260	*USP44*	0	p.Glu71Asp	LVO: ASD, mitral atresia, aortic atresia, HLHS	No	normal	25	25
1-02020	*SMAD2*	38	p.IVS6+1 G>A	HTX: Dextrocardia, ASD, CAVC-unbalanced, DORV, D-TGA, PS	Asplenia	normal	95	10
1-02621	*SMAD2*	38	p.Trp244Cys	HTX: Dextrocardia, LSVC to LA, PAPVR, CAVC unbalanced-right dominant, DORV, PS	Abnormal nose, foot syndactyly, malrotation	n/a	50	50
1-01451	*MED20*	25	p.IVS2+2 T>C	HTX: Dextrocardia, PAPVR, mitral atresia, HLHS, aortic atresia, hypoplastic AA	No	abnormal	<5	10
1-01151	*SUV420H1*	44	p.Arg143Cys	CTD: dbl AA	No	abnormal	50	10
1-00750	*HUWE1*	260	p.Arg3219Cys	LVO: Mitral stenosis, aortic stenosis, HLHS	No	mild abnormal	25	25
1-00577	*CUL3*	57	p.Iso144Phe fs*23	LVO: Hypoplastic mitral valve, hypoplastic aortic annulus, aortic stenosis, coarctation	Congenital hip dysplasia, congenital scoliosis	n/a	12	60
1-00116	*NUB1*	45	p.Asp310His	CTD: LSVC, sinus venosus ASD, truncus arteriosus, VSD-muscular	Spine lipoma	abnormal	<5	5
1-01828	*DAPK3*	55	p.Pro193Leu	CTD: TOF	No	n/a	n/a	n/a
1-03151	*SUPT5H*	133	p.Glu451Asp	LVO: BAV, aortic stenosis	No	n/a	75	90
1-00455	*NAA15*	214	p.Lys335Lys fs*6	HTX: Dextrocardia, TAPVR, LSVC, hypoplastic TV, DORV, hypoplastic RV, D-TGA, PS	Hydronephrosis, asplenia, malrotation	normal	50	50
1-00141	*NAA15*	214	p.Ser761*	CTD: TOF, single LCA	No	n/a	<5	20
1-01138	*USP34*	65	p.Leu432Pro	LVO: supra MS, BAV, CoA	No	n/a	25	>95
1-00448	*NF1*	55	p.IVS6+4 del A	CTD: PA VSD-MAPCAs	No	n/a	5	5
1-00802	*PTCH1*	32	p.Arg831Gln	LVO: HLHS	No	n/a	50	50
1-02458	*SOS1*	28	p.Thr266Lys	Other: ASD (multiple), dysplastic mitral, tricuspid and pulmonic valves	Macrocephaly, dolichocephaly,low-set ears, hyperextensible fingers, foot syndactyly, café-au-lait spots	abnormal	<5	5
1-02952	*PITX2*	18	p.Ala47Val	LVO: CoA	No	n/a	75	>95
1-01913	*RAB10*	119	p.Asn112Ser	Other: DILV, D-TGA, BAV, CoA	No	n/a	30	95

[†]Heart expression refers to # reads per million at murine e14.5. Mutation denotes the impact on encoded protein in three letter code; * denotes termination mutation. *Frameshift* mutation in *MLL2*, *CUL3 and NAA15*. 'IVS' stands for intervening sequence. 'fs' stands for frameshift. *Splice site* mutation in *KDM5B, MED20*, and *SMAD2* occur at 1st base of canonical splice donor of intron 12, at 2nd base of canonical splice donor of intron 2 and 1st base of canonical splice donor of intron 6 respectively.

[§]HLHS-hypoplastic left heart syndrome; Dbl AA-double aortic arch; TOF-tetralogy of Fallot; PAPVR-partial anomalous pulmonary venous return; LSVC-left superior vena cava; LA-left atrium; CAVC-complete atrioventricular canal defect; TAPVR- total anomalous pulmonary venous return; MV-mitral valve; BAV-bicuspid aortic valve; ASD-atrial septal defect; VSD- ventricular septal defect; PA-pulmonary atresia; RAI- right atrial isomerization.

Table S11. Genes with > 1 de novo mutation in CHD probands

ID	Gene	Heart Exp[†]	Mutation	Primary Classification: Specific Cardiovascular Diagnoses[§]	Extracardiac Anomalies	Neuro-Developmental Abnormal	Somatic Growth	
							HT(%)	Wt(%)
1-00455	*NAA15*	214	p.Lys335Lys fs*6	HTX: Dextrocardia, TAPVR, LSVC, hypoplastic TV, DORV, hypoplastic RV, D-TGA, PS	Hydronephrosis, asplenia, malrotation	Yes	50	50
1-00141	*NAA15*	214	p.Ser761*	CTD: TOF, single LCA	No	n/a	<5	20
1-02020	*SMAD2*	38	p.IVS6+1 G>A	HTX: Dextrocardia, ASD, CAVC-unbalanced, DORV, D-TGA, PS	Asplenia	No	95	10
1-02621	*SMAD2*	38	p.Trp244Cys	HTX: Dextrocardia, LSVC to LA, PAPVR, CAVC unbalanced-right dominant, DORV, PS	Abnormal nose, foot syndactyly, malrotation	n/a	50	50
1-02121	*DST*	122	p.Gly2936Asp	CTD: Right aortic arch-abn branching, vascular ring	Absent right kidney, dysplastic left kidney	Yes	<5	<5
1-02394	*DST*	122	p.Lys2653Ile	LVO: Coarctation	No	Yes	75	50
1-01538	*MKRN2*	19	p.Ala251Val	HTX: Mesocardia, atrial situs inversus, VSD-malalignment, D-TGA, PA	Abdominal situs inversus	No	50	50
1-00230	*MKRN2*	19	p.Arg50Trp	LVO: LSVC, primum ASD, cleft MV, sub-AS, BAV	No	No	<5	<5
1-01664	*OBSCN*	298	p.Phe5295Ser	HTX: Dextrocardia, LSVC, hypoplastic MV, sub AS, BAV, congenital coronary abnormality, coarctation	No	n/a	25	10
1-03190	*OBSCN*	298	p.Thr4421Met	CTD: TOF-PA with MAPCAs	No	No	75	75
1-01341	*UMODL1*	0	p.Val1090Met	CTD: TOF, ASD, LPA stenosis	No	No	n/a	n/a
1-02408	*UMODL1*	0	p.Lys1251Glu	HTX: Dextrocardia, mitral atresia, PA, L-TGA	Epicanthic folds, flat nasal bridge, short neck	No	10	10

[†]Heart expression refers to # reads per million in murine hearts at e14.5. Mutation denotes the impact on encoded protein in single letter code; * denotes termination mutation. *'*IVS*'* stands for intervening sequence. 'fs' stands for frameshift. *Frameshift* mutation in *NAA15*. *Splice site mutation* in *SMAD2* occurs at 1st base of cannonical splice donor of intron 6.

[§]LSVC-left superior vena cava; LA-left atrium; PAPVR-partial anomalous pulmonary venous return; CAVC-complete atrioventricular canal defect; TAPVR- total anomalous pulmonary venous return; ASD-atrial septal defect; DORV-double outlet right ventricle; D-TGA- dextro transposition of great arteries; PS-pulmonic stenosis; MV-mitral valve; BAV-bicuspid aortic valve; TOF-tetralogy of Fallot; LCA-left coronary artery, VSD- ventricular septal defect; LPA stenosis- late left pulmonary artery stenosis .

Table S12. Candidate genes for LVO, CTD and HTX

ACP6	CCDC40	FBN2	IGFBP4	MYH11	PPP3CA	SUPT3H
ACTA2	CCT4	FGF8	IGFBP5	MYH6	PQPB1	TBX1
ACTC1	CDH2	FGFR1	IHH	NEK2	PRKAB2	TBX20
ACVR1	CER1	FIBP1	INVERSIN	NF1	PROX1	TBX3
ACVR2B	CFC1	FLNA	IPPK	NFATC1	PTCH1	TBX5
AHSA2	CHD1L	FMO5	ISL1	NFATC3	PTCH2	TCF21
AKAP9	CHRAC1	FOXA2	JAG1	NFATC4	PTPN11	TDGF1
ANKRD1	CHRD	FOXC1	JAZF1	NIPBL	RAB10	TFAP2B
APOBEC2	CITED2	FOXH1	KCNE1	NKD1	RAB23	TGFBR2
ARL13B	CLDN7	FOXJ1	KCNJ2	NKX2-5	RAI1	TLL1
ASXL2	CLUL1	FOXL2	KCNQ1	NKX2-6	RAI2	TMBIM4
ATE1	CREBBP	FTO	KIAA1841	NKX2.5	RAPGEF5	TMEM195
ATP1A2	CRELD1	GADL1	KIF3A	NKX3-2	REL	TNFRSF21
ATP4A	CRHBP	GALNT11	KIF3B	NODAL	RFX2	TSC1
ATP4B	CRX	GATA4	KIF3C	NOTCH1	RFX3	TSEN15
BBS1	CSRP1	GATA5	KIFAP3	NOTCH2	ROCK2	TTC21B
BBS10	CTNNA3	GATA6	KLF13	NOTCH2NL	ROR2	TTC30A
BBS11	DAND5	GDF1	LBR	NOTCH3	ROTATIN	TWIST1
BBS12	DHCR7	GJA1	LEFTY1	NOTCH4	RPGRIP1L	TXNDC3
BBS2	DLL1	GJA5	LEFTY2	NOTO	RUNX2	UBR1
BBS3	DMRT2	GJA8	LEMD3	NPHP3	S100Z	USP34
BBS4	DNAH2	GJA9	LLPH	NPPA	SALL1	VANGL2
BBS5	DNAH5	GPC3	LPIN1	NSD1	SALL4	VEGFA
BBS6	DNAI1	GPR161	LRP2	NUMBL	SDC2	VEGFC
BBS7	DNAI2	GPRC6A	LRRC50	NUP188	SEMA3E	VIT
BBS8	DOLK	GSK3B	LRRC6	OFD1	SESN1	WNT3A
BBS9	DPPA4	HAND1	MARK2	OSR1	SHH	XPO1
BCL11A	DQ983818	HAND2	MAX	PAPOLG	SHOC2	ZAC1
BCL6	DVL1	HES1	MED13L	PCMTD2	SIL	ZEB2
BCL9	DVL2	HES4	MEF2A	PCNT	SLC2A10	ZFPM1
BCOR	DZIP1	HEY2	MEF2C	PCSK5	SMAD2	ZIC3
BICC1	EED	HOXA1	METT10D	PEX1	SMAD5	ZNF480
BMP4	EHMT1	ID2	MGAT1	PEX13	SMARCD3	ZNF528
BMP7	ELN	IER2	MGP	PHYHD1	SMO	ZNF534
BMPR1A	EP300	IFT122	MID1	PIFO	SNAI1	ZNF610
BMPR1B	ESCO2	IFT172	MKKS	PITX2	SOS1	ZNF638
BMPR2	EVC	IFT20	MKS1	PKD1L1	SOX17	ZNHIT3
BUB1B	EVC2	IFT57	MNDA	PKD2	SRF	
C1orf106	EZH1	IFT88	MSX1	PLAGL1	STIL	
CCDC39	FBN1	MYH10	MSX2	PPM1K	SUFU	

Table S13. *De novo* mutations in CHD cohort that occur in candidate gene set

CHD Cohort	Gene	AA Change
1-00638	FBN2	p.Asp2191Asn
1-00596	MLL2	p.Ser1722Argfs*9
1-00534	CHD7	p.Gln1599*
1-01913	RAB10	p.Asn112Ser
1-00197	BCL9	p.Met1395Lys
1-01138	USP34	p.Leu432Pro
1-00448	NF1	p.IVS6+4 delA
1-02020	SMAD2	p.IVS6+1 G>A
1-02621	SMAD2	p.Trp224Cys
1-00802	PTCH1	p.Arg831Gln
1-02458	SOS1	p.Thr266Lys
1-02952	PITX2	p.Ala47Val
1-02598	LRP2	p.Glu4372Lys

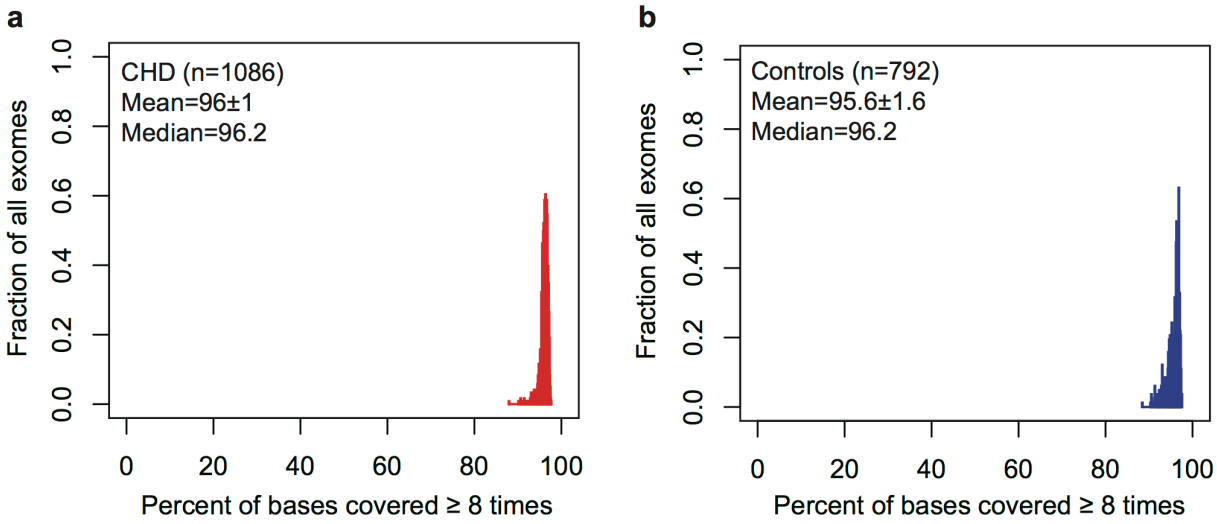

Figure S1. Distribution of 8X read coverage between cases and controls. Percent of bases covered ≥8 times plotted for CHD cases (a) and controls (b). Distribution, mean and median are highly concordant.

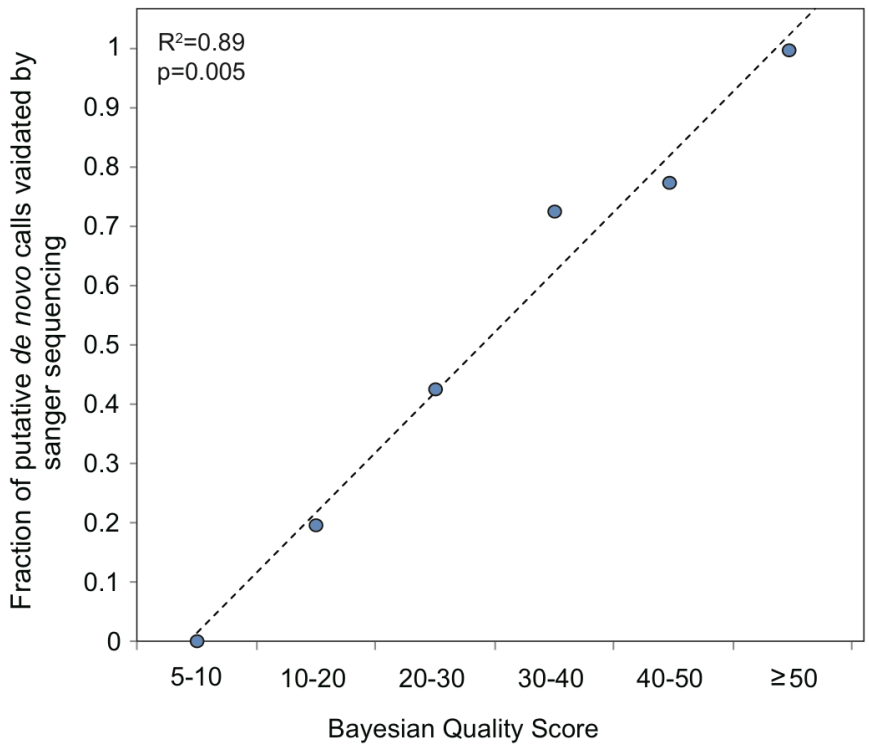

Figure S2. Correlation of Bayesian quality score and probability of Sanger validation. A strong correlation between the fraction of putative *de novo* variants and specific ranges of Bayesian quality scores, R^2=0.89. Notably, Sanger sequencing validated a subset (88) of all *de novo* calls with a Bayesian quality score ≥50, with a specificity of 100%.

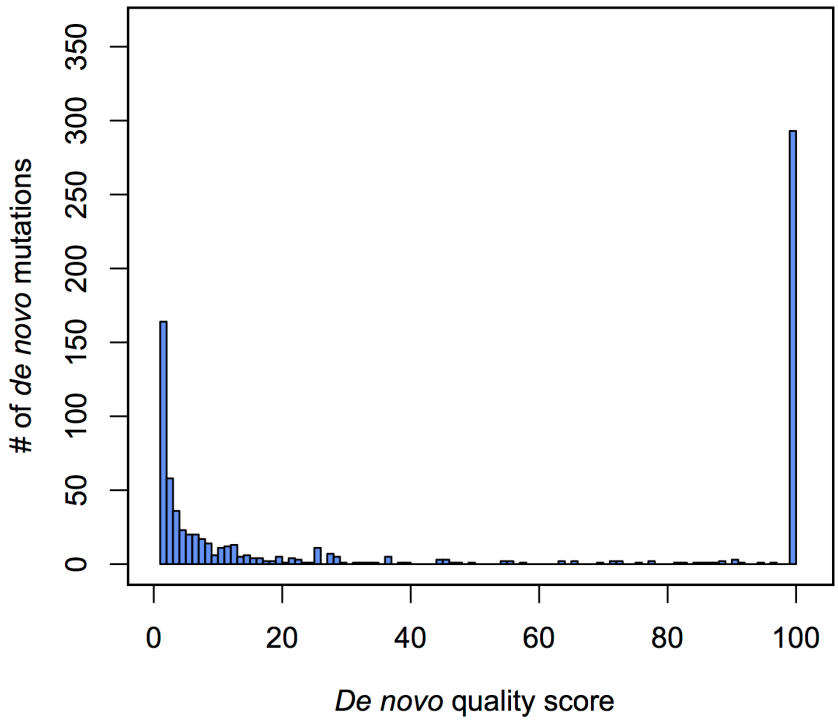

Figure S3. Distribution of *de novo* mutation quality scores. The frequency of potential *de novo* mutations in different bins of *de novo* quality scores are shown. 100% of variants with scores ≥ 50 confirmed as *de novo* mutations by Sanger sequencing. Of these, ~90% had the maximum QS of 100. (see Supplementary Table 3 and Supplementary Figure 2).

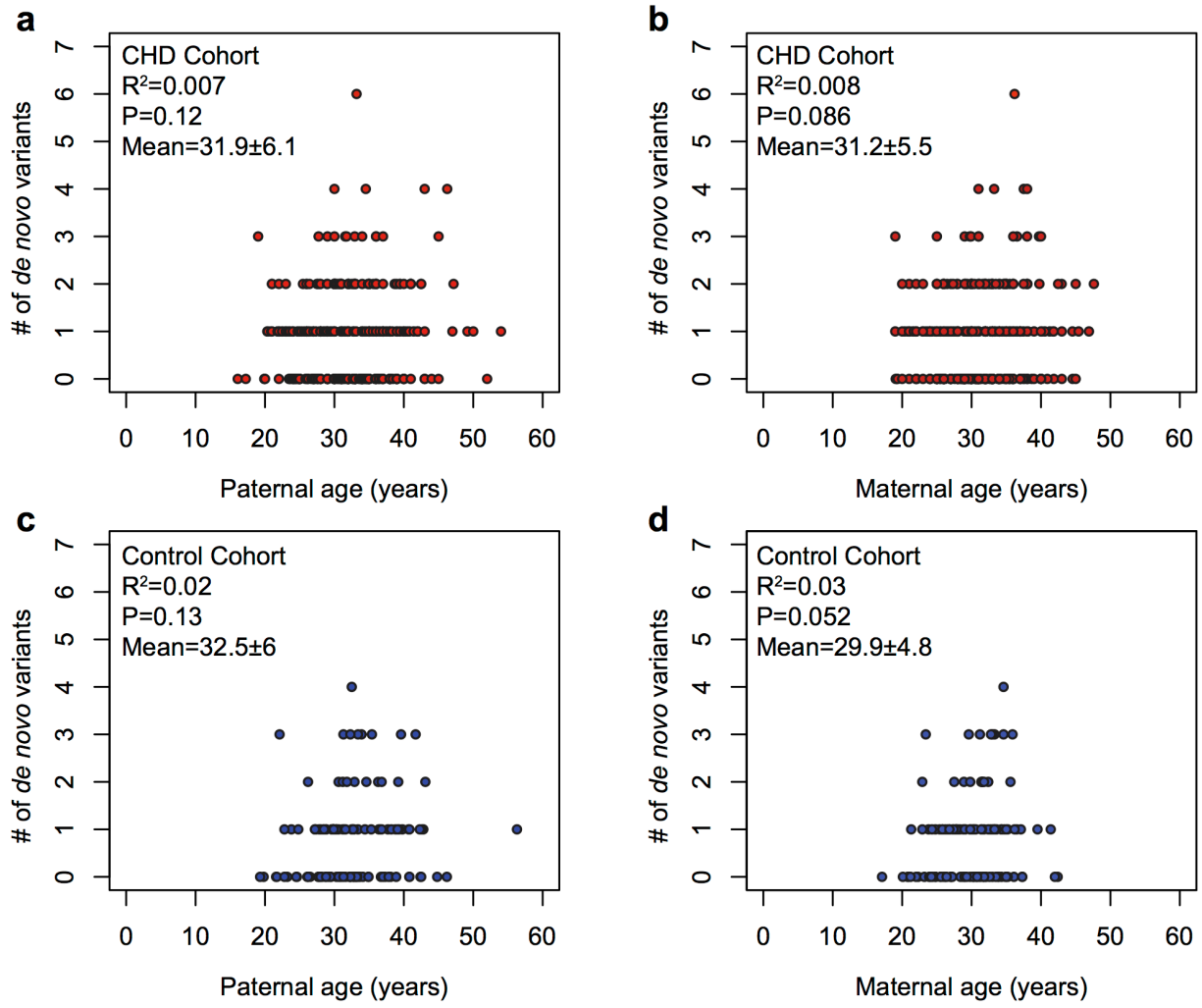

Figure S4. Correlation of paternal and maternal age with *de novo* mutation rate. Weak correlation between paternal (a,c) or maternal (b,d) age and number of *de novo* mutations per subject in the CHD (a,b) or control (c,d) cohorts.

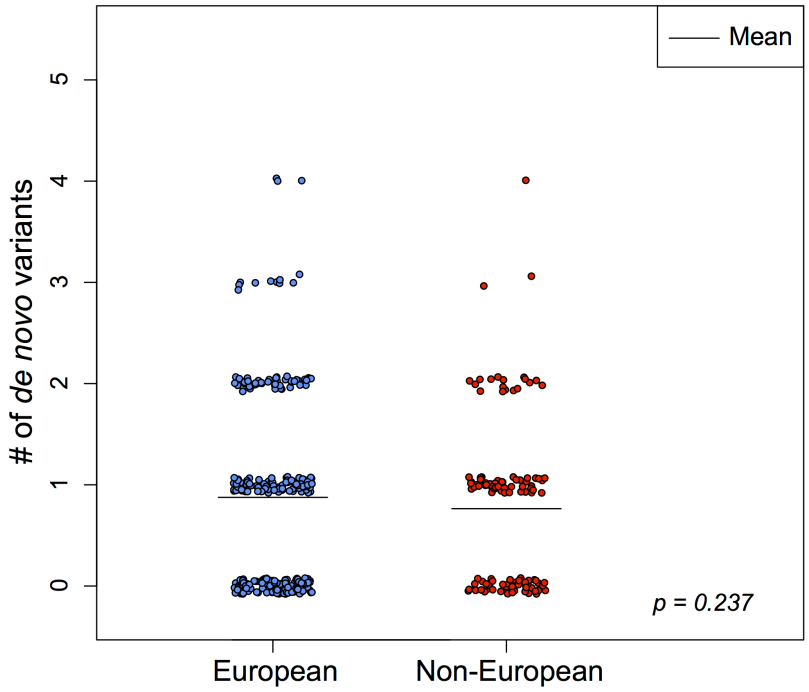

Figure S5. Effect of ancestry on *de novo* mutation rate. No significant difference in *de novo* mutation rate between European and Non-European (Indian, Mexican, African-American, and East Asian) ancestries, p=0.24.

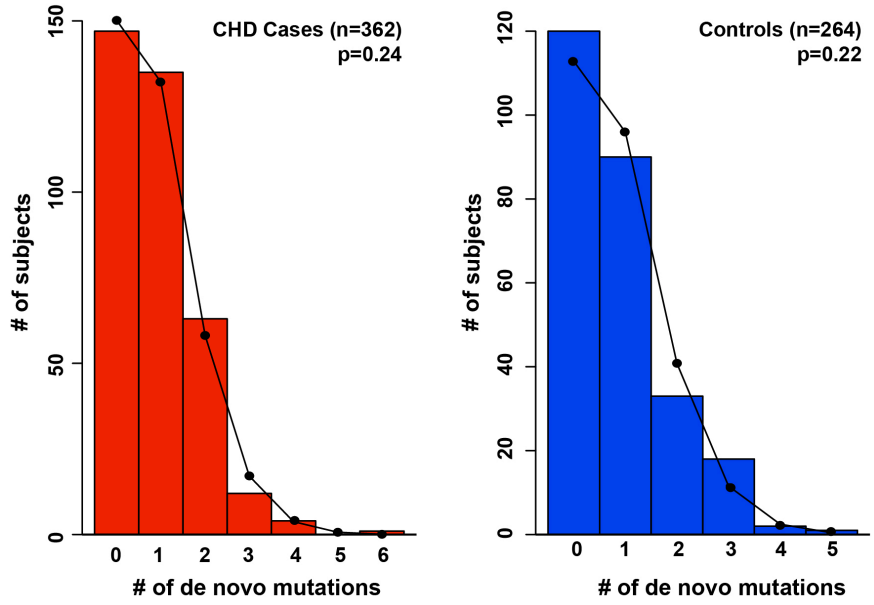

Figure S6. *De novo* mutation rate closely approximates Poisson distribution in CHD cases and controls. Observed number of *de novo* mutations per subject (bars) compared to the numbers expected (line) from the Poisson distribution in CHD (red) and control (blue) cohorts. ‘p' denotes Chi-squared value.

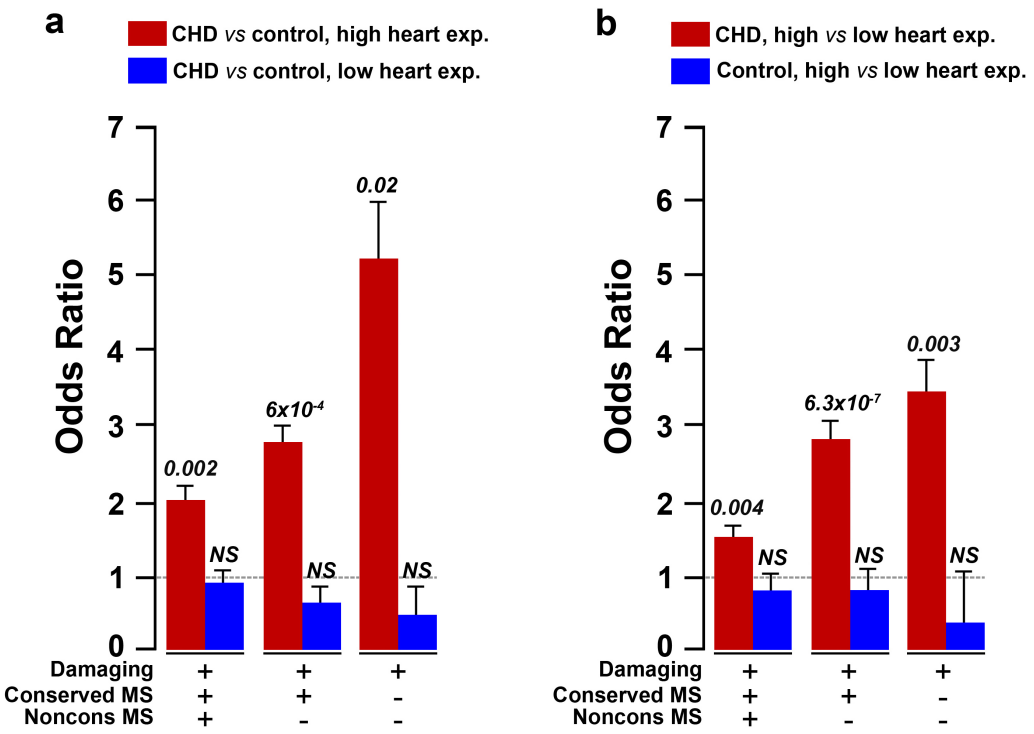

Figure S7. *De novo* mutations in CHD probands and controls stratified for gene expression in developing heart; categorical analysis of the presence or absence of any *de novo* mutation in probands a, Odds ratios (ORs) comparing fraction of patients with and without *de novo* mutations of indicated classes in CHD cases and controls for genes in top quartile (red bars) and bottom 75% (blue bars) of expression in developing heart. **b,** ORs comparing the presence of *de novo* mutations in genes in top quartile versus bottom 75% of expression in developing heart in CHD cases (red bars) and controls (blue bars). *De novo* mutations are classified as missense mutations at poorly conserved positions (noncons MS), missense mutations at highly conserved positions (cons MS), and damaging (nonsense, splice site, or frameshift mutations). Odds ratio + SEM is shown and significance of the difference between groups is indicated (*P* values from two-tailed Fisher exact test). Odds ratio compares the proportion of # of individuals with *de novo* mutations to the # of individuals without a *de novo mutation* in each specific category. NS, not significant.

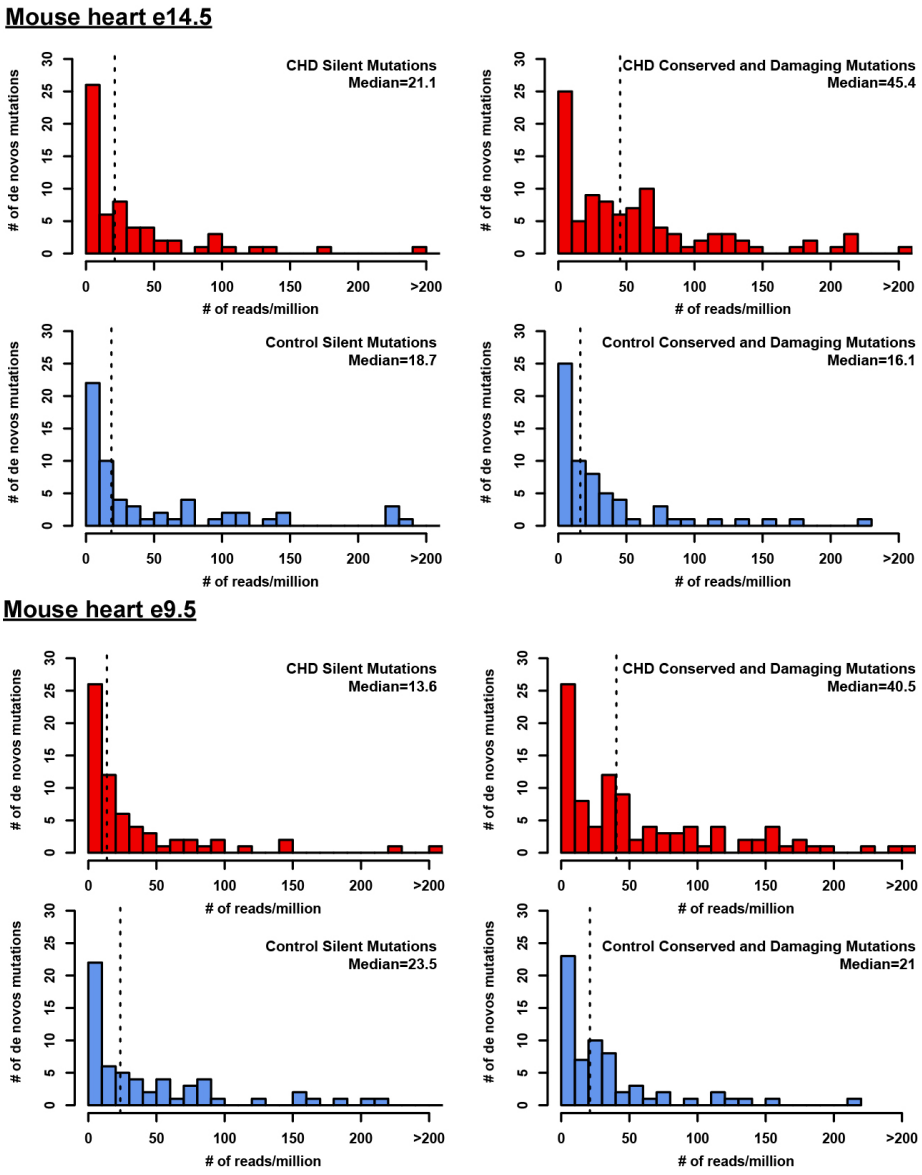

Figure S8. Expression of genes mutated in cases and controls. Expression levels (# of reads per million at e14.5 and e9.5) are shown for silent (a,c) and damaging and conserved (b,d) *de novo* mutations for cases (a,b) and controls (c,d). Vertical dashed lines indicate median values in each category. Conserved and damaging *de novo* mutations show higher expression in CHD cases than controls (P=5x10^{-4} at e14.5 and P= 1.6 x10^{-3} at e9.5), while silent mutations show no significant difference (P=0.7 at e14.5 and P=0.5 at e9.5) (comparison via Wilcoxon signed-ranked test).

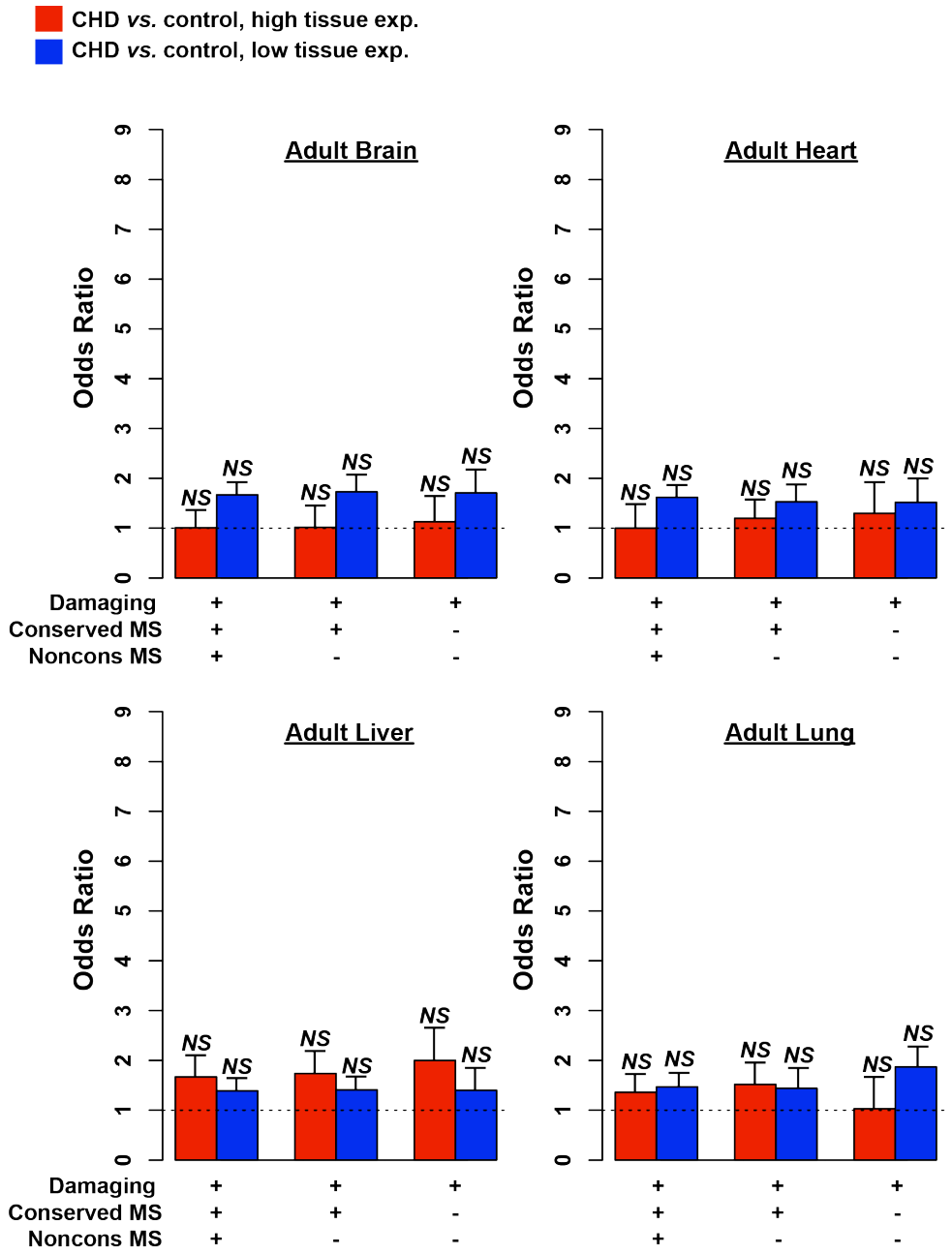

Figure S9. *De novo* mutations in CHD cases and controls stratified for gene expression in adult tissues. Odds ratios (ORs) comparing incidence of indicated classes of *de novo* mutations in CHD cases and controls for genes in top quartile (red bars) and bottom 75% (blue bars) of expression in different adult tissues. Odds ratio + SEM is shown and significance of the difference between groups is indicated (*P* values calculated from two-tailed binomial exact test). The odds ratio is the ratio of protein altering to silent variants in cases divided by the corresponding ratio in controls. NS, not significant.