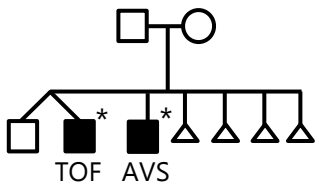
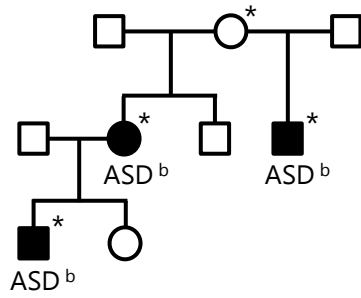


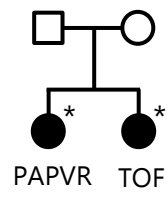
Family 49



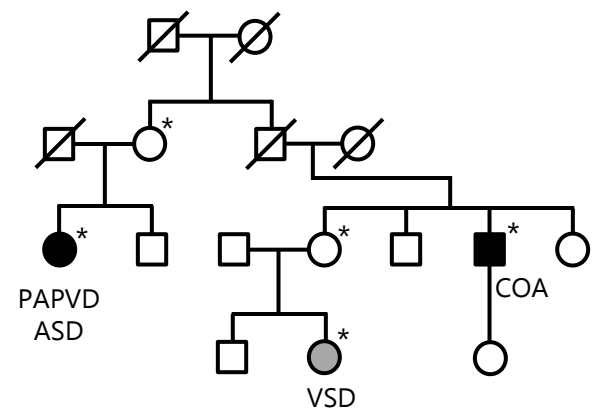
Family 62



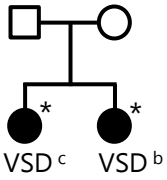
Family 226



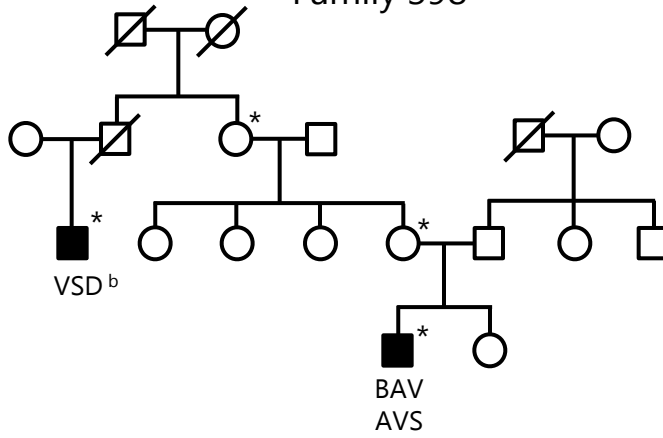
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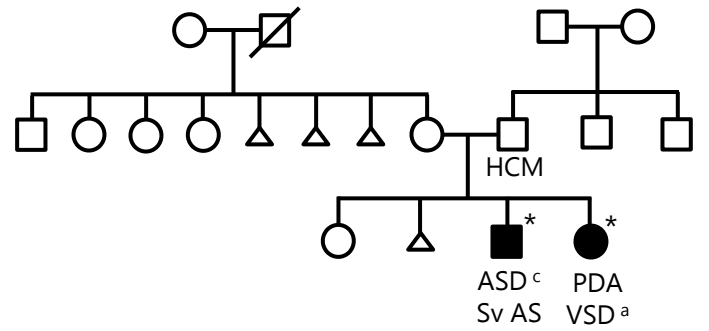
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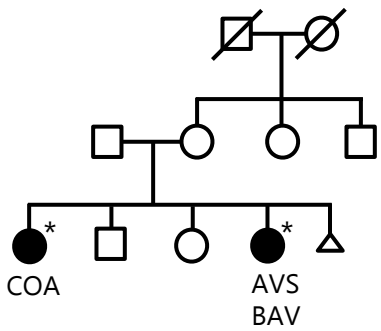
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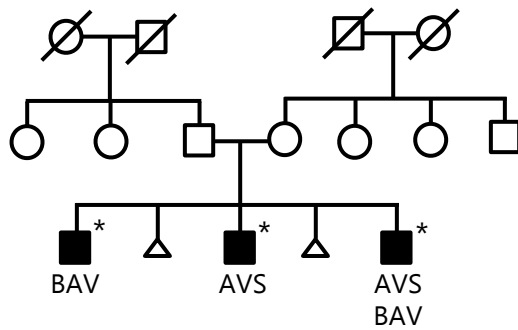
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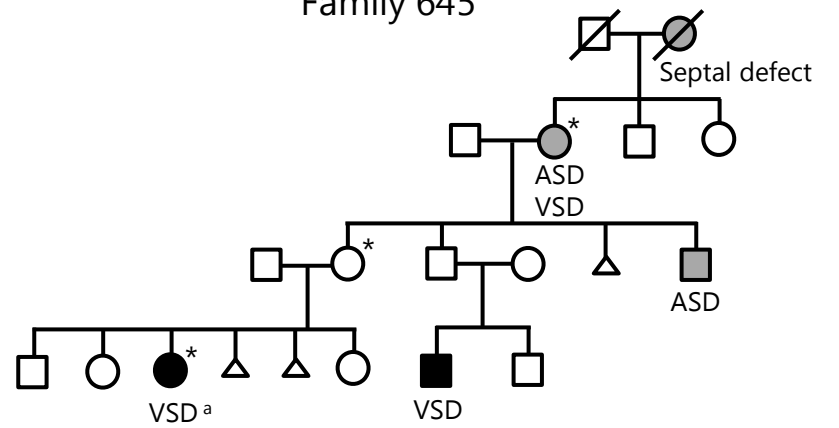
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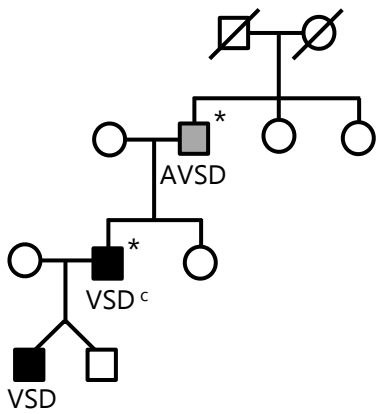
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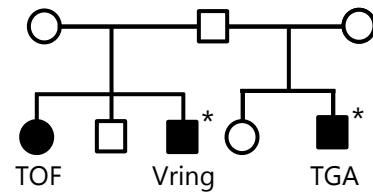
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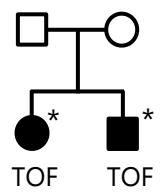
Family 702



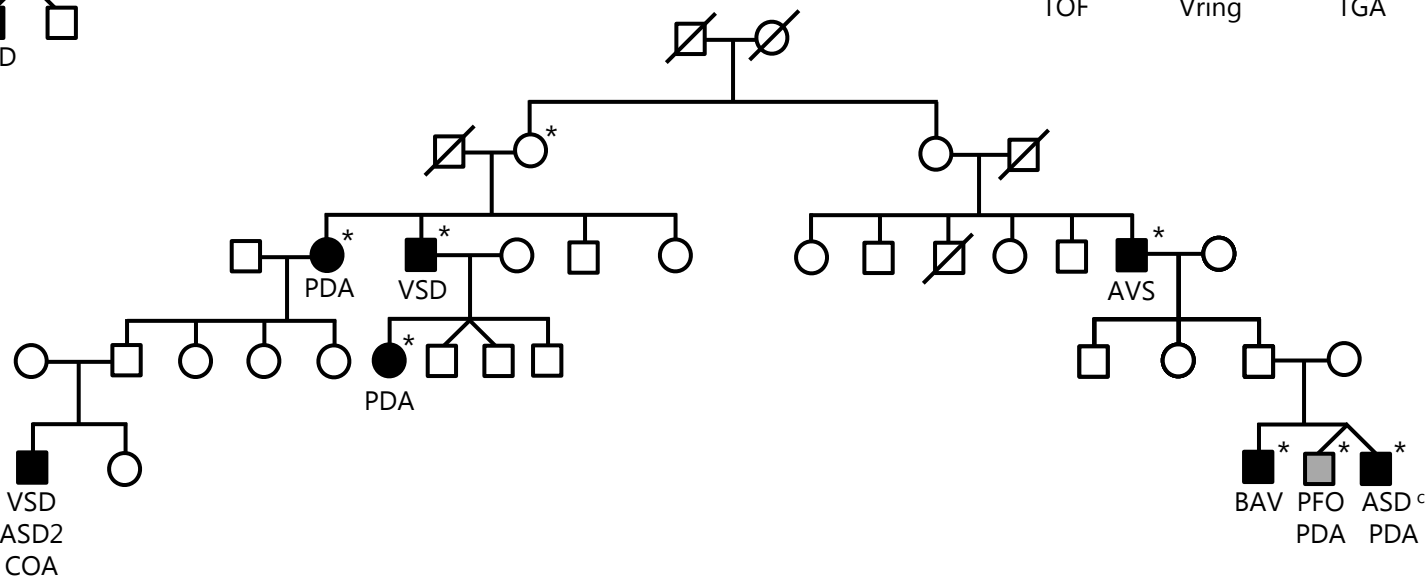
Family 720



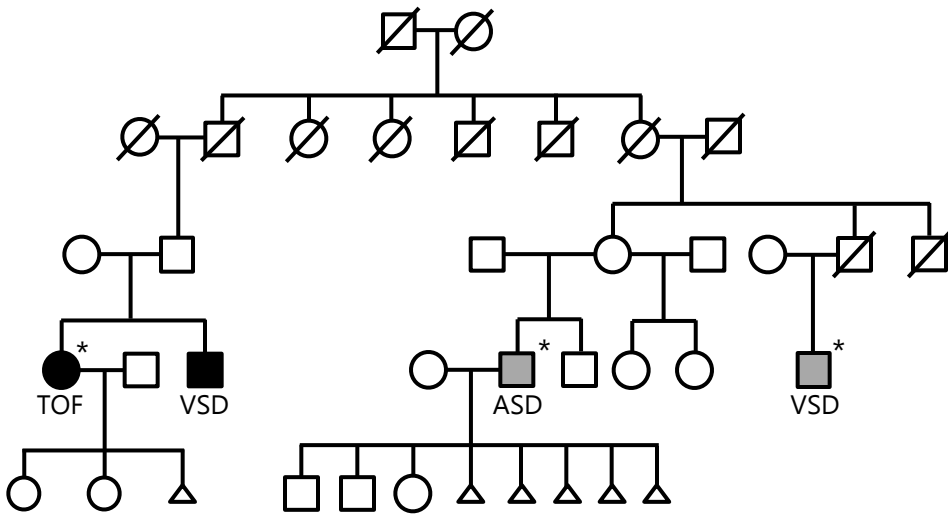
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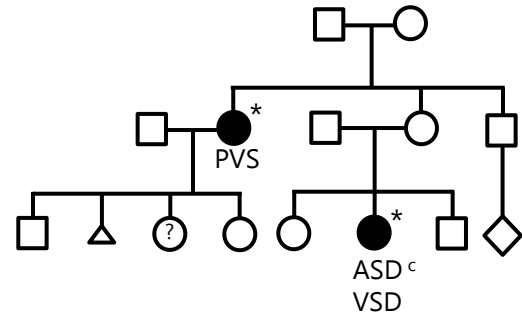
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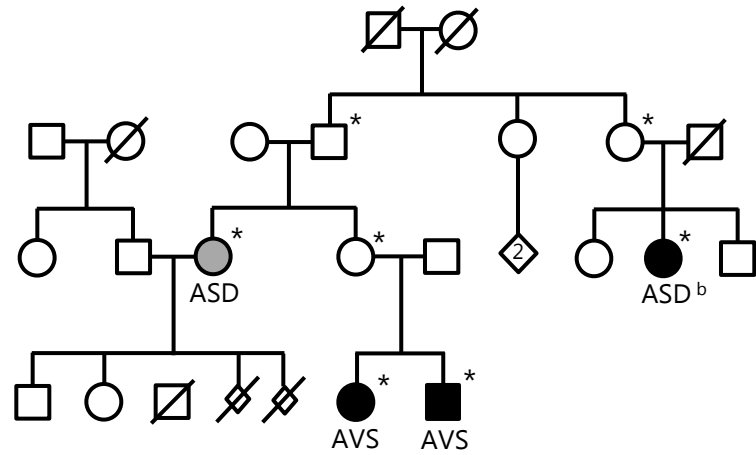
Family 1117



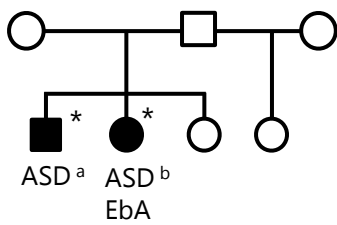
Family 1121



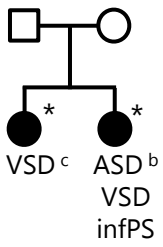
Family 1319



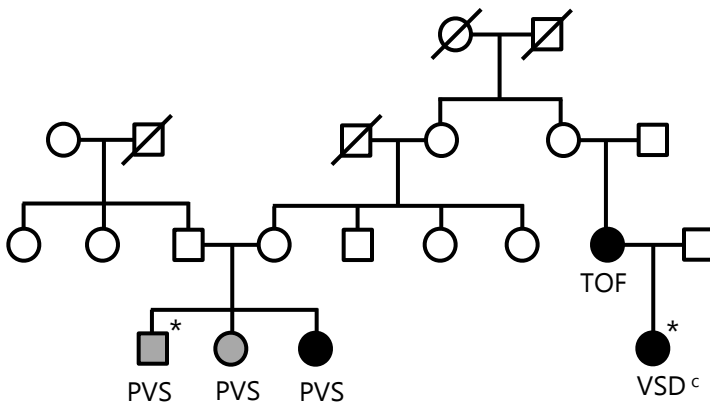
Family 1151



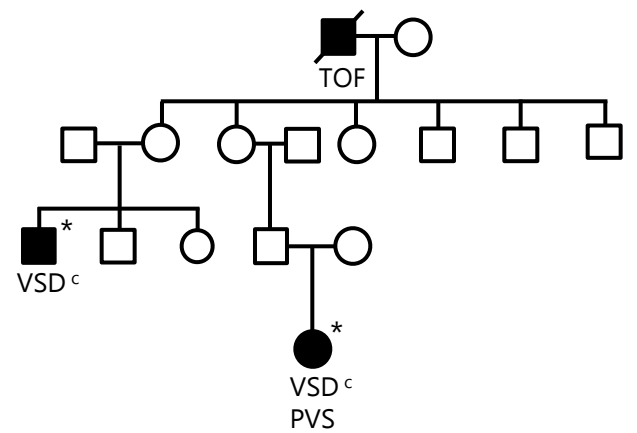
Family 1364



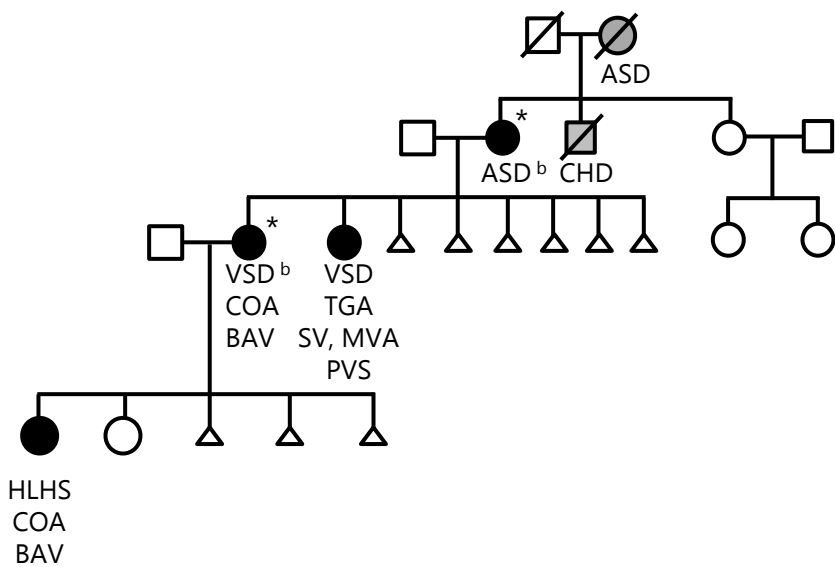
Family 1560



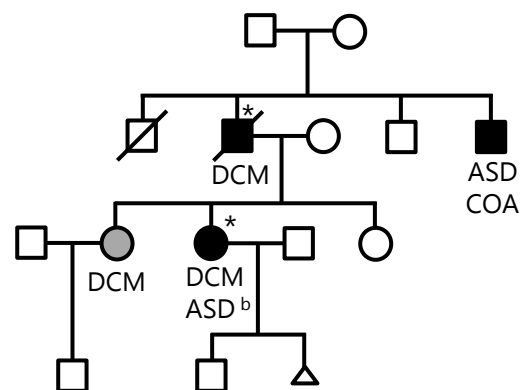
Family 1575



Family 1710



Family 1722



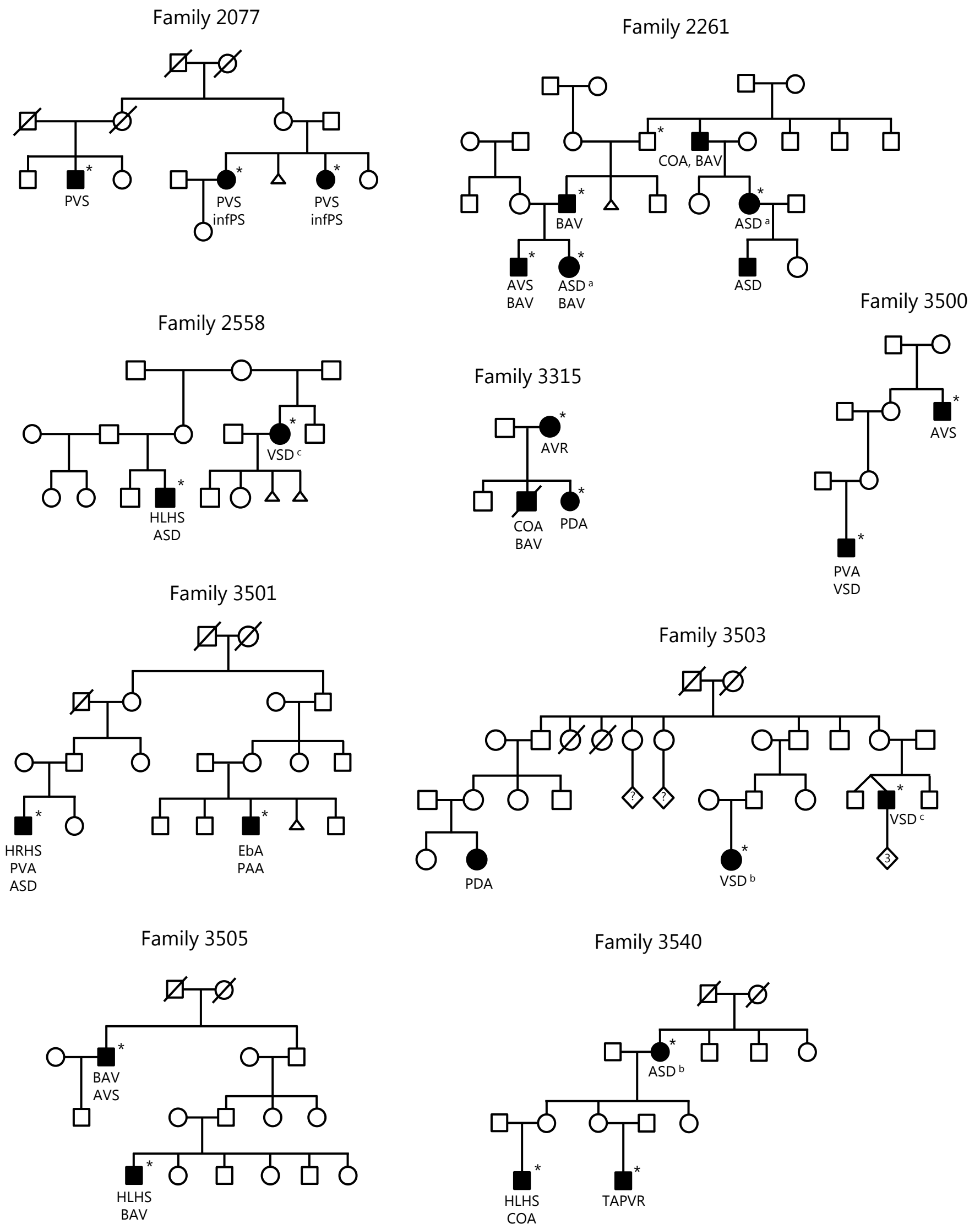
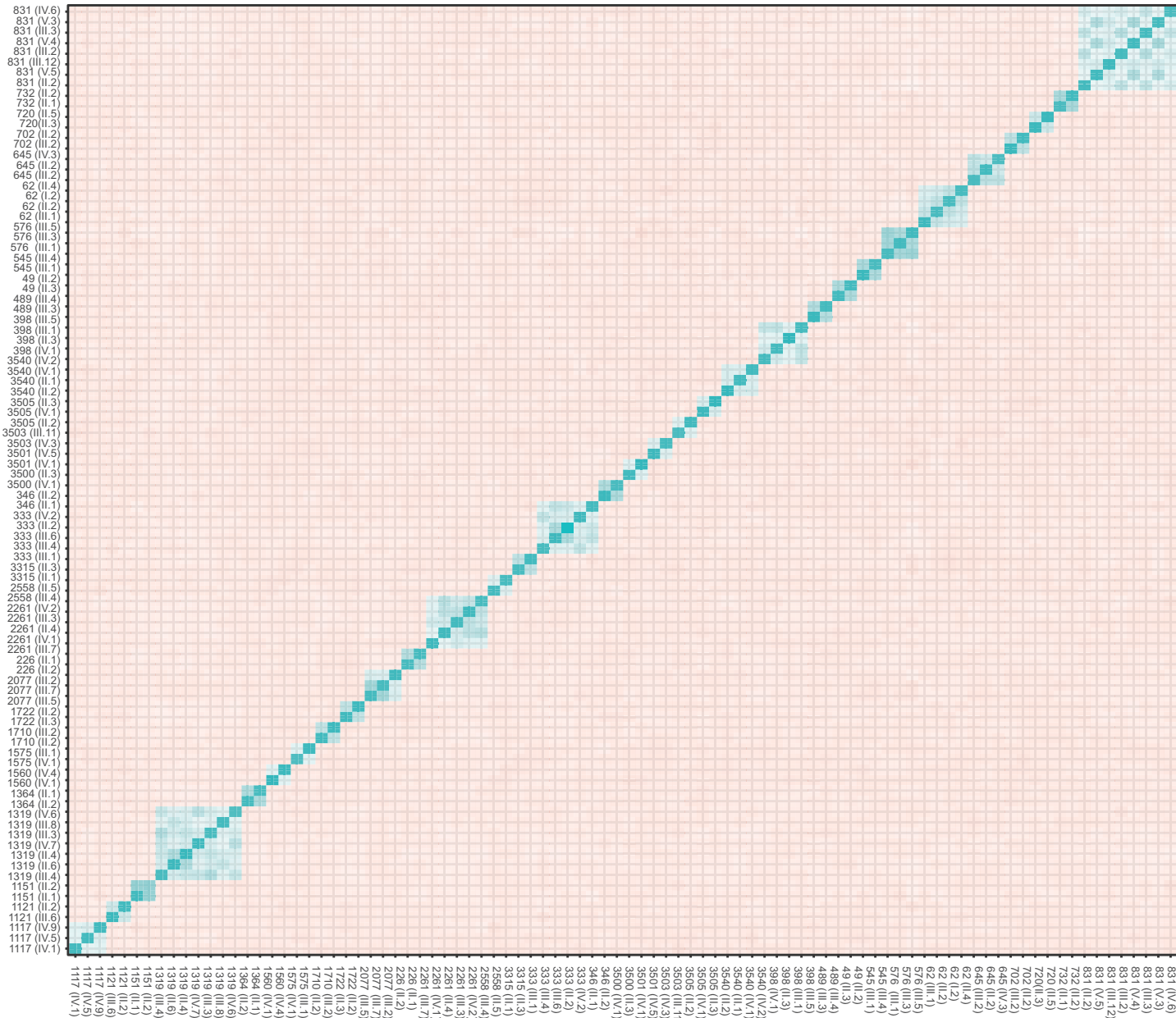


Fig S1. Pedigrees of 32 Danish multiplex CHD families. Circles: females. Squares: males. White symbols: unaffected family members. Filled symbols: affected family members. Triangles: abortion. CHD was determined by manual inspection of patient files (black) or data from DNPR/interview with family members (grey). Exome sequenced individuals are marked with asterix. The following heart defects were identified; Aortic Valve Regurgitation (AVR), Aortic Valve Stenosis (AVS), Atrial Septal Defect (ASD), Atrioventricular Septal Defect (AVSD), Bicuspid Aortic Valve(BAV), Coarctation of the Aorta (COA), Dilated Cardiomyopathy (DCM), Ebstein's Anomaly (EbA), Hypertrophic Cardiomyopathy (HCM), Hypoplastic Left Heart Syndrome (HLHS), Infundibular Pulmonary Stenosis (InfPS), Mitral Valve Atresia (MVA), Partial Anomalous Pulmonary Venous Return (PAPVR), Patent Ductus Arteriosus (PDA), Patent Foramen Ovale (PFO), Pulmonary Valve Atresia (PVA), Pulmonary Valve Stenosis (PVS), Single Ventricle (SV), Subvalvular Aortic Stenosis (SubAS), Tetralogy of Fallot (TOF), Total Anomalous Pulmonary Venous Return (TAPVR), Transposition of The Great Arteries (TGA), Vascular ring (Vring), Ventricular Septal Defect (VSD). For ASD and VSD spontaneous closure (a), intervention performed (b) and no intervention performed (c) is indicated if information was available.

A



B

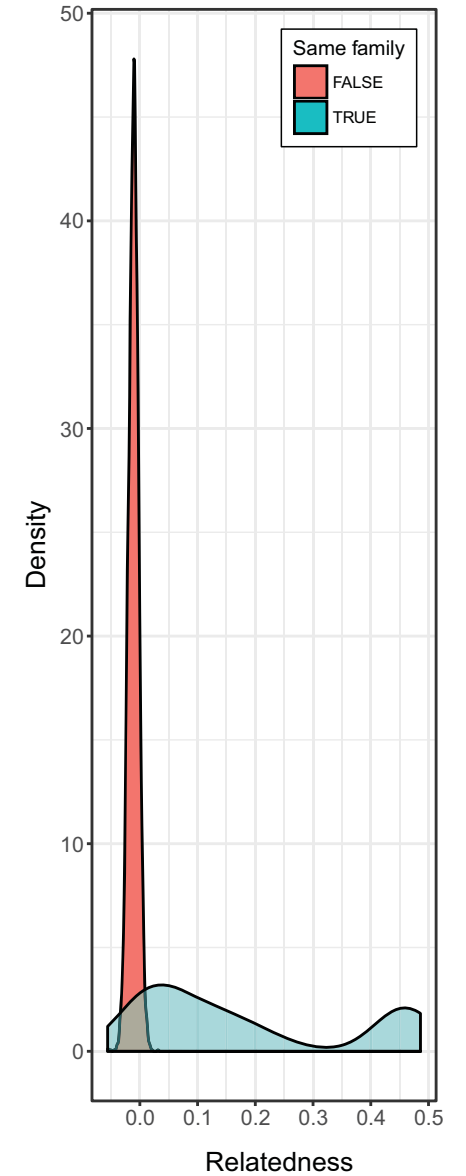


Fig. S2. Relatedness of the 90 individuals included in the study. A. Heatmap showing the relatedness score of pairs of individuals. The family number and position in the family is indicated left and below the heatmap. B. Density plot of relatedness score from the pairwise analysis. Individuals from same family is shown in blue, and individuals not from the same family is shown in red.

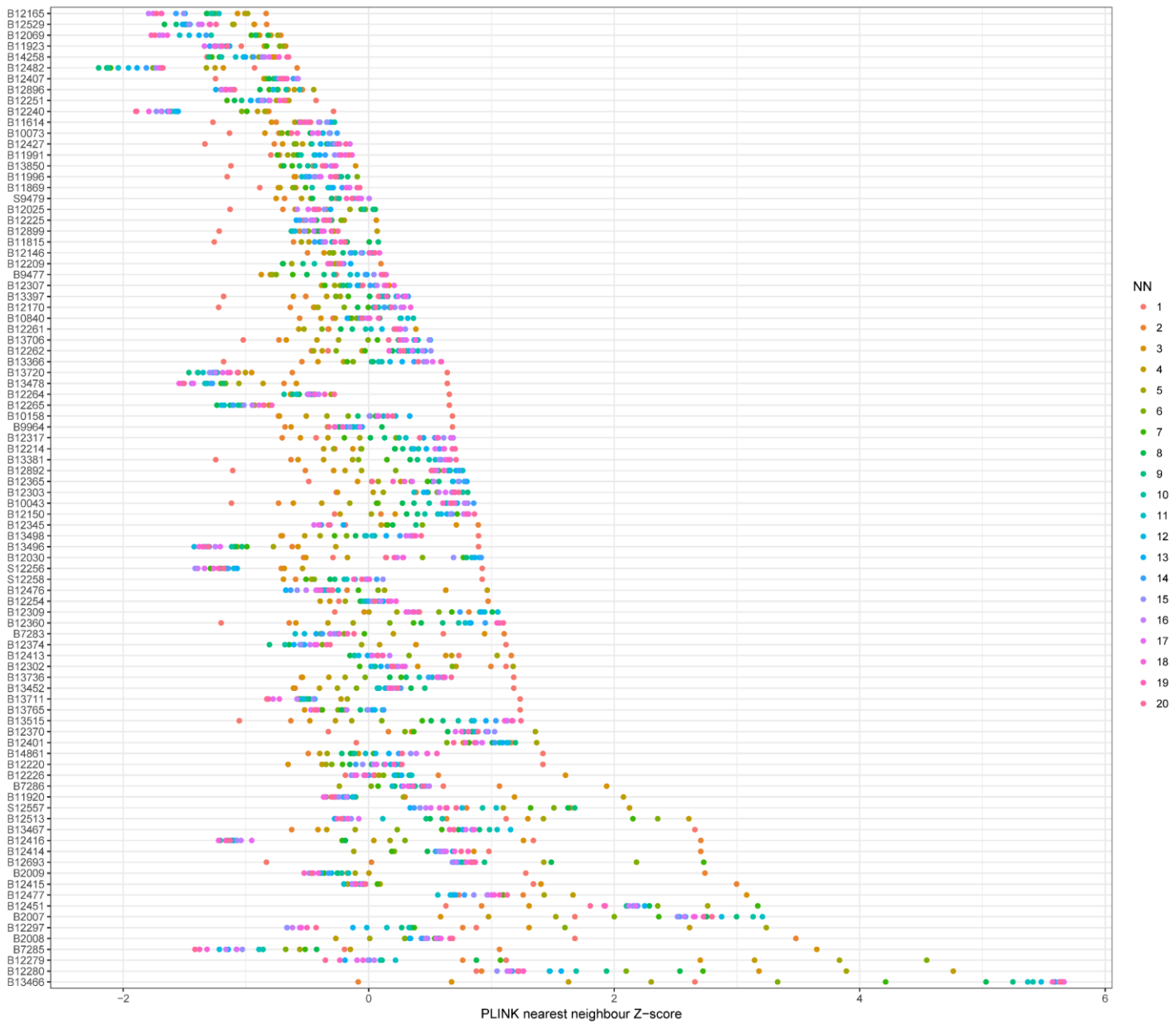


Fig. S3. Homogeneity of the cohort. Population distances from each of the 90 individuals in the cohort to the 20 nearest neighbors (NN) were calculated. The distances compared to the the mean of the population in terms of standard deviations (Z-scores) is plotted on the x-axis.

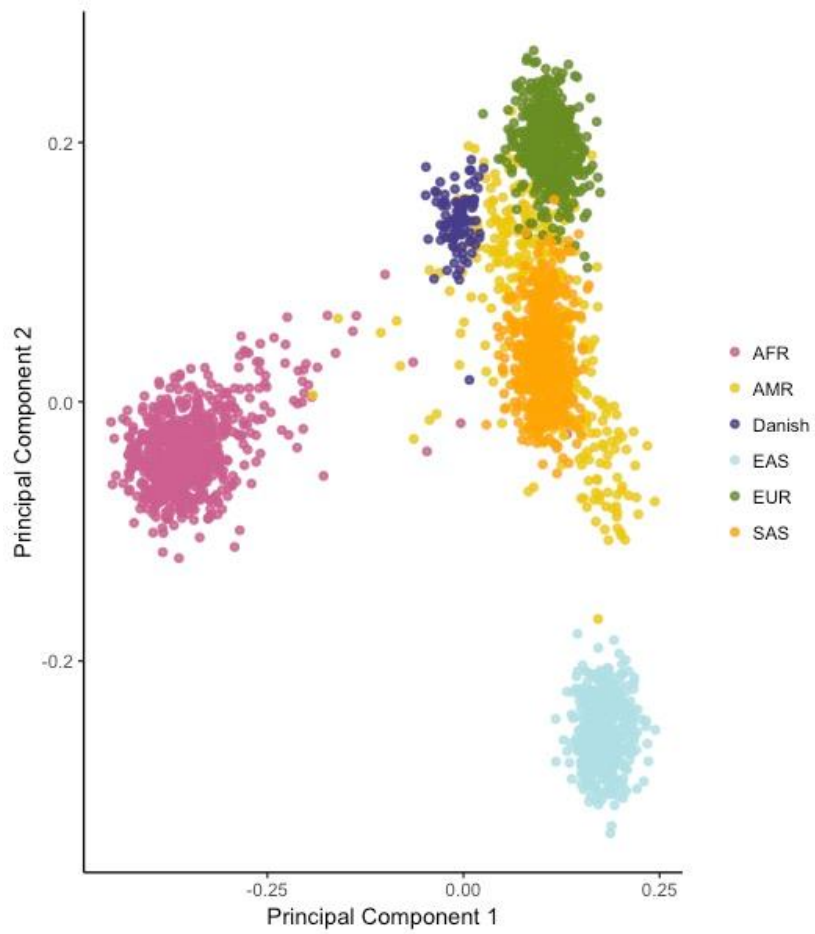


Fig. S4. Principle component analysis. The Danish samples were analyzed together with reference samples of different Super Populations from 1000 genomes (AFR= African, AMR= Admixed American, EAS= East Asian, EUR= European, SAS= South Asian).

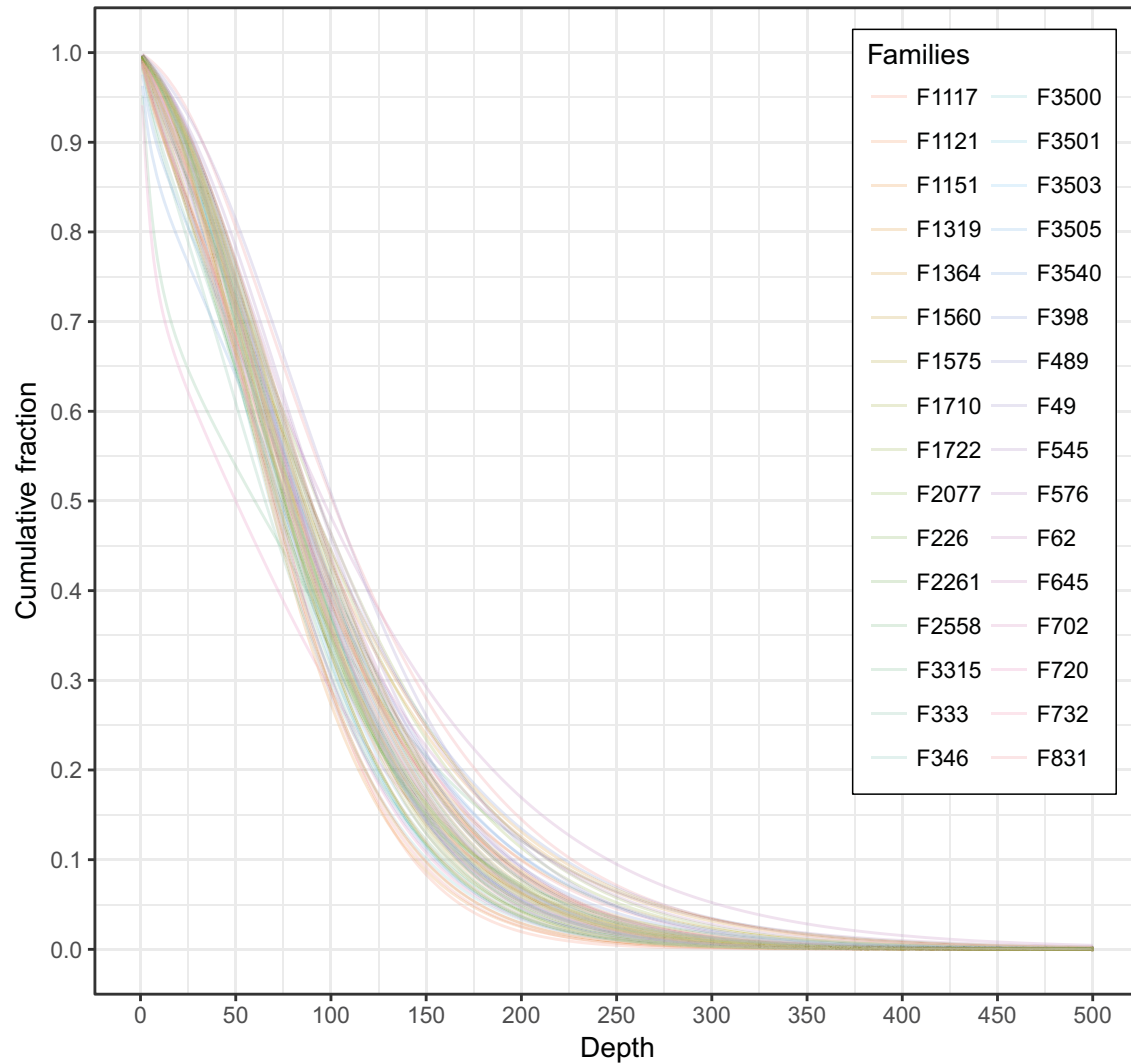
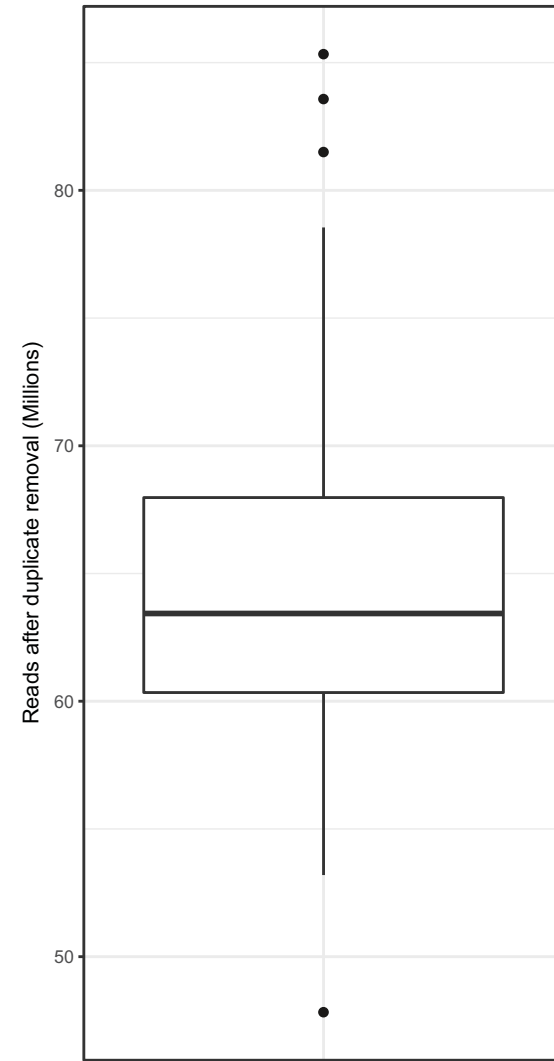
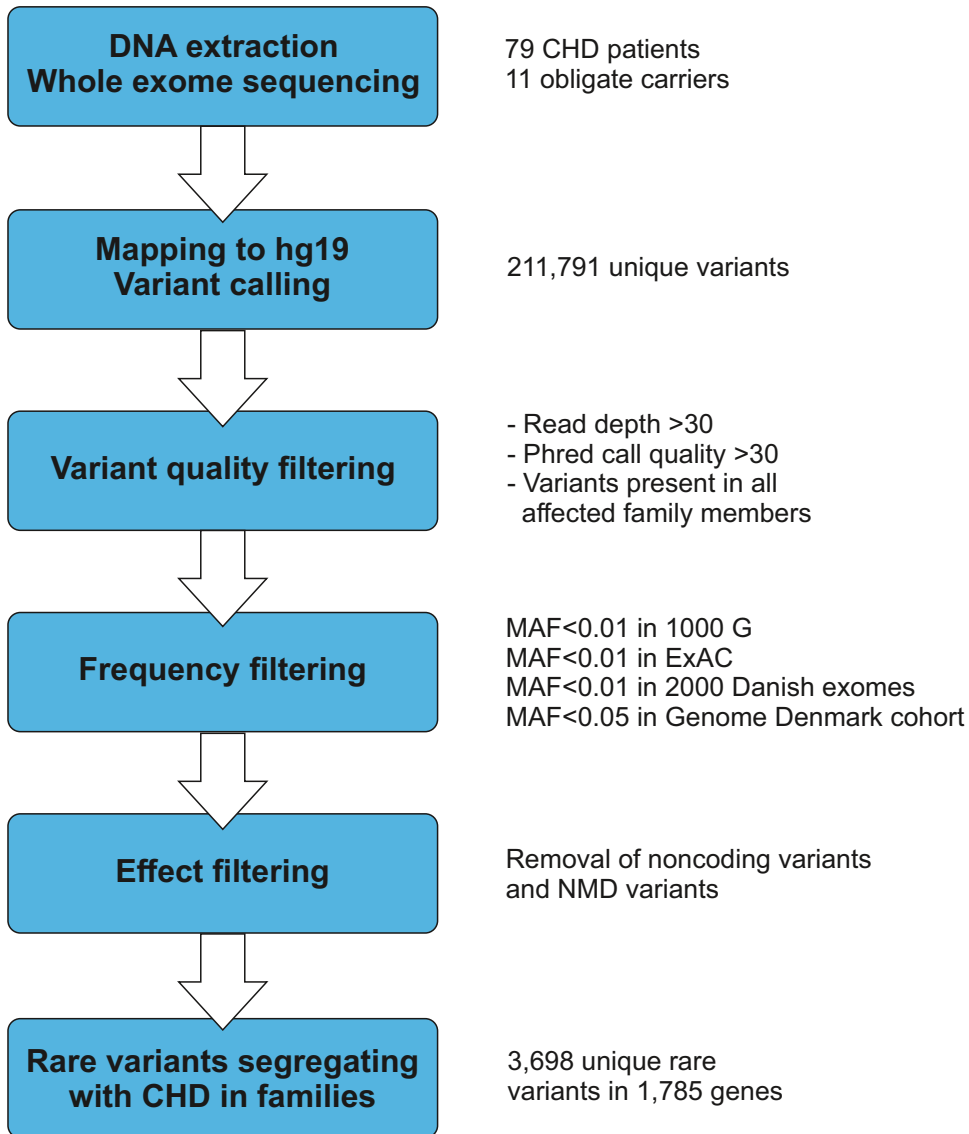
A**B**

Fig. S5. Sequencing coverage and number of reads. A. Cumulative depth of sequencing per family. B. Number of reads after removal of duplicates.

Whole exome sequencing



Systems analysis of CDGs

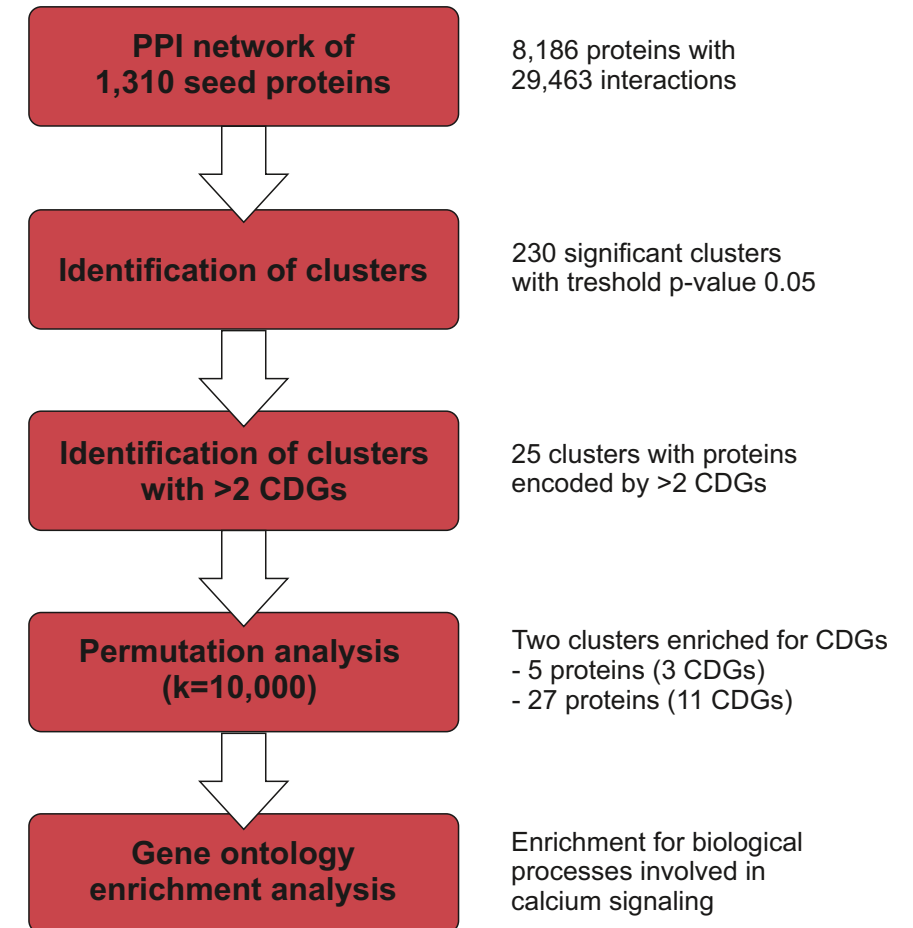


Fig. S6. Overview of the sequencing and data analysis processes.

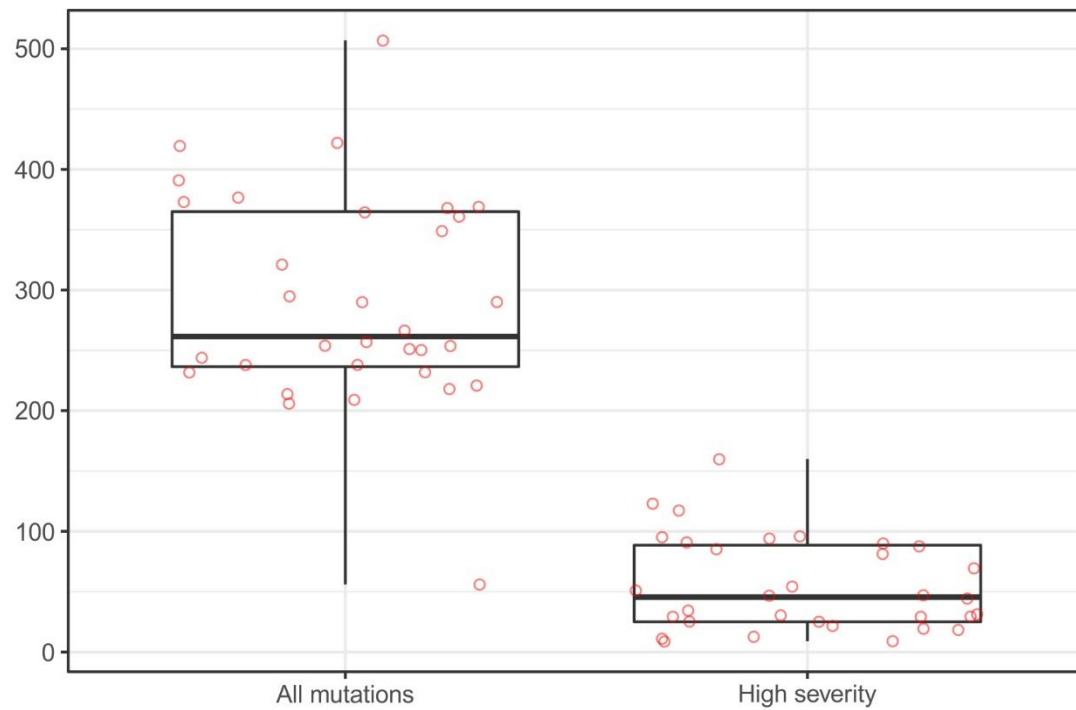


Fig. S7. Number of CDGs per family when all rare variants (left) or only high severity variants (right) were considered. High severity variants were defined as variants creating splicing defects, frameshifts, premature stop codons and missense variants predicted to be damaging by both Polyphen and SIFT.

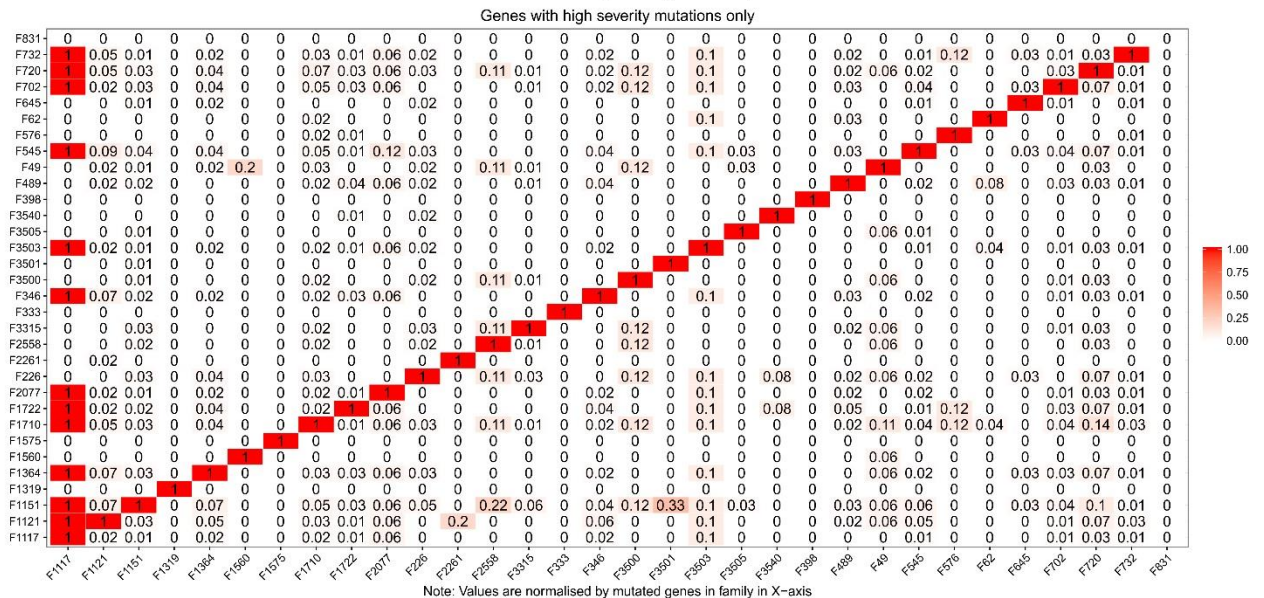
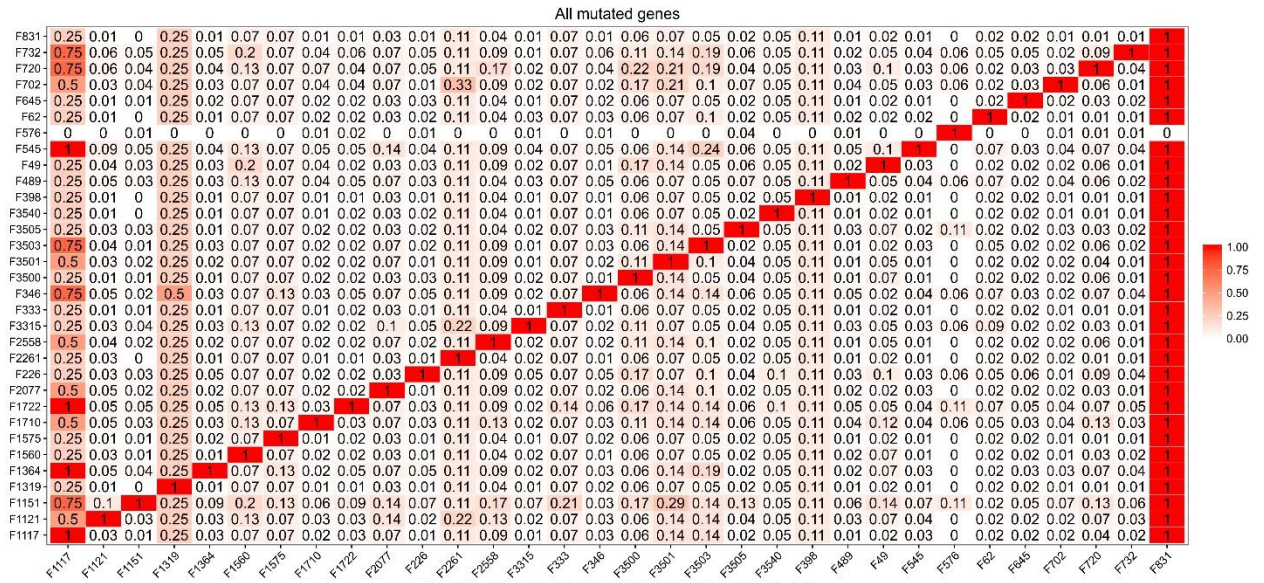
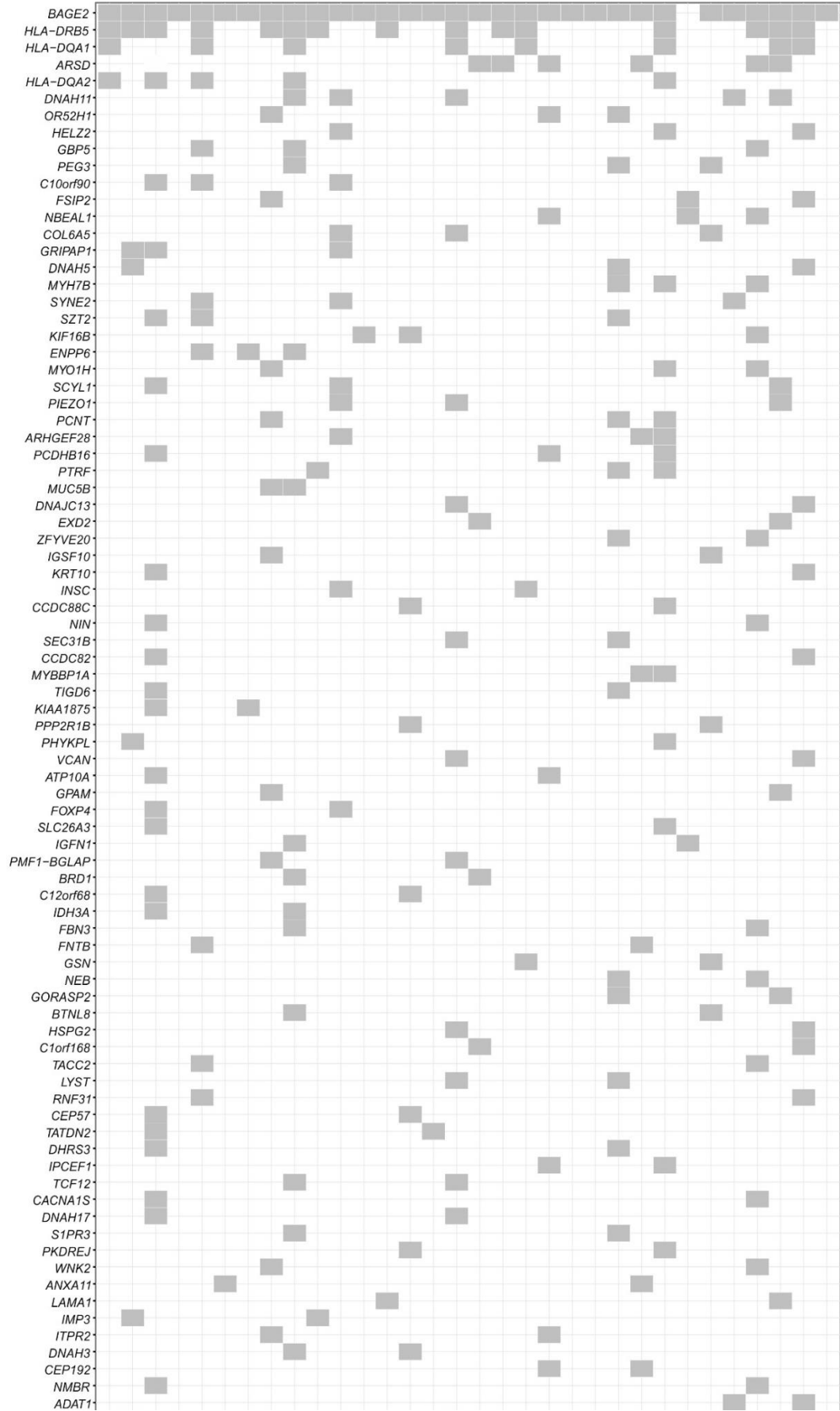


Fig. S8. Overlap between CDGs in pairs of families. The fraction of overlap (FO) between CDGs are shown for each pair of families. For example, 11% of the CDGs identified in family 49 overlaps with CDGs identified in family 2558, which is indicated by a FO value of 0.11.



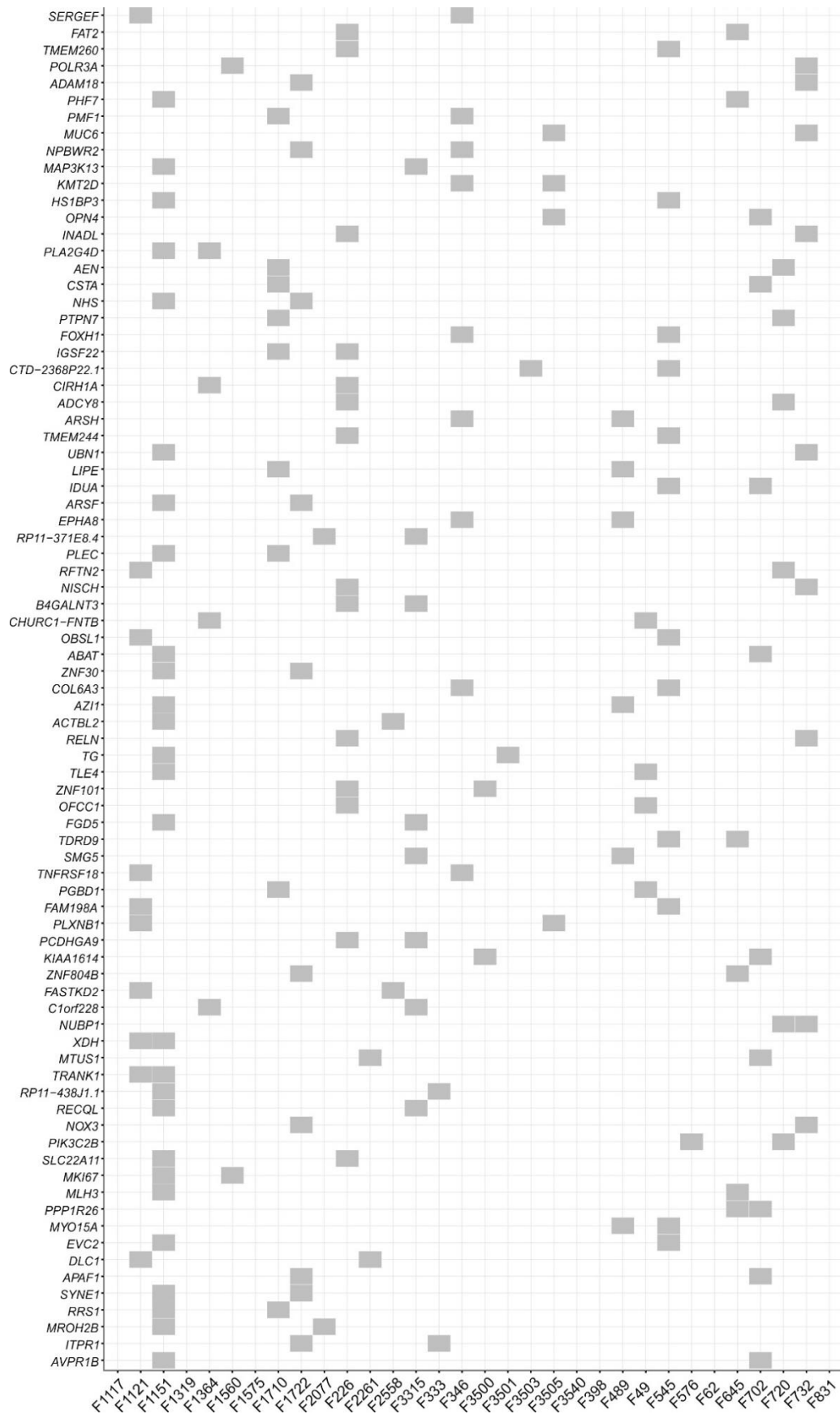
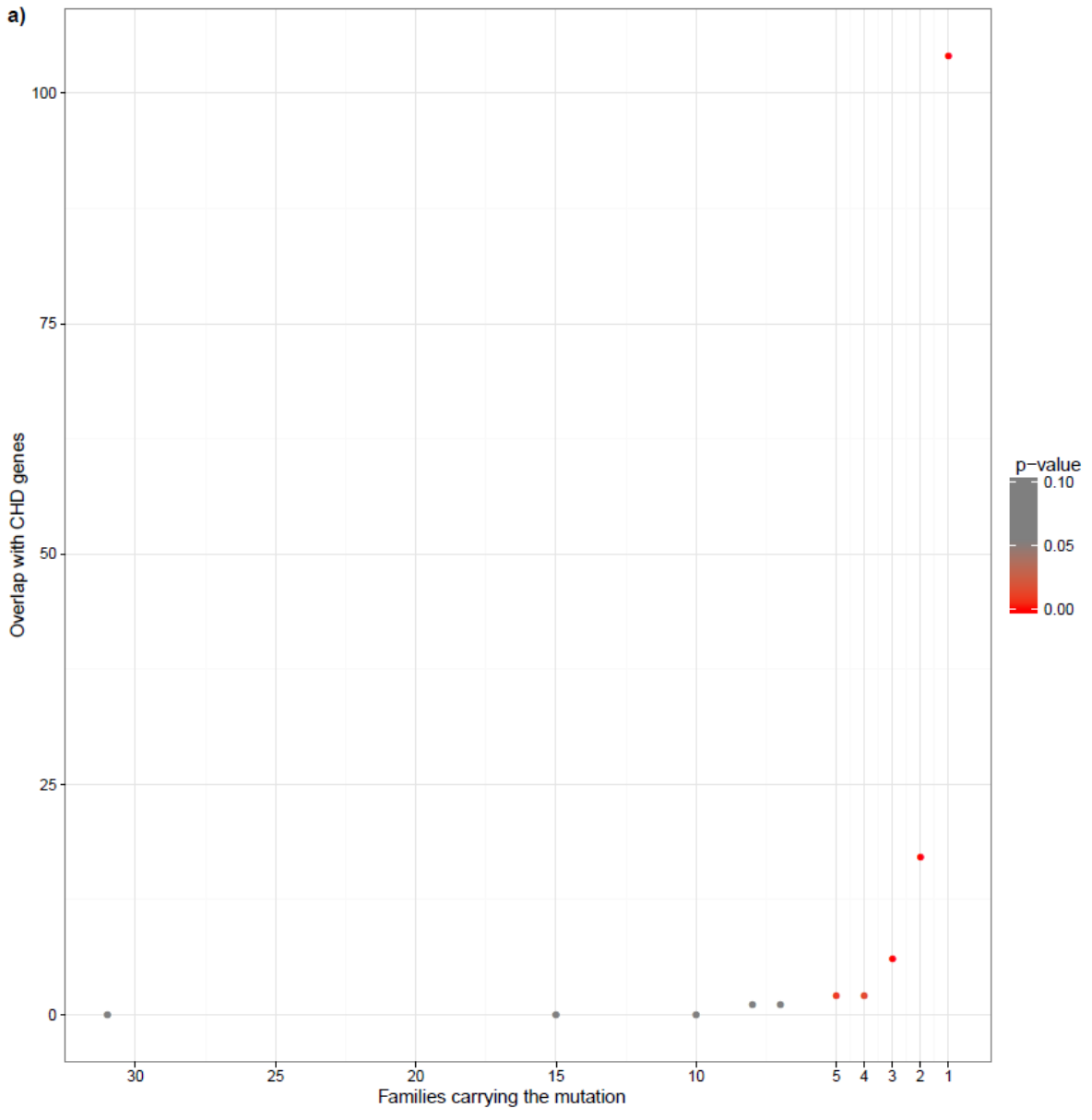


Fig. S9. Families with rare inherited variants in CDGs. Only genes mutated in two or more families are shown.



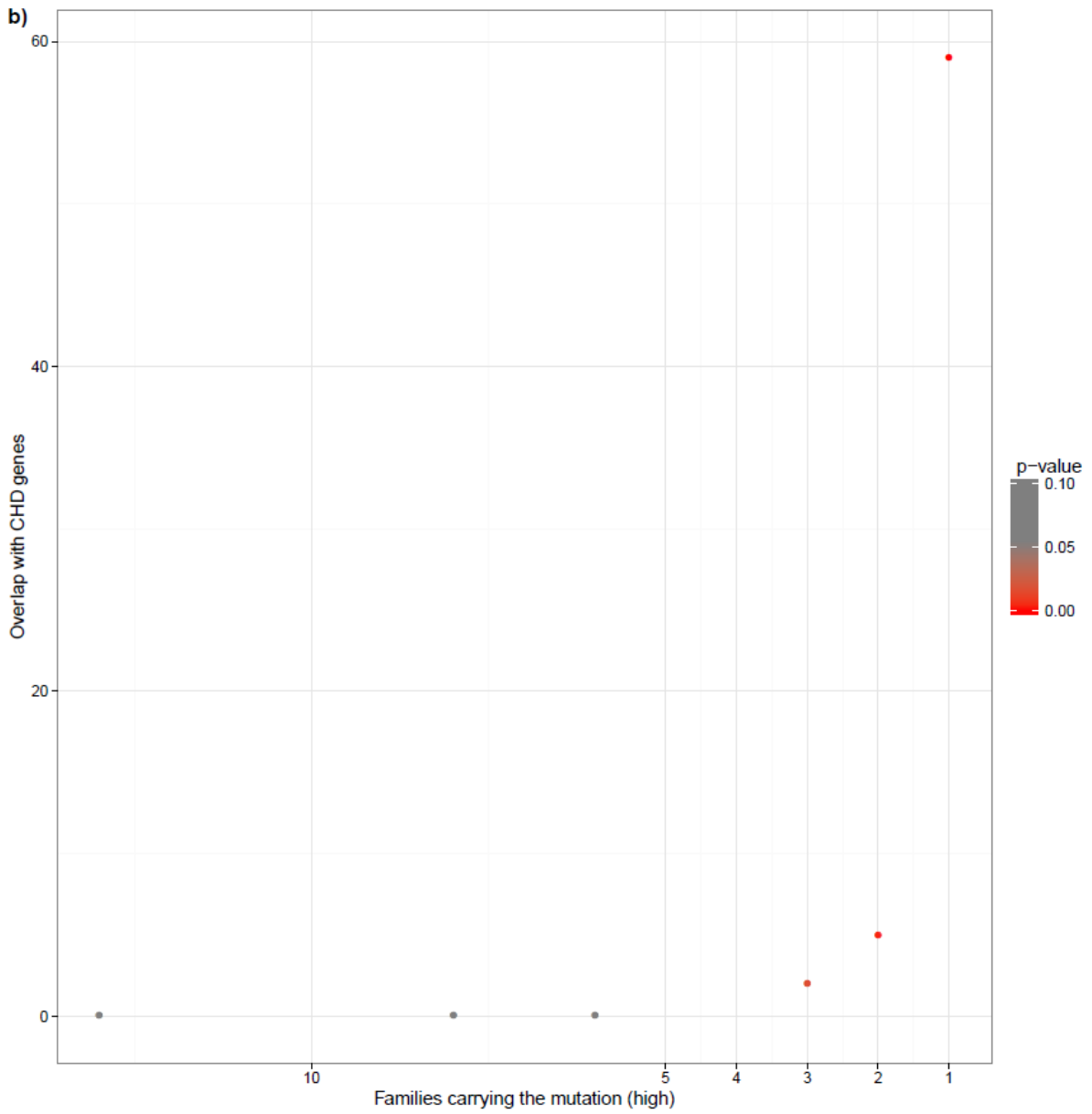


Fig. S10. Overlap between the 1,785 CDGs in our families and a curated list of 829 genes known to cause CHD in mice. A. Overlap with all 1,785 CDGs. B. Overlap with genes carrying mutations scored pathogenic by SIFT/Polyphen-2.

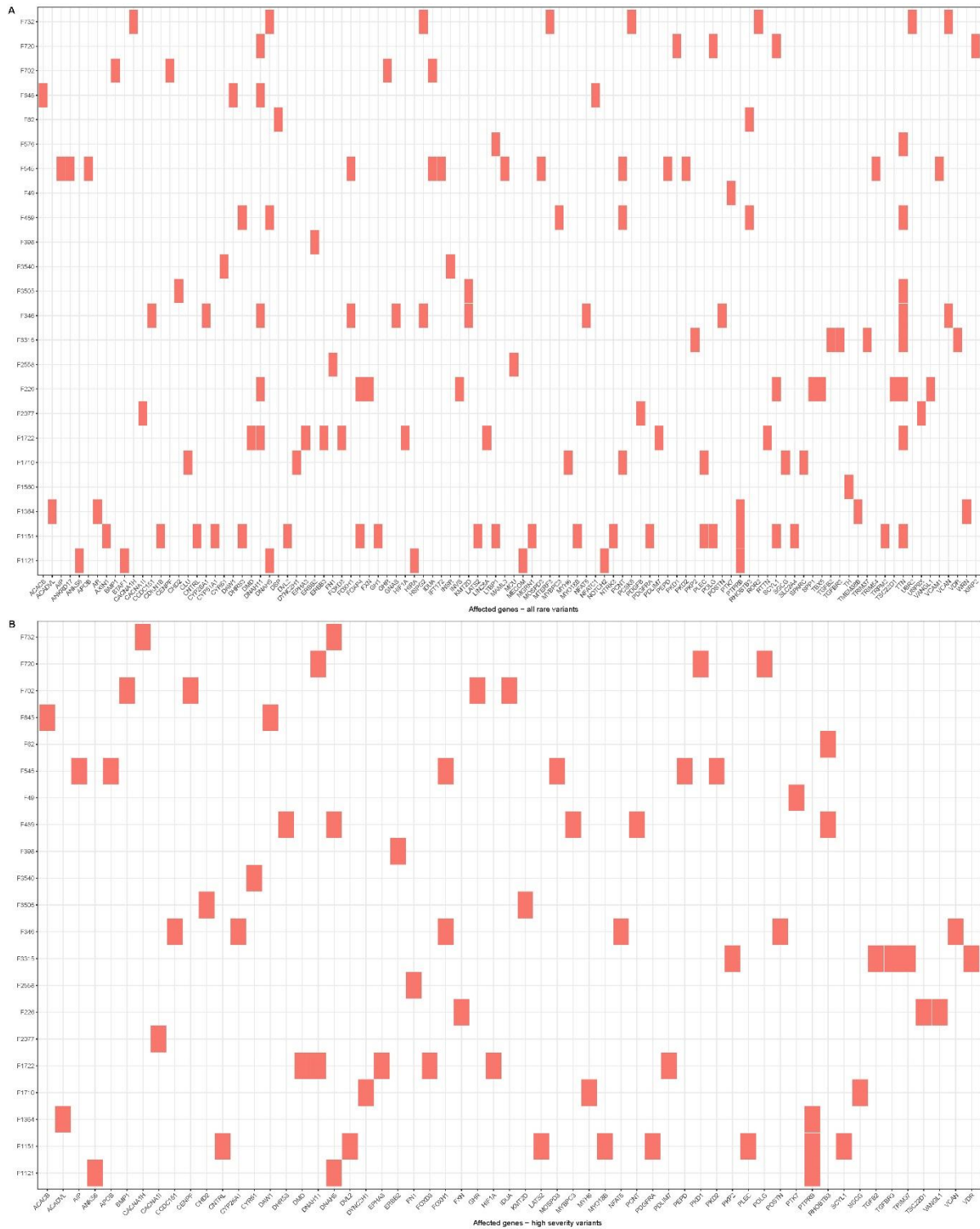


Fig. S11. Distribution of CHD genes across families. CHD genes identified in mouse models are shown here. The distribution of human CHD genes is shown in Supplemental Table 4. A. All CHD genes affected by rare variants. B. CHD genes affected by high severity variants. High severity variants were defined as variants creating splicing defects, frameshifts, premature stop codons and missense variants predicted to be damaging by both Polyphen and SIFT.

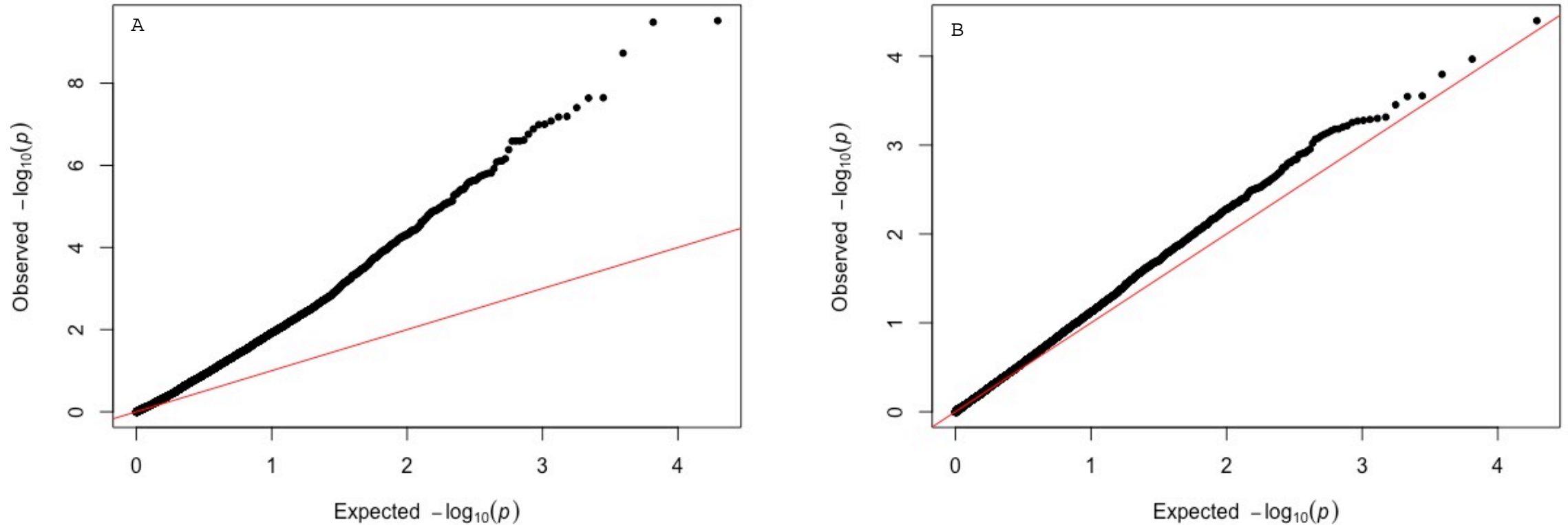


Fig. S12. Quantile-quantile plots. Plots were generated based on p-values from case control association testing (two-sided Fisher's Exact test) on the burden of synonymous mutations within 10,000 random genesets. The analysis was performed using different MAF cutoffs: A. 0.01 and B. 0.001.

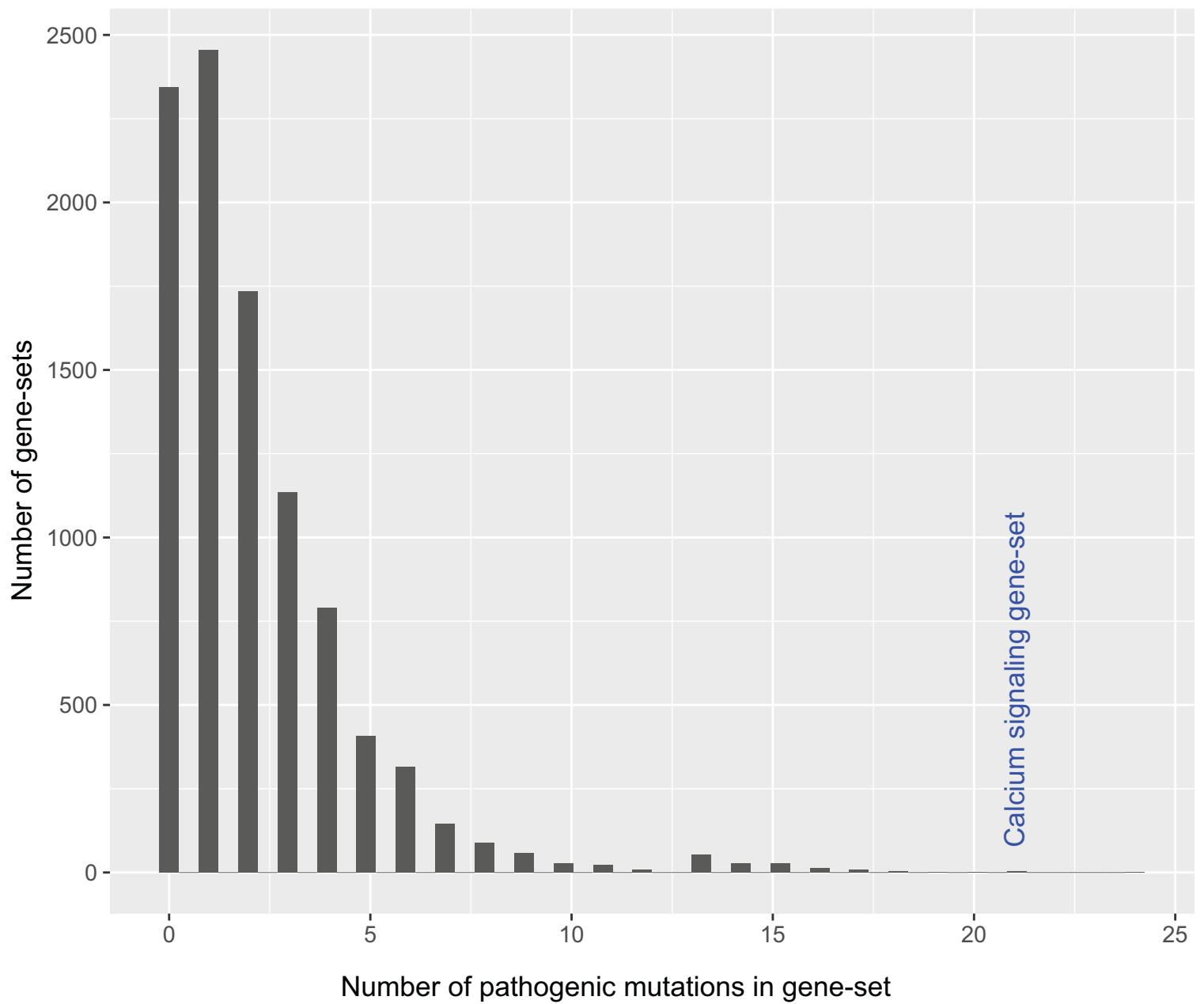


Fig. S13. Distribution of pathogenic mutations in random gene-sets. 10,000 random gene-sets of 10 genes were created. The size distribution of each set was similar to the calcium-signaling gene-set tested in the case-control study; *ADCY2* (6575 bp), *ADCY5* (7311 bp), *ITPR1* (10197 bp), *CACNAIS* (6166 bp), *CACNAII* (6740 bp), *CACNAIH* (8208 bp), *CACNAID* (8991 bp), *GRIA4* (5508 bp), *PLCB2* (4616 bp), and *NFAT5* (13362 bp). Pathogenic mutations were defined as mutations with MAF < 0.001 and MPC score >2.

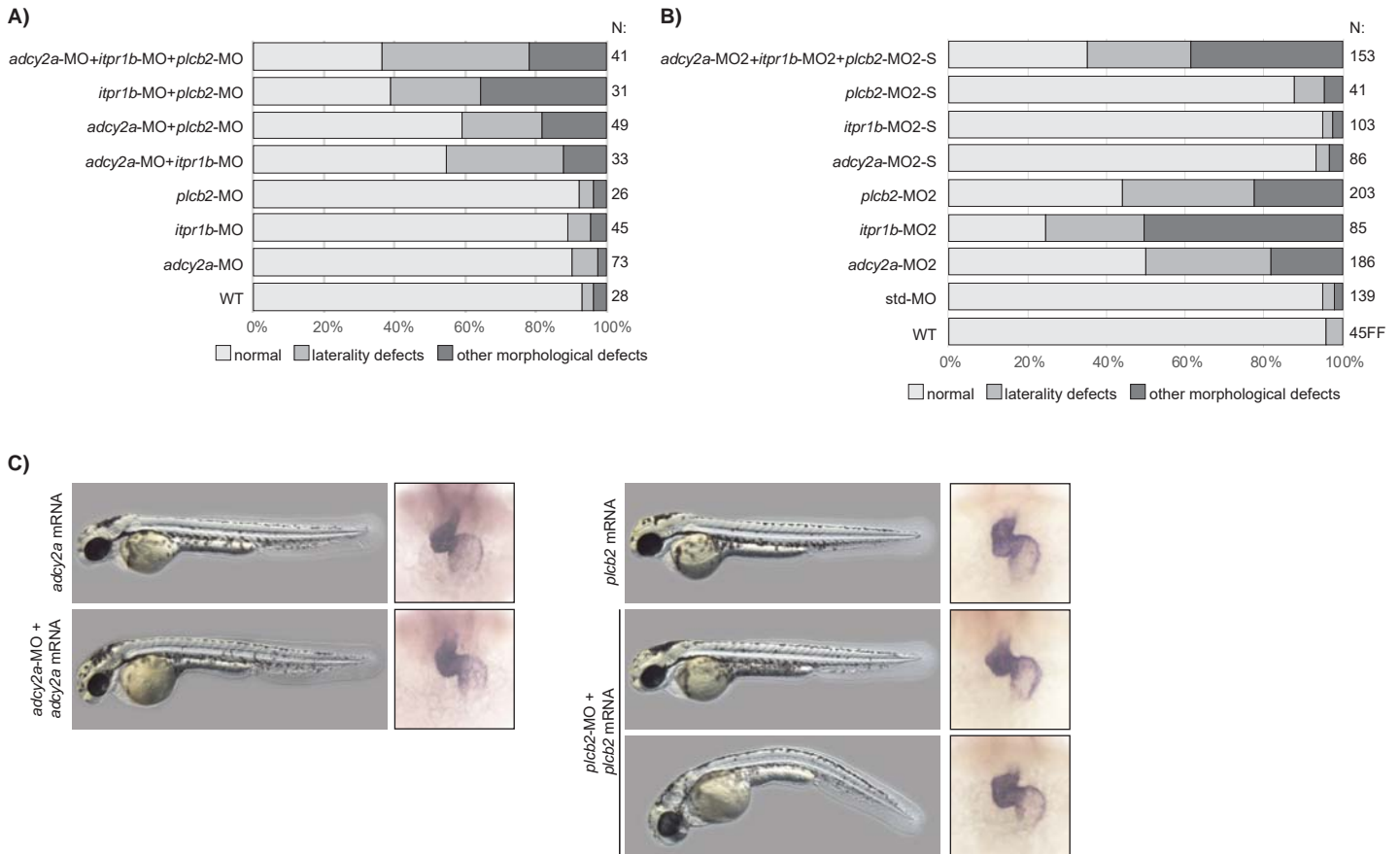


Fig. S14. A. Injection of sub-efficient doses of MOs. Note that only combinations of MOs result in significant numbers of heart defects among the embryos. B. Quantification of heart phenotypes in WT, controls and morphants injected with second set of MOs. S indicates that sub-efficient doses of MOs are injected. C. Rescue of *adcy2a* and *plcb2* knockdown. Embryos injected with *adcy2a* or *plcb2* RNA alone and in combinations with corresponding MOs, are displayed at 48 hpf. *myl7* in situ hybridization marks the heart in these embryos.

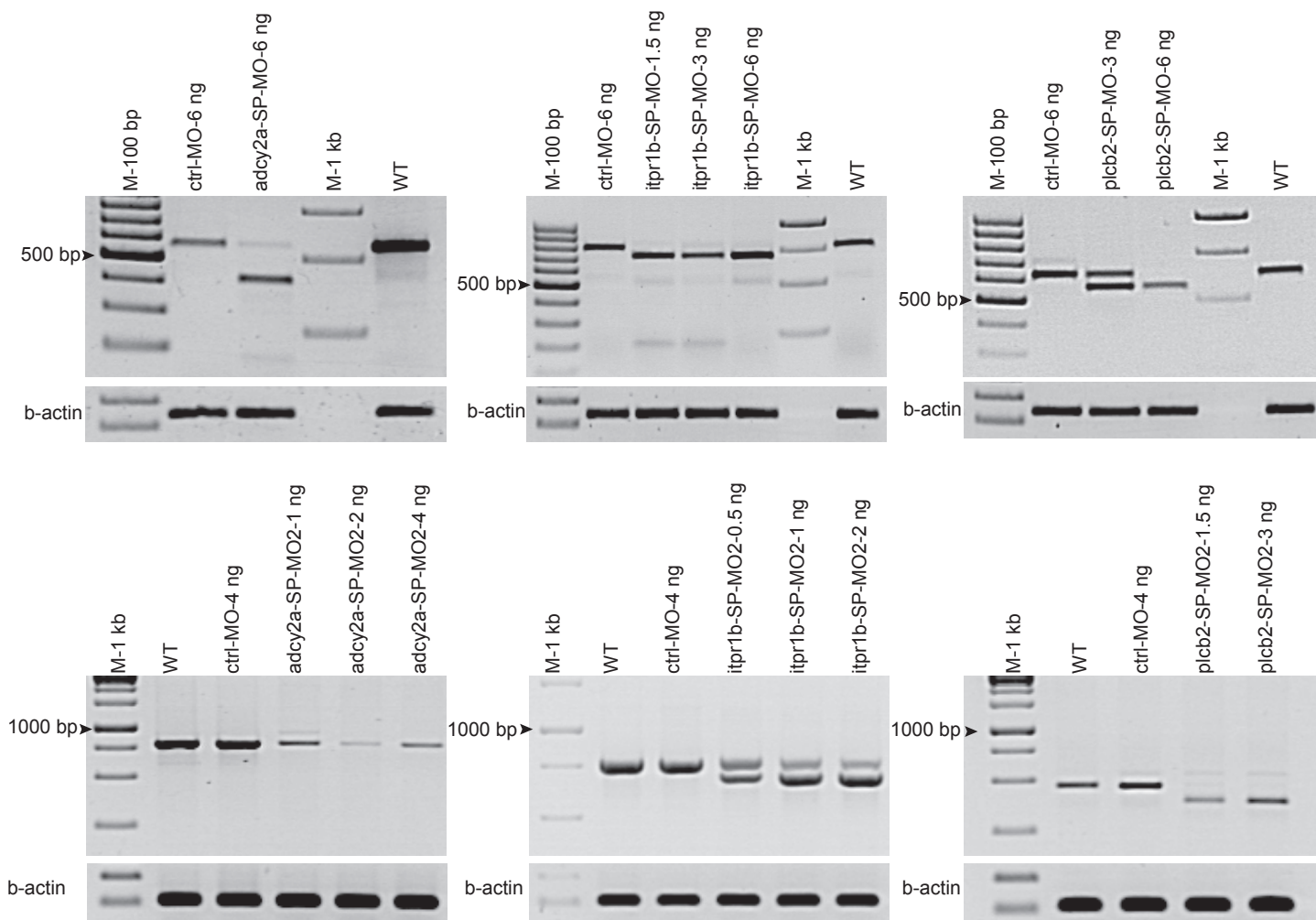


Fig. S15. Efficiency of the splice blocking morpholinos (MOs) used against *adcyl2a*, *itpr1b* and *plcb2*. Upper panel shows first set of MOs and lower panel shows second set of MOs. Aberrant splicing of *adcyl2a*, *itpr1b* and *plcb2* and decrease in corresponding WT mature mRNA is confirmed by RT-PCR analysis.

Table S1. Human orthologues to 829 genes known to be associated with CHD in mouse models. The list was compiled from data in the Mouse Genome Informatics database (<http://www.informatics.jax.org/>).

<i>ABCA5</i>	<i>CHD7</i>	<i>FGFRL1</i>	<i>INVS</i>	<i>MTERF3</i>	<i>PPARG</i>	<i>SPTBN1</i>
<i>ABCB8</i>	<i>CHMP5</i>	<i>FHOD3</i>	<i>IRAK1</i>	<i>MTERF4</i>	<i>PPARGC1A</i>	<i>SRF</i>
<i>ABI1</i>	<i>CHRD</i>	<i>FKBP1A</i>	<i>IRX3</i>	<i>MTMR12</i>	<i>PPARGC1B</i>	<i>SRSF1</i>
<i>ABL1</i>	<i>CHST14</i>	<i>FKBP1B</i>	<i>IRX5</i>	<i>MTO1</i>	<i>PPP1R13L</i>	<i>SRSF10</i>
<i>ACACB</i>	<i>CISD2</i>	<i>FLNA</i>	<i>ISL1</i>	<i>MUS81</i>	<i>PPP2R3A</i>	<i>SSBP2</i>
<i>ACADM</i>	<i>CITED2</i>	<i>FLRT3</i>	<i>ITCH</i>	<i>MYBPC3</i>	<i>PPP2R5C</i>	<i>SSR1</i>
<i>ACADVL</i>	<i>CLU</i>	<i>FMOD</i>	<i>ITGA5</i>	<i>MYCN</i>	<i>PPP3CB</i>	<i>STK39</i>
<i>ACE</i>	<i>CLUAP1</i>	<i>FN1</i>	<i>ITGAV</i>	<i>MYH10</i>	<i>PRDM1</i>	<i>SUFU</i>
<i>ACKR3</i>	<i>CNTRL</i>	<i>FOXA2</i>	<i>ITGB1</i>	<i>MYH6</i>	<i>PRDM16</i>	<i>T</i>
<i>ACSL4</i>	<i>COL18A1</i>	<i>FOXC1</i>	<i>ITPA</i>	<i>MYH7</i>	<i>PRDM6</i>	<i>TAB1</i>
<i>ACTC1</i>	<i>COL1A1</i>	<i>FOXC2</i>	<i>JAG1</i>	<i>MYL2</i>	<i>PRF1</i>	<i>TAL1</i>
<i>ACVR1</i>	<i>COL4A3BP</i>	<i>FOXD3</i>	<i>JARID2</i>	<i>MYL7</i>	<i>PRICKLE1</i>	<i>TBC1D32</i>
<i>ACVR2A</i>	<i>COMMD9</i>	<i>FOXG1</i>	<i>JMJD6</i>	<i>MYLK3</i>	<i>PRKARIA</i>	<i>TBX1</i>
<i>ACVR2B</i>	<i>CRB2</i>	<i>FOXH1</i>	<i>JPH2</i>	<i>MYO10</i>	<i>PRKCI</i>	<i>TBX18</i>
<i>ACVRL1</i>	<i>CREBBP</i>	<i>FOXJ1</i>	<i>JUN</i>	<i>MYO18B</i>	<i>PROC</i>	<i>TBX2</i>
<i>ADAM12</i>	<i>CRKL</i>	<i>FOXM1</i>	<i>JUND</i>	<i>MYOD1</i>	<i>PSEN1</i>	<i>TBX20</i>
<i>ADAM15</i>	<i>CSNK2A1</i>	<i>FOXO1</i>	<i>JUP</i>	<i>MYOZ2</i>	<i>PSEN2</i>	<i>TBX3</i>
<i>ADAM17</i>	<i>CSRP2</i>	<i>FOXP1</i>	<i>KAT6A</i>	<i>NACA</i>	<i>PSKH1</i>	<i>TBX5</i>
<i>ADAM19</i>	<i>CSRP3</i>	<i>FOXP4</i>	<i>KCNH2</i>	<i>NCKAP1</i>	<i>PTCD2</i>	<i>TBX6</i>
<i>ADAM9</i>	<i>CST9</i>	<i>FOXQ1</i>	<i>KCTD10</i>	<i>NCOA6</i>	<i>PTEN</i>	<i>TCF21</i>
<i>ADAMTS6</i>	<i>CTBP2</i>	<i>FRAS1</i>	<i>KDM1A</i>	<i>NCOR2</i>	<i>PTGS2</i>	<i>TCTN2</i>
<i>ADGRG6</i>	<i>CTNNA3</i>	<i>FREM2</i>	<i>KDM2A</i>	<i>NCSTN</i>	<i>PTK2</i>	<i>TDG</i>
<i>ADM</i>	<i>CTNNB1</i>	<i>FRS2</i>	<i>KDM4A</i>	<i>NDST1</i>	<i>PTK7</i>	<i>TEAD1</i>
<i>ADRA1A</i>	<i>CXADR</i>	<i>FSTL1</i>	<i>KDM6A</i>	<i>NEGR1</i>	<i>PTPN11</i>	<i>TEAD2</i>
<i>ADRA1B</i>	<i>CXCL12</i>	<i>FURIN</i>	<i>KDR</i>	<i>NEK6</i>	<i>PTPRB</i>	<i>TEK</i>
<i>AGT</i>	<i>CXCR4</i>	<i>FUS</i>	<i>KIF3A</i>	<i>NEK8</i>	<i>PTPRJ</i>	<i>TERC</i>
<i>AGTR1</i>	<i>CYBB</i>	<i>FUZ</i>	<i>KIF3B</i>	<i>NF1</i>	<i>RAC1</i>	<i>TFAP2A</i>
<i>AGTR2</i>	<i>CYBRD1</i>	<i>FXN</i>	<i>KIF7</i>	<i>NFAT5</i>	<i>RAF1</i>	<i>TFB1M</i>
<i>AIFM1</i>	<i>CYP11B2</i>	<i>FXR1</i>	<i>KIFAP3</i>	<i>NFATC1</i>	<i>RAMP2</i>	<i>TGFB1</i>
<i>AIP</i>	<i>CYP26A1</i>	<i>FZD1</i>	<i>KISS1R</i>	<i>NFATC3</i>	<i>RARA</i>	<i>TGFB2</i>
<i>AKAP12</i>	<i>CYP27B1</i>	<i>FZD2</i>	<i>KLF15</i>	<i>NFATC4</i>	<i>RARB</i>	<i>TGFBR2</i>
<i>AKAP13</i>	<i>CYP51A1</i>	<i>FZD7</i>	<i>KLF2</i>	<i>NGF</i>	<i>RARG</i>	<i>TGFBR3</i>
<i>AKAP6</i>	<i>CYR61</i>	<i>GAA</i>	<i>KLF3</i>	<i>NIPBL</i>	<i>RB1</i>	<i>TGIF1</i>
<i>AKT1</i>	<i>DAAM1</i>	<i>GAB1</i>	<i>KLF5</i>	<i>NKX2-5</i>	<i>RB1CC1</i>	<i>TGIF2</i>
<i>AKT3</i>	<i>DAG1</i>	<i>GAB2</i>	<i>KLHL40</i>	<i>NKX2-6</i>	<i>RBL2</i>	<i>TGM2</i>
<i>ALDH1A2</i>	<i>DAGLA</i>	<i>GAS1</i>	<i>KMT2B</i>	<i>NODAL</i>	<i>RBM20</i>	<i>TH</i>
<i>ALG13</i>	<i>DAND5</i>	<i>GATA4</i>	<i>KMT2D</i>	<i>NOG</i>	<i>RBP4</i>	<i>THBS1</i>
<i>ALPK3</i>	<i>DAWI</i>	<i>GATA5</i>	<i>KRAS</i>	<i>NOS1</i>	<i>RBPJ</i>	<i>THRA</i>
<i>AMN</i>	<i>DCHS1</i>	<i>GATA6</i>	<i>KRT19</i>	<i>NOS3</i>	<i>RCAN1</i>	<i>TIE1</i>
<i>ANGPT1</i>	<i>DCTN5</i>	<i>GBE1</i>	<i>LAMA4</i>	<i>NOTCH1</i>	<i>RCE1</i>	<i>TK2</i>

<i>ANKRD17</i>	<i>DDR2</i>	<i>GBX2</i>	<i>LAMA5</i>	<i>NOTCH2</i>	<i>RDH10</i>	<i>TKT</i>
<i>ANKS6</i>	<i>DDX11</i>	<i>GDF1</i>	<i>LARGE1</i>	<i>NOV</i>	<i>RERE</i>	<i>TLL1</i>
<i>AP2B1</i>	<i>DDX3X</i>	<i>GH1</i>	<i>LATS2</i>	<i>NPPB</i>	<i>RFX3</i>	<i>TLR2</i>
<i>AP4E1</i>	<i>DEDD</i>	<i>GHR</i>	<i>LBX1</i>	<i>NPR1</i>	<i>RHBDF1</i>	<i>TMED2</i>
<i>APC</i>	<i>DES</i>	<i>GHRHR</i>	<i>LDB3</i>	<i>NPRL3</i>	<i>RHBDF2</i>	<i>TMEM100</i>
<i>APOB</i>	<i>DHRS3</i>	<i>GIPC1</i>	<i>LDLR</i>	<i>NR1D2</i>	<i>RHEB</i>	<i>TMEM106B</i>
<i>APOE</i>	<i>DICER1</i>	<i>GJA1</i>	<i>LEFTY1</i>	<i>NR1H3</i>	<i>RHOBTB3</i>	<i>TMEM38A</i>
<i>AR</i>	<i>DISP1</i>	<i>GJA5</i>	<i>LEFTY2</i>	<i>NR2F2</i>	<i>RIC8B</i>	<i>TMEM38B</i>
<i>ARID1A</i>	<i>DLL1</i>	<i>GJC1</i>	<i>LEMD2</i>	<i>NR3C1</i>	<i>RIPPLY3</i>	<i>TMEM67</i>
<i>ARID2</i>	<i>DLL4</i>	<i>GNA11</i>	<i>LEP</i>	<i>NR3C2</i>	<i>RNF4</i>	<i>TMOD1</i>
<i>ARMC4</i>	<i>DMD</i>	<i>GNAQ</i>	<i>LEPR</i>	<i>NRG1</i>	<i>ROBO1</i>	<i>TMSB4X</i>
<i>ARNTL</i>	<i>DNAAF2</i>	<i>GNAS</i>	<i>LHCGR</i>	<i>NRP1</i>	<i>ROR1</i>	<i>TNNI3</i>
<i>ARSB</i>	<i>DNAAF3</i>	<i>GNG5</i>	<i>LHX1</i>	<i>NRP2</i>	<i>ROR2</i>	<i>TNNT2</i>
<i>ASL</i>	<i>DNAH11</i>	<i>GPC3</i>	<i>LIG3</i>	<i>NTF3</i>	<i>RPGRIP1L</i>	<i>TP53</i>
<i>ATE1</i>	<i>DNAH5</i>	<i>GRIN2D</i>	<i>LIMS1</i>	<i>NTRK3</i>	<i>RPS6KA2</i>	<i>TREX1</i>
<i>ATF2</i>	<i>DNAI1</i>	<i>GRK2</i>	<i>LIMS2</i>	<i>NXN</i>	<i>RSPO3</i>	<i>TRIM37</i>
<i>ATF7</i>	<i>DNAJA3</i>	<i>GSC</i>	<i>LIN28A</i>	<i>OFD1</i>	<i>RTTN</i>	<i>TRIM54</i>
<i>ATG5</i>	<i>DNASE1L2</i>	<i>GTF2I</i>	<i>LMNA</i>	<i>OPA3</i>	<i>RXRA</i>	<i>TRIM55</i>
<i>ATMIN</i>	<i>DNM1L</i>	<i>GTF2IRD1</i>	<i>LMOD2</i>	<i>OSR1</i>	<i>RXRB</i>	<i>TRIM63</i>
<i>ATP2A2</i>	<i>DNM2</i>	<i>GYS1</i>	<i>LOXL2</i>	<i>OTX2</i>	<i>RYR1</i>	<i>TRIP11</i>
<i>AXIN1</i>	<i>DNMT3B</i>	<i>GYS2</i>	<i>LRP1</i>	<i>OVOL2</i>	<i>RYR2</i>	<i>TRPM2</i>
<i>AXIN2</i>	<i>DOCK1</i>	<i>HADHB</i>	<i>LRP2</i>	<i>PAK1</i>	<i>SALL1</i>	<i>TSC1</i>
<i>B9D1</i>	<i>DOT1L</i>	<i>HAND1</i>	<i>LTBP1</i>	<i>PAK4</i>	<i>SALL4</i>	<i>TSC22D1</i>
<i>BAG3</i>	<i>DRC1</i>	<i>HAND2</i>	<i>LTBP4</i>	<i>PARD3</i>	<i>SCN5A</i>	<i>TTBK2</i>
<i>BAZ1B</i>	<i>DSP</i>	<i>HAS2</i>	<i>LUM</i>	<i>PARVA</i>	<i>SCN8A</i>	<i>TTC7A</i>
<i>BBX</i>	<i>DTNA</i>	<i>HBEGF</i>	<i>LUZP1</i>	<i>PATZ1</i>	<i>SCYL1</i>	<i>TTN</i>
<i>BCAR1</i>	<i>DUSP8</i>	<i>HCCS</i>	<i>LY6E</i>	<i>PAX3</i>	<i>SEC24B</i>	<i>TXNRD2</i>
<i>BCL6</i>	<i>DVL1</i>	<i>HDAC2</i>	<i>MAML1</i>	<i>PAXIP1</i>	<i>SEMA3C</i>	<i>UBE2U</i>
<i>BCOR</i>	<i>DVL2</i>	<i>HDAC5</i>	<i>MAML3</i>	<i>PBRM1</i>	<i>SEMA3D</i>	<i>UBE4B</i>
<i>BICC1</i>	<i>DVL3</i>	<i>HDAC9</i>	<i>MAP1S</i>	<i>PCNT</i>	<i>SEN2</i>	<i>UBP1</i>
<i>BIN1</i>	<i>DYNC2H1</i>	<i>HECTD1</i>	<i>MAP2K1</i>	<i>PCSK5</i>	<i>SGCB</i>	<i>UBR1</i>
<i>BMP1</i>	<i>DYNC2L1</i>	<i>HECTD3</i>	<i>MAP2K2</i>	<i>PCSK6</i>	<i>SGCD</i>	<i>UBR2</i>
<i>BMP10</i>	<i>DYNLL1</i>	<i>HEG1</i>	<i>MAP2K5</i>	<i>PDCD10</i>	<i>SGCG</i>	<i>UBR4</i>
<i>BMP2</i>	<i>DYRK1A</i>	<i>HERC4</i>	<i>MAP3K3</i>	<i>PDGFA</i>	<i>SGPL1</i>	<i>USP12</i>
<i>BMP4</i>	<i>DYX1C1</i>	<i>HEXIM1</i>	<i>MAP3K7</i>	<i>PDGFB</i>	<i>SH3PXD2B</i>	<i>USP24</i>
<i>BMPRIA</i>	<i>ECE1</i>	<i>HEY1</i>	<i>MAPK1</i>	<i>PDGFC</i>	<i>SHC1</i>	<i>USP9X</i>
<i>BMPR2</i>	<i>ECE2</i>	<i>HEY2</i>	<i>MAPK11</i>	<i>PDGFRA</i>	<i>SHH</i>	<i>UTF1</i>
<i>BRAF</i>	<i>EDN1</i>	<i>HEYL</i>	<i>MAPK14</i>	<i>PDGFRB</i>	<i>SHOC2</i>	<i>UTRN</i>
<i>BTA1F1</i>	<i>EDNRA</i>	<i>HGS</i>	<i>MAPK3</i>	<i>PDHA1</i>	<i>SHOX2</i>	<i>UVRAG</i>
<i>BTC</i>	<i>EDNRB</i>	<i>HHEX</i>	<i>MAPK6</i>	<i>PDLIM3</i>	<i>SIRT1</i>	<i>VANGL1</i>
<i>C2CD3</i>	<i>EFNA1</i>	<i>HIC2</i>	<i>MAPK7</i>	<i>PDLIM7</i>	<i>SIRT7</i>	<i>VANGL2</i>
<i>CACNA1C</i>	<i>EFNB2</i>	<i>HIF1A</i>	<i>MARVELD2</i>	<i>PDPK1</i>	<i>SIX1</i>	<i>VAV2</i>
<i>CACNA1H</i>	<i>EGFR</i>	<i>HIF3A</i>	<i>MATR3</i>	<i>PDPN</i>	<i>SLC22A8</i>	<i>VAV3</i>

<i>CACNA1I</i>	<i>EGLN1</i>	<i>HIRA</i>	<i>MB</i>	<i>PDS5A</i>	<i>SLC2A4</i>	<i>VCAM1</i>
<i>CACNB2</i>	<i>EGLN3</i>	<i>HMOX1</i>	<i>MBTD1</i>	<i>PDS5B</i>	<i>SLC38A10</i>	<i>VCAN</i>
<i>CALCRL</i>	<i>EGR2</i>	<i>HOPX</i>	<i>MCU</i>	<i>PELI1</i>	<i>SLC39A4</i>	<i>VCL</i>
<i>CALR</i>	<i>EHMT1</i>	<i>HOXA1</i>	<i>MDM2</i>	<i>PEPD</i>	<i>SLC4A1</i>	<i>VDR</i>
<i>CAPNS1</i>	<i>EIF2B5</i>	<i>HOXA3</i>	<i>MECOM</i>	<i>PHC1</i>	<i>SLC6A6</i>	<i>VEGFA</i>
<i>CASP8</i>	<i>ELN</i>	<i>HOXB4</i>	<i>MED1</i>	<i>PHIP</i>	<i>SLC8A1</i>	<i>VEGFB</i>
<i>CASQ2</i>	<i>EMC10</i>	<i>HPRT1</i>	<i>MED12</i>	<i>PIBF1</i>	<i>SLC9A1</i>	<i>VPS54</i>
<i>CAV1</i>	<i>EMD</i>	<i>HRAS</i>	<i>MED24</i>	<i>PIFO</i>	<i>SLIT3</i>	<i>WASF2</i>
<i>CAV3</i>	<i>ENG</i>	<i>HSD11B2</i>	<i>MED30</i>	<i>PIGV</i>	<i>SMAD1</i>	<i>WDPCP</i>
<i>CC2D2A</i>	<i>EP300</i>	<i>HSD17B7</i>	<i>MEF2C</i>	<i>PIK3CA</i>	<i>SMAD2</i>	<i>WDR1</i>
<i>CCDC124</i>	<i>EPHA3</i>	<i>HSPB11</i>	<i>MEF2D</i>	<i>PIK3CB</i>	<i>SMAD3</i>	<i>WDR35</i>
<i>CCDC151</i>	<i>EPHB4</i>	<i>HSPB8</i>	<i>MEGF8</i>	<i>PIK3CG</i>	<i>SMAD4</i>	<i>WHSC1</i>
<i>CCDC160</i>	<i>EPO</i>	<i>HSPG2</i>	<i>MEIS1</i>	<i>PIK3R1</i>	<i>SMAD5</i>	<i>WNK1</i>
<i>CCDC39</i>	<i>EPOR</i>	<i>HTR2B</i>	<i>MEN1</i>	<i>PIK3R2</i>	<i>SMAD6</i>	<i>WNT11</i>
<i>CCM2</i>	<i>ERBB2</i>	<i>HTRA2</i>	<i>MEOX2</i>	<i>PIKFYVE</i>	<i>SMAD7</i>	<i>WNT3A</i>
<i>CCM2L</i>	<i>ERBB3</i>	<i>HUWE1</i>	<i>MESP1</i>	<i>PINK1</i>	<i>SMARCA4</i>	<i>WNT5A</i>
<i>CCND1</i>	<i>ERBB4</i>	<i>IDUA</i>	<i>MEST</i>	<i>PIP5K1C</i>	<i>SMG1</i>	<i>WRN</i>
<i>CCND2</i>	<i>ESR1</i>	<i>IER3</i>	<i>MFN2</i>	<i>PITX2</i>	<i>SMG9</i>	<i>WT1</i>
<i>CCND3</i>	<i>ESR2</i>	<i>IFT122</i>	<i>MFSD8</i>	<i>PKD1</i>	<i>SMN1</i>	<i>WWTR1</i>
<i>CCNE1</i>	<i>EYA1</i>	<i>IFT140</i>	<i>MGAT1</i>	<i>PKD2</i>	<i>SMO</i>	<i>XBP1</i>
<i>CCNE2</i>	<i>EZH2</i>	<i>IFT172</i>	<i>MGP</i>	<i>PKP2</i>	<i>SMYD1</i>	<i>XIRP1</i>
<i>CDC73</i>	<i>F11</i>	<i>IFT27</i>	<i>MGRN1</i>	<i>PLA2G4A</i>	<i>SNAI1</i>	<i>XIRP2</i>
<i>CDH2</i>	<i>F2R</i>	<i>IFT46</i>	<i>MIB1</i>	<i>PLAGL1</i>	<i>SNAI2</i>	<i>YAP1</i>
<i>CDH5</i>	<i>FADD</i>	<i>IFT57</i>	<i>MIXL1</i>	<i>PLCE1</i>	<i>SNX17</i>	<i>YWHAE</i>
<i>CDK2</i>	<i>FAT4</i>	<i>IFT74</i>	<i>MKL1</i>	<i>PLEC</i>	<i>SNX27</i>	<i>ZBTB14</i>
<i>CDK4</i>	<i>FBLN1</i>	<i>IFT88</i>	<i>MKL2</i>	<i>PLN</i>	<i>SOD1</i>	<i>ZFP36L1</i>
<i>CDK6</i>	<i>FBN1</i>	<i>IGF1R</i>	<i>MKS1</i>	<i>PLVAP</i>	<i>SOD2</i>	<i>ZFPM1</i>
<i>CDKN1A</i>	<i>FENDRR</i>	<i>IGF2</i>	<i>MMP21</i>	<i>PLXND1</i>	<i>SOS1</i>	<i>ZFPM2</i>
<i>CDKN1B</i>	<i>FES</i>	<i>IGF2R</i>	<i>MMP9</i>	<i>PNN</i>	<i>SOX11</i>	<i>ZIC3</i>
<i>CENPF</i>	<i>FGF10</i>	<i>IGFBP2</i>	<i>MORF4L1</i>	<i>PNPLA2</i>	<i>SOX12</i>	<i>ZNF366</i>
<i>CENPJ</i>	<i>FGF16</i>	<i>IGHMBP2</i>	<i>MOSPD3</i>	<i>POFUT1</i>	<i>SOX4</i>	<i>ZNF521</i>
<i>CEP290</i>	<i>FGF2</i>	<i>IKBKAP</i>	<i>MPZL3</i>	<i>POGLUT1</i>	<i>SOX9</i>	<i>ZSCAN10</i>
<i>CFC1</i>	<i>FGF8</i>	<i>IL17RD</i>	<i>MSX1</i>	<i>POLG</i>	<i>SPARC</i>	
<i>CFL1</i>	<i>FGF9</i>	<i>IL6</i>	<i>MSX2</i>	<i>POR</i>	<i>SPP1</i>	
<i>CFLAR</i>	<i>FGFR1</i>	<i>INSR</i>	<i>MT-CO1</i>	<i>POSTN</i>	<i>SPTA1</i>	
<i>CHD2</i>	<i>FGFR2</i>	<i>INTU</i>	<i>MT-RNR2</i>	<i>PPARA</i>	<i>SPTAN1</i>	

Table S2. A list of 144 Human CHD disease genes. The list was curated from the literature (PMIDs 29106500, 23934094, 27058611, 28991257, 27479907, 28592524, 25996639, 29089047).

<i>ACTC1</i>	<i>CITED2</i>	<i>FLT4</i>	<i>KIAA0196</i>	<i>MYH7</i>	<i>PKD2</i>	<i>SOS1</i>
<i>ACVR1</i>	<i>COL2A1</i>	<i>FOXC1</i>	<i>KMT2A</i>	<i>NF1</i>	<i>PLRG1</i>	<i>SRCAP</i>
<i>ACVR2B</i>	<i>COL9A1</i>	<i>FOXC2</i>	<i>KMT2D</i>	<i>NFATC1</i>	<i>PRDM1</i>	<i>STK4</i>
<i>ADNP</i>	<i>CREBBP</i>	<i>FOXH1</i>	<i>KRAS</i>	<i>NIPBL</i>	<i>PRKD1</i>	<i>STRA6</i>
<i>ALDH1A2</i>	<i>CRELD1</i>	<i>FOXL2</i>	<i>LBR</i>	<i>NKX2</i>	<i>PTGFRA</i>	<i>TAB2</i>
<i>ALK2</i>	<i>CYR61</i>	<i>FOXP1</i>	<i>LEFTY2</i>	<i>NKX2-5</i>	<i>PTPN11</i>	<i>TBX1</i>
<i>ANKRD1</i>	<i>DDX3X</i>	<i>GATA4</i>	<i>MAP2K1</i>	<i>NKX2-6</i>	<i>RAF1</i>	<i>TBX20</i>
<i>ANKRD11</i>	<i>DHCR7</i>	<i>GATA5</i>	<i>MAP2K2</i>	<i>NODAL</i>	<i>RBFOX2</i>	<i>TBX3</i>
<i>B3GALTL</i>	<i>DNAH11</i>	<i>GATA6</i>	<i>MCTP2</i>	<i>NOTCH1</i>	<i>RBM10</i>	<i>TBX5</i>
<i>B3GAT3</i>	<i>DNAH5</i>	<i>GDF1</i>	<i>MDM4</i>	<i>NOTCH2</i>	<i>RIT1</i>	<i>TDGF1</i>
<i>BBS2</i>	<i>DNAI1</i>	<i>GDF3</i>	<i>MED13L</i>	<i>NPHP2</i>	<i>RNF20</i>	<i>TFAP2B</i>
<i>BCOR</i>	<i>DVL1</i>	<i>GJA5</i>	<i>MEK1</i>	<i>NPHP3</i>	<i>ROBO1</i>	<i>TNNI3</i>
<i>BMPRI1A</i>	<i>DYRK1A</i>	<i>GPC3</i>	<i>MESP1</i>	<i>NPHP4</i>	<i>ROR2</i>	<i>VCAN</i>
<i>BRAF</i>	<i>EHMT1</i>	<i>HAND2</i>	<i>MID1</i>	<i>NR1D2</i>	<i>SALL1</i>	<i>WNT5A</i>
<i>CBL</i>	<i>ELN</i>	<i>HAS2</i>	<i>MKKS</i>	<i>NR2F2</i>	<i>SALL4</i>	<i>ZEB2</i>
<i>CCDC11</i>	<i>EP300</i>	<i>HOXA1</i>	<i>MKRN2</i>	<i>NRAS</i>	<i>SEMA3D</i>	<i>ZFMP2</i>
<i>CDK13</i>	<i>EVC</i>	<i>HRAS</i>	<i>MKS1</i>	<i>NRP1</i>	<i>SH3PXD2B</i>	<i>ZFPM2</i>
<i>CEP152</i>	<i>EVC1</i>	<i>IRX4</i>	<i>MLL2</i>	<i>NSD1</i>	<i>SHOC2</i>	<i>ZIC3</i>
<i>CFC1</i>	<i>EVC2</i>	<i>JAG1</i>	<i>MMP21</i>	<i>PACS1</i>	<i>SHROOM3</i>	
<i>CHD4</i>	<i>FBN1</i>	<i>JARID2</i>	<i>MYH11</i>	<i>PDGFRA</i>	<i>SMAD6</i>	
<i>CHD7</i>	<i>FGFR3</i>	<i>KDM6A</i>	<i>MYH6</i>	<i>PITX2</i>	<i>SMURF1</i>	

Table S3. Primers used in the study

Name	5'-3' sequence
<i>Primers for test of morpholinos</i>	
zAdcy2a-SP-MO-test-F	GGAGTTTGAAAAGCGTCAGC
zAdcy2a-SP-MO-test-R	GGTACACCACCTGCTTCCAT
zItpr1b-SP-MO-test-F	TGTGGAGGTGGTGAGAAAGC
zItpr1b-SP-MO-test-R	AGAGGCTGTCCTGCGTTTAC
zPlcb2-SP-MO-test-F	ACGCTCTGCTTATCGACCTG
zPlcb2-SP-MO-test-R	GTCTGGTAGTTGAGGGCCAC
zAdcy2a-SP-MO2-test-F	GCCAGTCTGCAGTTCAACAT
zAdcy2a-SP-MO2-test-R	TGCCAGAATCTGCCATACCA
zPlcb2-SP-MO2-test-F	CTCTGATGAGGGAACGGCTG
zPlcb2-SP-MO2-test-R	AATCTTAATAGTGAGCGTGC
 <i>Primers for qRT-PCR</i>	
mef2cb-F	ACTCGGACATAGTGGAGACCCTG
mef2cb-R	TTCTTTGCAGGCCGTGGTGGG
actb1-F	AGATCTTCACTCCCCTTGTTTAC
actb1-R	AAACCGGCTTTGCACATACC
rpl13a-F	TCTGGAGGACTGTAAGAGGTATGC
rpl13a-R	AGACGCACAATCTTGAGAGCAG

Table S4. Variants identified in known human CHD genes.

Gene	Variant	Mutation	Family	MAF ^a	Sub population with MAF >0.01 ^b	CADD score ^c	ClinVar interpretation (N) [ID]
<i>ANKRD1</i>	10-92675322-G-A	p.Ala276Val	346	0.002638	AJ (0.02086)	23.00	Benign(5);Likely benign(5);Uncertain significance(1) [45639]
<i>CCDC11</i>	18-47765069-C-T	p.Arg407Gln	3315	0.000025	n/a	25.70	n/a
<i>CDK13</i>	7-40132656-G-A	p.Val1170Met	3315	0.005165	n/a	26.10	n/a
<i>CYR61</i>	1-86048526-C-G	p.Ser316Cys	3540	0.004706	n/a	24.60	n/a
<i>DNAH11</i>	7-21600738-A-G	p.Gln311Arg	226	0.000077	n/a	14.36	n/a
	7-21760479-G-A	c.7287+5 G>A	346	0.000074	n/a	14.54	n/a
	7-21789835-G-A	c.8798-5 G>A	645	0.003759	n/a	14.13	Benign(1);Likely benign(2);Uncertain significance(1) [163114]
	7-21788220-C-G	p.Arg2845Gly	720	0.001274	n/a	16.60	Likely benign(1);Uncertain significance(1) [359658]
	7-21640338-G-T	p.Glu1015Asp	1722	0.002989	n/a	22.70	Benign (2) [238905]
<i>DNAH5</i>	5-13850914-C-T	p.Arg1654Gln	489	0.0002617	n/a	32.00	Uncertain significance (2) [238977]
	5-13914751-C-T	p.Val400Met	732	0.0008012	n/a	34.00	Likely benign (1) [219733]

	5-13786351-C-G	p.Glu2919Asp	1121	0.003120	n/a	15.84	Benign(2);Likely benign(1);Uncertain significance(1) [188365]
<i>EVC2</i>	4-5642347-G-C	p.Thr455Arg	545	0.003836	n/a	23.90	Benign(3);Likely benign(1);Uncertain significance(1) [193762]
	4-5620263-G-A	p.Ala803Val	1151	0.003752	FIN (0.01365)	16.90	n/a
<i>FOXH1</i>	8-145700381-C-G	p.Ser113Thr	346	0.004183	n/a	22.70	Benign/Likely benign (5) [94385]
<i>KMT2A</i>	11-118352769-G-A	p.Ser1325Asn	49	0.000906	n/a	19.46	n/a
<i>KMT2D</i>	12-49426025-G-T ^d	p.Pro4155Thr	346	0.0000281	n/a	14.07	n/a
	12-49431844-G-A ^d	p.Arg3099Cys	3505	0.00002884	n/a	24.80	n/a
<i>MYH11</i>	16-15797967-T-A	p.Thr1934Ser	732	0.001347	n/a	16.85	Likely benign(5);Uncertain significance(1) [201044]
<i>MYH6</i>	14-23866451-G-T	Leu700Ile	1710	n/a	n/a	24.20	n/a
<i>NFATC1</i>	18-77246538-G-A	p.Gly795Arg	645	0.003186	n/a	14.95	n/a
<i>NOTCH2</i>	1-120468201-A-T	p.Leu1413His	1121	0.003299	n/a	15.52	Benign/Likely benign (5)[134975]
<i>NPHP4</i>	1-6012895-G-A	c.675 C>T (splice)	489	0.000086	n/a	5.49	n/a
<i>PACSI1</i>	11-66008953-G-A	Gly829Ser	1151	n/a	n/a	23.10	n/a

<i>PDGFRA</i>	4-55161324-C-T	p.Thr1052Met	1151	0.0001769	n/a	25.20	Uncertain significance (2) [39618]
<i>PKD2</i>	4-88967919-T-G	p.Phe482Cys	545	0.001984	n/a	24.90	Benign (3) [219481]
<i>ROR2</i>	9-94487187-C-T	p.Arg530Gln	732	0.001915	n/a	20.90	Likely benign (4) [284633]
<i>TBX5</i>	12-114837364-T-C	p.Ile106Val	226	0.000933	AJ (0.01119)	21.80	Likely benign (3) [213825]
<i>VCAN</i>	5-82868257-A-G	p.Asn3253Ser	346	0.0002088	n/a	26.10	n/a
	5-82816284-T-C	p.Val720Ala	732	0.001995	n/a	0.001	Likely benign (3) [354403]

^a Minor allele frequency in gnomAD v2.1.1 (141,456 individuals, <http://gnomad.broadinstitute.org>). ^b GnomAD sub population with MAF >0.01. AJ: Ashkenazi Jewish, FIN: European (Finnish). MAF in subpopulation is shown in parentheses. ^c CADD score according to the Combined Annotation-Dependent Depletion prediction algorithm. n/a: not available. ^d None of the patients with these variants in *KMT2D* had features of Kabuki syndrome.

Table S5. Significant protein-protein interaction clusters

with at least two CDGs. Statistical significance was determined by permutation analysis (k=10,000). *:P<0.05, **:P<0.01.

Cluster id.	Cluster size	Mut.Genes	p-value
cluster15	27	11	0.00331 **
cluster17	5	3	0.03872 *
cluster215	21	7	0.05427
cluster28	13	5	0.05775
cluster40	6	3	0.06705
cluster202	6	3	0.06807
cluster200	12	4	0.13646
cluster14	23	6	0.19057
cluster8	5	2	0.20428
cluster3	29	7	0.22072
cluster208	10	3	0.24048
cluster10	6	2	0.27304
cluster6	6	2	0.27311
cluster47	6	2	0.27341
cluster230	6	2	0.27429
cluster42	6	2	0.27477
cluster41	6	2	0.27558
cluster13	7	2	0.34492
cluster7	8	2	0.41017
cluster5	15	3	0.48476
cluster4	27	5	0.5071
cluster9	17	3	0.57995
cluster2	18	3	0.61678
cluster113	14	2	0.71902
cluster1	30	4	0.78055

Table S6. Rare calcium signaling gene variants shared among affected individuals in multiplex CHD families.

Family	Gene	Gene pLI ^a	Variant	Mutation ^b	MAF ^c	Sub pop. With MAF>0.01 ^d	CADD score ^e	MPC score	Heart malformations
333	<i>ITPR1^e</i>	1.000	3-4704816-G-A	V479I	0.004560	AJ (0.01023)	22.2	1.77	PAPVR, ASDsv, VSD, COA
346	<i>ADCY2^f</i>	1.000	5-7695981-G-A	IVS6+5 G>A	0.005025	AJ (0.01529), FIN (0.02375)	15.1	n/a	VSD
	<i>NFAT5^g</i>	1.000	16-69660329-A-G	K51E	0.000042	n/a	27.0	1.95	VSD
489	<i>PLCB2^h</i>	0.000	15-40584565-C-A	K802N	0.000004	n/a	29.7	n/a	ASD, subAS, VSD, PDA, HCM
	<i>PLCB2^h</i>	0.000	15-40584570-T-A	M801L	n/a	n/a	24.9	n/a	ASD, subAS, VSD, PDA, HCM
	<i>PLCB2^h</i>	0.000	15-40584571-C-A	E800D	n/a	n/a	21.1	n/a	ASD, subAS, VSD, PDA, HCM
645	<i>CACNA1Dⁱ</i>	1.000	3-53707750-C-T	A376V	0.00001591	n/a	32.0	2.04	ASD, VSD
702	<i>CACNA1Sⁱ</i>	0.000	1-201009430-G-T	P1767T	0.0002199	n/a	3.34	0.11	VSD, AVSD
732	<i>CACNA1Hⁱ</i>	0.756	16-1260783-G-A	IVS20-4 G>A	0.003458	AJ (0.03019)	22.9	n/a	TOF
	<i>CACNA1Hⁱ</i>	0.756	16-1270119-C-T	R2063W	0.00009303	n/a	0.07	n/a	TOF
	<i>GRIA4^j</i>	0.989	11-105776016-G-C	G383R	0.000004002	n/a	26.5	n/a	TOF
1121	<i>CACNA1Fⁱ</i>	0.874	X-49088240-T-C	T59A	0.00005878	n/a	0.12	0.54	ASD, VSD, PVS
1151	<i>CACNA1Sⁱ</i>	0.000	1-201009011-C-T	S1857N	0.002224	n/a	23.3	0.17	ASD, EbA
1722	<i>ADCY5^f</i>	0.990	3-123046510-C-G	E634D	0.004502	n/a	15.1	n/a	ASD, COA, DCM
	<i>ITPR1^e</i>	1.000	3-4842276-G-A	A2352T	0.0008309	n/a	23.5	0.92	ASD, COA, DCM
2077	<i>CACNA1Fⁱ</i>	1.000	22-40059828-G-C	Q1193H	0.003735	AJ (0.03569)	23.7	n/a	PVS, InfPS

^a Probability of loss of function intolerance of gene. ^b All 16 listed mutations were confirmed by Sanger sequencing. ^c Minor allele frequency in gnomAD v2.1.1 (141,456 individuals, <http://gnomad.broadinstitute.org>). n/a: not available in database. ^e GnomAD sub population with MAF >0.01. AJ: Ashkenazi Jewish, FIN: European (Finnish). MAF in subpopulation is shown in parentheses. ^d C score according to the Combined Annotation-Dependent Depletion (CADD) prediction algorithm ²⁷. ^d MPC-score: score based on regional missense constraint. ^e IP3 receptor. ^f Adenylate cyclase. ^g Transcription factor. ^h Phospholipase C. ⁱ Voltage Dependent Calcium Channel. ^j Glutamate receptor.

Table S7. Gene ontology term enrichment of 27 genes within the cluster shown in Figure 3A. P-values were adjusted for multiple testing.

Gene Ontology – Molecular Function	p-value
calcium-dependent protein kinase C activity (GO:0004698)	1.61E-06
calcium-dependent protein serine/threonine kinase activity (GO:0009931)	3.35E-05
calcium-dependent protein kinase activity (GO:0010857)	4.35E-05
protein kinase C activity (GO:0004697)	1.47E-04
histone kinase activity (H3-T6 specific) (GO:0035403)	9.01E-04
protein serine/threonine kinase activity (GO:0004674)	2.60E-03
ATP binding (GO:0005524)	2.99E-03
adenyl ribonucleotide binding (GO:0032559)	3.44E-03
adenyl nucleotide binding (GO:0030554)	3.57E-03
purine ribonucleoside triphosphate binding (GO:0035639)	1.03E-02
purine ribonucleoside binding (GO:0032550)	1.06E-02
purine nucleoside binding (GO:0001883)	1.07E-02
ribonucleoside binding (GO:0032549)	1.08E-02
cAMP-dependent protein kinase activity (GO:0004691)	1.10E-02
histone threonine kinase activity (GO:0035184)	1.10E-02
nucleoside binding (GO:0001882)	1.10E-02
purine ribonucleotide binding (GO:0032555)	1.18E-02
purine nucleotide binding (GO:0017076)	1.23E-02
ribonucleotide binding (GO:0032553)	1.31E-02
cyclic nucleotide-dependent protein kinase activity (GO:0004690)	1.82E-02
protein kinase activity (GO:0004672)	2.18E-02
adenylate cyclase activity (GO:0004016)	2.25E-02
carbohydrate derivative binding (GO:0097367)	3.51E-02
Gene Ontology – Biological Process	p-value
activation of phospholipase C activity (GO:0007202)	1.59E-07
positive regulation of phospholipase C activity (GO:0010863)	3.30E-07
regulation of phospholipase C activity (GO:1900274)	3.83E-07
positive regulation of phospholipase activity (GO:0010518)	1.03E-06
regulation of phospholipase activity (GO:0010517)	1.82E-06
positive regulation of lipase activity (GO:0060193)	2.14E-06
regulation of lipase activity (GO:0060191)	4.61E-06
activation of protein kinase A activity (GO:0034199)	3.76E-04
epidermal growth factor receptor signaling pathway (GO:0007173)	8.48E-04
fibroblast growth factor receptor signaling pathway (GO:0008543)	8.75E-04
regulation of body fluid levels (GO:0050878)	1.01E-03
ERBB signaling pathway (GO:0038127)	1.02E-03
cellular response to fibroblast growth factor stimulus (GO:0044344)	1.27E-03
response to fibroblast growth factor (GO:0071774)	1.38E-03
peptidyl-serine phosphorylation (GO:0018105)	2.19E-03

neurotrophin TRK receptor signaling pathway (GO:0048011)	2.37E-03
neurotrophin signaling pathway (GO:0038179)	2.46E-03
transmembrane receptor protein tyrosine kinase signaling pathway (GO:0007169)	2.51E-03
histone H3-T6 phosphorylation (GO:0035408)	2.74E-03
renal water homeostasis (GO:0003091)	2.74E-03
peptidyl-serine modification (GO:0018209)	3.18E-03
energy reserve metabolic process (GO:0006112)	3.27E-03
cellular response to glucagon stimulus (GO:0071377)	3.86E-03
activation of adenylate cyclase activity (GO:0007190)	4.52E-03
positive regulation of adenylate cyclase activity (GO:0045762)	8.41E-03
response to glucagon (GO:0033762)	8.41E-03
enzyme linked receptor protein signaling pathway (GO:0007167)	9.95E-03
synaptic signaling (GO:0099536)	1.11E-02
synaptic transmission (GO:0007268)	1.11E-02
trans-synaptic signaling (GO:0099537)	1.11E-02
multicellular organismal water homeostasis (GO:0050891)	1.48E-02
positive regulation of cyclase activity (GO:0031281)	1.72E-02
positive regulation of lyase activity (GO:0051349)	1.72E-02
water transport (GO:0006833)	1.90E-02
water homeostasis (GO:0030104)	1.99E-02
fluid transport (GO:0042044)	2.49E-02
regulation of adenylate cyclase activity (GO:0045761)	2.60E-02
histone-threonine phosphorylation (GO:0035405)	3.35E-02
positive regulation of cAMP biosynthetic process (GO:0030819)	3.87E-02
immune system process (GO:0002376)	3.91E-02
cellular response to forskolin (GO:1904322)	4.38E-02
response to forskolin (GO:1904321)	4.38E-02
regulation of cyclase activity (GO:0031279)	4.63E-02
regulation of lyase activity (GO:0051339)	4.97E-02

Table S8. Replication using WES data from 714 CHD cases and 4922 controls. The number of cases and controls with rare variants (MAF < 0.001) in any of the genes *ADCY2*, *ADCY5*, *CACNAID*, *CACNAIH*, *CACNAII*, *CACNAIS*, *GRIA4*, *ITPRI*, *NFAT5* and *PLCB2* was compared (see supplemental material for details). WES data was not available for *CACNAIF*.

Type of rare variant ^a	Cases	Controls	P-value ^b		Odds ratio
Protein sequence altering variants	116	877	3.18E-01	n.s.	0.89 [0.72-1.11]
Protein altering variants, MPC score >1	43	243	3.35E-01	n.s.	1.23 [0.86-1.73]
Protein altering variants, MPC score >2	21	55	3.69E-04	**	2.68 [1.53-4.54]
Protein truncating variants	3	16	7.25E-01	n.s.	1.29 [0.24-4.53]
Synonymous variants	94	630	7.65E-01	n.s.	1.03 [0.81-1.31]

^a Variants with pathogenic effect was defined by MPC score >2. ^b Fisher's exact test. **:P<0.01. n.s.: not significant.