Rare Genetic Variants Associated With Sudden Cardiac Death in Adults

ONLINE APPENDIX

Online Methods

Sudden cardiac death ascertainment

All 6 prospective cohorts employed similar methods to document the timing and mechanism of cardiovascular deaths as definite or probable sudden cardiac death (1) -- including ascertainment and review of medical records (inclusive of emergency medical services, emergency room, hospital, and autopsy reports as available) and standardized interviews with next-of-kin or witnesses to obtain detailed descriptions of the circumstances surrounding the death. Information from death certificates were not used in the assessment of sudden cardiac death, given limited concordance with rigorous endpoint adjudication in prior studies (2,3). Definite sudden cardiac deaths included cases in which the death or cardiac arrest occurred within 1 hour of symptom onset or those that fulfilled the Hinkle and Thaler criteria (4) for sudden cardiac death due to arrhythmia as documented in the medical records or witness reports. Unwitnessed deaths were additionally included as probable sudden cardiac deaths if the participant was documented to be symptom free when last observed within the preceding 24 hours, and circumstances suggested that the death could have been sudden as performed previously (1).

Intermediate cardiovascular phenotypes – MESA prospective cohort study

Within the Multi-Ethnic Study of Atherosclerosis (MESA) study, estimated untreated LDL cholesterol was determined by dividing measured LDL cholesterol by 0.7 among individuals taking a cholesterol-lowering statin medication as we and others have performed previously (5,6). Left ventricular ejection fraction was calculated based on cardiac magnetic resonance imaging as reported previously (7). QT interval was assessed based on a standard 12-lead electrocardiogram performed at the baseline study visit, corrected QT interval computed as reported previously and in accordance with current professional society guidelines (8,9). We set up a linear regression model with QT interval as the dependent variable and age (continuous), ethnicity (white, African American, Chinese American, and Hispanic), sex (female or male), and RR interval as independent variables. The RR interval was analyzed using restricted quadratic splines with knots at the 5th, 50th, and 95th percentiles to allow a more flexible and nonlinear relationship between the QT and RR intervals. Model residuals represent the component of QT interval duration that is not explained by the independent variables. Because the average of the

residuals is 0, we rescaled the residuals by adding the mean QT interval of the overall study population to calculate the final corrected QT interval.

Gene Sequencing

Whole exome sequencing was performed on 1200 samples of the sudden cardiac death nested case-control study at the Broad Institute of MIT and Harvard (Cambridge, MA) as previously described.(5) In brief, sequence data of all participants were aligned to a human reference genome build GRCh37.p13 using the Burrows-Wheeler Aligner algorithm. Aligned non- duplicate reads were locally realigned and base qualities were recalibrated using Genome Analysis Toolkit software.(10,11) Variants were jointly called using Genome Analysis Toolkit HaplotypeCaller software. The sensitivity of the selected variant quality score recalibration threshold was 99.6% for single-nucleotide polymorphisms as empirically assessed using HapMap controls with known genotypes included in the sequencing call set.

Sample quality control was performed to ensure adequate sample purity (contamination <10%), average sequencing depth >30x, concordance of reported and genotypic sex, no sample duplicates, and no variant metric count outliers. All 1200 (100%) of samples met these quality control criteria.

Whole genome sequencing of the MultiEthnic Study of Atherosclerosis (MESA) cohort was performed as part of the National Heart, Lung, and Blood Institute Trans-Omic for Precision Medicine (TOPMed) program as previously described. 4,632 participants underwent sequencing, of which 107 (2.3%) were excluded due to withdrawal of consent for genomics analyses, enrollment despite preexisting cardiovascular disease, excess DNA contamination, mean sequencing coverage less than 30x, or sample duplicates – resulting a final dataset of 4525 individuals with sequencing data available for the present analysis. Variants were jointly called using Genome Analysis Toolkit HaplotypeCaller software. The sensitivity of the selected variant quality score recalibration threshold was 99.8% for single-nucleotide polymorphisms as empirically assessed using HapMap controls with known genotypes included in the sequencing call set.

Online Table 1. Prospective cohorts included in the sudden cardiac death case-control analysis

Study cohort	Study description	Year of inception	Participants	Number with blood samples	Years of blood collection
Physicians Health Study I (12)	Randomized clinical trial of aspirin and beta-carotene	1982	22,071 male physicians, aged 40-84, free of prior cardiovascular disease or cancer	15,124	1982 – 1984
				1,218	1996 – 1998
Physicians Health Study II (13)	Randomized clinical trial of antioxidants and multivitamin	1997	14,641 male physicians, aged ≥ 50 years, including 7,641 Physicians Health Study I participants and 7,000 new participants	11,133*	1995 – 2002
Health Professionals Follow-up Study (14)	Prospective cohort study	1986	51,529 male health professionals, aged 40-75	18,018	1993 – 1994
Nurses' Health Study (15)	Prospective cohort study	1976	121,700 female registered nurses, aged 30-55	32,826	1989 – 1990
Women's Antioxidant Cardiovascular Study (16)	Randomized clinical trial of antioxidants and B-vitamins	1994	8,171 female health professionals, aged ≥ 40 years, with cardiovascular disease or at least 3 cardiovascular risk factors	5922	1993 – 1996
Women's Health Study (17)	Randomized clinical trial of aspirin and Vitamin E	1992	39,876 female health professionals, ≥ 40 years, free of prior cardiovascular disease or cancer	28,345	1993 – 1996

* Of these, 6,518 blood samples are from men who had not previously donated a blood sample in Physicians Health Study I.

Online Table 2. 49 Genes with Previous Evidence of Association with a Sudden Cardiac Death-Associated Condition

Disease Category	Gene Symbols
Cardiomyopathy	*ACTC1* (NM_005159.4), *DES* (NM_001927.3), *GLA* (NM_000169.2), *LMNA* (NM_170707.2), *MYBPC3* (NM_000256.3), *MYH7* (NM_000257.2), *MYL2* (NM_000432.3), *MYL3* (NM_000258.2), *PLN* (NM_002667.3), *PRKAG2* (NM_016203.3), *TNNI3* (NM_000363.4), *TNNT2* (NM_001001430.1), *TPM1* (NM_000366.5), *TTN* (NM_133378.4, except variant p.Glu4215ArgfsTer7 on transcript NM_133432.3)
Coronary artery disease	*APOB* (NM_000384.2), *LDLR* (NM_000527.4), *PCSK9* (NM_174936.3)
Arrhythmia syndrome	*CACNA1C* (NM_199460.2), *CACNB2* (NM_201596.2), *CAV3* (NM_033337.2), *DSC2** (NM_024422.3), *DSG2** (NM_001943.3), *DSP** (NM_004415.2), *GPD1L* (NM_015141.3), *HCN4* (NM_005477.2), *JUP* (NM_021991.2), *KCNE1* (NM_000219.3), *KCNE2* (NM_172201.1), *KCNE3* (NM_005472.4), *KCNH2* (NM_000238.3), *KCNJ2* (NM_000891.2), *KCNQ1* (NM_000218.2), *PKP2** (NM_004572.3), *RYR2* (NM_001035.2), *SCN1B* (NM_001037.4), *SCN3B* (NM_001040151.1), *SCN4B* (NM_174934.3), *SCN5A* (NM_198056.2), *SNTA1* (NM_003098.2), *TGFB3* (NM_003239.2), *TMEM43** (NM_024334.2)
Aortopathy/aortic dissection	*ACTA2* (NM_001613.2), *COL3A1* (NM_000090.3), *FBN1* (NM_000138.4), *MYH11* (NM_053025.3), *MYLK* (NM_053025.3), *SMAD3* (NM_005902.3), *TGFBR1* (NM_004612.2), *TGFBR2* (NM_003242.5)

* 5 genes predisposing to arrhythmogenic right ventricular cardiomyopathy were considered as related to an arrhythmia syndrome for the purposes of this study, acknowledging that this designation is somewhat arbitrary given associations with both arrhythmia and a cardiomyopathy phenotype.

Online Table 3. Consequences of variants in 49 known cardiovascular genes identified in the sudden cardiac death case control cohort

Variant Consequence	Number of Variants
3' untranslated region	152
5' untranslated region	44
Downstream gene	2
Frameshift	20
Inframe deletion	22
Inframe insertion	6
Intron	1,953
Missense	1,650
Splice acceptor	2
Splice donor	7
Splice region	205
Stop gained	13
Synonymous	1,096
Upstream gene	6
Total	**5,178**

A total of 5,178 variants in any of 49 known cardiovascular gene regions were identified in at least one of the 1,2000 participants of the sudden cardiac death case-control cohorts. Shown is the distribution of the consequences of these variants, as assessed by the 'Consequence' field of annotations produced using the Ensembl Variant Effect Predictor tool (18).

Online Table 4. Evidence in Support of Pathogenic or Likely Pathogenic Variant Assertions in the Sudden Cardiac Death Nested Case-Control Cohort

Variant* (Case ID)	Gene (Variant Type)	Amino acid or cDNA change	Associated disease	Evidence in Support of Pathogenicity Assessment
Cardiomyopathy variants				
2:179417587:C>A (P1)	*TTN* Premature stop	p.Gly27446Ter	Dilated cardiomyopathy	The p.Gly27446Ter variant in TTN has not been previously reported in individuals with cardiomyopathy and was absent from large population studies. This nonsense variant leads to a premature termination codon at position 27446, which is predicted to lead to a truncated or absent protein. Nonsense and other truncating variants in TTN are strongly associated with DCM if they impact the exons encoding for the A-band (Herman 2012, Pugh 2014). The p.Gly27446Ter variant is located in A-band in the highly expressed exon 284. In summary, although additional studies are required to fully establish its clinical significance, the p.Gly27446Ter variant is **likely pathogenic**. ACMG/AMP Criteria applied: PVS1; PM2.
2:179433438:G>T (P2)	*TTN* Premature stop	p.Tyr23239Ter	Dilated cardiomyopathy	The p.Tyr23239Ter variant in TTN has not been previously reported in individuals with DCM and was absent from large population studies. This nonsense variant leads to a premature termination codon at position 23239, which is predicted to lead to a truncated or absent protein. Nonsense and other truncating variants in TTN are strongly associated with DCM if they impact the exons encoding for the A-band (Herman 2012, Pugh 2014). The p.Tyr23239Ter variant is located in A-band in the highly expressed exon 275. In summary, although additional studies are required to fully establish its clinical significance, the p.Tyr23239Ter variant is **likely pathogenic**. ACMG/AMP Criteria applied: PVS1; PM2.
2:179444577:T>G (P3)	*TTN* Splice site	c.59645-2A>C	Dilated cardiomyopathy	The c.59645-2A>C variant in TTN has been reported in compound heterozygous state in 1 individual with congenital muscular dystrophy and DCM (reported as c.67349-2A>C, O'Grady 2016). It has also been identified in 1/109468 of European chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org; dbSNP rs753948675) and has been reported in ClinVar (Variation ID: 424832). This variant occurs in the invariant region (+/- 1,2) of the splice consensus sequence and is predicted to cause altered splicing leading to an abnormal or absent protein. Truncating variants in TTN are strongly associated with DCM if they impact the exons encoding for the A-band (Herman 2012, Pugh 2014). The c.59645-2A>C variant is located in A-band adjacent to the highly expressed exon 267. In summary, although additional studies are required to fully establish its clinical significance, the c.59645-2A>C variant is **likely pathogenic**. ACMG/AMP Criteria applied: PM2; PM3; PVS1_Moderate.
2:179486244:G>A (P4)	*TTN* Premature stop	p.Arg12535Ter	Dilated cardiomyopathy	The p.Arg12535Ter variant in TTN has been identified by our laboratory in 1 individual with DCM and segregated with disease in 2 affected relatives, including one obligate carrier. This variant was absent from large population studies. This nonsense variant leads to a premature termination codon at position 12535, which is predicted to lead to a truncated or absent protein. Nonsense and other truncating variants in TTN are strongly associated with DCM if they impact the exons encoding the A-band (Herman 2012, Pugh 2014) and/or are located in an exon that is highly expressed in the heart (Roberts 2015). The p.Arg12535Ter variant is located in the I-band in the highly expressed exon 194. In summary, although additional studies are required to fully establish its clinical significance, the p.Arg12535X variant is **likely pathogenic**. ACMG/AMP Criteria applied: PVS1_Strong; PM2.
2:179604601:TTC>T (P5)	*TTN* Frameshift	p.Glu4215ArgfsTer7	Dilated cardiomyopathy	The p.Glu4215fs variant in TTN has not been previously reported in individuals with DCM and was absent from large population studies, though the ability of these studies to accurately detect indels may be limited. This variant is predicted to cause a frameshift, which alters the protein's amino acid sequence beginning at position 4215 and leads to a premature termination codon 7 amino acids downstream. This alteration is then predicted to lead to a truncated or absent protein. Frameshift and other truncating variants in TTN are strongly associated with DCM if they impact the exons encoding for the A-band (Herman 2012, Pugh 2014) and/or are located in an exon that is highly expressed in the heart (Roberts 2015). The p.Glu4215fs variant is located in the highly expressed exon 45 in the I band. In summary, although additional studies are required to fully establish its clinical significance, the p.Glu4215fs variant is **likely pathogenic.** ACMG/AMP Criteria applied: PVS1_Strong; PM2.

19:55665525:C>T (P6)	*TNNI3* Missense	p.Arg141Gln	Hypertrophic cardiomyopathy	The p.Arg141Gln variant in TNNI3 has been reported in >14 individuals with HCM and segregated with disease in 3 affected relatives from 3 families (Richard 2003, Van Driest 2003, Mogensen 2004, Morita 2008, Curila 2009, van den Wijngaard 2011, Rani 2012, Santos 2012, Kapplinger 2014, LMM data). Of note, this variant was homozygous in one individual with severe disease (Mogensen 2004), and compound heterozygous in 2 individuals with early-onset disease (Morita 2008, LMM data, Santos 2012). Our laboratory has also detected this variant in an additional individual with Wolff-Parkinson-White syndrome. In addition, this variant has been reported by other clinical laboratories in ClinVar (Variation ID 43381) and has been identified in 1/8728 of African chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org/; dbSNP rs397516347). Arginine (Arg) at position 141 is highly conserved in mammals and across evolutionarily distant species and the change to glutamine (Gln) was predicted to be pathogenic using a computational tool clinically validated by our laboratory. This tool's pathogenic prediction is estimated to be correct 94% of the time (Jordan 2011). In summary, although additional studies are required to fully establish its clinical significance, the p.Arg141Gln variant is **likely pathogenic**. ACMG/AMP Criteria applied: PS4; PP1; PM2; PP3.
Coronary artery disease variants				
19:11213408:T>G (P7 & P8)	*LDLR* Missense	p.Trp87Gly	Familial hypercholesterolemia	The p.Trp87Gly variant in LDLR is a well-established pathogenic variant for familial hypercholesterolemia (FH; Leitersdorf 1990, Jensen 1996, Vohl 1997, Tybjaerg-Hansen 2005, Futema 2013), and is a known founder mutation in the French Canadian population where it has been reported in >400 individuals with FH, including >15 homozygous individuals (Vohl 1997). It has also been identified in 7/126722European chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org; dbSNP rs121908025); however, this frequency is low enough to be consistent with the frequency of FH in the general population. Additionally, in vitro functional studies provide some evidence that the p.Trp87Gly variant may impact protein function (Leitersdorf 1990). In summary, this variant meets our criteria to be classified as **pathogenic** for FH in an autosomal dominant manner based upon its identification in a large number of affected individuals and low frequency in controls. ACMG/AMP Criteria applied: PS4; PM3; PM2_Supporting; PP3; PS3_Supporting.
19:11221435:C>T (P9)	*LDLR* Premature stop	p.Arg350Ter	Familial hypercholesterolemia	The p.Arg350Ter variant in LDLR (also described as p.Arg329Ter in the literature) has been reported in >15 individuals with hypercholesterolemia and segregated with disease in >15 affected relatives from 8 families (Day 1997, Humphries 2006, Kubalska 2008, Dušková 2011, van der Graaf 2011, Tichý 2012, Huijgen 2012, Radovica-Spalvina 2015, Do 2015, Fan 2015). This variant has also been identified in 1/111552 European chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org; dbSNP rs769737896) and in ClinVar (Variation ID: 226342). This nonsense variant leads to a premature termination codon at position 350, which is predicted to lead to a truncated or absent protein. Heterozygous loss of function of the LDLR gene is an established disease mechanism in individuals with familial hypercholesterolemia (FH). In summary, this variant meets criteria to be classified as **pathogenic** for FH in an autosomal dominant manner. ACMG/AMP Criteria applied: PVS1; PS4; PP1_Strong; PM2.
19:11224299:T>C (P10)	*LDLR* Missense	p.Trp483Arg	Familial hypercholesterolemia	The p.Trp483Arg variant in LDLR has been reported in at least 7 individuals with hypercholesterolemia and segregated with disease in 6 affected members of 1 family (Ward 1995, Fouchier 2005, Taylor 2007, Martin 2016). It was absent from large population studies. Computational prediction tools and conservation analysis suggest that the variant may impact the protein, though this information is not predictive enough to determine pathogenicity. In summary, although additional studies are required to fully establish its clinical significance, the p.Trp483Arg variant is **likely pathogenic**. ACMG/AMP Criteria applied: PM2; PS4_Moderate; PP1_Moderate; PP3.
Inherited arrhythmia syndrome variants				
6:7580603:C>T (P11)	*DSP* Premature stop	p.Gln1394Ter	Arrhythmogenic right ventricular cardiomyopathy	The p.Gln1394Ter variant in DSP has not been previously reported in individuals with cardiomyopathy, but has been identified in 1/111616 European chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org; dbSNP rs140474226). This variant is located within exon 23 of DSP which undergoes alternative splicing resulting into three isoforms: a short (DSPII), an intermediate (DSPIa) and a long (DSPI) form of this

				exon. This variant is only located in the coding region of the longer isoform (DSPI). The DSPI transcript is the predominant isoform in cardiac tissue (Uzumcu 2006, Cabral 2010). This nonsense variant leads to a premature termination codon at position 1394, which is predicted to lead to a truncated or absent protein. Loss-of-function variants in the longer form of exon 23 have been observed in individuals with ARVC, DCM, and/or Carvajal syndrome, suggesting that loss-of-function variants in this region are disease causing (Uzumcu 2006, LMM data). In summary, although additional studies are required to fully establish its clinical significance, the p.Gln1394Ter variant is **likely pathogenic**. ACMG/AMP Criteria applied: PVS1_Strong; PM2.
7:150647424:G>A (P12)	*KCNH2* Premature stop	p.Arg744Ter	Long QT syndrome	The p.Arg744Ter variant in KCNH2 has been reported in 1 Caucasian individual with sudden unexpected death in epilepsy (Bagnall 2016), 8 individuals with Long QT syndrome (Ko 2001, Moss 2002, Schwartz 2009, Kapplinger 2009, Crotti 2012), and 1 individual with LQTS and Charcot-Marie-Tooth disease, who also carried a duplication of 17p11.2 (Losito 2009). This variant segregated with LQTS in 13 relatives from multiple families. This variant has also been reported in ClinVar (Variation ID 180383) and was absent from large population studies. This nonsense variant leads to a premature termination codon at position 744, which is predicted to lead to a truncated or absent protein. Heterozygous loss of function of the KCNH2 gene is an established disease mechanism in LQTS. In summary, this variant meets criteria to be classified as **pathogenic** for LQTS in an autosomal dominant manner based upon segregation studies, absence from controls, and predicted impact on protein. ACMG/AMP Criteria applied: PVS1; PP1_Strong; PM2; PS4_Moderate.
7:150655169:CG>C (P13)	*KCNH2* Frameshift	p.Pro298ArgfsTer62	Long QT syndrome	The p.Pro298fs variant in KCNH2 has not been previously reported in individuals with long QT syndrome, but has been identified in 1/330 European chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org;). This variant is predicted to cause a frameshift, which alters the protein's amino acid sequence beginning at position 298 and leads to a premature termination codon 62 amino acids downstream. This alteration is then predicted to lead to a truncated or absent protein. In summary, although additional studies are required to fully establish its clinical significance, the p.Pro298fs variant is **likely pathogenic**. ACMG/AMP Criteria applied: PVS1.
Aortopathy/aortic dissection variants				
2:189855742:C>T (P14)	*COL3A1* Premature stop	p.Arg271Ter	Vascular Ehlers-Danlos syndrome	The p.Arg271Ter variant in COL3A1 has been reported in two Caucasian individuals with clinical features of vascular Ehlers-Danlos syndrome (Frank 2015, Campens 2015). It was also reported in one fetus with multiple limb defects, father (clinical status not reported), and paternal uncle with limb defects (Pangalos 2016). It was absent from large population studies. This nonsense variant leads to a premature termination codon at position 271, which is predicted to lead to a truncated or absent protein. Heterozygous loss of function of the COL3A1 gene is an established disease mechanism in Ehlers-Danlos syndrome and is associated with late-onset, reduced penetrance, and possibly a milder clinical course (Leistritz 2011, Frank 2015). In summary, this variant is **pathogenic**. ACMG/AMP Criteria applied: PVS1; PM2; PS4_Supporting.
15:48892335:C>T (P15)	*FBN1* Splice site	c.442+1G>A	Marfan syndrome	The c.442+1G>A variant in FBN1 has not been previously reported in individuals with Marfan syndrome, but was identified in 1/15008 European chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org; dbSNP rs86840373). This variant occurs in the invariant region (+/- 1,2) of the splice consensus sequence and is predicted to cause altered splicing leading to an abnormal or absent protein. In summary, although additional studies are required to fully establish its clinical significance, the c.442+1G>A variant is **likely pathogenic** base on predicted impact to protein. ACMG/AMP Criteria applied: PVS1; PM2.

* Variant is described based on' chromosome:position reference allele>alternate allele' formatting, with chromosome positions based on the GRCh37 genome assembly.

Online Table 5. Characteristics of sudden cardiac death cases according to pathogenic variant

Characteristics	No pathogenic or likely pathogenic variant (N=585)	Pathogenic or likely pathogenic variant (N= 15)
Age at time of blood sample – yr	64 ± 9	63 ± 8
Age at sudden death – yr	72 ± 9	70 ± 8
Sudden cardiac death		
Definite	425 (73%)	11 (73%)
Probable	160 (27%)	4 (27%)
Male sex – no. (%)	387 (66%)	8 (53%)
White race – no. (%)	566 (97%)	15 (100%)
Atherosclerotic CVD at time of blood sample – no. (%)	141 (24%)	4 (27%)
Atherosclerotic CVD at time of sudden death – no. (%)	227 (39%)	5 (33%)
Congestive heart failure at time of sudden death – no. (%)	103 (18%)	4 (27%)
Smoking status		
Never – no. (%)	219 (37%)	6 (40%)
Former – no. (%)	269 (46%)	6 (40%)
Current – no. (%)	91 (16%)	2 (20%)
Study cohort		
Physicians Health Study I	152	4
Physicians Health Study II	110	2
Health Professionals Follow-up Study	125	2
Nurses' Health Study	124	2
Women's Antioxidant Cardiovascular Study	38	3
Women's Health Study	36	2

Plus-minus values are mean ± SD.
CVD – cardiovascular disease

Online Table 6. Concordance of Pathogenic or Likely Pathogenic Mutations and Clinical Characteristics

Case ID	Age*/ Sex	Variant §	Gene (Variant Type)	Amino acid or cDNA change	Relevant Clinical Findings	Assessment of Concordance
P1	64M	2:179417587:C>A	*TTN* Premature stop	p.Gly27446Ter	History of ventricular tachycardia	Possibly consistent
P2	81M	2:179433438:G>T	*TTN* Premature stop	p.Tyr23239Ter	History of congestive heart failure, cardiomyopathy, and ventricular tachycardia	Probably consistent
P3	64F	2:179444577:T>G	*TTN* Splice site	c.59645-2A>C	History of coronary revascularization, but no known cardiomyopathy	Uncertain
P4	75M	2:179486244:G>A	*TTN* Premature stop	p.Arg12535Ter	None documented	Uncertain
P5	61M	2:179604601:TTC>T	*TTN* Frameshift	p.Glu4215ArgfsTer7	History of cardiomyopathy, palpitations	Probably consistent
P6	78F	19:55665525:C>T	*TNNI3* Missense	p.Arg141Gln	None documented	Uncertain
P7	78M	19:11213408:T>G	*LDLR* Missense	p.Trp87Gly	History of hypercholesterolemia and coronary revascularization, chest discomfort prior to death	Probably consistent
P8	70M	19:11213408:T>G	*LDLR* Missense	p.Trp87Gly	History of hypercholesterolemia but no known CAD prior to death; severe CAD and acute coronary thrombus on autopsy	Probably consistent
P9	69F	19:11221435:C>T	*LDLR* Premature stop	p.Arg350Ter	History of multivessel CAD, hypercholesterolemia, ischemic cardiomyopathy	Probably consistent
P10	73F	19:11224299:T>C	*LDLR* Missense	p.Trp483Arg	History of lipid-lowering medication, exertional chest discomfort prior to death	Probably consistent
P11	53F	6:7580603:C>T	*DSP* Premature stop	p.Gln1394Ter	Autopsy with biventricular and fibrous hypertrophy, increased RV fat but no definitive fibrofatty replacement.	Uncertain
P12	60M	7:150647424:G>A	*KCNH2* Premature stop	p.Arg744Ter	None documented	Uncertain
P13	67M	7:150655169:CG>C	*KCNH2* Frameshift	p.Pro298ArgfsTer62	None documented	Uncertain
P14	75F	2:189855742:C>T	*COL3A1* Premature stop	p.Arg271Ter	None documented	Uncertain
P15	80F	15:48892335:C>T	*FBN1* Splice site	c.442+1G>A	History of prior CABG and neck discomfort prior to death	Possibly consistent

* Age refers to age at sudden cardiac death event

The concordance between a given pathogenic or likely pathogenic variant and clinical findings at time of or prior to sudden cardiac death was assessed based on manual chart review by the consensus of two cardiologists (AVK, CMA) as probably consistent, possibly consistent, or uncertain.

Online Table 6. Evidence in Support of Pathogenic or Likely Pathogenic Variant Assertions in the Multi-Ethnic Study of Atherosclerosis Cohort

Age/ Sex/Race	Variant §	Gene (Variant Type)	Amino acid or cDNA change	Associated disease	Evidence in Support of Pathogenicity Assessment
Cardiomyopathy variants					
58/M/Black	2:179395588:G>A	*TTN* Premature stop	p.Arg32684Ter	Dilated cardiomyopathy	The p.Arg32684Ter variant in TTN has not been previously reported in individuals with cardiomyopathy, but has been identified in 1/15278 African and 1/9842 Ashkenazi Jewish chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org/; dbSNP rs886043924) and in ClinVar (Variation ID: 288511). This nonsense variant leads to a premature termination codon at position 32684, which is predicted to lead to a truncated or absent protein. This variant is located in M-band. Truncating variants in this domain are prevalent in the general population (Pugh 2014) but there is also some evidence linking them to disease. Homozygous frameshift variants have been described in two families with early onset myopathy and DCM (Carmignac 2007) and heterozygous truncating variants have been reported in individuals with tibial muscular dystrophy without cardiomyopathy (Hackman 2002, Hackman 2008). In summary, although additional studies are required to fully establish its clinical significance, the p.Arg32684Ter variant is **likely pathogenic**. ACMG/AMP Criteria applied: PVS1_Strong; PM2.
49/F/Hispanic	2:179413670:G>A	*TTN* Premature stop	p.Arg28327Ter	Dilated cardiomyopathy	The p.Arg28327Ter variant in TTN has been reported in 1 individual with DCM (Roberts 2015) and 1 individual with Wolff-Parkinson-White syndrome (ClinVar ID# 223300), and was absent from large population studies. This nonsense variant leads to a premature termination codon at position 28327, which is predicted to lead to a truncated or absent protein. Nonsense and other truncating variants in TTN are strongly associated with DCM if they impact the exons encoding for the A-band (Herman 2012, Pugh 2014). This variant is located in A-band in the highly expressed exon 288. In summary, although additional studies are required to fully establish its clinical significance, the p.Arg28327Ter variant is **likely pathogenic**. ACMG/AMP Criteria applied: PVS1; PM2.
51/F/White	2:179431880:G>A	*TTN* Premature stop	p.Arg23759Ter	Dilated cardiomyopathy	The p.Arg23759Ter variant in TTN has not been previously reported in individuals with DCM, but has been identified 1/15000 European chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org) and in ClinVar (Variation ID 498278). This nonsense variant leads to a premature termination codon at position 23759, which is predicted to lead to a truncated or absent protein. Nonsense and other truncating variants in TTN are strongly associated with DCM if they impact the exons encoding for the A-band (Herman 2012, Pugh 2014). The p.Arg23759Ter variant is located in A-band in the highly expressed exon 275. In summary, although additional studies are required to fully establish its clinical significance, the p.Arg23759Ter variant

					is **likely pathogenic**. ACMG/AMP criteria applied: PVS1; PM2.
73/M/White	2:179437013:G>A	*TTN* Premature stop	p.Arg22048Ter	Dilated cardiomyopathy	The p.Arg22048* variant in TTN has not been previously reported in individuals with DCM, but has been identified 1/30736 South Asian chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org, dbSNP rs794729284). This variant has been reported in ClinVar (Variation ID 202404). This nonsense variant leads to a premature termination codon at position 22048, which is predicted to lead to a truncated or absent protein. Nonsense and other truncating variants in TTN are strongly associated with DCM if they impact the exons encoding for the A-band (Herman 2012, Pugh 2014). The p.Arg22048Ter variant is located in A-band in the highly expressed exon 275. In summary, although additional studies are required to fully establish its clinical significance, the p.Arg22048Ter variant is **likely pathogenic**. ACMG/AMP criteria applied: PVS1; PM2.
66/M/White	2:179444577:T>G	*TTN* Splice site	c.59645-2A>C	Dilated cardiomyopathy	The c.59645-2A>C variant in TTN has been reported in compound heterozygous state in 1 individual with congenital muscular dystrophy and DCM (reported as c.67349-2A>C, O'Grady 2016). It has also been identified in 1/109468 of European chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org; dbSNP rs753948675) and has been reported in ClinVar (Variation ID: 424832). This variant occurs in the invariant region (+/- 1,2) of the splice consensus sequence and is predicted to cause altered splicing leading to an abnormal or absent protein. Truncating variants in TTN are strongly associated with DCM if they impact the exons encoding for the A-band (Herman 2012, Pugh 2014). The c.59645-2A>C variant is located in A-band adjacent to the highly expressed exon 267. In summary, although additional studies are required to fully establish its clinical significance, the c.59645-2A>C variant is **likely pathogenic**. ACMG/AMP Criteria applied: PM2; PM3; PVS1_Moderate.
56/M/White	2:179453701:CA>C	*TTN* Frameshift	p.Val18350SerfsTer2	Dilated cardiomyopathy	The p.Val18350fs variant in TTN has not been previously reported in individuals with DCM and was absent from large population studies. This variant is predicted to cause a frameshift, which alters the protein's amino acid sequence beginning at position 18350 and leads to a premature termination codon 2 amino acids downstream. This alteration is then predicted to lead to a truncated or absent protein. Frameshift and other truncating variants in TTN are strongly associated with DCM if they impact the exons encoding for the A-band (Herman 2012, Pugh 2014). The p.Val18350fs variant is located in A-band in the highly expressed exon 253. In summary, although additional studies are required to fully establish its clinical significance, the p.Val18350fs variant is **likely pathogenic**. ACMG/AMP criteria applied: PVS1; PM2.
61/M/Black	2:179658178:C>A	*TTN* Premature stop	p.Glu497Ter	Dilated cardiomyopathy	The p.Glu497Ter variant in TTN has not been previously reported in individuals with DCM and was absent from large population studies. This nonsense variant leads to a premature termination codon at position 497, which is predicted to lead to a truncated or absent protein. Nonsense and other truncating variants in TTN are strongly associated

					with DCM if they are located in an exon that is highly expressed in the heart (Roberts 2015). The p.Glu497Ter variant is located in Z-band in the highly expressed exon 9. In summary, although additional studies are required to fully establish its clinical significance, the p.Glu497Ter variant is **likely pathogenic**. ACMG/AMP criteria applied: PVS1; PM2.
82/M/White	11:47364129:C>G	*MYBPC3* Missense	p.Glu542Gln	Hypertrophic cardiomyopathy	The p.Glu542Gln variant in MYBPC3 has been identified in >50 individuals with HCM and segregated with disease in >10 affected relatives from multiple families (Carrier 1997, Page 2012, Fokstuen 2011, Rodriguez-Garcia 2010, Barriales-Villa 2010, Garcia-Castro 2009, Olivotto 2008, Girolami 2006, Ingles 2005, Van Driest 2004, Richard 2003, Crehalet 2012, Walsh 2017, LMM data). This variant has also been identified in 4/117922 European chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org; dbSNP rs121909374). This variant is located in the last three bases of the exon, which is part of the 5' splice region. Computational tools suggest an impact to splicing, which was confirmed by in vitro studies (Carrier 1997, Crehalet 2012, Ito 2017). Pathogenic splice variants in the MYBPC3 gene are common in HCM patients. In summary, this variant meets criteria to be classified as **pathogenic** for HCM in an autosomal dominant manner based on prevalence among affected individuals, segregation studies, and observed impact on splicing. ACMG/AMP Criteria applied: PS4; PP1_Strong; PVS1_Strong; PM2.
57/M/White	11:47373058:T>C	*MYBPC3* Splice site	c.26-2A>G	Hypertrophic cardiomyopathy	The c.26-2A>G variant in MYBPC3 has been reported in >10 individuals with HCM, segregated with disease in 4 affected relatives from 3 families, including 1 obligate carrier (Van Driest 2004, Ehlermann 2008, Waldmuller 2011, Page 2012, Kapplinger 2014, LMM unpublished data). It has also been reported in ClinVar (Variation ID 42644), in 7/114350 of European chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org; dbSNP rs376395543), and in 2 unaffected adults (Natarajan 2016, LMM unpublished data). For diseases with clinical variability and reduced penetrance, pathogenic variants may be present at a low frequency in the general population. Finally, this variant occurs in the invariant region (+/- 1,2) of the splice consensus sequence, and the variant is predicted to damage the canonical splice site. Heterozygous splice variants in MYBPC3 are prevalent in cases of HCM. However, an alternate splice site is predicted to occur 6 bps downstream, and, if used, would lead to an in-frame deletion of 2 amino acids, though these computational tools are not predictive enough to suggest this alternative splice site would be use in vivo. In summary, this variant meets criteria to be classified as **pathogenic** for HCM in an autosomal dominant manner based upon predicted impact to the protein, presence in affected individuals and segregation studies. ACMG/AMP Criteria applied: PS4_Moderate; PM4; PP1_Supporting; PM2_Supporting.
83/F/White	14:23894048:C>T	*MYH7* Missense	p.Arg870His	Hypertrophic cardiomyopathy	The p.Arg870His variant in MYH7 has been reported in >15 individuals with HCM and segregated with disease in >15

					affected relatives (Nishi 1995, Erdmann 2003, Tanjore 2006, Laredo 2006, Bashyam 2007, Garcia-Castro 2009, Capek 2011, Santos 2012, Zou 2013, Meyer 2013, LMM data). This variant has been identified in 2/126674 European chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org/; dbSNP rs36211715). In vitro studies provide additional evidence that the p.Arg870His variant impacts protein function (Cuda 1997, Gruen 1999). In summary, this variant meets criteria to be classified as **pathogenic** for HCM in an autosomal dominant manner based upon number of probands, segregation studies, and functional evidence. ACMG/AMP Criteria applied: PS4; PP1_Strong; PP3; PM2; PS3_Supporting.
51/F/White	14:23895023:G>A	*MYH7* Missense	p.Arg723Cys	Hypertrophic cardiomyopathy	The p.Arg723Cys variant in MYH7 has been reported in at least 14 individuals with HCM, segregated with disease in 7 affected relatives from 3 families, and was reported to have occurred de novo in 1 affected individual with confirmed parental identities (Watkins 1992, Tesson 1998, Richard 2003, Ingles 2005, Girolami 2010, Olivotto 2011, LMM data). Moreover, another variant at this position (p.Arg723Gly) is pathogenic based on extensive segregation with disease. Of note, the p.Arg723Cys variant was identified in multiple unaffected relatives at older ages and is likely associated with reduced penetrance and a variable disease course (Tesson 1998, Richard 2003, Girolami 2010, LMM data). This variant has been reported by other clinical laboratories in ClinVar and was also identified in 2/111468 European chromosomes and 1/30782 South Asian chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org; dbSNP rs121913630). In summary, this variant meets criteria to be classified as **pathogenic** for HCM in an autosomal dominant manner based upon presence in multiple affected individuals, segregation studies, de novo occurrence, and low allele frequency in the general population. ACMG/AMP Criteria applied: PS4; PM2; PS2; PP1_Strong.
54/F/White	19:55665463:G>A	*TNNI3* Missense	p.Arg162Trp	Hypertrophic cardiomyopathy	The p.Arg162Trp variant in TNNI3 has been reported in the heterozygous state in >5 individuals with HCM (Kimura 1997, Garcia-Pavia 2011, Kubo 2011, Santos 2012, LMM data). It was also identified in the homozygous state in 1 Indian and 1 Jordanian individual with HCM and segregated with disease in the homozygous state in 3 affected relatives, but none of the heterozygous relatives were affected (Gray 2013, Das 2014, LMM data). The variant has been identified in 3/111,610 European chromosomes and 3/30,779 South Asian chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org/; dbSNP rs368861241). In vitro functional studies provide some evidence that the p.Arg162Trp variant may impact protein function (Elliott 2000, Takahashi-Yanaga 2001). However, these types of assays may not accurately represent biological function. Computational prediction tools and conservation analysis do not provide strong support for or against an impact to the protein. However, two other likely pathogenic variants have been identified at this position (p.Arg162Pro, p.Arg162Gln), suggesting changes at this position are not

					tolerated. The available data on the p.Arg162Gln suggests that it may be a mild variant, with reduced penetrance. In summary, the p.Arg162Trp variant is **likely pathogenic**; however, this variant may have a milder role suggested by the incomplete penetrance seen in some family members and the individuals who were homozygous, similar to that observed for p.Arg162Gln. ACMG/AMP Criteria applied: PS4_Moderate; PM2_Supporting; PM5_Strong; PP1.
56/F/Black	19:55665525:C>T	*TNNI3* Missense	p.Arg141Gln	Hypertrophic cardiomyopathy	The p.Arg141Gln variant in TNNI3 has been reported in >14 individuals with HCM and segregated with disease in 3 affected relatives from 3 families (Richard 2003, Van Driest 2003, Mogensen 2004, Morita 2008, Curila 2009, van den Wijngaard 2011, Rani 2012, Santos 2012, Kapplinger 2014, LMM data). Of note, this variant was homozygous in one individual with severe disease (Mogensen 2004), and compound heterozygous in 2 individuals with early-onset disease (Morita 2008, LMM data, Santos 2012). Our laboratory has also detected this variant in an additional individual with Wolff-Parkinson-White syndrome. In addition, this variant has been reported by other clinical laboratories in ClinVar (Variation ID 43381) and has been identified in 1/8728 of African chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org/; dbSNP rs397516347). Arginine (Arg) at position 141 is highly conserved in mammals and across evolutionarily distant species and the change to glutamine (Gln) was predicted to be pathogenic using a computational tool clinically validated by our laboratory. This tool's pathogenic prediction is estimated to be correct 94% of the time (Jordan 2011). In summary, although additional studies are required to fully establish its clinical significance, the p.Arg141Gln variant is **likely pathogenic**. ACMG/AMP Criteria applied: PS4; PP1; PM2; PP3.
Coronary artery disease variants					
54/F/Asian	1:55505604:G>A	*PCSK9* Missense	p.Glu32Lys	Familial hypercholesterolemia	The p.Glu32Lys variant in PCSK9 has been reported in >40 Japanese and Korean individuals with hypercholesterolemia, including 2 homozygous individuals and 9 double heterozygotes who had a variant in LDLR (Miyake 2008, Mabuchi 2011, Noguchi 2010, Mabuchi 2014, Han 2015, Hopkins 2015). Homozygotes and double heterozygotes had more severe disease on average than heterozygotes, and heterozygotes for this variant had milder disease than heterozygotes for other variants associated to familial hypercholesterolemia (Mabuchi 2014, Hopkins 2015). Additionally, this variant segregated with disease in >20 affected relatives from >5 families (Noguchi 2010, Mabuchi 2014). In vitro functional studies provide some evidence that the p.Glu32Lys variant may impact protein function (Noguchi 2010). This variant has been identified in 4/12338 East Asian chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org/; dbSNP rs564427867) and is present in ClinVar (variation ID: 297692). Please note that for diseases with clinical variability or reduced penetrance, pathogenic variants may

					be present at a low frequency in the general population. In summary, the p.Glu32Lys variant is **pathogenic** for autosomal dominant familial hypercholesterolemia based upon presence in affected individuals and segregation with disease. ACMG/AMP Criteria applied: PS4; PP1_Strong; PS3_Supporting.
73/F/White	2:21229160:C>T	*APOB* Missense	p.Arg3527Gln	Familial hypercholesterolemia	The p.Arg3527Gln variant in APOB is a well-established pathogenic variant that is mainly found in individuals of European descent. It has been previously reported in >500 individuals with familial hypercholesterolemia (FH) and segregated with disease in >50 affected relatives (Soria 1989, März 1993, Leren 1997, Ludwig 1990, Bednarska-Makaruk 2001, Horvath 2001, Kalina 2001). It has been reported in ClinVar (Variation ID 17890) and identified in 53/126056 of European chromosomes, including 1 homozygote, by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org/; dbSNP rs5742904). This frequency is low enough to be consistent with the frequency of FH in the general population. In summary, this variant meets criteria to be classified as **pathogenic** for autosomal dominant familial hypercholesterolemia based upon presence in multiple affected individuals and segregation studies. Please note that pathogenic variants in APOB can have reduced penetrance and a less severe phenotype than disease-causing LDLR or PCSK9 variants (Youngblom and Knowles, GeneReviews). ACMG/AMP Criteria applied: PS4_Strong; PP1_Strong.
63/F/White	2:21229160:C>T	*APOB* Missense	p.Arg3527Gln	Familial hypercholesterolemia	See above.
59/F/Asian	2:21229161:G>A	*APOB* Missense	p.Arg3527Trp	Familial hypercholesterolemia	The p.Arg3527Trp variant (also referred to in the literature as p.Arg3500Trp) in APOB has been reported in at least 33 individuals with familial hypercholesterolemia, the majority of whom are of East Asian ancestry, and segregated with disease in at least 15 affected relatives from 4 families (Gaffney 1995, Choong 1997, Tai 1998, Fisher 1999, Tai 2001, Yang 2007, Hollandt 2012, Chiou 2010, Chiou 2011, Chiou 2012, Bertolini 2013). This variant has been reported in ClinVar (Variation ID 40223) and has also been identified in 22/18848 East Asian chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org; dbSNP rs144467873). This frequency is low enough to be consistent with the frequency of familial hypercholesterolemia (FH) in the general population. In vitro functional studies provide some evidence that the p.Arg3527Trp variant may impact protein function (Gaffney 1995, Fisher 1999, Tai 2001). Additionally, another variant at this position, p.Arg3527Gln, is a well-established pathogenic variant for FH. In summary, this variant meets criteria to be classified as **pathogenic** for FH in an autosomal dominant manner based upon segregation studies, increased prevalence in affected individuals, and pathogenicity of other variants at this position. Please note that pathogenic variants in APOB can have reduced penetrance and a less severe phenotype than disease-causing LDLR or PCSK9 variants (Youngblom and Knowles, GeneReviews). ACMG/AMP Criteria applied: PS4; PP1_Strong; PM5; PS3_Supporting.

49/F/Black	19:11200292:G>A	*LDLR* Splice site	c.67+1G>A	Familial hypercholesterolemia	The c.67+1G>A variant in LDLR has been reported in 1 individual with hypercholesterolemia (Nauck 1998; http://www.ucl.ac.uk/fh-old/muttab.html). This variant has also been identified in 1/107726 European chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org/) and is reported in ClinVar (Variation ID: 431507). This variant occurs in the invariant region (+/- 1,2) of the splice consensus sequence and is predicted to cause altered splicing leading to an abnormal or absent protein. Heterozygous loss of function of the LDLR gene is an established disease mechanism in individuals with familial hypercholesterolemia (FH). In summary, this variant meets criteria to be classified as **pathogenic** for FH in an autosomal dominant manner based upon predicted impact to the protein. ACMG/AMP Criteria applied: PVS1; PM2.
60/F/Hispanic	19:11216172:G>A	*LDLR* Missense	p.Cys197Tyr	Familial hypercholesterolemia	The p.Cys197Tyr variant in LDLR (also described as p.Cys176Tyr in the literature) has been reported in at least 3 individuals with familial hypercholesterolemia (FH) in the heterozygous, homozygous, and compound heterozygous states (Hobbs 1992, Chiou 2010, Setia 2016, ClinVar: Variation ID 200919). The variant segregated with disease in at least 1 affected relative (homozygous FH) from 1 family (Setia 2016). This variant was absent from large population studies. Computational prediction tools and conservation analysis suggest that the p.Cys197Tyr variant may impact the protein. In addition, other variants at this position (p.Cys197Trp, p.Cys197Cys, p.Cys197Arg, p.Cys197Phe, p.Cys197Gly) have been reported in individuals with FH (Clinvar, HGMD database; Stenson 2017), suggesting that changes at this position are not tolerated. In summary, although additional studies are required to fully establish its clinical significance, the p.Cys197Tyr variant is **likely pathogenic**. ACMG/AMP Criteria applied: PS4_Supporting; PP3; PM2; PM5.
71/F/Black	19:11223968:C>G	*LDLR* Missense	p.Leu401Val	Familial hypercholesterolemia	The p.Leu401Val variant in LDLR has been reported in at least 10 individuals with familial hypercholesterolemia and segregated with disease in 8 relatives (Leren 1997, Vaca 2011, Lange 2014, Jannes 2015, Amendola 2015). This variant has been identified in 5/24008 African chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org; dbSNP rs146200173). Computational prediction tools and conservation analysis suggest that the p.Leu401Val variant may impact the protein, though this information is not predictive enough to determine pathogenicity. In summary, although additional studies are required to fully establish its clinical significance, the p.Leu401Val variant is **likely pathogenic**. ACMG/AMP Criteria applied: PP1_Strong; PS4_Moderate; PM2_Supporting; PP3.
53/M/Asian	19:11227576:C>T	*LDLR* Missense	p.His583Tyr	Familial hypercholesterolemia	The p.His583Tyr variant in LDLR has been reported in at 15 heterozygous individuals and 3 compound heterozygous individuals with hypercholesterolemia and segregated with disease in 9 affected relatives from 3 families (Sun 1994, Punzalan 2005, Chiou 2012, Yao 2012, Ma 2017). Compound heterozygotes were more severely affected than heterozygotes in the same families. In vitro functional

					studies provide some evidence that the p.His583Tyr variant may impact protein processing (Sun 1994). This variant has been identified in 0.13% (24/18868) of East Asian chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org/; dbSNP rs730882109) and is reported in ClinVar (Variation ID: 200921). Please note that for diseases with clinical variability or reduced penetrance, pathogenic variants may be present at a low frequency in the general population. Computational prediction tools and conservation analysis do not provide strong support for or against an impact to the protein. In summary, this variant meets criteria to be classified as **pathogenic** for hypercholesterolemia in an autosomal dominant manner based upon segregation studies and functional evidence. ACMG/AMP Criteria applied: PS4; PP1_Strong; PS3_Supporting.
56/F/Asian	19:11227576:C>T	*LDLR* Missense	p.His583Tyr	Familial hypercholesterolemia	See above
57/F/White	19:11227604:G>A	*LDLR* Missense	p.Gly592Glu	Familial hypercholesterolemia	The p.Gly592Glu variant in LDLR has been reported in >200 Caucasian individuals with familial hypercholesterolemia (FH) and segregated with disease in >30 affected relatives from >3 families (Gorski 1998, Miltiadous 2001, Kuhrova 2002, Kublaska 2008, Bourbon 2008, Chmara 2010, Diakou 2011, Tichy 2012, Bertolini 2013, Do 2015, Medeiros 2015, Jannes 2015, Braenne 2015). This variant has also been identified in 15/126710 European chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org; dbSNP rs137929307). However, this frequency is low enough to be consistent with the frequency of FH in the general population. Furthermore, in vitro functional studies provide some evidence that the p.Gly592Glu variant may impact protein function (Romano 2011). In summary, this variant meets criteria to be classified as **pathogenic** for familial hypercholesterolemia in an autosomal dominant manner based upon presence in multiple affected individuals and segregation studies. ACMG/AMP Criteria applied: PS4; PP1_Strong.
73/F/White	19:11230819:C>T	*LDLR* Missense	p.Arg633Cys	Familial hypercholesterolemia	The p.Arg633Cys variant in LDLR has been reported in at least 9 individuals with familial hypercholesterolemia (FH), including one homozygote (Day 1997, Mozas 2004, Guardamagna 2009, Taylor 2009, Chiou 2011, Tichy 2012, Bertolini 2013). This variant has been reported by other clinical laboratories in ClinVar (Variation ID: 226379) and has also been identified in 1/30782 South Asian chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org/; dbSNP rs746118995). This frequency is low enough to be consistent with the frequency of FH in the general population. Computational prediction tools and conservation analysis suggest that the p.Arg833Cys variant may impact the protein. Two other variants at this amino acid position have been reported in at least 4 individuals with FH (p.Arg633Leu and p.Arg633His). In summary, although additional studies are required to fully establish its clinical significance, the p.Arg633Cys variant is **likely pathogenic**.

					ACMG/AMP Criteria applied: PS4_Moderate; PM2; PM5_Supporting; PP3.
58/M/Black	19:11231154:C>T	*LDLR* Missense	p.Pro699Leu	Familial hypercholesterolemia	The p.Pro699Leu variant in LDLR (also reported as p.Pro678Leu in the literature) has been reported in the heterozygous state at least 11 individuals with familial hypercholesterolemia (FH) and 7 individuals with suspected FH (Thiart 2000, Fouchier 2001, Van Gaal 2001, Huijgen 2011, Tichy 2012, Bertolini 2013, Jannes 2015, Wang 2016, Sharif 2016). It was also identified in 1 individual with homozygous FH who had a second pathogenic loss of function variant in LDLR (Schuster 1995). This variant was also present in this individual's father who had normal cholesterol levels, suggesting reduced penetrance. The p.Pro699Leu variant has been reported by other clinical laboratories in ClinVar (Variation ID# 252219) and has been identified in 9/24032 African chromosomes by the Genome Aggregation Database (GnomAD, http://gnomad.broadinstitute.org; dbSNP rs201573863). This frequency is low enough to be consistent with the frequency of FH in the general population. Computational prediction tools and conservation analysis suggest that the p.Pro699Leu variant may impact the protein. In summary, although additional studies are required to fully establish its clinical significance, the p.Pro699Leu variant is **likely pathogenic**. ACMG/AMP Criteria applied: PS4; PM2_supporting; PP3.
Arrhythmia syndrome variants					
65/M/Black	3:38592513:C>T	*SCN5A* Missense	p.Glu1784Lys	Long QT /Brugada syndrome	The p.Glu1784Lys variant in SCN5A has been previously reported in >20 individuals with prolonged QT intervals, Long QT syndrome (LQTS), and/or Brugada syndrome, including 1 de novo occurrence (Wei 1999, Makita 2008, Deschenes 2000, Nakajima 2011, Takahashi 2014). Furthermore, the variant segregated with disease in many affected relatives (LQTS, Brugada syndrome, or prolonged QT intervals; Makita 2008, Wei 1999, Deschenes 2000, Shim 2005, Veltmann 2016). It has been identified in 1/111718 of European chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org/; rs137854601). In vitro functional studies provide some evidence that the p.Glu1784Lys variant may impact protein function (Deschenes 2000, Makita 2008). This variant has been classified by other clinical laboratories as pathogenic in ClinVar (Variation ID:251679). In summary, this variant meets criteria to be classified as **pathogenic** for LQTS and Brugada syndrome in an autosomal dominant manner based upon presence in multiple affected individuals, segregation studies, very low frequency in controls and functional studies. ACMG/AMP criteria applied: PS4_Strong; PP1_Strong; PS3_Supporting.
65/M/White	3:38603913:C>A	*SCN5A* Missense	p.Gly1319Val	Brugada syndrome	The p.Gly1319Val variant in SCN5A has been previously reported in >10 individuals with Brugada syndrome and 1 individual with DCM, and segregated with disease in 3 affected individuals from at least 2 families (Smits 2002, Meregalli 2009, Kapplinger 2010, Amin 2011, Nannenberg 2012, Golbus 2014). This variant has also been reported by

					other clinical laboratories in ClinVar (Variation ID 67838). It has also been identified in 9/121862 European chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org; dbSNP rs6199473220). Functional studies provide some evidence that this variant may impact protein function (Casini 2007, Hoshi 2014). In summary, although additional studies are required to fully establish its clinical significance, the p.Gly1319Val variant is **likely pathogenic**. ACMG/AMP Criteria applied: PP1; PS4_Moderate; PM2_Supporting; PS3_Supporting; PP3.
53/F/Black	3:38640496:TG>T	*SCN5A* Missense	p.Gln646ArgfsTer5	Brugada syndrome	The p.Gln646fs variant in SCN5A has been reported in 5 individuals with Brugada syndrome (Kapplinger 2010, Kante 2012, Park 2012) and segregated with disease in 21 affected relatives (clinical manifestations included arrhythmia, syncope, cardiac arrest, or sudden death) from one family (Park 2012). It was absent from large population studies. This variant is predicted to cause a frameshift, which alters the protein's amino acid sequence beginning at position 646 and leads to a premature termination codon 5 amino acids downstream. This alteration is then predicted to lead to a truncated or absent protein. Heterozygous loss of function variants in the SCN5A gene have been reported in individuals with Brugada syndrome (Kapplinger 2010), DCM (Olson 2005), ventricular fibrillation (Chen 1998), as well as AV block and cardiac conduction defects (Baruteau 2012). In summary, the p.Gln646fs variant meets our criteria to be classified as **pathogenic** for autosomal dominant Brugada syndrome based upon predicted impact to the protein, segregation with disease and absence from controls. ACMG/AMP Criteria applied: PVS1; PS4_Moderate; PP1_Strong; PM2.
69/F/Hispanic	7:150647400:G>A	*KCNH2* Missense	p.Arg752Trp	Long QT syndrome	The p.Arg752Trp variant in KCNH2 has been reported in the heterozygous state in at least 5 individuals with long QT syndrome (LQTS; Splawski et al. 2000; Ficker et al. 2000; Nagaoka et al. 2008; Stattin et al. 2012, Itoh et al. 2016), and segregated with disease in 23 individuals in one family (Ficker et al. 2000). In vitro functional studies provide evidence that the p.Arg752Trp variant may impact protein function by causing decreased trafficking to the cell membrane due to its retention in the endoplasmic reticulum (Ficker et al. 2000; Anderson et al. 2006). Additionally, animal models in zebrafish have shown that this variant causes decreased cardiac repolarization (Jou et al. 2013). This variant has also been reported by other clinical laboratories in ClinVar (Variation ID# 67379), and was absent from large population studies. Other variants at this amino acid position (p.Arg752Gln, Arg752Pro) have been reported in association with LQTS (HGMD, Stenson 2017). In summary, this variant meets criteria to be classified as **pathogenic** for LQTS in an autosomal dominant manner based upon presence in multiple affected individuals, segregation studies, absence from controls, and functional evidence. ACMG/AMP criteria applied: PS3_Moderate; PS4_Supporting; PP1_Strong; PM5; PM2; PP3.
54/F/White	11:2591900:C>T	*KCNQ1* Missense	p.Arg174Cys	Long QT syndrome	The p.Arg174Cys variant in KCNQ1 has been reported in 4 heterozygous individuals, 1 double compound individual,

					and 1 homozygous individual (normal hearing) with long QT syndrome (LQTS), and 1 compound heterozygous individual with Jervell and Lange-Nielsen syndrome (Donger 1997, Tester 2005, Couderc 2012, Giudicessi 2013, Uysal 2017, Wu 2018). Multiple heterozygous carriers for this variant were asymptomatic, suggesting reduced penetrance (Uysal 2017, Wu 2018). This variant has been reported in ClinVar (Variation ID:53058) and has been identified in 1/33564 of Latino chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org; dbSNP rs199472696). In vitro functional studies provide some evidence that the p.Arg174Cys variant may impact protein expression and cellular localization, and cause dominant-negative effect in function (Chouabe 1997, Matavel 2010, Wu 2018, Huang 2018). Computational prediction tools and conservation analysis suggest that the p.Arg174Cys variant may impact the protein. However, the functional and computational data may not accurately represent biological function. Other missense variants at this position (p.Arg174His, p.Arg174Leu and p.Arg174Pro) have been previously reported in individuals with LQTS (Donger 1997, Splawski 2000, Napolitano 2005, Aziz 2011), suggesting changes at this position are not tolerated. In summary, although additional studies are required to fully establish its clinical significance, the p.Arg174Cys variant is **likely pathogenic**. ACMG/AMP criteria applied: PS3_Moderate; PS4_Moderate; PM3; PM2; PP3.
46/M/Hispanic	11:2594125:C>T	*KCNQ1* Missense	p.Ser277Leu	Long QT syndrome	The p.Ser277Leu variant in KCNQ1 has been reported in 14 heterozygous individuals with long QT syndrome (LQTS) and segregated with disease in at least 8 affected relatives from multiple families (Liu 2002, Chen2011, Aidery 2011, Yoshinaga 2014, Yagi 2018). This variant has also been reported in ClinVar (Variation ID: 53116), but was absent from large population databases. Computational prediction tools and conservation analysis suggest that the p.Ser277Leu variant may impact the protein. In vitro functional studies provide evidence that the p.Ser277Leu variant may have a dominant-negative effect on protein's expression and function (Aidery 2011, Chen 2011). Other missense variants at this codon (p.Ser277Pro and p.Ser277Trp) have also been associated with LQTS (Kapplinger 2009, Napolitano 2005), suggesting that a change at this position might not be tolerated. In summary, this variant meets criteria to be classified as **pathogenic** for long QT syndrome in an autosomal dominant manner based upon clinical data, segregation studies and functional evidence. ACMG/AMP criteria applied: PP1_Strong; PM2; PS4_Moderate; PP3; PS3_Supporting.
46/M/Black	11:2594203:T>TG	*KCNQ1* Frameshift	p.Trp304ValfsTer159	Long QT syndrome	The p.Trp304ValfsTer159 variant in KCNQ1 has not been previously reported in individuals with KCNQ1-associated disorders and was absent from large population studies. This variant is predicted to cause a frameshift, which alters the protein's amino acid sequence beginning at position 304 and leads to a premature termination codon 159 amino acids downstream. This alteration is then predicted to lead to a truncated or absent protein. Heterozygous loss of function of

					the KCNQ1 gene is an established disease mechanism in Romano-Ward syndrome, while homozygous loss of function is an established disease mechanism in Jervell and Lange-Nielsen syndrome. In summary, although additional studies are required to fully establish its clinical significance, the p.Trp304ValfsTer159 variant is **likely pathogenic**. ACMG/AMP Criteria applied: PVS1; PM2.
47/F/White	11:2604775:G>A	*KCNQ1* Missense	p.Ala344Ala	Long QT syndrome	The p.Ala344Ala variant in KCNQ1 has been reported in >20 heterozygous individuals with Long QT syndrome (LQTS) and in one compound heterozygous individual with Jervell and Lange-Nielsen syndrome (JLNS). It segregated with LQTS in >10 relatives from multiple families (Kanters 1998, Struijk 2006, Tsuji 2007, Gao 2012, Itoh 2015, Vyas 2016, Robyns 2017). This variant has also been reported in ClinVar (Variation ID: 3135) and has been identified in 1/111614 European chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org/; dbSNP rs1800171). This variant is located at the last base of exon 7, which is part of the 5' splice region. Computational tools and functional studies using patient mRNAs suggest that the p.Ala344ala variant impacts splicing, resulting mainly in the skipping of exon 7 but other aberrant mRNA transcripts were also reported (Wuriyanghai 2018, Tsuji 2007). In summary, this variant meets criteria to be classified as **pathogenic** for LQTS in an autosomal dominant manner based upon presence in multiple affected individuals, segregation studies, predicted impact to the protein, and functional studies. ACMG/AMP criteria applied: PS4; PP1_Strong; PM4; PS3_Moderate; PM2.
72/F/Black	11:2606494:A>G	*KCNQ1* Missense	p.Lys362Arg	Long QT syndrome	The p.Lys362Arg variant in KCNQ1 has been reported in the heterozygous state in at least 3 individuals that have been referred for long QT syndrome (LQTS) testing (Tester 2005, Kapplinger 2009, Kapa 2009, Giudicessi 2012, Natarajan 2016) and in the compound heterozygous state with another pathogenic variant in 2 individuals with an early-onset, more severe LQTS phenotype without hearing loss (Giudicessi 2013). It was also identified in one compound heterozygous individual with Jervell-Lange-Nielsen syndrome (JLNS; Darbar 2005). Additionally, the p.Lys362Arg variant has also been reported by other clinical laboratories in ClinVar (Variation ID: 52953). In vitro functional studies provide some evidence that the p.Lys362Arg variant may impact the protein (Slaats 2015). This variant has been identified in 9/111678 European chromosomes by the Genome Aggregation Consortium (GnomAD, http://gnomad.broadinstitute.org; dbSNP rs12720458). Computational prediction tools and conservation analysis suggest that the p.Lys362Arg variant may impact the protein. In summary, although additional studies are required to fully establish its clinical significance, the p.Lys362Arg variant is **likely pathogenic**. ACMG/AMP Criteria applied: PM3_Strong; PP3; PS3_Supporting; PS4_Supporting.
63/M/White	12:32955491:C>G	*SCN5A* Missense	p.Glu1784Lys	Long QT /Brugada syndrome	The p.Glu1784Lys variant in SCN5A has been previously reported in >20 individuals with prolonged QT intervals, Long QT syndrome (LQTS), and/or Brugada syndrome,

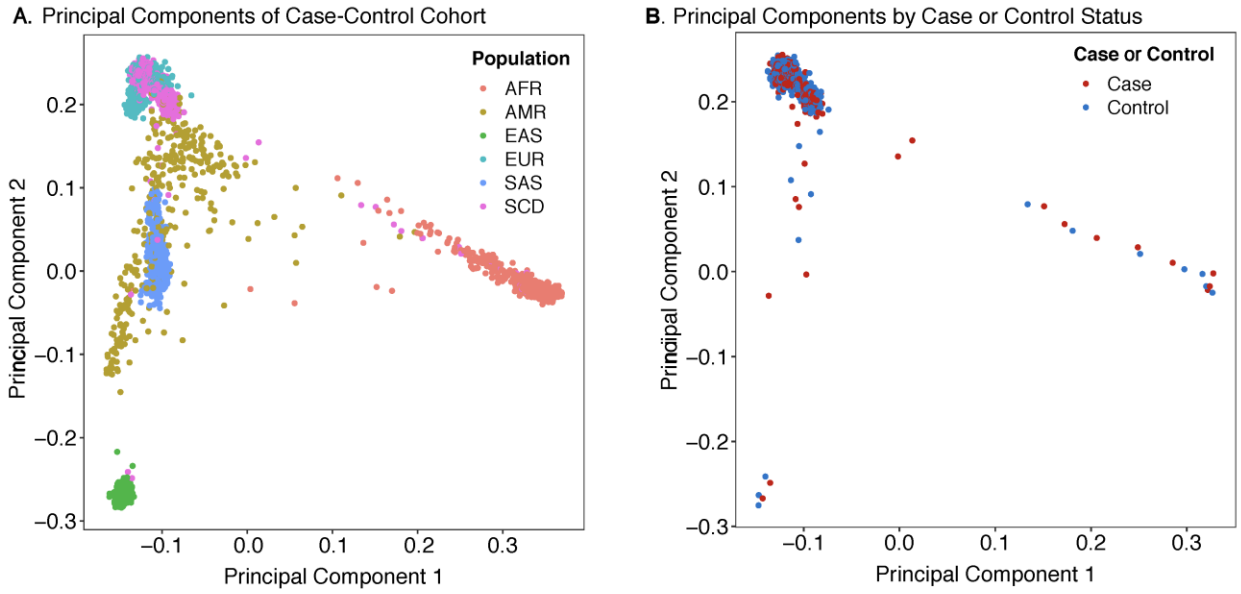
					including 1 de novo occurrence (Wei 1999, Makita 2008, Deschenes 2000, Nakajima 2011, Takahashi 2014). Furthermore, the variant segregated with disease in many affected relatives (LQTS, Brugada syndrome, or prolonged QT intervals; Makita 2008, Wei 1999, Deschenes 2000, Shim 2005, Veltmann 2016). It has been identified in 1/111718 of European chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org/; rs137854601). In vitro functional studies provide some evidence that the p.Glu1784Lys variant may impact protein function (Deschenes 2000, Makita 2008). This variant has been classified by other clinical laboratories as pathogenic in ClinVar (Variation ID:251679). In summary, this variant meets criteria to be classified as **pathogenic** for LQTS and Brugada syndrome in an autosomal dominant manner based upon presence in multiple affected individuals, segregation studies, very low frequency in controls and functional studies. ACMG/AMP criteria applied: PS4_Strong; PP1_Strong; PS3_Supporting.
53/M/Black	12:32977097:C>G	*SCN5A* Missense	p.Gly1319Val	Long QT /Brugada syndrome	The p.Gly1319Val variant in SCN5A has been previously reported in >10 individuals with Brugada syndrome and 1 individual with DCM, and segregated with disease in 3 affected individuals from at least 2 families (Smits 2002, Meregalli 2009, Kapplinger 2010, Amin 2011, Nannenberg 2012, Golbus 2014). It has also been identified in 9/121862 European chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org; dbSNP rs6199473220) and in ClinVar (Variation ID 67838). Functional studies provide some evidence that this variant may impact protein function (Casini 2007, Hoshi 2014). In summary, although additional studies are required to fully establish its clinical significance, the p.Gly1319Val variant is **likely pathogenic**. ACMG/AMP Criteria applied: PP1; PS4_Moderate; PM2_Supporting; PS3_Supporting; PP3.
67/M/Black	12:32994006:AC>A	*PKP2* Frameshift	p.Gly548ValfsTer15	Arrhythmogenic right ventricular cardiomyopathy	The p.Gly548ValfsTer15 variant in PKP2 has been reported in at least 7 individuals with either a possible or definitive diagnosis of ARVC and segregated with disease in 1 affected relative (Gerull 2004, Dalal 2006, Perrin 2013, Fressart 2010, Dalal 2006, LMM data). This variant is absent from large population studies. This variant is predicted to cause a frameshift, which alters the protein's amino acid sequence beginning at position 548 and leads to a premature termination codon 15 amino acids downstream. This alteration is then predicted to lead to a truncated or absent protein. Heterozygous loss of function of the PKP2 gene is an established disease mechanism in ARVC. In summary, this variant is **pathogenic** in an autosomal dominant manner. ACMG/AMP Criteria applied: PVS1; PS4_Moderate; PM2.
76/F/White	12:33003700:C>T	*PKP2* Missense	p.Asp460Asn	Arrhythmogenic right ventricular cardiomyopathy	The p.Asp460Asn variant in PKP2 has been reported in at least 5 individuals with ARVC and segregated with disease in 2 affected relatives from 2 families (La Gerche 2010, Cox 2011, Munoz Calero 2012, Alcalde 2014, Groeneweg 2014). It was absent from large population studies. This variant is located in the last base of exon 5, which is part of the 5' splice region. RNA studies and computational tools suggest

					an impact to splicing by activating a cryptic splice site, resulting in an out-of-frame transcript that is likely degraded by NMD (Groeneweg 2014). In summary, although additional studies are required to fully establish its clinical significance, the p.Asp460Asn variant is **likely pathogenic**. ACMG/AMP Criteria applied: PM2; PM4; PS3_Supporting; PS4_Supporting.
67/M/Black	12:33031151:G>T	*PKP2* Premature stop	p.Tyr221Ter	Arrhythmogenic right ventricular cardiomyopathy	The p.Tyr221Ter variant in PKP2 has been reported in 6 individuals with clinical features of ARVC, 1 individual with primary electrical disease, and 1 individual with sudden cardiac death (Groeneweg 2015, Lin 2017, Orgeron 2017, Proost 2017, Rigato 2013, Te Riele 2013, Walsh 2017). It also segregated with disease in 1 affected relative. This variant has been identified in 2/24030 African chromosomes by the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org; dbSNP rs767987619) and has been reported in ClinVar (Variation ID 201976). This nonsense variant leads to a premature termination codon at position 221, which is predicted to lead to a truncated or absent protein. Heterozygous loss of function of the PKP2 gene is an established disease mechanism in ARVC. In summary, this variant meets criteria to be classified as **pathogenic** for ARVC in an autosomal dominant manner. ACMG/AMP Criteria applied: PVS1; PS4_Moderate; PM2_Supporting.
71/F/Black	12:33031429:G>A	*PKP2* Premature stop	p.Gln129Ter	Arrhythmogenic right ventricular cardiomyopathy	The p.Gln129Ter variant in PKP2 has not been previously reported in individuals with ARVC and was absent from large population studies. This nonsense variant leads to a premature termination codon at position 129, which is predicted to lead to a truncated or absent protein. Heterozygous loss of function of the PKP2 gene is an established disease mechanism in ARVC. In summary, although additional studies are required to fully establish its clinical significance, the p.Gln129Ter variant is **likely pathogenic**. ACMG/AMP Criteria applied: PVS1; PM2.
47/F/White	21:35821707:C>T	*KCNE1* Premature stop	p.Asp76Asn	Long QT syndrome	The p.Asp76Asn variant in KCNE1 (ClinVar ID# 13477) has been reported in the heterozygous state in at least 18 individuals with Long QT syndrome (LQTS; Splawski 1997, Duggal 1998, Tester 2005, Tester 2006, Kapplinger 2009, Christiansen 2014, Weeke 2014, Li 2015) as well as segregated in one affected relative (Splawski 1997). This variant has also been identified in 14/126618 European chromosomes by the Genome Aggregation Database (GnomAD, http://gnomad.broadinstitute.org). This frequency is high given the prevalence of LQTS (Orphanet: ~1/2,500 individuals) and genetic heterogeneity of the disease but does not rule out pathogenicity as LQTS is known to have clinical variability and reduced penetrance. A pathogenic role is supported by two families with Jervell and Lange-Nielsen syndrome (JLNS), which includes prolonged QT as one of several features. In one family, the variant was present in the compound heterozygous state in three affected siblings (Schulze-Bar 1997). In the other family, the proband was homozygous for the p.Asp76Asn variant and two heterozygous family members were reported to have LQTS (Duggal 1998). In vitro functional studies provide some evidence that the p.Asp76Asn variant may impact

					protein function (Splawski 1997, Kurokawa 2003, Seebohm 2008, Haitin 2009, Chen 2009, Du 2013). However, these types of assays may not accurately represent biological function. In vivo studies in guinea-pigs have shown that this variant affects cardiac repolarization in myocytes (Hoppe 2001). Computational prediction tools and conservation analysis do not provide strong support for or against an impact to the protein. In summary, this variant meets criteria to be classified as **likely pathogenic** for LQTS in an autosomal dominant manner and for JLNS in an autosomal recessive manner based upon segregation studies and functional evidence. ACMG/AMP criteria applied: PM3; PS3_Moderate; PP1_Moderate.

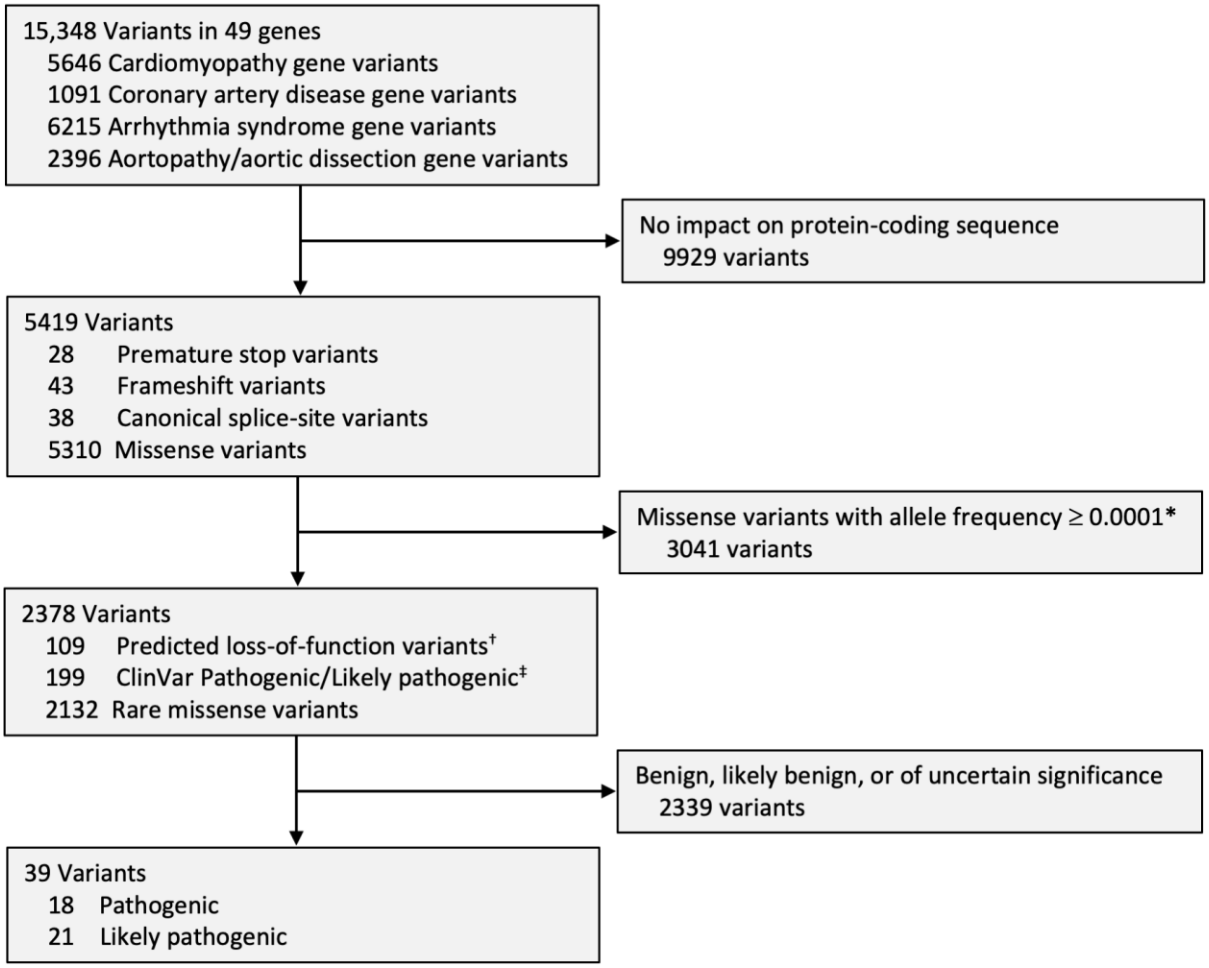
* Variant is described based on' chromosome:position reference allele>alternate allele' formatting, with chromosome positions based on the GRCh37 genome assembly.

Online Figure 1. Genetic ancestry of sudden cardiac death cases versus controls as assessed by principal components of ancestry



Principal components of ancestry are a commonly used method in human genetics to assess for important differences in genetic background between cases and controls of a given cohort (19). We quantified principal components of ancestry in 2,504 samples from the 1000 Genomes project using the FlashPCA2 software package (20,21), including 661 individuals of African (AFR) ancestry, 347 Admixed Americans (AMR), 504 East Asians (EAS), 503 Europeans (EUR), and 489 South Asians (SAS). The 1,200 participants of our sudden cardiac death (SCD) case-control cohort were then projected onto these principal components. **A.** The first two principal components of ancestry are plotted, noting that the majority of the SCD participants were of European ancestry. **B.** The first two principal components of ancestry are plotted according to SCD case versus control status, confirming that the cases and controls were well matched with respect to genetic background.

Online Figure 2. Filtration and curation of variants identified by whole genome sequencing, Multi-Ethnic Study of Atherosclerosis Study



* Missense variants filtered out if present with allele frequency ≥0.0001 in any racial subpopulation of the Genome Aggregation Database, a publicly available allele frequency database derived from 123,136 exome sequences.(22)
† Predicted loss-of-function variants do not necessarily all meet current standards to be classified as pathogenic or likely pathogenic. Representative examples include variants in the gene encoding titin (*TTN*) that occur in exons not found in cardiac-specific isoforms(23), variants in the gene encoding apolipoprotein B (*APOB*) that are associated with *lower* risk of hypercholesterolemia and coronary artery disease(24), and variants in the gene encoding tyrosine-protein phosphatase (*DSP2*), where only those variants that have previously been proven to segregate with disease are classified as pathogenic or likely pathogenic (25).
‡ The ClinVar database provides open access to variant classifications shared by many individuals and clinical laboratories. While an important means of communicating and coordinating efforts, many variant pathogenicity assertions in ClinVar do not warrant this designation based on current ACMG/AMP criteria (26).

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