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Investigating Unexplained Deaths through Molecular Autopsies

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Abstract

Sudden Unexplained Death (SUD) is natural death in a previously healthy individual whose cause remains undetermined after scene investigation, complete autopsy, and medical record review. SUD affects children and adults, devastating families, challenging medical examiners, and is a focus of research for cardiologists, neurologists, clinical geneticists, and scientists. As sequencing technology advanced rapidly from single gene testing to gene panels, genetic study has emerged as a leading tool in postmortem diagnostics in SUD. Testing yields from larger SUD cohort studies varied 10% to 30%. Besides demographic and geographic differences of cohorts, differences in number of genes tested, forensic approaches in the SUD investigation in medical examiner offices, and the subjectivity of variant interpretation contribute to the variation in testing yield. Conflicting variant classification is widespread in the literature and in ClinVar, and is recognized in the molecular diagnostics. A statistical framework of "high-resolution" variant interpretation, accounting for disease inheritance mode, prevalence, allelic heterogeneity, and reduced penetrance, can help to determine the maximum tolerated allele counts (AC) in ExAC for a likely disease-contributing variant. This framework removed two-thirds of variants from consideration compared to the lenient frequency of 0.1% for autosomal dominant diseases, without discarding true pathogenic variants that would have been missed if filtering for singleton variants exclusively.

We present our application of this statistical framework in classifying the variants identified through panel testing of cardiac arrhythmogenic genes in a large demographically diverse cohort where all cases underwent comprehensive, standardized investigation in the United States' largest office of chief medical examiner. Applying maximum tolerated reference AC in gnomAD which contains 277264 sequenced chromosomes, we reclassified those high AC variants that are currently called mostly as "pathogenic" in ClinVar. Combining literature review, we classified pathogenic variants, novel, and rare variants of uncertain significance (VUS) in the cohort. We present the demographic variations of testing yields in this cohort and emphasize the importance of conducting additional research on novel and rare VUS uncovered in under-represented populations.

The demographics of the 296 decedents are: 147 Blacks/African Americans (AA), 64 Hispanics, 49 Whites , 22 Asians, and 14 mixed ethnicities (MIX); 142 infants (1-11m), 39 children (1-17 years), 74 young adults (18-34 years), and 41 adults (35-55 years). Genomic DNA from postmortem tissue samples preserved in *RNALater*® and bloodstain cards were extracted. The testing method involved the HaloPlex custom kit (Agilent Technologies) for the target genes enrichment, and Illumina Miseq for deep sequencing of the targeted genes according to the manufacturers' protocols. 89 cardiac disease genes were among the 114 genes tested.

Combining a statistic framework to calculate the maximum allele counts in gnomAD and literature review, 24 variants in cardiac genes were classified as pathogenic or likely pathogenic (P/LP), 41 novel, and 124 rare variants of uncertain significance (VUS). We also reclassified 12 P/LP or VUS in ClinVar as benign. Overall, 23 (7.8%) cases had P/LP (positive cases), 112 (37.8%) had VUS, and 161 (54.4%) had negative results. The yields of positive testing results by were: 14.6% in adults, 12.8% children, 6.8% young adults, and 4.9% infants; 28.6% in MIX, 14.3% Whites, 7.8% Hispanics, 4.5% Asians, and 4.1% AA. The percentages of uncertain cases

were: 45.5% in Asians, 41.5% AA, 40.6% Hispanics, 28.6% Whites, and 7.1% in MIX. P/PL and VUS are frequent in *SCN5A* and *RYR2.* All variants classified as pathogenic or likely pathogenic were confirmed by Sanger Sequencing.

Postmortem genetic testing requires standardization of variant interpretation to lower rates of false positives and avoid the risk of false negatives. Differences of testing yields between demographic groups highlights the need for the inclusion of un-represented populations in future studies.The feasibility and utility of broad molecular autopsy provides the first needed step in personalized diagnostics and precision preventive medicine for families of victims of SUD. Future direction will be focused on improving our understanding of the large number of novel and rare variants of uncertain significance, using family studies and the functional characterizations.

Keywords: statistic framework, variant interpretation, testing yields, cardiac arrhythmia

Contents

Executive Summary

Backgrounds and Objectives

Sudden Unexplained Death (SUD) is natural death in a previously healthy individual whose cause remains undetermined after scene investigation, complete autopsy, and medical record review. SUD affects children and adults, devastating families, challenging medical examiners, and is a focus of research for cardiologists, neurologists, clinical geneticists, and scientists[1, 2]. As sequencing technology advanced rapidly from single gene testing to gene panels, genetic study has emerged as a leading tool in postmortem diagnostics in SUD. Testing yields from larger SUD cohort studies varied 10% to 30%. [3,4-7] Besides demographic and geographic differences of cohorts, differences in number of genes tested, forensic approaches in the SUD investigation in medical examiner offices, and the subjectivity of variant interpretation contribute to the variation in testing yield. Conflicting variant classification is widespread in the literature and in ClinVar, and is recognized in the molecular diagnostics.[8, 9] A statistical framework of "high-resolution" variant interpretation, accounting for disease inheritance mode, prevalence, allelic heterogeneity, and reduced penetrance, can help to determine the maximum tolerated allele counts (AC) in ExAC for a likely disease-contributing variant[9]. This framework removed two-thirds of variants from consideration compared to the lenient frequency of 0.1% for autosomal dominant diseases, without discarding true pathogenic variants that would have been missed if filtering for singleton variants exclusively.

We present our application of this statistical framework[9] in classifying the variants identified through panel testing of cardiac arrhythmogenic genes in a large demographically diverse cohort where all cases underwent comprehensive, standardized investigation in the United States' largest office of chief medical examiner. Applying maximum tolerated reference AC in gnomAD which contains 277264 sequenced chromosomes, we reclassified those high AC variants that are currently called mostly as "pathogenic" in ClinVar. Combining literature review, we classified pathogenic variants, novel, and rare variants of uncertain significance (VUS) in the cohort. We present the demographic variations of testing yields in this cohort and emphasize the importance of conducting additional research on novel and rare VUS uncovered in under-represented populations.

Materials and Methods

SUD Cases

In the New York City Office of Chief Medical Examiner (NYC-OCME), forensic investigations in sudden death include: scene investigation and family interview (by certified physician assistants), complete gross autopsy, cardiac pathology and neuropathology examinations, toxicological tests, microbiological tests (in infants), metabolic screen tests (in infants), and medical record reviews. Cases defined as SUD had negative or unremarkable results from the studies described above. 296 SUD cases investigated in NYC-OCME from 2001 to 2014 met these criteria. Data collected for each case include age, ethnicity, gender, body mass index

(BMI), death circumstance (sleeping or strenuous activities), a family history of sudden death or cardiac arrhythmia, a personal history of seizure, fainting, heart murmur, sleep apnea, or cardiac arrhythmia. Race/ethnicity as defined by the U.S. Census, indicates the interpretation of what families of a decedent considered himself or herself to be: Black/African American, Hispanic (Mexican, Puerto Rican, Cuban, Dominican, etc.), White, Asian, and mixed ethnicity. This study is not regulated by 45 CFR Part 46 because only cadaver specimens were used. OCME approved this study for diagnosis of the underlying cause of SUD.

Deep Next-generation Sequencing of Targeted Genes and Variant Confirmation by Sanger Sequencing

Genome DNA was extracted from postmortem tissue samples or bloodstain cards from the 296 SUD cases using the Qiagen DNA kit and the M48 Biorobot (Qiagen, Germany). HaloPlex custom kit (Agilent Technologies) was used for target gene enrichment and Illumina Miseq for deep sequencing of the 114 targeted genes according to the manufacturers' protocols. These genes included 89 *cardiac disease genes* responsible for cardiac channelopathy and cardiomyopathy and 25 *non cardiac-disease genes* identified through Genome-Wide Association (GWA) studies that increase heart rate or PR intervals, associated with seizure disorders, in the serotonin pathway, or associated with central hypoventilation syndrome (Table S1). Paired-end sequencing (150bp) by Illumina Miseq produced a mean sequencing coverage of 1000x, more than 98% of the target base coverage ≥ 50x, and less than 0.1% have zero coverage. Sanger Sequencing confirmed all variants classified as pathogenic or likely pathogenic, as well as novel VUS in the cardiac disease genes.

Sequencing Data Analysis

Agilent SureCall software (version 2.0) was used to perform adaptor trimming and alignment to the human-genome reference (GRCh37/hg19). Strand-ngs® (version 2.5) was used for sequencing data quality filtering, reads local realignment, base quality recalibration and variant calling. Modified GATK is used for SNP calling along with comparison to dbSNP138. The confidence of variant calling was set at the minimal coverage of 50 reads and the heterozygote variants' percentage between 30% and 70%. Non-synonymous (NS) variants - missense and equivalent (including in-frame indels, start lost, stop lost, and mature miRNA-altering), and protein-truncating variants (nonsense, essential splice site, and frameshift) were annotated with Ensembl-GRCh37.

Variant Interpretation

Variants from 89 cardiac disease genes were classified as pathogenic or likely pathogenic variants (P/LP), variants of uncertain significance (VUS), or benign or likely benign (B/LB). We applied Whiffin et al's [9] statistical framework to compute the maximum credible population allele frequency (AF) in gnomAD[10], given disease prevalence, estimated allelic contribution and the penetrance, to identify candidate variants for further analysis. The maximum credible population AF in the genes for autosomal dominant inherited long QT syndrome, Brugada syndrome, catecholaminergic polymorphic ventricular tachycardia (CPVT), hypertrophic cardiomyopathy, dilated cardiomypathy, or arrhythmogenic cardiomyopathy were calculated using the following equation: maximum credible population AF = prevalence × maximum allelic contribution × 1/penetrance (http://cardiodb.org/allelefrequencyapp).

To calculate the maximum allele counts (AC) in gnomAD which contains total sequenced 277264 chromosomes of 138632 individuals (123,136 whole exome sequencing and 15,496 whole genome sequencing), a one-tailed 95% confidence interval of a Poisson distribution was applied. For example, the prevalence of long QT type 1 due to *KCNQ1* gene mutations is approximately 1/2000, the allelic contribution of a common variant is set at 2%, and penetrance

of the disease is set at 0.5, the maximum AC in gnomAD for a disease-causing variant in the KCNQ1 gene should be less than 6 (see chart below)

Disease Prevalence	Allelic Heterogeneity	Confidence	Penetrance	Reference population Size (alleles)	Maximum credible population AF (range*)	Maximum tolerated reference AC (range*)
1/500	0.01-0.02	0.95	0.5	277264	0.00002-0.00004	10-17
1/1000	0.01-0.02	0.95	0.5	277264	0.00001-0.00002	6-10
1/2000	0.01-0.02	0.95	0.5	277264	0.000005-0.00001	4-6
1/4000	0.01-0.02	0.95	0.5	277264	0.0000025-0.000005	2-4

*range is calculated based on allelic contribution 0.01 to 0.02;

We removed variants whose AF in the sub-population in gnomAD was enriched (i.e. if a variant is relatively common in an ethnic group, it is unlikely to be pathogenic, unless the disease prevalence is also enriched in that population). By applying this statistical framework and calculation, we downgraded those "pathogenic" variants and VUS reported in ClinVar[11] and the literature to "benign" without removing truly likely pathogenic variants.

In addition to allele frequency, literature from both the Human Gene Mutation Database (HGMD) and PubMed on family or *in vitro* functional studies, cohort testing, as well as protein structural studies was evaluated for the level of evidence supporting pathogenicity according to criteria of the American College for Medical Genetics and Genomics (ACMG). *In silico* predictions were performed by dbNSFPv2.6 (e.g. SIFT, PolyPhen 2, LRT, MutationTaster, Fathmm, Gerp++ and Phylop) in the context of the inheritance mode of a disease gene. For cardiomyopathy gene variants, cadiopathological finding is used for phenotype correlation.

P/LP variants are: 1) novel or low frequency variants with supporting evidence from previous studies, 2) variants affecting the same amino acid position previously studied, or 3) supported by cardio-pathological findings. VUS are novel or rare variants, but lack supporting evidence from previous studies. Likely benign/benign variants are either previously classified or common variants with AC far higher than the maximum AC in gnomAD.

Results

Characteristics of the Cases

The overall demographics of the 296 decedents are (Table 1): 147 Blacks/African Americans (49.7%), 64 Hispanics (21.6%), 49 Whites (16.6%), 22 Asians (7.4%), and 14 mixed ethnicities (4.7%); 142 infants (birth to under one year old), 39 children (one year to less than 18 years), 74 young adults (18 to 34 years), and 41 adults (35 to 55 years); 125 females and 171 males.

High Resolution Classification of Pathogenic, Novel and Rare VUS, and Benign Variants

We re-classified 12 variants (previously reported as P/LP or VUS in ClinVar) that well exceeded the maximum AC in gnomAD (Table 2) [9]. For example, the g.4:114269433A>G (NP_001120965.1:p.Glu1449Gly) variant in *ANK2*, occured in 96 of 126426 Europeans and 143 of 276786 chromosomes tested in gnomAD, yet this variant was called twice as pathogenic, twice as likely pathogenic, twice as VUS and once as likely benign in ClinVar. Another example is the g.1:112323335G>A variant (NP_004971.2:p.Leu450Phe) in *KCND3*, found in 7 of the10048 Ashkenazi Jewish and 36 of total 273382 chromosomes tested in gnomAD, yet it was called as pathogenic/likely pathogenic variants by two submitters in ClinVar.

In our cohort, we identified 24 *pathogenic and likely pathogenic variants* that were previously studied (referenced by PMID number in PubMed in Table 3); all are below the maximum AC in gnomAD.

We also identified 41 *novel* variants of uncertain significance (Table 4) that are located within high coverage regions (>30x) in gnomAD. Only one novel variant, g.2:220283414C>T (NP_001918.3:p.Thr77Ile) in *DES*, for which the nonsynonymous change affecting the same amino acid position, p.Thr77Ala, occurred 24 times in the gnomAD; for the remaining 40 novel variants, a nonsynonymous change affecting the same amino acid position is either not present or present at low frequency (≤5) in gnomAD.

In addition, we found 124 *rare VUS* present at low AC in gnomAD (Table S3) in this study.

Demographic Distribution of Positive, Uncertain, and Negative Cases

Among the 296 cases, 23 (7.8%) cases had at least one P/LP variant (positive cases, Table S2a), 112 (37.8%) cases had at least one novel or rare VUS (uncertain cases, Table S2b), and 161 (54.4%) cases had negative results (negative cases, Table S2c). The testing yield (percentage of positive cases) from high to low by age (Figure 1a) is: 14.6% in adults, 12.8% children, 6.8% young adults, and 4.9% infants. The testing yield (percentage of positive cases) from high to low by ethnicity (Figure 1b) was: 28.6% in mixed ethnicity, 14.3% Whites, 7.8% Hispanics, 4.5% Asians, and 4.1% Blacks/African Americans.

Furthermore, the percentage of uncertain cases varied sharply by ethnicity (Figure 1b): 45.5% in Asians, 41.5% Blacks/African Americans, 40.6% Hispanics, 28.6% Whites, and 7.1% in mixed ethnicity.

Genes affected by Pathogenic or Likely Pathogenic Variants and Variants of Uncertain Significance

P/LP and VUS were found in 56 panel genes. Two genes, *SCN5A* and *RYR2*, were enriched with P/LP, novel and rare VUS. We found 8 P/LP, 2 novel, and 7 rare VUS in *SCN5A* and 7 P/LP, 3 novel, and 9 rare VUS in *RYR2*. Cases that had P/LP variants in *SCN5A* and *RYR2* were found in 34.8% and 30.4% of the positive cases, 2.7% and 2.4% of the total cases, respectively (Table S4). Schematic representations of the amino acid positions affected by P/LP variants and VUS are shown in Figure 2, and the correlations of those variants in the functional domains of the respectively encoded proteins are described below.

SCN5A encodes α-subunit of the cardiac Na^+ channel, Nav1.5, which mediates the voltage-dependent sodium ion permeability of excitable membranes and is responsible for the initial upstroke of the action potential[12]. Genetic variation in *SCN5A* is linked to a spectrum of arrhythmogenic disorders[13], including Brugada syndrome, progressive and non-progressive heart block, Long QT syndrome-3, familial ventricular fibrillation, and susceptibility to sudden infant death syndrome. The Nav1.5 subunit is a polypeptide that self-assembles in the membrane with repeats of four similar domains, each of these domains consists of six transmembrane α-helices arranged in a tetrameric symmetry (S1 to S6, Fig. 2a). The S4 α-helices are positively charged and they form the "voltage sensor"; the Nav1.5 subunit also has regions interacting with other proteins, such as syntrophins, desmosomal proteins, FGF13, calmudulin-binding, or Nedd4-like E3 ubiquitin ligases (Figure 1a). Most of the variants we uncovered are located in regions important for either ion transport or interaction with other proteins. For example, R225Q and T1316P map to S4 α-helices and they are likely to disrupt the voltage-dependence of the Nav1.5 Na^+ channel. Some mapped to the outer vestibule (e.g. R340Q and R893H) or the S5-S6 helices (I849N) and these may disrupt channel permeation or gating properties. Variants in the DIII-DIV interdomain linker (e.g. M1487L) are likely to have major effects on channel inactivation. Many of the residues in intracellular regions have the potential to disrupt interaction with other regulatory proteins. For example, the E1890K mutation occurs at the interface of Nav1.5/ FGF12B interaction (Figure 2a) and this mutation has previously been demonstrated to disrupt this interaction[14].

RYR2 encodes the cardiac ryanodine receptor type 2 (RyR2) protein, a large, highly conserved component of the Ca^{2+} ion channel complex in the sarcoplasmic reticulum (SR) membrane. The calcium channel complex consists of a tetramer of the ryanodine receptor proteins and a tetramer of FK506 binding protein 1B proteins (FKBP12), which supplies calcium to cardiac muscle[15]. Pathogenic variants in the RYR2 gene cause CPVT, characterized by exertional polymorphic ventricular tachycardia (VT) in structurally normal heart [16], through affecting the channel function of RyR2 protein, or its sensitivity to cytosolic or luminal Ca^{2+}, and/or its interaction with regulatory proteins (such as FKBP12.6). Most of the RYR2 variants associated with sudden death in our study cluster with known clinical CPVT mutations[17], especially those in the N-terminal domain, the central domain, and the channel domain (Fig. 2b), which strongly suggests a shared clinical phenotype. Notably, several variants mapped to residues outside of these common regions, with a hotspot in the helical domain. Two variants (N2629H and R2772H) were mapped to this helical domain are likely to introduce structural abnormalities that lead to previously unidentified RyR2 channel defects (Figure 2b).

The ExAC dataset also showed high degree of intolerance of variation in *RYR2* and *SCN5A* (Figure S1), with the Z scores 5.21 and 2.53 for missense variants, respectively, and pLI = 1 for both genes. (pLI >= 0.9 is for extremely LoF intolerant set of genes.)

Genotype and History Correlation

A family history of sudden death or cardiac arrhythmia was found in 17.6% of infants, 9.8% in adults, 7.7% in children, and 6.8% in young adults. In addition, a personal history of seizure was found in 25.6% of children and 12.2% of adults, and a personal history of fainting, heart murmur, sleep apnea, or cardiac arrhythmia was found in 19.5% of adults, 17.9% of children, 12.2% of young adults (Figure S2). When correlating the family or personal history with the positive genotype, only two positive cases had a pertinent family history (a young adult and an adult case), nine positive cases older than one year had a personal history (40%), while none of the infant cases had a personal history (Table 3). When correlating the death circumstances with the genotype, we found that all, except two individuals, were at rest (either in bed or sitting) and not engaging in any moderate to vigorous activities.

Among seven positive infant cases (Table 3), four had P/LP variants in *SCN5A*, one each in *PKP2* (supported by our unpublished functional study), *RYR2*, and *MYBPC3*. Among five positive children cases, two had P/LP variants in *RYR2* and one each in *SCN5A, KCNH2*, and *CACNA1C.* Among five positive young adult cases, two had P/LP variants in *RYR2,* and one each in *SCN10A, MYBPC3,* and *KCNH2*. Among seven positive adult cases, three had P/LP variants in *SCN5A,* two in *RYR2,* and one each in *GLA, and ACTN2*.

Discussion

We present the results of high-resolution variant classification using a statistical framework recently developed [9]and we believe that, while there is still inherent subjectivity in variant calling and this framework may not be applicable for ubiquitous use, this approach allows for the culling of variants with appreciable frequency to avoid inflation with false-positives without running the risk of a false negative by exclusively considering pathogenicity amongst novel variants.

We reclassified 12 variants (originally submitted to ClinVar as pathogenic variants or VUS) as benign or likely benign variants (Table 2). It is important for the original submitters to re-evaluate their calling, as a false-positive calling from a reputable academic lab presents challenges to other diagnostic labs in reporting the significance of the same variant, and even more concerning, a physicians may treat families with benign variants whose status as P/LP variants remain outdated.

Alongside the small number of pathogenic variants (Table 3) we identified in the cohort, we uncovered a large number of novel (Table 4) and rare VUS (Table S3) that should not be discarded as genetic noise before clinical and function studies prove they are benign. However, we emphasize that these novel variants should not be considered diagnostic and lead to treatment decisions for asymtomatic family members who carry them, or for making prenatal decisions. This is particularly important in non-Whites, as our low testing yield in non-White minorities (Figure 1b) correlates with the dearth of reported SUD studies in those populations, since populations of European descent have been most extensively studied.[3, 7,4-6] Our study highlights the critical need for more studies of ethnic minorities in future population genetic and clinical research, both for more ethnicity matched normative controls in the face of the big genomic data boom as well as genotype/phenotype information to utilize for the creation of clinical guidelines in the current personalized medicine climate that can span all ethnicities.

A study by Lahrouchi etc[7], where 77 primary electrical disorder and cardiomyopathy genes were tested in 302 SUD individuals, 88% of whom were European descent between age 1 to 64 years. Clinically actionable P/LP variants were present in 13% of cases, similar to the 14.3% testing yield in Whites of our cohort. They also reported the genes that frequently mutated in SUD cohort are *RYR2, KCNH2, KCNQ1,* and *SCN5A*, whereas we found that *SCN5A* and *RYR2* are frequently mutated. The difference of the ranking of *SCN5A* in the two cohorts might be due to the inclusion of infants in our study.

The identification of incidental findings in large-scale genetic testing is among the challenges facing molecular diagnostics in SUD. Interpreting the contribution of an age-penetrant, well-studied pathogenic variant which typically manifests disease in adulthood, to "the" cause of death in infants and children is an example of this challenge. In our study, we found a pathogenic variant in *MYBPC3*, splice variant (g.11:47360070C>T) (Table 3), which typically does not manifest as hypertrophic cardiomyopathy (HCM) or sudden death in people younger than 20 years[18]. We found this variant in a one-month old infant who had negative autopsy findings including a normal heart. Although the positive testing result is useful to guide any at-risk adult family members for clinical screening of HCM and prevent sudden death, it is *unlikely* that this variant is "the" cause of the death in the infant through the mechanisms that cause death in adults. The distinction between "testing positive" cases and "solved" cases where "the" cause of death is found should be made cautiously; in the previously described example, "the" cause of death remained undetermined. As the testing yield using this panel is low in infants (57% negative cases among tested infants), additional research is greatly needed.

The high-resolution statistical framework we used to classify variants has limitations. We lack accurate knowledge of disease prevalence, penetrance, and allelic heterogeneity for all the genes tested as previously discussed [9]. In addition, although gnomAD is believed to be depleted of severe childhood onset disease, it includes sequencing data from several heart studies, such as Framingham Heart Study, Jackson Heart Study, Myocardial Infarction Genetics Consortium (MIGen), and the clinical evaluation of hearts in the individuals who had a low frequency variant is unknown to us. However, this method is superior than a framework commonly utilized in population genetics which filters variants so that only singleton variants are considered candidates for pathogenicity, as that would removed low frequency true pathogenic variants leading to false negative results.

Conclusion

Variant interpretation needs to be standardized in molecular diagnostics to lower rates of false positives and avoid the risk of false negatives. Differences of testing yields between demographic groups highlights the need for the inclusion of un-represented populations in future studies.

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Table 1. Characteristics of the 296 Sudden Unexplained Death Cases

Ethnicity	Age cases, n (%)				Gender cases, n (%)		Subtotal cases, n (%)
	Infants (<12 months)	Children (1-17 years)	Young Adults (18-34 years)	Adults (35-55 years)	Males	Females	
African Americans	86 (60.6%)	24 (61.5%)	26 (35.1%)	11 (26.8%)	77 (45.0%)	70 (56.0%)	147 (49.7%)
Hispanics	27 (19.0%)	6 (15.4%)	19 (25.7%)	12 (29.3%)	53 (31.0%)	11 (8.8%)	64 (21.6%)
Whites	12 (8.5%)	8 (20.5%)	15 (20.3%)	14 (34.1%)	21 (12.3%)	28 (22.4%)	49 (16.6%)
Asians	4 (2.8%)	1 (2.6%)	13 (17.6%)	4 (9.8%)	13 (7.6%)	9 (7.2%)	22 (7.4%)
Mixed Ethnicities	13 (9.2%)	0	1 (1.4%)	0	7 (4.1%)	7 (5.6%)	14 (4.7%)
Subtotal cases, n	142	39	74	41	171	125	296 (100%)

Table 2. Reclassified "Benign" Variants due to high AC in gnomAD

Gene	HGVSGenomic (GRCh37)	HGVSProtein	Highest AC in gnomAD (subpopulation; total) †,	ClinVar Classification (n)*
ANK2	g.4:114269433A>G	NP_001120965.1:p.Glu1449Gly	96 in 126426 European; 143 in total	Likely pathogenic(2);Pathogenic(2);Uncertain significance(2);Likely benign(1)
SCN5A	g.3:38603958G>A	NP_932173.1:p.Thr1304Met	35 in 126116 "European (Non-Finnish); 42 in total	Likely pathogenic(2);Uncertain significance(2)
KCNQ1	g.11:2790111C>T	NP_000209.2:p.Arg518Ter	21 in 111682 "European (Non-Finnish); 26 in total	Pathogenic(4);Uncertain significance(1)
KCNJ2	g.17:68171457G>A	NP_000882.1:p.Val93Ile	2 in 6468 "other"; 41 in total	Likely pathogenic(1);Pathogenic(1);Uncertain significance(1)
KCND3	g.1:112323335G>A	NP_004971.2:p.Leu450Phe	7 in 10048"Ashkenazi Jewish; 36 in total	Pathogenic/Likely pathogenic (2)
ACTN2	g.1:236906323C>T C>T	NP_001094.1:p.Thr412Met	13 in 24028 AA; 28 in total	Uncertain significance(5);Likely benign(1)

KCNJ5	g.11:128786525G>C	NP_000881.3:p.Gly387Arg	47 in 18848 Asian; 47 in total	Pathogenic(2);Likely benign(2);
KCNH2	g.7:150647283G>A	NP_000229.1:p.Arg791Trp	24 in 23796 AA; 26 in total	Uncertain significance(2);Likely benign(1);
VCL	g. 1075849898C>G	NP_054706.1:p.Leu432Val	41 European; 43 in total	Uncertain significance (4)
MYL2	g.12:111350901	NP_000423.2:p.Glu134Ala	42 in 126684 European; 54 in total	Likely pathogenic(1);Uncertain significance(4)
SCN5A	g.3:38674719C>T	NP_000326.2:p.Arg27His	54 in 34410 Latino; 67 in total	Pathogenic(1);Uncertain significance(1);Likely benign(2)
SCN3B	g.11:123524481A>G	NP_001035241.1:p.Leu10Pro	51 in 126696 European; 55 in total	Likely pathogenic(2);Pathogenic(4);Uncertain significance(2)

*Reported "Pathogenic Variants" or "VUS" in ClinVar, access on May 26, 2017; n, number of submitters; †gnomAD, accessed on May 26, 2017

Table 3. Pathogenic and Likely Pathogenic Variants

Gene	HGVSGenomic (GRCh37)	HGVSProtein	Highest AC in gnomAD (subpopulation; total) †	Age (y)	Gender	Ethnicity*	Family History	Personal History of Seizure or Arrhythmia	Death Circumstances	Autopsy	References and Comments
SCN5A	g.3:38627291 C>T	NP_932173.1:p.Arg893His	1 in 22300 "European (Finnish)" ; 1 in 246262 total	41	M	Hispanic	Negative	Positive	eating	slight myocyte hypertrophy	PMID: 20129283 (cohort study)
SCN5A	g.3:38655263 C>T	NP_932173.1:p.Arg225Gln	absent	41	M	Asian	Negative	Positive	in bed	No	PMID: 16922724 (family study); 24573164 (Function study); 25624448 (function study); 19716085 (cohort study)
SCN5A	g.3:38592914 A>T	NP_932173.1:p.Leu1650His	absent	37	F	White	Negative	Negative	on couch	No	PMID: 24631775 (cohort study); 19716085 (Cohort study on p.L1650F)

SCN5A	g.3:38592033 G>A	NP_932173.1:p. Arg1944Ter	1 in 18796 "East Asian"; 4 in total 276108	0.09	F	MIX	Negati ve	Negativ e	Sleeping	No	PMID: 24631775 (cohort study; stop-codon variant)
SCN5A	g.3:38597230 T>G	NP_932173.1:p. Met1487Leu	3 in 24024 "African"; 3 in total 277160	0.1	F	AA	Negati ve	Negativ e	Sleeping	No	PMID: 19716085 (Cohort study)
SCN5A	g.3:38592195 C>T	NP_932173.1:p. Glu1890Lys	2 in 111714 "European (Non-Finnish)"; 2 in total 246244	0.2	M	MIX	Negati ve	Negativ e	Sleeping	No	PMID: 24631775 (cohort study); 22705208 (structure study); PMCID: PMC4603502 (function/struct ure study)
SCN5A	g.3:38646236 T>C	NP_932173.1:p. Asp501Gly	absent	0.3	M	AA	Negati ve	Negativ e	Sleeping	No	PMID: 20129283 (cohort study)
SCN5A	g.3:38627423 A>T	NP_932173.1:p. Ile849Asn	absent	1.8	F	White	Negati ve	Positive	Sleeping	No	PMID: 24631775 (cohort study) . 19716085 (reported p. I848F)

SCN10A	g.3:38798293G>T	NP_006505.2:p.Leu388Met	absent	24	M	AA	Negative	Negative	playing fire hydrant	No	PMID: 24998131 (reported for p.L388P & p. F386C)
RYR2	g.1:237942017G>A	NP_001026.2:p.Ala3943Thr	1 in 33526 "Latino"; 1 in total 245642	41	M	Hispanic	Negative	Positive	in bed	slight myocyte hypertrophy	PMID: 24631775 (cohort study), mutation hotspot
RYR2	g.1:237947427A>G	NP_001026.2:p.Met4139Val	absent	23	M	White	Positve	Positive	in music festive	slight myocyte hypertrophy with occasional perivascular firosis	PMID: 24631775 (cohort study), mutation hotspot
RYR2	g.1:237957206C>T	NP_001026.2:p.Arg4608Trp	absent	34	M	White	Negative	Positive	shopping	No	PMID: 24631775 (cohort study); PMID: 25194972 (cohort study for p.R4608Q)
RYR2	g.1:237817634A>C	NP_001026.2:p.Asn2629His	1 in 982 "other" 0.001018; 1	0.2	M	White	Negative	Negative	Sleeping	No	PMID: 23595086 (reported for p.

			in total 30970								G2628E)
RYR2	g.1:237824126G>A	NP_001026.2:p.Arg2772His	1 in 22426 "South Asian" 0.00004459; 1 in total 149144	38	F	White	Negative	Negative	Sleeping	No	PMID: 27532257 (reported for p.I2770T)
RYR2	g.1:237991699T>C	NP_001026.2:p.Phe4870Ser	absent	15	F	AA	Negative	Negative	spleeping	No	novel, in mutation hot spot; predicted deleterious; PMID: 12093772, 16239587 (cohort and functional reports for p.I4867M); PMID: 23973953 (reported for p.I4867V)
RYR2	g.1:237935377G>A	NP_001026.2:p.Val3875Ile	absent	15	M	Hispanic	Negative	Positive	witnessed collapsing	No	PMID: 23595086 (reported for p.D3876E)
PKP2	g.12:33031151G>T	NP_004563.2:p.Tyr221Ter	2 in 24030 "African" 0.00008323;	0.3	F	MIX	Negative	Negative	Sleeping	No	PMID: 24070718 (cohort study);

			2 in total 277206								unpublished functional study
MYBPC3	g.11:47355201G>A	NP_000247.2:p.Arg1033Trp	1 in 15296 "African" 0.00006538; 2 in 243384	23	M	Hispanic	Negative	Negative	in bed	cardiac hypertrophy	PMID: 23283745 (cohort study); PMID: 20474083 (reported for p.Arg1033Gln); cardiac pathology finding
MYBPC3	g.11:47360070C>T	splice-site variant	absent	0.1	M	MIX	Negative	Negative	Sleeping	No	PMID: 9048664 (family study); PMID: 16199542 (family study); PMID: 26914223 (cohort study genotype-phenotype); PMID: 21088121 (family study Genotype-phenotype); PMID: 25525159 (induce a large

											splicing change)
KCNH2	g.7:150646089C>A	NP_000229.1:p.Gly816Val	absent	27	F	White	Negative	Positive	on toilet bowl	No	PMID: 21951015 (family and function study)
KCNH2	g.7:150646032C>T	NP_000229.1:p.Arg835Gln	2 in 24030 "African" 0.00008323; 2 in total 277194	16	F	AA	Negative	Negative	Sleeping	No	PMID: 25140878 (family and function assay)
GLA	g.X:100658929C>T	NP_000160.1:p.Gly80Asp	absent	42	M	Hispanic	Positve	Positive	in bed	hypertrophied cardiac myocytes with enlarged nuclei. Focal area with architectural disarry of the myocyte	PMID: 26415523; cardiac phenotype

										s.	
CACNA1C	g.12:2676833C>T	NP_000710.5:p.Arg590Cys	1 in 17216 "East Asican" 0.00005809; 3 in total 244754	17	F	AA	Negative	Negative	in bed	No	PMID: 24981977 (cohort study for p.R590S)
ACTN2	g.1:236917325C>T	NP_001094.1:p.Arg640Cys	1 in 6464 "other" 0.00016; 4 in total 276964	38	M	Hispanic	Negative	Negative	at home	slightly cardiac myocyte hypertrophy	cardiac phenotype

* AA: African American; MIX: mixed ethnicity; †gnomAD, accessed on May 26, 2017

Table 4. Novel VUS

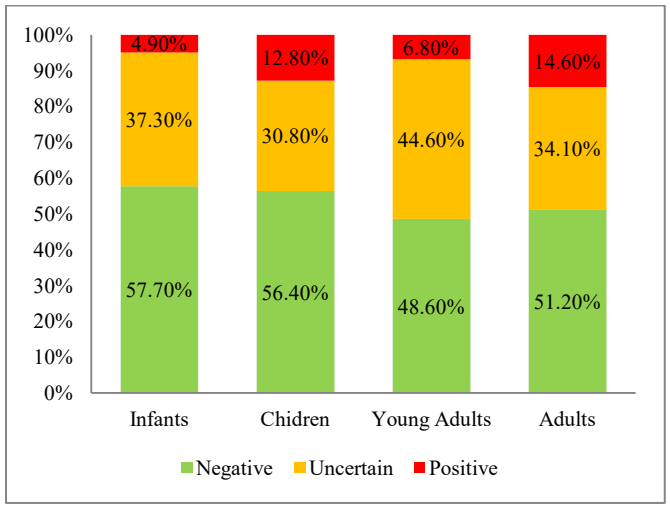
Gene	HGVSGenomic	HGVSProtein	nonsynonymous change affecting the same AA position in gnomAD*
ACTN2	g.1:236925779G>A	NP_001094.1:p.Glu849Lys	no
AKAP9	g.7:91709239G>A	NP_005742.4:p.Gly2598Arg	no
AKAP9	g.7:91631686G>A	NP_005742.4:p.Glu819Lys	no
ANK2	g.4:114120221A>G	NP_001139.3:p.Lys114Glu	no
ANK2	g.4:114288743A>T	NP_001139.3:p.Glu3685Val	no
CACNA1C	g.12:2786915T>C	NP_000710.5:p.Leu1658Pro	no
DES	g.2:220283414C>T	NP_001918.3:p.Thr77Ile	p.Thr77Ala (24 in 202100)
DPP6	g.7:154519540G>A	NP_001927.3:p.Val222Ile	no
DPP6	g.7:154585829A>C	NP_001927.3:p.Lys339Gln	no
DSG2	g.18:29126149A>G	NP_001934.2:p.Ile934Val	no
DSG2	g.18:29122531A>G	NP_001934.2:p.Arg684Gly	no
FHL2	g.2:105990179G>T	NP_963849.1:p.Tyr56Ter	no
GLA	g.X:100658867C>T	NP_000160.1:p.Asp101Asn	no
GPD1L	g.3:32169635A>C	NP_055956.1:p.Lys39Gln	no
KCNA5	g.12:5154087G>T	NP_002225.2:p.Leu258Phe	no
KCND3	g.1:112318793G>T	NP_004971.2:p.Pro625Gln	no

KCNJ8	g.12:21919175G>C	NP_004973.1:p.Pro253Ala	no
KCNQ1	g.11:2608876G>A	NP_000209.2:p.Ser402Asn	No
KCNQ1	g.11:2683193A>G	NP_000209.2:p.Arg466Gly	no
LAMA4	g.6:112451220T>A	NP_002281.3:p.Ser1324Cys	no
LAMA4	g.6:112493818G>T	NP_002281.3:p.His509Asn	no
LDB3	g.10:88446951C>T	NP_001073585.1:p.Pro157Leu	no
MYBPC3	g.11:47367814A>G	NP_000247.2:p.Leu345Pro	no
MYH6	g.14:23869568T>C	NP_002462.2:p.His493Arg	no
MYL2	g.12:111348948T>C	NP_000423.2:p.Asp145Gly	p.Asp145Asn (5 in total 243768)
MYPN	g.10:69881661G>A	NP_115967.2:p.Asp156Asn	p.Asp156Tyr (1 total 246042)
NEBL	g.10:21139412T>C	NP_006384.1:p.Tyr343Cys	no
PRKAG2	g.7:151372520C>T	NP_057287.2:p.Ala224Thr	p.Ala224Gly (2 in 245846)
RBM20	g.10:112583345G>A	NP_001127835.1:p.Val1142Met	no
RBM20	g.10:112572241A>G	NP_001127835.1:p.Asn696Asp	no
RBM20	g.10:112581297G>A	NP_001127835.1:p.Ala974Thr	no
RYR2	g.1:237806735A>G	NP_001026.2:p.Thr2444Ala	no
RYR2	g.1:237796939T>C	NP_001026.2:p.Ile2206Thr	no
RYR2	g.1:237586481A>T	NP_001026.2:p.Asn313Ile	no
SCN10A	g.3:38753726C>T	NP_006505.2:p.Gly1339Ser	p.Gly1339Val (1 in 30972)

SCN5A	g.3:38663927G>A	NP_932173.1:p.Ala149Val	no
SCN5A	g.3:38604016T>C	NP_932173.1:p.Ser1285Gly	no
TMEM43	g.3:14176656insGGTG	ESSENTIAL_SPLICE_SITE	no
TPM1	g.15:63336331G>T	NP_001018005.1:p.Ala74Ser	no
TRPM4	g.19:49669350A>G	NP_060106.2:p.Met49Val	no
TRPM4	g.19:49713579T>G	NP_060106.2:p.Ile1082Ser	no

*Absent in gnomAD and the coverage of the region is higher than 30x; accessed on May 26, 2017

A.



B.

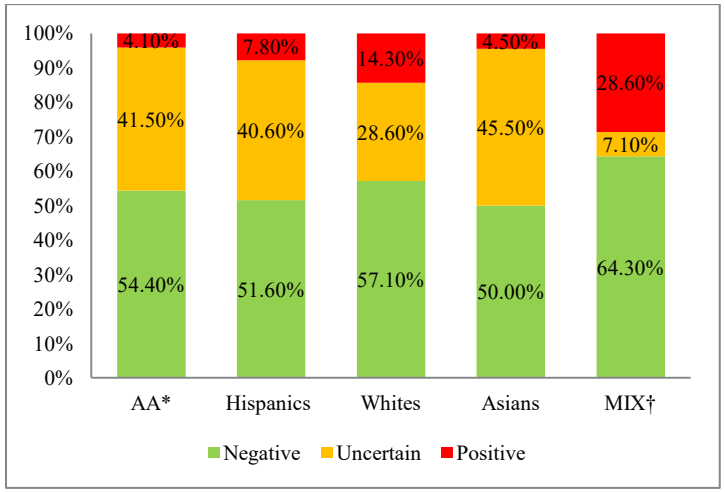


Figure 1. Demographic distributions of positive, uncertain, and negative cases by age (A) and by ethnicity (B). * AA, African Americans. MIX, mixed ethnicities

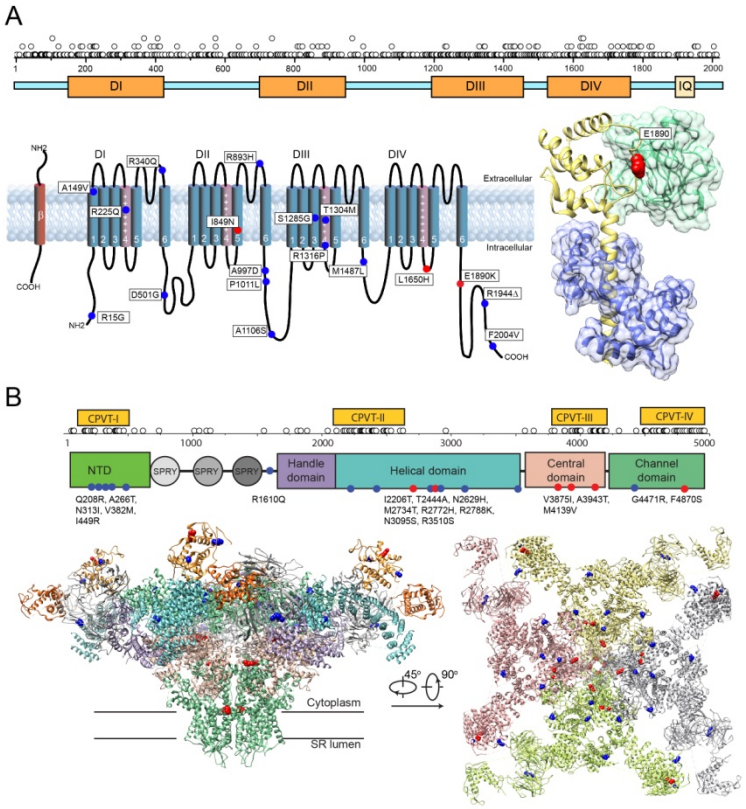


Figure 2: Schematic representation of the amino acid positions affected by the identified variants.

Variants of unknown significance are indicated in blue, whereas likely pathogenic variants are colored in red. Known clinical variants (from The Human Gene Mutation Database and from literature searches) are indicated in open symbols above the scale bars. (A) The domain structure and membrane topology of SCN5A encoded Nav1.5 (Uniprot ID Q14524), which consists of four domains (DI-DIV), each consisting of 6 transmembrane transmembrane segments (S1-S6). The right hand panel depicts a 3D crystal structure of the Nav1.5 C-terminus in yellow (PDB: 4JQ0, residues 1787-1926), interacting with calmodulin (blue) and FGF12B (green). The E1890K mutation occurs at the Nav1.5/FGF12B interaction interface. (B) The domain structure of RYR2, which consists of: N-terminal domain (NTD), SPRY1 domains, P1 and P2 domains, the

handle domain, helical domains, the U Motif and the channel domain. Also shown is the recently published cryo-EM structure of the *Sus scrofa* RYR2 in the closed state (PDB: 5GO9), which has a 98% amino acid identity with human RYR2 (NP_001026.2). The structure on the left is viewed from the side and is colored by functional domains, whereas the structure on the right shown from the SR lumen perspective and is colored by subunit.

SUPPLEMENTAL MATERIAL

Table S1. 114 genes in our SUD panel

Disease	# Gene	Gene Name	Gene Category
LQT	14	CAV3,SCN5A,AKAP9,ANK2,CACNA1C,KCNE1,KCNE2,KCNH2, KCNJ2,KCNJ5,KCNQ1,NOS1AP,SCN4B,SNTA1	Arrhythmia genes
SQT	3	KCNH2 ,KCNJ2,KCNQ1	Arrhythmia genes
BrS	15	ABCC9,SCN5A,CACNA1C,CACNB2,GPD1L,HCN4,KCND3,KCNE3, KCNJ8,RANGRF,SCN1B,SCN2B,SCN3B,SCN10A,TRPM4	Arrhythmia genes
CPVT	3	CASQ2,RyR2,KCNJ2	Arrhythmia genes
AF	8	SCN5A,KCNA5,KCNE1L,KCNE2,KCNE4,KCNJ2,KCNQ1,SCN2B	Arrhythmia genes
Conduction, Other	9	AKAP10,ARHGAP24,BCAT1,CACNA2D1,CALM1,CAML2,DPP6,FLRT2,HAND1,CAV 1,CDKN1A,STRN	Arrhythmia genes
HCM	29	ACTC1,ACTN2,ANKRD1,BAG3,CAV3,CSRP3,GLA,LAMP2,LDB3, MYBPC3,MYH6,MYH7,MYL2,MYL3,MYLK2,MYOZ2,NEXN,PLN, PRKAG2,RyR2,TCAP,TNNC1,TNNI3,TNNT2,TPM1,TTN,TTR,VCL,MYPN	Cardiomyopathy
DCM	40	ABCC9,ACTC1,ACTN2,ANKRD1,BAG3,CAV3,CRYAB,CSRP3, CTF1,DES,DSC2,DSG2,DSP,EMD,FHL2,GATAD1,LAMA4,LAMP2,LDB3,LMNA,MYB PC3,MYH6,MYH7,NEBL,NEXN,PKP2,PLN,RBM20,SCN5A,SGCD,TAZ,TCAP,TMPO, TNNC1,TNNI3,TNNT2,TPM1,TTN,VCL,MYPN	Cardiomyopathy
ARVC	10	DES,DSC2,DSG2,DSP,JUP,PKP2,RyR2,TMEM43,TTN,TGFB3	Cardiomyopathy
LVNC	10	ACTC1,CASQ2,DTNA,LDB3,LMNA,MYBPC3,MYH7,TAZ,TNNT2, VCL	Cardiomyopathy
RCM	5	ACTC1,BAG3,DES,MYH7,TNNT2	Cardiomyopathy

Central Nervous System	7	CDKL5,KCNA1,PHOX2a,PHOX2b,RET,SCN9A,SLC6A4,	Central Nervous system
Metabolic	2	CHRM2,SLC9A3	others
Global Regulation	9	C1orf77,CACHD1,CALM1,CAML2,CAV1,CDKN1A,STRN,TRPM4, Yip1A	others

Table S2a. 23 cases (7.8%) with P/LP variants

		infant	children	young adult	adult	male	female
cases# carried 1/3a	23 (7.8%)	7 (4.9%)	5 (12.8%)	5 (6.8%)	6 (14.6%)	14 (8.2%)	10 (8.0%)
AA*	6 (4.1%)	2 (2.3%)	3 (12.5%)	1 (3.8%)	0	2 (2.6%)	4 (5.7%)
Hispanics	5 (7.8%)	0	1 (16.7%)	1 (5.3%)	3 (25.0%)	5 (9.4%)	0
Whites	7 (14.3%)	1 (8.3%)	1 (12.5%)	3 (20.0%)	2 (14.3%)	4 (19.0%)	4 (14.3%)
Asians	1 (4.5%)	0	0	0	1 (25.0%)	1 (7.7%)	0
MIX†	4 (28.6%)	4 (30.8%)	0	0	0	2 (28.6%)	2 (28.6%)

Table S2b. 112 cases (37.8%) with VUS (3bN/3bL), but not P/LP

		infant	children	young adult	adult	male	female

cases# carried VUS	112 (37.8%)	53 (37.3%)	12 (30.8%)	33 (44.6%)	14 (34.1%)	65 (38.0%)	47 (37.6%)
AA*	61 (41.5%)	37 (43%)	9 (37.5%)	11 (42.3%)	4 (36.4%)	25 (32.5%)	36 (51.4%)
Hispanics	26 (40.6%)	9 (33.3%)	1 (16.7%)	12 (63.2%)	4 (33.3%)	24 (45.3%)	2 (18.2%)
Whites	14 (28.6%)	5 (41.7%)	2 (25.0%)	3 (20.0%)	4 (28.6%)	7 (33.3%)	7 (25.0%)
Asians	10 (45.5%)	1 (25.0%)	0	7 (53.8%)	2 (50.0%)	8 (61.5%)	2 (22.2%)
MIX†	1 (7.1%)	1 (7.7%)	0	0	0	1 (14.3%)	0

Table S2c. 161 cases (54.4%) are negative cases

		infant	children	young adult	adult	male	female
negative cases# (without P/LP or VUS)	161 (54.4%)	82 (57.7%)	22 (56.4%)	36 (48.6%)	21 (51.2%)	93 (54.4%)	68 (54.4%)
AA*	80 (54.4%)	47 (54.7%)	12 (50.0%)	14 (53.8%)	7 (63.6%)	50 (64.9%)	30 (42.9%)
Hispanics	33 (51.6%)	18 (66.7)	4 (66.7%)	6 (31.6%)	5 (41.7%)	24 (45.3%)	9 (81.8%)
Whites	28 (57.1%)	6 (50.0%)	5 (62.5%)	9 (60.0%)	8 (57.1%)	11 (52.4%)	17 (60.7%)
Asians	11 (50.0%)	3 (75.0%)	1 (100%)	6 (46.2%)	1 (25.0%)	4 (30.8%)	7 (77.8%)
MIX†	9 (64.3%)	8 (61.5%)	0	1 (100%)	0	4 (57.1%)	5 (71.4%)

*AA = African Americans; †MIX = mixed ethnicities

Table S3. VUS (rare) variants found in this study

Chromosome	HGVSGenomic	Gene	Exon Number	HGVSCoding	HGVSProtein
1	g.236890997C>T	ACTN2	6	NM_001103.3:c.556C>T	NP_001094.1:p.Leu186Phe
1	g.156106163G>A	LMNA	7	NM_170707.3:c.1316G>A	NP_733821.1:p.Arg439His
1	g.78392549G>A	NEXN	8	NM_144573.3:c.836G>A	NP_653174.3:p.Arg279His
1	g.78395146T>C	NEXN	9	NM_144573.3:c.1010T>C	NP_653174.3:p.Ile337Thr
1	g.78383881G>A	NEXN	5	NM_144573.3:c.370G>A	NP_653174.3:p.Glu124Lys
1	g.237918364G>A	RYR2	6	uc010pya.2:c.424G>A	p.Asp113Asn
1	g.237550627A>G	RYR2	9	NM_001035.2:c.623A>G	NP_001026.2:p.Gln208Arg
1	g.237774207G>A	RYR2	36	NM_001035.2:c.4829G>A	NP_001026.2:p.Arg1610Gln
1	g.237881795C>A	RYR2	73	NM_001035.2:c.10528C>A	NP_001026.2:p.Arg3510Ser
1	g.237580371G>A	RYR2	11	NM_001035.2:c.796G>A	NP_001026.2:p.Ala266Thr
1	g.237863684A>G	RYR2	65	NM_001035.2:c.9284A>G	NP_001026.2:p.Asn3095Ser
1	g.237821315T>C	RYR2	54	NM_001035.2:c.8201T>C	NP_001026.2:p.Met2734Thr
1	g.237824174G>A	RYR2	56	NM_001035.2:c.8363G>A	NP_001026.2:p.Arg2788Lys
1	g.237951370G>A	RYR2	92	NM_001035.2:c.13411G>A	NP_001026.2:p.Gly4471Arg
1	g.201331083C>T	TNNT2	13	NM_001001430.2:c.647G>A	NP_001001430.1:p.Arg216Lys
2	g.220283290C>T	DES	1	NM_001927.3:c.106C>T	NP_001918.3:p.Pro36Ser
2	g.220285661G>A	DES	5	NM_001927.3:c.1009G>A	NP_001918.3:p.Ala337Thr

2	g.220286086C>T	DES	6	NM_001927.3:c.1048C>T	NP_001918.3:p.Arg350Trp
2	g.105977756C>T	FHL2	8	NM_201555.1:c.824G>A	NP_963849.1:p.Cys275Tyr
3	g.32200587G>A	GPD1L	6	NM_015141.3:c.838G>A	NP_055956.1:p.Ala280Thr
3	g.46901072T>C	MYL3	4	NM_000258.2:c.374A>G	NP_000249.1:p.Lys125Arg
3	g.38781020G>A	SCN10A	14	NM_006514.2:c.2266C>T	NP_006505.2:p.Arg756Trp
3	g.38793942C>T	SCN10A	11	NM_006514.2:c.1523G>A	NP_006505.2:p.Arg508Gln
3	g.38743348C>A	SCN10A	26	NM_006514.2:c.4639G>T	NP_006505.2:p.Val1547Leu
3	g.38752365G>C	SCN10A	23	NM_006514.2:c.4113C>G	NP_006505.2:p.Asp1371Glu
3	g.38812830T>C	SCN10A	4	NM_006514.2:c.539A>G	NP_006505.2:p.Asn180Ser
3	g.38739398-38739403delGAGAGT	SCN10A	27	uc003ciq.3:c.5308-5313delGAGAGT	N/A
3	g.38603922C>G	SCN5A	22	NM_198056.2:c.3947G>C	NP_932173.1:p.Arg1316Pro
3	g.38620899C>A	SCN5A	18	NM_198056.2:c.3316G>T	NP_932173.1:p.Ala1106Ser
3	g.38622618G>A	SCN5A	17	NM_198056.2:c.3032C>T	NP_932173.1:p.Pro1011Leu
3	g.38622660G>T	SCN5A	17	NM_198056.2:c.2990C>A	NP_932173.1:p.Ala997Asp
3	g.38674756T>C	SCN5A	2	NM_198056.2:c.43A>G	NP_932173.1:p.Arg15Gly
3	g.38618271 G>A	SCN5A	19	NM_198056.2:c.3392C>T	NP_932173.1:p. Thr1131Ile
3	g.38648281C>T	SCN5A	9	NM_198056.2:c.1019G>A	NP_932173.1:p.Arg340Gln
3	g.14180743T>C	TMEM43	11	NM_024334.2:c.946T>C	NP_077310.1:p.Trp316Arg
3	g.14177385C>T	TMEM43	10	NM_024334.2:c.859C>T	NP_077310.1:p.His287Tyr

3	g.52485835A>G	TNNC1	4	NM_003280.2:c.242T>C	NP_003271.1:p.Met81Thr
4	g.114302755C>T	ANK2	3' UTR	NM_001148.4:c.*128C>T	N/A
4	g.114279510G>A	ANK2	38	NM_001148.4:c.9736G>A	NP_001139.3:p.Asp3246Asn
4	g.114280401A>G	ANK2	38	NM_001148.4:c.10627A>G	NP_001139.3:p.Ile3543Val
4	g.114277815C>G	ANK2	38	NM_001148.4:c.8041C>G	NP_001139.3:p.Leu2681Val
4	g.114277990C>G	ANK2	38	NM_001148.4:c.8216C>G	NP_001139.3:p.Ser2739Cys
4	g.114288919A>C	ANK2	42	NM_001148.4:c.11230A>C	NP_001139.3:p.Thr3744Pro
4	g.114288805T>G	ANK2	42	NM_001148.4:c.11116T>G	NP_001139.3:p.Cys3706Gly
4	g.114278935C>A	ANK2	38	NM_001148.4:c.9161C>A	NP_001139.3:p.Ala3054Asp
4	g.114276526C>G	ANK2	38	NM_001148.4:c.6752C>G	NP_001139.3:p.Pro2251Arg
4	g.114296016G>A	ANK2	19	uc003ibg.4:c.2866G>A	p. Val916Met
6	g.7585273T>G	DSP	24	NM_004415.2:c.7778T>G	NP_004406.2:p.Ile2593Ser
6	g.7563008G>A	DSP	5	NM_004415.2:c.721G>A	NP_004406.2:p.Asp241Asn
6	g.7585059-7585062delAGAA	DSP	24	uc003mxp.1:c.7843-7846delAGAA	frameshift
6	g.7580647-7580649delAAT	DSP	23	uc003mxp.1:c.4503-4505delAAT	N/A
6	g.112437143T>C	LAMA4	36	NM_002290.4:c.5014A>G	NP_002281.3:p.Arg1672Gly
6	g.112454085C>T	LAMA4	28	NM_002290.4:c.3683G>A	NP_002281.3:p.Arg1228His
7	g.91708901C>A	AKAP9	31	NM_005751.4:c.7454C>A	NP_005742.4:p.Ser2485Tyr
7	g.91622340G>A	AKAP9	5	NM_005751.4:c.547G>A	NP_005742.4:p.Val183Ile

7	g.91670060G>A	AKAP9	18	NM_005751.4:c.4765G>A	NP_005742.4:p.Asp1589Asn
7	g.91726154G>A	AKAP9	41	NM_005751.4:c.9881G>A	NP_005742.4:p.Arg3294Gln
7	g.91712904G>A	AKAP9	33	NM_005751.4:c.8581G>A	NP_005742.4:p.Val2861Ile
7	g.81589052T>C	CACNA2D1	37	NM_000722.2:c.3060A>G	NP_000713.2:p.Ile1020Met
7	g.81591804G>T	CACNA2D1	35	NM_000722.2:c.2788C>A	NP_000713.2:p.Leu930Ile
7	g.154561183G>A	DPP6	9	NM_001936.3:c.778G>A	NP_001927.3:p.Ala260Thr
7	g.150655400G>T	KCNH2	4	NM_000238.3:c.663C>A	NP_000229.1:p.His221Gln
7	g.151483576C>T	PRKAG2	2	NM_016203.3:c.166G>A	NP_057287.2:p.Gly56Arg
10	g.121436277A>G	BAG3	4	NM_004281.3:c.1211A>G	NP_004272.2:p.Glu404Gly
10	g.121429667C>T	BAG3	2	NM_004281.3:c.485C>T	NP_004272.2:p.Pro162Leu
10	g.18439837A>G	CACNB2	2	uc001ipr.2 :c.206A>G	p. Lys49Arg
10	g.18828565A>C	CACNB2	13	NM_201590.2:c.1733A>C	NP_963884.2:p.Asp578Ala
10	g.18787370C>G	CACNB2	3	NM_201590.2:c.258C>G	NP_963884.2:p.Ile86Met
10	g.18828550G>A	CACNB2	13	NM_201590.2:c.1718G>A	NP_963884.2:p.Arg573His
10	g.88476339T>C	LDB3	9	uc001kdu.3:c.180T>C	p. Phe386Ser
10	g.69881946A>G	MYPN	2	NM_032578.3:c.751A>G	NP_115967.2:p.Thr251Ala
10	g.69935159G>A	MYPN	12	NM_032578.3:c.2644G>A	NP_115967.2:p.Ala882Thr
10	g.21098812C>T	NEBL	25	NM_006393.2:c.2534G>A	NP_006384.1:p.Arg845His
10	g.112572050G>A	RBM20	9	NM_001134363.1:c.1895G>A	NP_001127835.1:p.Arg632Lys

10	g.112404226C>T	RBM20	1	NM_001134363.1:c.14C>T	NP_001127835.1:p.Ala5Val
10	g.112540731C>G	RBM20	2	NM_001134363.1:c.364C>G	NP_001127835.1:p.Gln122Glu
10	g.112540911C>A	RBM20	2	NM_001134363.1:c.544C>A	NP_001127835.1:p.Pro182Thr
10	g.75849809C>T	VCL	10	NM_014000.2:c.1205C>T	NP_054706.1:p.Pro402Leu
11	g.111781031insA	CRYAB	2	uc010rwp.1:c.369insA	frameshift
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11	g.74168313C>T	KCNE3	3	NM_005472.4:c.296G>A	NP_005463.1:p.Arg99His
11	g.2466624C>G	KCNQ1	1	NM_000218.2:c.296C>G	NP_000209.2:p.Pro99Arg
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11	g.47358984T>C	MYBPC3	25	NM_000256.3:c.2560A>G	NP_000247.2:p.Met854Val
11	g.47354752T>G	MYBPC3	30	NM_000256.3:c.3323A>C	NP_000247.2:p.Lys1108Thr
11	g.47371612G>A	MYBPC3	4	NM_000256.3:c.458C>T	NP_000247.2:p.Pro153Leu
11	g.47355487G>A	MYBPC3	28	NM_000256.3:c.2980C>T	NP_000247.2:p.Leu994Phe
11	g.47367916G>A	MYBPC3	12	NM_000256.3:c.932C>T	NP_000247.2:p.Ser311Leu
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12	g.2694586C>T	CACNA1C	17	NM_000719.6:c.2384C>T	NP_000710.5:p.Ala795Val
12	g.2789609A>G	CACNA1C	41	uc001qki.1:c.5154A>G	p.Gln1578Arg
12	g.5154003C>G	KCNA5	1	NM_002234.3:c.690C>G	NP_002225.2:p.Asn230Lys

12	g.32996157C>T	PKP2	6	NM_004572.3:c.1469G>A	NP_004563.2:p.Arg490Gln
12	g.33030834C>A	PKP2	3	NM_004572.3:c.980G>T	NP_004563.2:p.Gly327Val
12	g.98926810A>C	TMPO	4	NM_003276.2:c.775A>C	NP_003267.1:p.Thr259Pro
12	g.98938066G>A	TMPO	5	uc001tfj.3:c.*1017G>A	p.Gly241Glu
12	g.98927638A>G	TMPO	4	NM_003276.2:c.1603A>G	NP_003267.1:p.Lys535Glu
12	g.98927009-98927011delTAG	TMPO	4	uc001tfh.2:c.1269-1271delTAG	N/A
14	g.86089509G>T	FLRT2	1	uc021rxf.1:c.1651G>T	p.Val551Leu
14	g.86088699C>G	FLRT2	2	uc001xvr.3:c.1608C>G	p.Leu281Val
14	g.23855205G>A	MYH6	34	NM_002471.3:c.5095C>T	NP_002462.2:p.Arg1699Trp
14	g.23858246G>T	MYH6	29	NM_002471.3:c.3997C>A	NP_002462.2:p.His1333Asn
14	g.23857410T>A	MYH6	30	NM_002471.3:c.4313A>T	NP_002462.2:p.Asn1438Ile
14	g.23856761G>T	MYH6	32	NM_002471.3:c.4627C>A	NP_002462.2:p.Gln1543Lys
14	g.23863408C>T	MYH6	21	NM_002471.3:c.2554G>A	NP_002462.2:p.Ala852Thr
14	g.23870143G>T	MYH6	13	NM_002471.3:c.1185C>A	NP_002462.2:p.Asp395Glu
14	g.23858078C>T	MYH6	29	NM_002471.3:c.4165G>A	NP_002462.2:p.Glu1389Lys
14	g.23883098T>C	MYH7	38	NM_000257.2:c.5660A>G	NP_000248.2:p.Glu1887Gly
14	g.76447140C>T	TGFB3	1	NM_003239.2:c.97G>A	NP_003230.1:p.Gly33Ser
15	g.73617740C>T	HCN4	5	NM_005477.2:c.1636G>A	NP_005468.1:p.Asp546Asn
17	g.19835188A>G	AKAP10	10	NM_007202.3:c.1571T>C	NP_009133.2:p.Leu524Pro

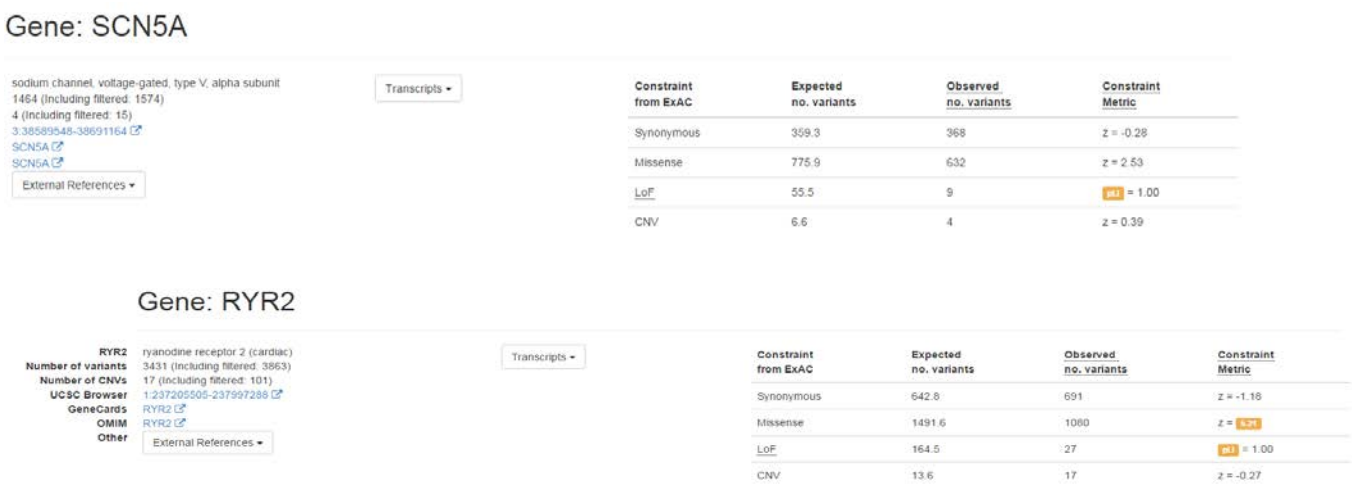
17	g.39915055G>A	JUP	9	NM_002230.2:c.1565C>T	NP_002221.1:p.Ala522Val
17	g.39913717C>T	JUP	12	NM_002230.2:c.1996G>A	NP_002221.1:p.Val666Met
18	g.32391989G>A	DTNA	5	NM_001390.4:c.515G>A	NP_001381.2:p.Arg172Gln
19	g.49703983G>A	TRPM4	19	NM_017636.3:c.2894G>A	NP_060106.2:p.Arg965His
19	g.49671652G>A	TRPM4	5	NM_017636.3:c.584G>A	NP_060106.2:p.Arg195Gln
19	g.49686061A>G	TRPM4	11	NM_017636.3:c.1490A>G	NP_060106.2:p.Glu497Gly
19	g.49661482T>A	TRPM4	2	NM_017636.3:c.58T>A	NP_060106.2:p.Cys20Ser
20	g.30414425G>C	MYLK2	7	NM_033118.3:c.990G>C	NP_149109.1:p.Glu330Asp
20	g.32031326C>T	SNTA1	1	NM_003098.2:c.101G>A	NP_003089.1:p.Ser34Asn
20	g.32000135C>T	SNTA1	5	NM_003098.2:c.1007G>A	NP_003089.1:p.Arg336Gln
21	g.35821734G>A	KCNE1	4	NM_000219.4:c.199C>T	NP_000210.2:p.Arg67Cys
X	g.153641558C>T	TAZ	3	NM_000116.3:c.253C>T	NP_000107.1:p.Arg85Cys

*preferred transcript that is commonly used in the literature is used for most variants, except for a few variants affecting coding or splice-site based on non-preferred transcript.

Table S4. Tope genes with pathogenic/likely pathogenic variants in our panel study

	Cases with P/LP variants	*% in Positive Cases*	*% in all cases*
SCN5A	*8*	*34.8%*	*2.7%*
RYR2	*7*	*30.4%*	*2.4%*
MYBPC3	*2*	*8.7%*	*0.7%*
KCNH2	*2*	*8.7%*	*0.7%*

Figure S1. *SCN5A* and *RYR2* Statistical from ExAC (data source: exac.broadinstitude.org)



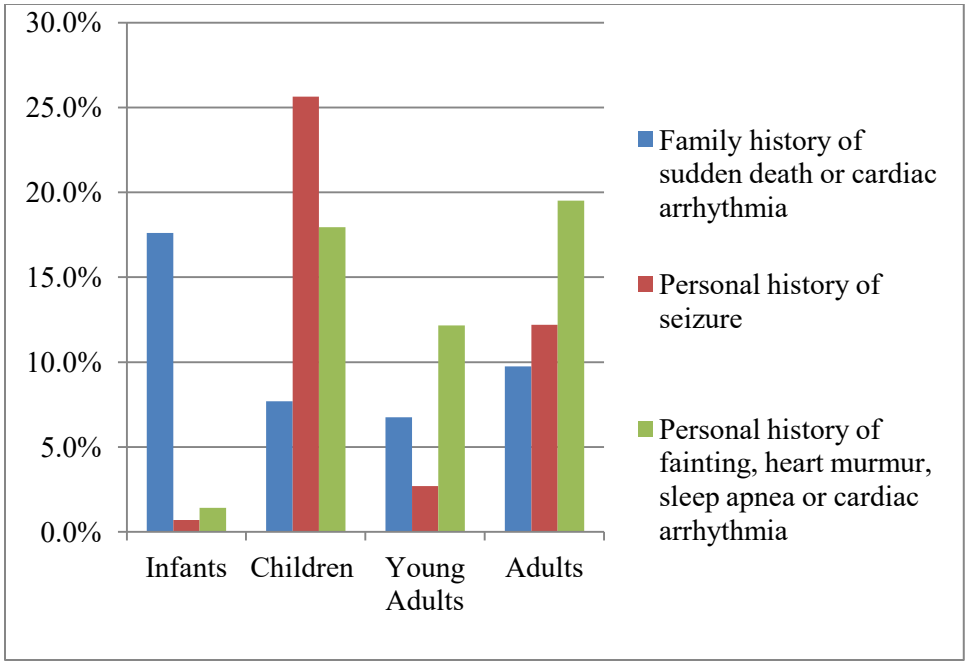


Figure S2. Family and Personal History by Age