**SUPPLEMENTAL MATERIAL**

SUPPLEMENTAL METHODS

**Study populations and definitions**

In this retrospective Institutional Review Board-approved study (IRB 1216-97), the yield of P/LP variants in 24 ClinGen adjudicated definitive/strong evidence arrhythmogenic cardiomyopathy (ACM)-, dilated cardiomyopathy (DCM)-, and hypertrophic cardiomyopathy (HCM)-susceptibility genes was assessed in a referral cohort of 38 young unexplained SCA survivors and 68 autopsy-inconclusive/negative SUDY cases that remained genotype negative following comprehensive commercial and/or laboratory-based LQTS*,* BrS, and CPVT genetic testing (*AKAP9*, *CACNA1C*, *CALM1*, *CALM2*, *CALM3*, *CASQ2*, *CAV3*, *KCNE1*, *KCNE2*, *KCNH2*, *KCNJ2*, *KCNQ1*, *RYR2*, *SCN4B*, *SCN5A*, *SNTA1*, *TRDN*). For autopsy-inconclusive SUDY cases, basic demographics, clinical history (including prior symptomatology), circumstance of death, and cardiovascular pathology (gross and microscopic) were obtained from next of kin and/or medical examiner reports. SUDY decedents without gross and microscopic cardiovascular pathological evaluation were excluded. Research laboratory-based exome sequencing (ES) was performed on 32 SCA survivors and 68 SUDY cases. Additionally, commercially available pan-cardio genetic testing in a CLIA-approved laboratory was performed on 6 SCA survivors.

**Control Population**

The 973 controls (509 females, 464 males) from the ICR1000 UK exome series and the 1958 Birth Cohort study were included for case-control analysis. As previously reported, ES was performed using the Illumina TruSeq and Illumina instruments.

**Exome Sequencing (ES)**

Genomic DNA samples were submitted to Mayo Clinic’s Advanced Genomics Technology Center for ES. The Bravo liquid handler and Aligent’s protocol was used to prepare paired-end libraries, and DNA was fragmented using a Covaris E210 sonicator. Agencourt AMPure SPRI beads were used to purify the constructs. SureSelect forward and Agilent SureSelect ILM Pre-Capture Indexing reverse primers were used to enrich the DNA fragment libraries, which were analyzed with Agilent Bioanalyzer DNA 1000 chip.

Exome capture was performed with the SureSelect XT Human All Exon V5 plus UTR Target Enrichment System (Agilent, Santa Clara, California). Dynal Dynabeads MyOne Streptavidin T1 captured the DNA:RNA hybrids, and Agencourt Ampure XZP beads eluted DNA from the beads, which were amplified with Agilent Sure Select Post-Capture Indexing forward and Index PCR reverse primers. ES of the exome libraries was completed with Illumina HiSeq 2000 platform (San Diego, California) and TruSeq SBS sequencing kit V3 reagents.

**Variant Filtering and Pathogenicity Assessment**

Following ES, variants were filtered using Qiagen’s Ingenuity® Variant Analysis™ software (Qiagen Bioinformatics, Redwood City, California). Variants were included only if they met the following filtering parameters: 1) had a high quality score (read depth > 10 reads, call quality > 20, genotype quality > 20), 2) were non-synonymous variants (i.e. missense, nonsense, frameshift insertion/deletion [INDEL], in-frame INDEL, or splice-error), and 3) met our rarity threshold (minor allele frequency [MAF] ≤ 0.00005 in any ethnic group within Genome Aggregation Database [gnomAD, n=141,456]). Variants meeting the above criteria underwent a further gene-specific surveillance for all known ClinGen adjudicated definitive/ strong evidence ACM-, DCM, and HCM-susceptibility genes (N=24). The ACMG guidelines for the interpretation of sequence variants were used to classify identified variants as P, LP, or variant of uncertain significance (VUS).Candidate disease-causing P/LP variants identified through post-mortem genetic testing were confirmed in the decedents’ genomic DNA using standard polymerase chain reaction (PCR) and Sanger sequencing methods. PCR primers, conditions, and sequencing methods are available upon request.

**Statistical Analysis**

Fisher’s exact tests were performed to determine statistical significance between two groups. A p<0.05 was considered to be significant.

SUPPLEMENTAL TABLES

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| **Supplemental Table I |** Pathogenic/Likely Pathogenic Variants in Sudden Cardiac Death-Susceptibility Genes Identified in Idiopathic Ventricular Fibrillation Cases | | | | | | |
| Gene | Variant (Coding) | Variant (Protein) | gnomAD MAF | CADD Score | ACMG Criteria | ACMG Classification |
| *FLNC* | c.1444C>T | p.R482\* | Absent | 38.0 | PVS1, PM2, and PP3 | Pathogenic |
| *TTN* | c.46843dupA | p.T15615fs\*4 | Absent | N/A | PVS1, PM2, and PM6 | Pathogenic |
| *TTN* | c.47943\_47946delAGAA | p.K23405fs\*8 | Absent | N/A | PVS1 and PM2 | Likely pathogenic |
| **Abbreviations:** ACMG, American College of Medical Genetics and Genomics; CADD, combined annotation dependent depletion; PM, pathogenic moderate; PS, pathogenic strong; PVS, pathogenic very strong; PP, pathogenic supporting. | | | | | | |

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| **Supplemental Table II |** Pathogenic/Likely Pathogenic Variants in Sudden Cardiac Death-Susceptibility Genes Identified in Autopsy-Negative Sudden Cardiac Death Cases | | | | | | |
| Gene | Variant (Coding) | Variant (Protein) | gnomAD MAF | CADD Score | ACMG Criteria | ACMG Classification |
| *BAG3* | c.331\_332delTT | p.F111fs\*14 | Absent | 24.3 | PVS1, PM2, and PP3 | Pathogenic |
| *DSP* | c.808C>T | p.R270\* | Absent | 38.0 | PVS1, PM2, and PP3 | Pathogenic |
| *DSP* | c.3865C>T | p.Q1289\* | Absent | 38.0 | PVS1, PM2, PP3, and PP5 | Pathogenic |
| *MYBPC3* | c.2500C>T | p.R834W | 3/249090 | 27.5 | PS4, PM1, and PP3 | Likely pathogenic |
| *MYH7* | c.550A>C | p.K184Q | Absent | 25.1 | PM1, PM2, PP2, PP3, and PP5 | Likely pathogenic |
| *PKP2* | c.1901delA | p.N634fs\*22 | Absent | 33.0 | PVS1, PM2, and PP3 | Pathogenic |
| *TTN* | c.325C>T | p.R109\* | 1/247160 | 36.0 | PVS1, PM2, and PP3 | Pathogenic |
| *TTN* | c.66500delA | p.D22167fs\*7 | Absent | N/A | PVS1 and PM2 | Likely pathogenic |
| **Abbreviations:** ACMG, American College of Medical Genetics and Genomics; CADD, combined annotation dependent depletion; PM, pathogenic moderate; PS, pathogenic strong; PVS, pathogenic very strong; and PP, pathogenic supporting. | | | | | | |

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| **Supplemental Table III |** Pathogenic/Likely Pathogenic Variants in Sudden Cardiac Death-Susceptibility Genes Identified in Controls | | | | | | |
| Gene | Variant (Coding) | Variant (Protein) | gnomAD MAF | CADD Score | ACMG Criteria1 | ACMG Classification |
| *DSP* | c.1197C>G | p.I399M | 7/251198 | 16.22 | PS4, PM1 | Likely Pathogenic |
| *DSP* | c.5472delA | p.D1825fs\*12 | 1/251156 | 33 | PVS1, PM2 | Likely Pathogenic |
| *JUP* | c.909+1G>A |  | 1/251152 | 34 | PVS1, PM2, PP3 | Likely Pathogenic |
| *MYBPC3* | c.1090+1G>T |  | 1/248814 | 35 | PVS1, PS4, PM2, PP5 | Pathogenic |
| *MYBPC3* | c.1504C>T | p.R502W | 13/280632 | 28.6 | PS4, PM1, PM5, PP1, PP3, PP5, BS4, BP2, BP5 | Pathogenic |
| *MYH7* | c.1456C>T | p.Q486\* | 2/251472 | 38 | PVS1, PM2 | Likely Pathogenic |
| *MYH7* | c.3740delA | p.K1247fs\*10 | 3/251216 | 34 | PVS1 | Uncertain |
| *MYH7* | c.5029C>T | p.R1677C | 3/251376 | 31 | PS4, PM1, PM2, PP2, PP3, BP2 | Pathogenic |
| *MYH7* | c.5504A>G | p.E1835G | 4/250548 | 25.8 | PM1, PM2, PP2, PP3 | Likely Pathogenic |
| *MYH7* | c.5657A>G | p.E1886G | 1/251436 | 33 | PM1, PM2, PP2, PP3 | Likely Pathogenic |
| *MYL2* | c.337dupG | p.V113fs\*5 | 1/251494 | 33 | PVS1, PM2 | Likely Pathogenic |
| *TNNT2* | c.856C>T | p.R286C | 6/242990 |  | PS4, PP1, PP3 | Likely Pathogenic |
| *TPM1* | c.241-1G>C |  | 1/251466 | 35 | PVS1, PM2, PP3 | Likely Pathogenic |
| *TTN* | c.8608C>T | p.Q2870\* | 1/251216 | 37 | PVS1, PM2, PP3 | Likely Pathogenic |
| *TTN* | c.24850\_24856delCAGTGTA | p.Q8284fs\*36 | 1/244224 | 37 | PVS1, PM2, PP3 | Likely Pathogenic |
| *TTN* | c.48160+1G>C |  | 2/247624 | 34 | PVS1, PP3 | Likely Pathogenic |
| *TTN* | c.58034\_58035delCT | p.T19345fs\*2 | 1/248526 | 53 | PVS1, PM2, PP3 | Likely Pathogenic |
| *TTN* | c.39818delA | p.K22146fs\*28 | 1/248358 | 56 | PVS1, PM2, PP3 | Likely Pathogenic |
| *TTN* | c.86076dupA | p.S28693fs\*2 | 2/247202 | 61 | PVS1, PS4, PP3 | Likely Pathogenic |
| *TTN* | c.107800G>T | p.G35934\* | 1/249118 | 74 | PVS1, PM2, PP3 | Likely Pathogenic |
| **Abbreviations:** ACMG, American College of Medical Genetics and Genomics; CADD, combined annotation dependent depletion; PM, pathogenic moderate; PS, pathogenic strong; PVS, pathogenic very strong; and PP, pathogenic supporting. | | | | | | |