**Title:** **Exome Sequencing Highlights a Potential Role for Concealed Cardiomyopathies in Youthful Sudden Cardiac Death**

**Running Title:** Neves et al. – Concealed Cardiomyopathy and Unexplained SCA/SCD

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**ABSTRACT**

**Background:** Sudden cardiac arrest (SCA) and sudden unexplained death (SUD) are feared sequelae of many genetic heart diseases (GHDs). In rare circumstances, pathogenic variants in cardiomyopathy-susceptibility genes may result in electrical instability leading to SCA/SUD before any structural manifestations of an underlying cardiomyopathy are evident.

**Methods:** Collectively, 38 unexplained SCA survivors (21 males; mean age at SCA 26.4 ± 13.1 years), 68 autopsy-inconclusive SUD cases (49 males; mean age at death 20.4 ± 9.0 years) without disease-causative variants in the channelopathy genes and 973 ostensibly healthy controls were included. Following exome sequencing (ES), ultra-rare (minor allele frequency ≤ 0.00005 in any ethnic group within Genome Aggregation Database [gnomAD, n=141,456 individuals]) nonsynonymous variants identified in 24 ClinGen adjudicated definitive/strong evidence cardiomyopathy-susceptibility genes were analyzed. Eligible variants were adjudicated as pathogenic (P), likely pathogenic (LP), or variant of uncertain significance (VUS) in accordance with current ACMG guidelines.

**Results:** Overall, 7/38 (18.4%) SCA survivors and 14/68 (20.5%) autopsy-inconclusive, channelopathic-negative SUD cases had at least one pathogenic/likely pathogenic (P/LP) or a variant of uncertain significance (VUS) nonsynonymous variant within a strong evidence, cardiomyopathy-susceptibility gene. Following ACMG-criterion variant adjudication, a P or LP variant was identified in 3/38 (7.9%; p=0.05) SCA survivors and 8/68 (11.8%; p=0.0002) autopsy-inconclusive SUD cases compared to 20/973 (2.1%) European controls. Interestingly, the yield of P/LP variants was significantly greater in autopsy-inconclusive SUD cases with documented interstitial fibrosis (4/11, 36%) compared to only 4/57 (7%, p<0.02) SUD cases without ventricular fibrosis.

**Conclusion:** Our data further supports the inclusion of strong evidence cardiomyopathy-susceptibility genes on the genetic testing panels used to evaluate unexplained SCA survivors and autopsy-inconclusive/negative SUD decedents. However, to avoid diagnostic miscues, the careful interpretation of genetic test results in patients without overt phenotypes is vital.

**Keywords:** Concealed cardiomyopathy; Genetic testing; Multi-phenotype genetic testing; Sudden cardiac arrest, Sudden unexplained death

**Abbreviations:**

ACM = arrhythmogenic cardiomyopathy

 ACMG = American College of Medical Genetics and Genomics

 BrS = Brugada syndrome

 CPVT = catecholaminergic polymorphic ventricular tachycardia

 DCM = dilated cardiomyopathy

 HCM = hypertrophic cardiomyopathy

 LQTS = long QT syndrome

 PVC = premature ventricular contraction

 PTVF = PVC-triggered ventricular fibrillaiton

 P/LP = pathogenic/likely pathogenic

 SCA = sudden cardiac arrest

 SCVF = short-coupled ventricular fibrillation

 SUD = sudden unexplained death

 TTNtv = TTN-encoded titin truncating variants

 VUS = variant of uncertain significance

**INTRODUCTION**

Sudden cardiac death (SCD) remains the most common cause of death in the industrialized world. For example, in the United States around 350,000 individuals die suddenly each year 1. Ventricular fibrillation (VF) due to coronary artery disease is the leading cause of sudden cardiac arrest (SCA) or SCD, especially among the elderly 2. Following a comprehensive clinical evaluation, including electrocardiogram (ECG), cardiac imaging, coronary angiography, and pharmacological challenges, a definitive diagnosis occurs in approximately 90% of all SCA survivors.3 The remaining unexplained cases often receive a default designation as idiopathic ventricular fibrillation (IVF)4. These events are relatively infrequent in individuals with a structural and electrically normal heart. In fact, a diagnosis of IVF represents only approximately 1.2% to 6.8% of all out-of-hospital SCA survivors presenting with a shockable rhythm5 or presenting with VF as first rhythm6. Notably, the mean age of these patients is 43 years old7 and 1 out 10 of IVF survivors are ≤ 16 years of age at time of the arrhythmic event8.

Annually, approximately 2,000 to 5,000 people between 1 to 35 years of age die suddenly in the United States 9. For many, a comprehensive investigation including a conventional autopsy examination identifies a clear cause of death. However, gross and microscopic evaluation of the heart and other organs fails to reveal a definite etiology in up to 40% of these sudden death cases which are often categorized as autopsy-negative or autopsy-inconclusive sudden unexplained death (SUD) 10.

 Potentially lethal and heritable cardiac channelopathies like long QT syndrome (LQTS), catecholaminergic polymorphic ventricular tachycardia (CPVT), and Brugada syndrome (BrS), are associated typically with grossly and histologically normal hearts and may account for a significant portion of autopsy-inconclusive SUD and SCA survivors11. In fact, these channelopathies serve as the pathogenic basis in up to 35% of SUD in the young2, 4, 12-14 and up to 54% of pediatric SCA 15.

Additionally, heritable cardiomyopathies, including hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and arrhythmogenic cardiomyopathy (ACM), may present with a mild structural phenotype that could escape detection at the time of either clinical evaluation in the SCA survivor or autopsy in the SCD victim or potentially present in a pre-cardiomyopathic, arrhythmogenic phase that leaves the individual prone to ventricular arrhythmias before the underlying cardiomyopathic substrate is easily detected15-17. Moreover, myocardial fibrosis in the absence of an identifiable cause was observed commonly in a recent post-mortem study of sudden unexplained death in the young (SUDY) decedents.18 Interestingly, pathogenic (P) or likely pathogenic (LP) variants in cardiomyopathy-susceptibility genes, in the absence of pathologic features consistent with HCM, DCM, or ACM, were detected in 27% of SUDY decedents with so-called primary myocardial fibrosis.18

As a result, we sought to determine the prevalence of disease-causative variants (i.e. those adjudicated as either a pathogenic (P) variant or a likely pathogenic (LP) variant according to the 2015 American College of Medical Genetics and Genomics (ACMG) guidelines) in strong evidence cardiomyopathy-susceptibility genes within a referral cohort of unexplained SCA survivors and autopsy-inconclusive/negative SCD cases that have remained genotype-negative following cardiac channelopathy genetic testing.

**METHODS**

The data, analytic methods, and study materials will not be made available to other researchers for purposes of reproducing the results or replicating the procedure in order to protect patient privacy. The data that support the findings of this study are available from the corresponding author upon reasonable request. In this retrospective Institutional Review Board-approved study (IRB 1216-97), the yield of P/LP variants in 24 ClinGen adjudicated definitive/strong evidence cardiomyopathy-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. The list of 24 ClinGen adjudicated definitive/strong evidence ACM, DCM and HCM cardiomyopathic genes and the ClinGen gene classifications are summarized in **Table 1**. 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 19. The ACMG guidelines for the interpretation of sequence variants were used to classify identified variants as P, LP, or variant of uncertain significance (VUS) 20. For the purposes of this manuscript, percent yield refers to the number of variant-positive individuals. Detailed methods are available in the **online supplement**.

**RESULTS**

**Unexplained Sudden Cardiac Arrest Survivor and Sudden Unexplained Death Cohorts**

The demographics for the 38 young SCA survivors and 68 SUDY decedents are summarized in **Tables 2 and 3.** For the 38 SCA survivors,the average age at SCA was 26.4 ± 13.1 years. There were 21 males (55.3%) and 17 females (44.7%). Thirteen (34.2%) had a history of syncope/seizure and 7 (18.4%) had a family history of SCD. The SCA occurred during the following circumstances: rest (29; 76.3%), exertion (8; 21.1%), and nonspecific (1; 2.6%). The average QTc was 422 ± 37 ms and the average left ventricular ejection fraction (LVEF) was 60.8 ± 4.5%. An implantable cardioverter defibrillator (ICD) was implanted in 38 (100%) and 17 (44.7%) have received > 1 appropriate ICD shock.

 For the 68 SUDY cases, the average age at death was 20.4 ± 9.0 years. There were 46 (70.6%) males and 22 (32.4%) females. The activity during their SCD were: rest (21; 30.9%), exertion (13; 19.1%), non-specific (24; 35.3%), and unknown (10; 17.7%). Although the autopsies were deemed as negative or inconclusive by the performing pathologist/medical examiner in all cases, interstitial fibrosis involving either the left ventricle (LV), right ventricle (RV), or both the LV and RV was noted in 11 (16.2%) cases.

**Yield of Pathogenic/Likely Pathogenic Variants and Variants of Uncertain Significance in Cardiomyopathy-Susceptibility Genes**

Overall, 7/38 (18.4%) SCA survivors, 14/68 (20.5%) autopsy-inconclusive SUDY cases, and 116/973 (11.9%) of controls had at least one P/LP or VUS (MAF<0.005% in gnomAD) nonsynonymous variant within a strong-evidence cardiomyopathy-susceptibility gene. TTN missense variants were not included in this study. One (2.6%) of the SCA survivors, three (4.4%) of the autopsy-inconclusive SUDY cases, and six (0.6%) of the controls had multiple nonsynonymous variants. Following ACMG-criterion variant adjudication, a pathogenic (P) or likely pathogenic (LP) variant was identified in 3/38 (7.9%; p.Arg482\*-FLNC, p.Thr15615fs\*4-TTN, and p.Lys23405fs\*8-TTN; p=0.05) SCA survivors and 8/68 (11.8%; p.Phe111fs\*14-BAG3, p.Gln1289\*-DSP, p.Arg270\*-DSP, p.Arg834Trp-MYBPC3, p.Lys184Gln-MYH7, p.Asn634fs\*22-PKP2, p.Asp22167fs\*7-TTN, and p.Arg109\*-TTN) autopsy-inconclusive SUDY cases compared to only 20/973 (2.1%; p=0.0002) European controls (**Figure 1**, **Table 4**, and **Table 5**). However, the VUS rate observed in these 24 genes was not different between the SCA survivors (4/38, 10.5%), the autopsy-inconclusive SUDY cases (7/68, 10.3%), and the controls (104/973, 10.7%). The specific ACMG criteria that were used to determine the P or LP status for each variant are shown in **Supplemental Tables 1, 2, and 3**. Interestingly, the yield of P/LP variants was significantly greater in autopsy-inconclusive SUDY cases with documented LV/RV interstitial fibrosis (4/11, 36.4%) compared to only 4/57 (7%, p=0.02) SUDY cases that were fibrosis negative (**Figure 2**).

Of note, in comparison to controls (n=973), an excess number of truncating variants in TTN-encoded titin (TTNtvs) was observed in the combined young SCA/SCD cohort (n=106) (7/973 [0.7%] vs 4/106 [3.8%], p<0.02). Furthermore, whereas TTNtvs localizing to the A-band region of titin were observed in 2.8% (3/106) of young SCA/SCD cases, the prevalence of A-band localizing TTNtvs was only 0.4% (4/973) in controls (p=0.02; **Table 4** and **Table 5**).

 The clinical and genetic characteristics of the 3 P/LP variant-positive unexplained SCA survivors [2 females and average age at SCA 22 ± 12 years (11-39)] are summarized in **Table 4**. Of note, all three cases had normal cardiac evaluations, including at a minimum a normal electrocardiogram, exercise stress test, and transthoracic echocardiogram, and experienced SCA at rest (**Table 4**).

Interestingly, 2 out of the 3 (67%) unexplained SCA survivors with a P/LP variant in a strong evidence cardiomyopathy-susceptibility genes (cases 1 and 3) were diagnosed with so-called premature ventricular contraction (PVC)-triggered ventricular fibrillation [PTVF; also referred to as short-coupled ventricular fibrillation (SCVF)] based on the observation of PVC-triggered polymorphic VT or VF on ambulatory Holter monitoring (**Figure 3a** and **Figure 3b**) and device electrograms (**Figure 3c** and **Figure 3d**). Following targeted ablation for clinical PVCs originating from the right ventricular outflow tract (p.Arg482\*-FLNC-positive case 1) and the left posterior fascicle (p.Lys23405fs\*8-TTN-positive case 3), both unexplained SCA survivors with presumed PTVF/SCVF have remained cardiac event free.

The clinical and genetic characteristics of the 8 [3 females and average age at the time of death 22 ± 10 years (6-36)] P/LP variant-positive SUD victims are summarized in **Table 5**. Four of the decedents (50%) were found unresponsive in bed, 3 (37.5%) died following an exertional collapse, and 1 (12.5%) was found unresponsive following an unknown circumstance. The toxicology was negative in all cases. The gross and ventricular histopathology was normal in 4 (50%) of the P/LP variant-positive cases. However, mild-to-moderate interstitial fibrosis and myocyte hypertrophy was noted in 4 patients (**Table 5**). Importantly, the presiding medical examiner concluded that each of these autopsies was negative and the findings of interstitial fibrosis was deemed inconclusive.

Cascade genetic testing was pursed for 2 families amongst the 3 SCA survivors. The p.Lys23405fs\*- 8-TTN variant was found by research based next generation sequencing after the patient was lost to follow-up. The p.Thr15615fs\*4-TTN was found to have arisen de novo and the p.Arg482\*-FLNC was found to be paternally inherited. Besides, from frequent PVCs, the p.Arg482\*-FLNC-positive proband’s father, currently in his 40’s, has no evidence of cardiomyopathy. Unfortunately, due the referral nature of our SUD cohort we have limited clinical data available on variant-positive family members.

**DISCUSSION**

According to the recently released 2020 Asia Pacific Heart Rhythm Society (APHRS) and Heart Rhythm Society (HRS) expert consensus guidelines on the investigation of SUD decedents and SCA survivors, genetic evaluation is recommended in those unexplained SCA survivors/SUD decedents with a suspected SCA/SCD-predisposing genetic heart disease phenotype (class I; only genes linked to the phenotype observed), SUD decedents < 40 years of age (i.e. SUDY) or any age when the death circumstance is suspicious for an inherited arrhythmia syndrome (class I; only strong-evidence channelopathy genes) and may be considered in unexplained SCA survivors in certain circumstances (class IIb; only strong-evidence channelopathy/cardiomyopathy genes).21 In regards to unexplained SCA survivors, the latter is a slight departure from the 2013 APHRS/EHRA/HRS expert consensus guidelines12 on the diagnosis and management of inherited arrhythmia syndromes which recommend against the use of large gene panels (APHRS/EHRA/HRS) in unexplained SCA survivors that received an ambiguous diagnosis of IVF following clinical evaluation.

 Initial concern regarding the use of so-called pan-cardiac (i.e. large gene panels with a large number of cardiomyopathy- and channelopathy-susceptibility) in the assessment of SCA survivors and SUD decedents with ambiguous phenotypes is rooted in the high probability of finding ≥ 1 VUS, particularly in **limited** evidence cardiomyopathy- or channelopathy susceptibility genes12, 23. Indeed, the use of pan-cardiac/multi-phenotype genetic testing panels revealed ≥ 1 ambiguous VUS in as many as 55% of unexplained SCA cases24.

However, examples of cardiomyopathy-susceptibility genes resulting in a “concealed” cardiomyopathy phenotype that can cause electrical instability/ventricular arrhythmia susceptibility prior to the development of overt cardiomyopathic structural changes detectable by commonly employed imaging modalities (i.e. echocardiography and cardiac MRI) exist17, 24-26. During the genetic evaluation of unexplained SCA survivors in the CASPER registry, P/LP variants in cardiomyopathy-susceptibility genes were identified in 5 out of 102 (4.9%) SCA survivors who remained classified as unexplained or idiopathic (i.e. structurally normal hearts) despite the rigorous, protocolized approach to clinical evaluation employed in the CASPER registry.24

Interestingly, in a long-term follow-up of patients with a default diagnosis of IVF, Visser and colleagues demonstrated that a diagnosis was eventually rendered in 22 out of 107 (21%), including 11/22 (50%) patients found to have an underlying cardiomyopathy (ACM = 5, DCM = 3 and HCM =3).25 This observation lends suggests that an arrhythmogenic event in the absence of imaging-detected structural abnormalities can be the first presentation in some SCA survivors with an underlying genetic cardiomyopathy, ACM in particular.25 This concept is perhaps best illustrated by *PKP2*-mediated ACM. In a recent study by our group, 2-5% of patients diagnosed clinically with CPVT or who suffered an exercise-associated autopsy-negative SUDY hosted P/LP variants in *PKP2*-encoded plakophilin-2.17 Although the *PKP2* P/LP variant-positive unexplained SCA survivors in this study lacked the necessary imaging and electrocardiographic findings to fulfill ARVC task force criteria, they all displayed some degree of ventricular ectopy at rest with more complex ventricular ectopy patterns (i.e. bigeminy), consistent with CPVT, emerging during stress.17 As resting ectopy is rare in CPVT, it seems more likely that *PKP2* is capable of generating an “concealed” pre-cardiomyopathic electrical state that, at least for a period time, is capable of predisposing to SCA/SCD and generating a convincing CPVT phenocopy in some cases.17

Of note, this phenomenon does not appear to be limited to *PKP2*. In the recent study by Isbister et al, a P/LP variant in cardiomyopathy-susceptibility gene was discovered in 7/36 (19%) of unexplained SCA survivors that underwent extended genetic testing as part of their evaluation within a specialized genetic heart disease clinic in Sydney, Australia.26 In addition to *PKP2* (2 patients: c.2489+1G>A-PKP2; p.Leu744Serfs\*3-PKP2), P/LP variants were also found in *MYBPC3 (*p.Glu828Serfs\*2-MYBPC3), *DES* (p.Arg454Trp-DES), *MYH7 (*p.Gly425Arg-MYH7), *ACTN2* (p.Ala119Thr-ACTN2), and *DSP* (p.Tyr2547\*-DSP).26

However, in comparison to the current study (3/38; 7.9%) and other prior unexplained SCA studies that examined yield of genetic testing including, most notably the CASPER registry study by Mellor et al (5/102; 4.9%)24, the impressive yield of P/LP variants in cardiomyopathy-susceptibility genes in the study by Isbister et al (7/36; 19%)26 appears to be an outlier. Ultimately, we suspect the differences in P/LP variant yield across these studies is multifactorial and potentially related to i) demographic differences (e.g. the average age in the study by Isbister et al26 was 36.9 ± 16.9 years compared to 26.4 ± 13.1 years in the current study), ii) utilization rates of cardiac MRI and other advanced diagnostics (standardized in the CASPER registry study by Mellor et al24, but highly variable in Isbister et al26 and the current study), and iii) the reflection of a referral bias inherent to the presence of specialized Genetic Heart Disease clinics at the University of Sydney and Mayo Clinic.

Similarly, prior molecular autopsy studies have also demonstrated that P/LP variants in cardiomyopathy-susceptibility genes can appear in autopsy-negative or autopsy-inconclusive decedents. Most notably, the recent study by Lahrouchi et al identified a P/LP variant in a cardiomyopathy-susceptibility gene in 6/268 (2.2%) decedents following the exclusion of individuals with P/LP variants in channelopathy-susceptibility genes.16 Moreover, Lahrouchi et al found 1/28 (3%) sudden death victims with a LP variant in cardiomyopathy-susceptibility genes and also had a non-specific findings (idiopathic fibrosis) at the autopsy27. Interestingly, a similar percentage [22/973 (2.3%)] of controls in the current study hosted a P/LP variant in a cardiomyopathy-susceptibility gene. However, unlike the study by Lahrouchi et al, in the current study the burden of P/LP variants in cardiomyopathy-susceptibility genes was significantly higher in SUDS decedents than in controls [8/68 (11.8%) vs 22/973 (2.3%); p= p=0.0004].

In both the current study and the aforementioned autopsy-inconclusive SUDY study by Lahrouchi et al, the initial gross and histological cardiac autopsy was performed locally. However, unlike the current study, the author-adjudicated autopsy findings in the study by Lahrouchi et al were over-read/confirmed by a single expert cardiac pathologist. In theory, the re-classification of author-adjudicated findings in favor of an underlying cardiomyopathy and the anticipated reduction in inter-observer variability associated this extra step could, at least in part, underlie the higher yield of P/LP variants in cardiomyopathy-susceptibility genes observed in the current study.

Interestingly, 36% of our inconclusive-autopsy SUD cases with presumed idiopathic ventricular fibrosis were positive for a P/LP variant in a cardiomyopathy-susceptibility gene compared to only 7% of those cases without this autopsy finding. This finding is consistent with the recent post-mortem study by Junttila et al that identified cardiomyopathy-susceptibility gene variants in 26/96 (27%) SUD decedents with so-called primary myocardial fibrosis as the only pathologic finding identified during autopsy18. Collectively, these data suggest that genetic testing for P/LP variants in strong-evidence cardiomyopathy-susceptibility genes should be considered in SUD cases with ventricular interstitial fibrosis on autopsy.

In this context, it is noteworthy that P/LP A-band-localizing TTNtvs were over-represented in our combined young SCA/SCD cohort [3/106 (2.8%) SCA/SCD cases versus 4/973 (0.4%) controls; p=0.02]. Of note, TTNtvs represent the most common cause of genetic/familial DCM accounting for ~20-25% of cases28 29 . However, the background rate of TTNtvs in controls and public exomes/genomes is ~2-3%, suggesting that the penetrance of TTNtvs may be relatively low and the expressivity highly variable28 29. Nevertheless, Corden and colleagues identified that TTNtv-positive DCM patients are at greater risk of receiving an appropriate ICD therapy as well as the development of persistent atrial fibrillation (AF) than those without30. Moreover, Akhtar et al. cohort showed one-third of patients that are TTNtvs positive developed atrial arrhythmias and one-half developed non-sustained ventricular tachycardia (NSVT)31. Importantly, around 10% of TTNtvs positive patients with preserved left ventricular ejection fraction (LVEF) developed AF or NSVT31.

Despite the statistical over-representation of TTNtvs in variety of clinical settings (DCM, secondary cardiomyopathies, and now unexplained SCA/SCD), caution must still be exercised when interpreting the clinical significance of TTNtvs in SCA/SUDY cases32. Given that the penetrance of TTNtvs appears to be relatively low, suggesting a critical role for environmental and genetic modifiers, the potential for diagnostic miscues in patients with ambiguous or non-DCM/cardiomyopathy phenotypes appears to be high.

 However, the over-representation of TTNtvs as well as the pathology/clinical phenotype (interstitial fibrosis, PTVF/SCVF phenotype, etc.) observed in TTNtv-positive individuals/decedents in the current study does suggest that TTNvs are capable of generating a potentially lethal pro-arrhythmic substrate before overt structural changes are readily appreciable by imaging or gross pathology assessment. Ultimately, large, multicenter registries of TTNtv-positive individuals/families and individuals with unexplained SCA, particularly those with a PTVF/SCVF endophenotype, are needed to understand the mechanism(s) driving the over-representation of P/LP variants in strong evidence cardiomyopathy-susceptibility genes observed in this and other unexplained SCA/SUDY studies33.

The identification of a causative pathogenic variant in an individual that has suffered an unexplained SCA or SUD can help determine, or provide additional evidence, regarding the root cause for the SCA/SUD, inform the clinical management of SCA survivors, and guide family screening to assure at-risk relatives are identified and appropriately treated. The majority of current expert consensus guidelines/statements support the utility of genetic testing in those unexplained SCA survivors/SUD decedents with a suspected SCA/SCD-predisposing genetic heart disease phenotype21 with the assessment of strong-evidence channelopathy genes receiving the strongest support.12, 21, 22 However, within our combined single center referral cohort of unexplained SCA survivors and autopsy-inconclusive SUD decedents, we demonstrate that the collective burden of P/LP variants in strong evidence cardiomyopathy-susceptibility genes is 10% (11/106). In other words, 1 in 10 unexplained SCA survivors/autopsy-inconclusive SUD decedents may have an underlying genetic cardiomyopathy in a subclinical or concealed pre-cardiomyopathic arrhythmogenic state, the inclusion of strong-evidence cardiomyopathy-susceptibility genes in the genetic evaluation of these patient populations merits serious re-consideration. However, due to the high likelihood of finding an ambiguous VUS24 with this approach as well as the resulting downstream risk of diagnostic and management miscues, such an approach is best pursued in the context of dedicated cardiovascular genetic clinics with the resources and expertise to carefully interpret genetic testing results obtained in individuals without clear evidence of an underlying clinical phenotype.

**LIMITATIONS**

As a referral cohort, the clinical evaluation of some SCA survivors may have been incomplete. For example, those unexplained SCA survivors who received an ICD prior to their evaluation at our institution often did not receive a cardiac MRI. Given cardiac MRI’s role in identifying more subtle findings (i.e. late gadolinium enhancement) suggestive of an underlying cardiomyopathy, it is possible, at least in some cases, that evidence that would have led to increased clinical suspicion for ACM, DCM or HCM was not available. In addition, all SCA survivors and SUDY decedents self-reported or were reported by referring medical examiners/pathologists as being of European descent. However, a principle component analysis was not performed and in theory could complicate comparison to the European control cohort. Moreover, an autopsy performed by a board certified cardiovascular pathologist was not required for enrollment in our research-based SUDY registry. As a result, the gross and microscopic pathology data contained within this study was sourced from available autopsy and pathology reports provided by each referring medical examiner. Therefore, the heterogeneity and inter-individual variability of the pathologist represent a limitation on this study.

 Lastly, the use of pan-arrhythmia, pan-cardiac, and exome-based genetic testing is discouraged because of the significantly increased possibility of finding VUS in disputed/refuted/limited evidence channelopathy- or cardiomyopathy-susceptibility genes12, 23. Therefore, we only included the 24 **strong evidence** cardiomyopathy-susceptibility genes in this study. Additionally, only ACMG guideline criteria specified P and LP variants were considered. None of the ultra-rare VUS identified even in the strong evidence genes were included in the calculated yields. Our conservative approach could have caused us to under-estimate the true prevalence of SCA/SUD-predisposing variants as some of the VUS may be upgraded to LP variants in the future.

**CONCLUSION**

The ability to establish a definitive diagnosis after a SCA/SUD provides important benefits to the SCA survivor and to the surviving family. However, interpretation of genetic results must be performed cautiously, especially in the absence of an overt phenotype. That said, our data from a combined unexplained SCA and autopsy-inconclusive SUD cohort suggest that strong evidence cardiomyopathy-susceptibility genes merit consideration in the genetic evaluation of SCA survivors and SCD decedents with either ambiguous or negative clinical phenotypes.

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**SUPPLEMENTAL MATERIALS:**

Supplemental Methods and Data

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**FIGURE LEGENDS**

**Figure 1 | Yield of ACMG Guideline-Designated Pathogenic/Likely Pathogenic Variants Identified in Sudden Cardiac Arrest Survivors and Sudden Unexplained Death Victims-** Shown is a bar graph indicating the percent yield of “ultra-rare” (i.e. minor allele frequency < 0.005%) nonsynonymous variants and ACMG guideline predicated “pathogenic” (P) / “likely pathogenic” (LP) variants detected among 24 strong evidence cardiac cardiomyopathy-susceptibility genes for our European controls, sudden cardiac arrest (SCA) survivors, and autopsy-inconclusive sudden unexplained death (SUD) victims. \*For the purposes of this manuscript, percent yield refers to the number of variant-positive individuals.

**Figure 2 | Effect of Left / Right Ventricular Interstitial Fibrosis Observed at Autopsy on the Yield of ACMG Guideline-Designated Pathogenic/Likely Pathogenic Variants Identified in Autopsy-inconclusive Sudden Unexplained Death Victims -** Shown is a bar graph indicating the percent yield of “ultra-rare” (i.e. minor allele frequency < 0.005%) nonsynonymous variants and ACMG guideline predicated “pathogenic” (P) / “likely pathogenic” (LP) variants detected among 24 strong evidence cardiac cardiomyopathy-susceptibility genes for our autopsy-inconclusive sudden unexplained death (SUD) victims with (positive) or without (negative) for left ventricular (LV)/right ventricular (RV) interstitial fibrosis. \*For the purposes of this manuscript, percent yield refers to the number of variant-positive individuals.

**Figure 3** | Electrocardiographic evidence of underlying premature ventricular contraction (PVC)-triggered ventricular tachycardia/ventricular fibrillation in unexplained sudden cardiac arrest survivors with likely pathogenic variants in strong evidence cardiomyopathy-susceptibility genes. a) 12-lead ambulatory Holter monitor tracing displaying probable right ventricular outflow tract localizing PVC-triggered non-sustained ventricular tachycardia in a 11-year-old p.Arg482\*-FLNC-positive unexplained SCA survivor. b) 3-lead ambulatory Holter monitor tracing displaying probable left posterior fascicle-localizing PVC-triggered non-sustained ventricular tachycardia in a p.Lys23405fs\*8-TTN-positive unexplained SCA survivor. c) Device electrocardiogram displaying spontaneously terminating polymorphic ventricular tachycardia in a 11-year-old p.Arg482\*-FLNC-positive unexplained SCA survivor. d) Device electrocardiogram displaying the appropriate termination of PVC-triggered polymorphic ventricular tachycardia/ventricular fibrillation by an implantable cardioverter defibrillator shock.

**TABLES**

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| **Table 1 |** Strong Evidence Cardiomyopathy Genes |
| **Gene** | **Gene-Encoded Protein** | **RefSeqTranscript**  | **ClinGen ACM** | **ClinGen DCM** | **ClinGen HCM** |
| *ACTC1* | actin alpha cardiac muscle 1 | NM\_005159.4 |  |  | Definitive |
| *ALPK3* | alpha kinase 3 | NM\_020778.5 |  |  | Strong |
| *BAG3* | BAG cochaperone 3 | NM\_004281.3 |  | Definitive |  |
| *DES* | desmin | NM\_001927.3 |  | Definitive |  |
| *DSC2* | desmocollin 2 | NM\_024422.4 | Definitive |  |  |
| *DSG2* | desmoglein 2 | NM\_001943.3 | Definitive |  |  |
| *DSP* | desmoplakin | NM\_004415.2 | Definitive | Strong |  |
| *FLNC* | filamin C | NM\_001458.4 |  | Definitive | Definitive |
| *JUP* | Junctiojn plakoglobin | NM\_002230.2 | Definitive |  |  |
| *LMNA* | Lamin A/C | NM\_170707.3 |  | Definitive |  |
| *MYBPC3* | myosin-binding protein C | NM\_000256.3 |  |  | Definitive |
| *MYH7* | myosin heavy chain 7 | NM\_000257.3 |  | Definitive | Definitive |
| *MYL2* | myosin light chain 2 | NM\_000432.3 |  |  | Definitive |
| *MYL3* | myosin light chain 3 | NM\_000258.2 |  |  | Definitive |
| *PKP2* | Plakophilin 2 | NM\_004572.3 | Definitive |  |  |
| *PLN* | phospholamban | NM\_002667.3 |  |  | Definitive |
| *PRKAG2* | protein kinase AMP-activated non-catalytic subunit gamma 2 | NM\_016203.3 |  |  | Definitive |
| *RBM20* | RNA binding motif protein 20 | NM\_001134363.3 |  | Definitive |  |
| *TMEM43* | transmembrane protein 43 | NM\_024334.3 | Definitive |  |  |
| *TNNC1* | troponin C1 | NM\_003280.4 |  | Definitive |  |
| *TNNI3* | troponin I3 | NM\_000363.4 |  |  | Definitive |
| *TNNT2* | troponin T2 | NM\_001001430.2 |  | Definitive | Definitive |
| *TPM1* | tropomyosin 1 | NM\_001018005.1 |  |  | Definitive |
| *TTN\** | titin | NM\_001267550.2 |  | Definitive |  |

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| **Table 2 |** Cohort Demographics for Sudden Cardiac Arrest (SCA) Survivors |
| **Demographics** | **SCA Cohort (n=38)** |
|  Female; n (%) | 17 (45) |
|  Average age at SCA (years) | 26.4 ± 13.1 |
|  Age range (years) | 1 to 56 |
|  European descent\*; n (%) | 33 (87) |
|  Recurrent cardiac events; n (%) | 21 (55) |
|  History of syncope/seizure; n (%) | 13 (34) |
|  Family history of SCD; n (%) | 7 (18) |
| **Device therapy** |  |
|  ICD implanted; n (%) | 38 (100) |
|  Appropriate ICD shocks; n (%) | 17 (45) |
| **Activity at SCA**  |  |
|  Rest; n (%) | 29 (76) |
|  Exertion; n (%) | 8 (21) |
|  N/A; n (%) | 1 (2.6) |
| **Electrocardiographic findings** |  |
|  Average QTc (ms) | 422 + 37 |
|  QTc range (ms) | 367 to 549 |
|  Documented VF; n (%) | 38 (100) |
| **Echocardiographic findings** |  |
|  Average LVEF (%) | 60.8±4.5 |
|  LVEF Range (%) | 45 to 68 |
| **Additional work-up** |  |
|  Coronary Angiography (Invasive or CT); n (%) | 28 (74) |
|  Cardiac MRI; n (%) | 30 (79) |
|  EP study; n (%) | 26 (68) |
|  Drug provocation; n (%) | 21 (55) |
| \*Represents self-reported race/ethnicity.**Abbreviations:** CT, computed tomography; EP, electrophysiological; MRI, magnetic resonance imaging; LVEF, left ventricular ejection fraction; ICD, implantable cardioverter defibrillator; QTc, heart rate-corrected QT interval; SCA, sudden cardiac arrest; SCD, sudden cardiac death; and VF, ventricular fibrillation. |

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| **Table 3 |** Cohort Demographics for Autopsy inconclusive Sudden Unexplained Death Victims |
| **Demographics** | **SUD Cohort (n=68)** |
|  Female; n (%) | 22 (32) |
|  Average age at SCD (years) | 20.4 ± 9.0 |
|  European descent\*, n (%) | 55 (81 ) |
| **Activity at SCD** |  |
|  Rest; n (%) | 21 (31) |
|  Exertion; n (%) | 13 (19) |
|  Non-specific; n (%) | 24 (35) |
|  Unknown; n (%) | 10 (18) |
| **Autopsy findings** |  |
|  LV/RV interstitial fibrosis; n (%) | 11 (16) |
| \*Represents self-reported race/ethnicity.**Abbreviations:** LV, left ventricle; RV, right ventricle; and SCD, sudden cardiac death.  |

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| **Table 4 |** Clinical, Electrocardiographic, Imaging, and Genetic Findings in Unexplained Sudden Cardiac Arrest Survivors |
| **Demographic** | **Electrocardiographic** | **Holter** | **Echocardiogram** | **MRI** | **EP Study** | **Coronary Angiography (Invasive or CT)** | **ACMG P/LP Variant(s)** |
| Case | Age at SCA | Sex | HR | QTc(ms) | ECG Stress | VE/HR | NSVT | LVEF(%) | LVEDD(mm) |  | HV (ms) | VT/VF |  |  |
| 1 | 11 | M | 57 | 426 | Neg | 227.3 | Yes | 63 | 49 | N/A | 43 | Yes | Normal coronaries | p.Arg482\*-FLNC (LP) |
| 2 | 16 | F | 80 | 443 | Neg | 0.2 | No | 62 | 44 | Neg | 43 | No | N/A | p.Thr15615fs\*4-TTN (LP) |
| 3 | 39 | F | 84 | 468 | Neg | 28.4 | Yes | 55 | 46 | N/A | 75 | Yes | Normal coronaries | p.Lys23405fs\*8-TTN (LP) |
| **Abbreviations:** ECG, electrocardiogram; HR, heart rate; LP, likely pathogenic; LV, left ventricle; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end diastolic dimension; N/A, not available; Neg, negative; NSVT, non-sustained ventricular tachycardia; P, pathogenic; QTc, heart rate-corrected QT interval; SCA, sudden cardiac arrest; VE, ventricular ectopics; VF, ventricular fibrillation; and VT, ventricular tachycardia.  |

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| **Table 5 |** Gross and Histopathologic Findings in the Hearts of Sudden Unexplained Death Victims with Pathogenic/Likely Pathogenic Variants in Cardiomyopathy-Susceptibility Genes.  |
| **Case** | **Age****at Death** | **Sex** | **Death Scene** | **Toxicology** | **Heart Weight****(g)** | **Gross Pathology** | **Ventricular****Histopathology** | **Medical Examiner Conclusion** | **ACMG P/LP Variant(s)** |
| 1 | 25 | F | Exertional collapse | Negative | 411 | Mild LV dilation | RV fibrofatty infiltrate | Autopsy-negative | p.Phe111fs\*14-BAG3 (LP) |
| 2 | 29 | M | Found unresponsive | Negative | 370 | Normal | Subendocardial fibrosis | Autopsy-negative | p.Gln1289\*-DSP (P) |
| 3 | 6 | F | Unresponsive in bed | Negative | 137 | Normal | Normal | Autopsy-negative | p.Arg270\*-DSP (LP) |
| 4 | 12 | M | Exertional collapse | Negative | N/A | Normal | Normal | Autopsy-negative | p.Arg834Trp-MYBPC3 (LP) |
| 5 | 21 | M | Unresponsive in bed | Negative | 365 | Normal | Normal | Autopsy-negative | p.Lys184Gln-MYH7 (LP ) |
| 6 | 16 | M | Exertional collapse | Negative | 490 | Normal | Normal | Autopsy-negative | p.Asn634fs\*22-PKP2 (P) |
| 7 | 36 | F | Unresponsive in bed | Negative | 350 | Normal | Mild-to-moderate interstitial fibrosis; myocyte hypertrophy | Autopsy-negative | p.Asp22167fs\*7-TTN (LP) |
| 8 | 32 | M | Unresponsive in bed | Negative | 400 | Myxomatous MV leaflets; biventricular dilation | LV focal fibrofatty infiltration; myocyte hypertrophy | Arrhythmia due to mitral valve prolapse | p.Arg109\*-TTN (LP) |
| **Abbreviations:** ACMG, American College of Medical Genetics; LP, likely pathogenic; LV, left ventricle; MV, mitral valve; P, pathogenic; and RV, right ventricle |