Sudden Cardiac Death with Autopsy Findings of Uncertain Significance: Potential for Erroneous Interpretation

Running title: Papadakis et al.; Autopsy Findings of Uncertain Significance in SCD

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Abstract:

**Background** - The sudden death of young individuals is commonly attributed to inherited cardiac disorders and familial evaluation is advocated. The identification of pathognomonic histopathological findings, or the absence of cardiac pathology (Sudden Arrhythmic Death Syndrome; SADS) at post-mortem, directs familial evaluation targeting structural disorders or primary arrhythmogenic syndromes, respectively. In a proportion of autopsies, structural abnormalities of uncertain significance are reported. We explored the hypothesis that such sudden cardiac deaths (SCD) represent SADS.

**Methods and Results** - Families (n=340) of index cases of SCD who underwent post-mortem evaluation were evaluated in specialist cardiogenetics clinics. Families in whom the deceased exhibited structural abnormalities of uncertain significance (n=41) such as ventricular hypertrophy, myocardial fibrosis and minor coronary artery disease were included in the study. Results were compared to 163 families with normal post-mortem (SADS). Relatives underwent comprehensive cardiac evaluation. Twenty-one families (51%) with autopsy findings of uncertain significance received a diagnosis based on the identification of an inherited cardiac condition phenotype in ≥1 relatives: 14 Brugada syndrome; 4 long-QT syndrome; 1 catecholaminergic polymorphic ventricular tachycardia; 2 cardiomyopathy. A similar proportion of families (47.2%) received a diagnosis in the SADS cohort (p=0.727). An arrhythmogenic syndrome was the predominant diagnosis in both cohorts (46% versus 45%, p=0.863).

**Conclusions** - Familial evaluation following SCD with autopsy findings of uncertain significance identified a similar proportion of primary arrhythmogenic syndromes to a contemporary series of SADS. Our study highlights the need for accurate interpretation of autopsy findings to avoid erroneous diagnoses, with potentially devastating implications.

**Key words:** sudden cardiac death, pathology, arrhythmia (heart rhythm disorders), cardiomyopathy, ion channel
Introduction

The majority of sudden cardiac deaths (SCD) are attributable to atherosclerotic coronary artery disease and manifest in the older population, whereas cardiomyopathies predominate in the young (<35 years). In a proportion of sudden cardiac deaths (SCDs), a cardiac abnormality cannot be identified despite detailed histopathological examination and toxicology screen; such cases are classified as sudden arrhythmic death syndrome (SADS). The recognition of SADS is imperative, since evaluation of blood relatives of the deceased identifies a hereditary arrhythmogenic syndrome in almost 50% of families, thereby providing a likely cause of death and identifying surviving relatives at risk from the same fate.

The interpretation of the results of post-mortem evaluation of SCD cases is a complex task and uncertainty may exist regarding the causal relationship between the pathological findings and the sudden death. The significance of myxoid degeneration of the mitral valve with prolapse, stable atherosclerotic coronary plaque with limited (<50%) luminal stenosis and focal myocarditis, which are relatively prevalent in the general population, may be erroneously overestimated. Not infrequently, post-mortem diagnoses of hypertrophic cardiomyopathy (HCM) and arrhythmogenic right ventricular cardiomyopathy (ARVC) are based solely on the presence of left ventricular hypertrophy (LVH) and fatty infiltration of the right ventricular wall, respectively, in the absence of pathognomonic histological changes. Left ventricular hypertrophy however, is a recognized feature of physiological adaptation to exercise and fatty infiltration of the right ventricle is commonly present in obese individuals. The distinction between pathology and normal variants may therefore be challenging in the context of SCD.

This study explored the hypothesis that a proportion of SCDs with autopsy findings of uncertain significance may represent part of the SADS spectrum, and in particular inherited
arrhythmogenic syndromes.

Methods

Setting

The SCD of several young individuals prompted the United Kingdom (UK) government to commission the 8th chapter of the National Service Framework for heart disease, aimed at facilitating early identification of individuals at risk of SCD. St George’s Hospital and University Hospital Lewisham (London, UK) have implemented dedicated inherited cardiac diseases clinics, serving relatives of individuals who experienced SCD, from throughout the UK. Family members undergo comprehensive cardiac evaluation aimed at identifying those at risk and prevent further tragedies.

Study Cohort

Between 2003 and 2009, 368 families of cases of premature SCD (aged between 4 and 64 years), were evaluated in our inherited cardiac diseases clinics. Criteria for inclusion in the study comprised of: 1. Unexpected death of an apparently healthy individual; 2. Death from natural causes; 3. Last seen alive and well within 12 hours; 4. Complete post-mortem report; 5. The absence of an extra-cardiac cause of death; and 6. Negative toxicology screen. Twenty-eight families were excluded from further analysis based on the absence of a complete post-mortem report (n=4), positive toxicology (n=14) and the presence of documented past medical history prior to death (n=10).

Post-mortem reports of the 340 SCD cases were scrutinized by two authors and divided into three groups: Group 1: Autopsy findings highly suggestive of structural cardiac pathology accounting for the SCD (n=136); Group 2: No identifiable structural cardiac pathology, consistent with a SADS death (n=163); and Group 3: Autopsy findings with structural
abnormalities of uncertain causal effect (n=41). In cases of disagreement a third, senior author was consulted. The main study cohort consisted of 41 families, comprising 157 blood relatives, where the post-mortem report was classified into group 3. The 163 families in group 2 (SADS cohort) were used as controls for comparison (Figure 1).

**Autopsy Evaluation**

All cases of SCD included in the study had undergone a full coroners’ pathologist post-mortem and in 39% of cases a specialist cardiac pathologist had performed additional assessment. The diagnostic criteria for specific structural cardiac diseases and examples of autopsy findings of uncertain significance are outlined in table 1.

**Familial Cardiological Evaluation**

All relatives underwent comprehensive cardiac evaluation according to a previously published protocol. Baseline ECG, echocardiography, holter monitoring and exercise tolerance testing were conducted routinely. Ajmaline provocation testing to identify the type-1 Brugada phenotype was performed in the event of normal ECG recordings and echocardiograms or in the presence of type-2 or type-3 Brugada ECG patterns. Ajmaline testing was conducted by placing leads V1 and V2 in the conventional 4th intercostal space as well as the higher 3rd and 2nd intercostal spaces. Ajmaline testing was not performed in relatives ≤16 years of age (n=28) who did not have sinister cardiac symptoms or in patients (n=7) who refused consent.

Cardiac magnetic resonance imaging (CMR) with gadolinium was performed in all relatives with ECG or echocardiographic features suggestive of cardiomyopathy. All relatives diagnosed with an arrhythmogenic syndrome where the deceased’s post-mortem findings could be interpreted to represent a cardiomyopathy (LVH, myocardial fibrosis, ventricular dilatation and fatty infiltration of the myocardium) also underwent CMR. Further investigations were based
on clinical need.

**Genetic Testing**

Mutation analysis was offered to all relatives with phenotypic abnormalities suggestive of inherited arrhythmogenic syndromes or cardiomyopathies, after appropriate counselling. Following consent, targeted mutation analysis was performed in one phenotypically affected member of each family, dependent upon the suspected clinical condition: KCNQ1, KCNH2, SCN5A, KCNE1 and KCNE2 in long QT syndrome (LQTS); SCN5A in Brugada syndrome (BrS); selected exons (7–9, 13–16, 43–50, 82–84, and 87–105) of RYR2 in catecholaminergic polymorphic ventricular tachycardia (CPVT). Exons and flanking intronic regions were amplified from genomic deoxyribonucleic acid (DNA), and bi-directionally sequenced to identify coding variants. Variants were labelled as pathogenic if they were previously reported to be associated with disease-susceptibility; in-frame or frameshift-causing insertions or deletions; affecting splice sites; missense mutations likely to be pathogenic, as identified by two in-silico models [affect protein function by a tolerance index score of <0.05 in sorting intolerant from tolerant (SIFT) and classified “probably damaging” by polymorphism phenotyping (PolyPhen)]. If a pathogenic mutation was identified in a phenotypically affected member, other family members were offered cascade screening. Segregation analysis was utilized to confirm mutation pathogenicity.

**Aetiology of Sudden Cardiac Death**

An inherited condition was deemed the most likely cause of SCD if ≥ 1 blood relatives of the deceased exhibited phenotypic evidence of the disease. Standard criteria for the diagnosis of LQTS were utilized. The second consensus criteria for the diagnosis of BrS were employed. Only the presence of the type-1 Brugada pattern (coved ST-segment elevation ≥ 2mm followed
by a negative T-wave) in > 1 right pre-cordial leads, including higher intercostal leads,\textsuperscript{7} was considered diagnostic. Standard criteria were applied for the diagnosis of CPVT.\textsuperscript{15}

Cardiomyopathies were diagnosed based on published diagnostic criteria.\textsuperscript{16}

**Statistical Analysis**

Data analysis was undertaken using R v2.15.2 (R Development Core Team). Data are expressed in means and standard deviations. Comparison of population proportions utilized Fisher’s exact test with Donner’s adjustment as necessary to account for clustered data.

**Results**

**Characteristics of Cases of SCD and Blood Relatives**

The characteristics of the cases of SCD are depicted in table 2. Of the 157 blood relatives evaluated 48\% were male, with a mean age of 33.7±17.9 years, (range 9-70 years). Almost a quarter (23\%) of the evaluated relatives reported cardiac symptoms with 10\% having experienced at least one episode of syncope in the past.

**Autopsy Findings and Results of Familial Evaluation**

The post-mortem findings of uncertain aetiological significance in the 41 cases are illustrated in figure 2. Following familial evaluation, 21 (51\%) out of the 41 SCD cases were considered to have died from a definite or probable inherited cardiac disorder (Figure 1). Of the 157 relatives who underwent cardiac evaluation, 36 (23\%) were diagnosed with a cardiac condition, which had not been previously identified.

**Diagnosis of Arrhythmogenic Syndromes**

A hereditary arrhythmogenic syndrome was diagnosed in 19 of 21 families in whom an underlying inherited cardiac condition was identified. Brugada syndrome (n=14) was the predominant diagnosis, followed by LQTS (n=4) and a single case of CPVT.
Following familial evaluation, an arrhythmogenic syndrome was detected in 42% (11/26) of cases where the autopsy findings were suggestive of a possible cardiomyopathy (LVH, myocardial fibrosis, ventricular dilatation, myocardial fatty infiltration), (Figure 2). In these cases, all relatives with an arrhythmogenic syndrome phenotype underwent CMR scans, in addition to standard evaluation, to exclude co-existent myocardial disease. All CMR scans were reported as normal. Of interest, in the 19 SCD cases where LVH or myocardial fibrosis was reported at post-mortem, (isolated LVH: n=10; myocardial fibrosis alone: n=6 or in conjunction with LVH: n=3) evaluation of family relatives identified an arrhythmogenic syndrome in almost 50% of families (5 out of 10 cases with isolated LVH and 4 out of 9 cases with myocardial fibrosis). A cardiomyopathy was diagnosed in only one case in either group. In the remaining 8 (42%) cases we were unable to identify any features of inherited cardiac pathology.

Brugada syndrome was also diagnosed in one of the three families whose proband exhibited isolated fatty infiltration of the right ventricle (Figure 3.4). One of the families where the pathologist reported marked right ventricular dilatation was subsequently diagnosed with CPVT, based on the identification of typical bi-directional ventricular tachycardia on exercise testing in two relatives.

Moreover, 2 out of the 6 families whose probands exhibited atheromatous disease at post-mortem were diagnosed with BrS. Both probands were young, aged 28 and 34 years-old respectively, and exhibited up to 50% coronary artery lesions in the left anterior descending and right coronary arteries (Figure 3.1). In one of the two families where an inflammatory infiltrate commonly attributed to myocarditis was present, BrS was diagnosed during Ajmaline provocation testing in the deceased’s father. In similar fashion, one of the three families whose proband exhibited pathological features of mitral valve prolapse was subsequently diagnosed.
with BrS based on the presence of type-1 Brugada ECG in two relatives.

**Diagnosis of Cardiomyopathy**

Only 2 families were diagnosed with an inherited cardiomyopathy; 1 dilated cardiomyopathy (DCM); and 1 HCM. The first case was of a 17-year-old boy who died in his sleep. The post-mortem revealed circumferential subendocardial haemorrhage with extensive myocardial fibrosis of the left ventricle. Evaluation of his relatives revealed a dilated, globally hypokinetic left ventricle in his mother and one of his sisters. The second case was of a 20-year-old male who died at rest. The post-mortem revealed a heavy heart (>500g) with LVH but no evidence of myocardial fibrosis or myocyte disarray. There was no history of hypertension or regular exercise. Familial evaluation revealed asymmetric septal hypertrophy in the context of a non-dilated left ventricular cavity in his father, raising suspicion of HCM. Unfortunately the father declined further investigations.

**Mutation Analysis**

We had the opportunity to undertake mutation analysis in relatives with phenotypes suggestive of inherited cardiac conditions in 17 out of the potential 21 families. In 2 families (1:HCM, 1:BrS), individuals declined genetic testing after counselling. In 2 LQTS families genetic testing was performed by their local geneticist. Due to the absence of co-existing atrio-ventricular block, mutation analysis was not performed in the DCM family.\(^8\) Of the 13 families with BrS who underwent genetic testing, three carried pathogenic SCN5A mutations (R376H, H558fs, A1680T). Pathogenic mutations were also identified in the two LQTS families tested (E1784K and G840R in SCN5A) and in the CPVT family (A4556T in RYR2). Four of the identified mutations are previously reported as disease-associated (SCN5A R376H, A1680T, E1784K and RYR2 A4556T).\(^9,10\) One novel SCN5A mutation (H558fs) is a deletion resulting in a frame-shift,
while the other (G840R) is a missense mutation with in-silico confirmation of disease-causation.11,12 A detailed description of the 6 families with a positive genotype is tabulated in the supplemental table provided on-line.

Immediate Management

All relatives affected received appropriate life style modification and drug avoidance advice. Eleven patients were prescribed beta-blockers and two angiotensin converting enzyme inhibitors. Prophylactic cardioverter defibrillators were implanted in five patients: 3 BrS; 2 LQTS and two LQTS patients received a pacemaker.

Comparison of diagnostic yield with the SADS cohort

The SADS cohort consisted of 163 families, comprising 463 relatives. The characteristics of the SADS victims are described in table 2. The diagnostic yield in the SADS cohort was similar to that of individuals with autopsy findings of uncertain significance (47.2% versus 51%, p=0.727). In both cohorts the predominant diagnosis was of a primary arrhythmogenic syndrome (Figure 4). A similar proportion of the relatives evaluated in both the SADS and the autopsy findings of uncertain significance cohorts were diagnosed with a cardiac condition (24.6% versus 22.9%, p=0.715).

Discussion

Sudden cardiac death in young, previously healthy individuals instigates cardiac evaluation of first-degree relatives aimed at identifying potentially inherited cardiac pathology to minimize the risk of further tragedies.2,3 In a significant proportion of SCDs the pathologist may observe findings that are relatively common in the general population, or findings that partially fulfil diagnostic criteria for structural cardiac disease, leaving uncertainty with regard to causality and management of surviving relatives. In this study of 41 families with post-mortem findings of
uncertain significance, almost 50% were diagnosed with a hereditary arrhythmogenic syndrome
and the causes of SCD were similar with those observed in a true SADS cohort. This finding is
of particular importance since by convention the absence of any cardiac pathology is regarded a
prerequisite for the definition of a death as SADS.1

**Implications of Autopsy Findings of Uncertain Significance**

The causal effect of the autopsy findings is unclear. The authors offer four plausible hypotheses:

(a) **Innocent Bystander**

Bicuspid aortic valve and floppy mitral valve are present in 1-2% of the general population and
may represent innocent bystanders. Likewise, coronary atherosclerosis without significant
narrowing of the arterial lumen and without evidence of acute or chronic ischaemia is common.
Moreover, it is well documented that the degree of coronary artery stenosis can be overestimated
by the pathologists as a result of post-mortem collapse of the vessel wall.17 Finally, foci of
lymphocytes are common in the normal heart and a degree of myocardial inflammation may be
the effect of prolonged resuscitation efforts rather than evidence of myocarditis resulting in
SCD.18

(b) **Primary Cause of Sudden Cardiac Death**

Most of the conditions identified at autopsy in our cohort have been associated with ventricular
arrhythmias and sudden death.5 Similarly the absence of severe luminal narrowing of the
coronary arteries does not preclude ventricular arrhythmias due to myocardial ischaemia,
particularly as a result of coronary artery vasospasm19 and isolated fatty infiltration involving the
cardiac conduction system has been implicated in SCD of obese people.20

(c) **Trigger in the Context of an Arrhythmogenic Syndrome**

Consideration must also be given to the fact that structural cardiac disorders may serve as
triggers for arrhythmias in the context of a coexistent inherited arrhythmogenic syndrome. One-third of SCDs in our cohort with minor coronary disease were subsequently attributed to an arrhythmogenic syndrome. Current evidence suggest that the presence of coronary artery disease is an independent risk factor for LQTS-related symptomatic events. It appears likely that transient ischaemia alters the arrhythmic substrate by reducing the threshold for after-depolarisations or increasing transmural dispersion of repolarization, both recognized mechanisms for arrhythmogenesis in ion-channel disease.

(d) Spectrum of Arrhythmogenic Syndromes

There is mounting evidence that individuals with ion-channel defects may exhibit structural cardiac changes. Although the majority of BrS patients possess a structurally normal heart, a small proportion, appears to exhibit evidence of ventricular wall motion abnormalities, ventricular dilatation and fibrosis. Such structural abnormalities may be subtle requiring sophisticated diagnostic tools. Several theories have been postulated to correlate ion-channel dysfunction with structural abnormalities, ranging from impaired excitation-contraction coupling and energy production, to a hibernation-like state which over time may even lead to intracellular lipid accumulation. Support for potential structural abnormalities in patients with BrS is also provided by the study of Nademanee et al. where the authors identified the anterior aspect of the RVOT epicardium as the substrate for the Brugada ECG pattern.

Additionally, there are reports in the literature of identical mutations presenting with either a cardiomyopathy or an arrhythmogenic syndrome phenotype, suggesting that structural and ion-channel defects may be part of a spectrum incorporating myocardial disease and primary arrhythmogenic syndromes. Mutations in the cardiac ryanodine receptor gene (Ryr2), commonly implicated in CPVT, have been reported in individuals exhibiting an ARVC
phenotype.\textsuperscript{27} Mutations in the SCN5A gene, implicated in BrS, may present with arrhythmia, conduction disease and atrial or ventricular dilatation.\textsuperscript{28} Heritable SCN5A defects have also been associated with early-onset DCM and atrial fibrillation.\textsuperscript{29}

\textit{Left Ventricular Hypertrophy and Myocardial Fibrosis}

In our cohort isolated LVH and myocardial fibrosis were the most prevalent findings. Idiopathic LVH is an increasingly recognized entity in cases of SCD. It remains unclear whether it represents an innocent bystander, a pathological variant of physiological LVH in genetically predisposed individuals or part of the HCM spectrum.\textsuperscript{5} Although LVH is a well-recognized feature of cardiovascular adaptation to exercise, in our study only 4 out of the 10 individuals exhibiting isolated LVH exercised on a regular basis. Data from the Framingham study also indicate that LVH confers a four-fold risk of sudden death.\textsuperscript{30} In addition, experimental studies suggest that LVH alters ion-channel expression and function predisposing to re-entry arrhythmias and ventricular fibrillation.\textsuperscript{31} Although in the majority of individuals such adaptations are unlikely to result in increased risk of arrhythmias, the development of LVH in an individual with an underlying arrhythmogenic syndrome, may exacerbate electrical instability and predispose to sudden death.

The amount of myocardial fibrosis and the collagen texture appear to play a role in vulnerability to arrhythmia.\textsuperscript{32} Moreover, myocardial fibrosis may represent incomplete expression of underlying cardiomyopathy. Myocardial fibrosis has also been reported in marathon runners and in cases of SCD in athletic individuals raising concerns whether prolonged arduous exercise can lead to repeated myocardial injury, necrosis and subsequent fibrosis.\textsuperscript{8} Finally, animal models have demonstrated that SCN5A mutations cause progressive impairment of atrial and ventricular conduction associated with myocardial rearrangements and fibrosis.\textsuperscript{33}
The Role of the Cardiac Pathologist

This study highlights the importance of accurate interpretation of the autopsy findings since false conclusions may misguide familial evaluation or offer false reassurance to surviving relatives and dissuade physicians from initiating familial screening. Given the relative rarity of SCDs from inherited conditions and the challenges associated with their diagnosis, the authors propose that all cases of SCDs, and particularly SCDs in young (≤ 35 years) individuals, where an inherited condition is suspected or diagnostic uncertainty remains as to the cause of death, should be referred for further evaluation to an expert cardiac pathologist.

Limitations

The predominant diagnosis in our cohort was of BrS, reflecting the victims’ demographics (80% males, mean age of 30 years), predominant mode of death (60% asleep/ at rest) and the routine use of Ajmaline provocation testing. It is plausible that some Ajmaline-based diagnoses may be erroneous. Although currently there are no large series of normal subjects undergoing Ajmaline test, existing literature in SCN5A positive families suggests that the specificity of the Ajmaline challenge exceeds 94%. Therefore, in the absence of an alternative gold standard, provocation testing with a sodium channel blocker remains an integral part of the evaluation of individuals with suspected BrS.

The authors also concede that given the relative novelty of the condition and the association of the Brugada phenotype with several structural cardiac abnormalities, it is possible that some of the relatives exhibiting the Brugada phenotype did not have a genuine arrhythmogenic syndrome. However, all individuals who were diagnosed with BrS underwent comprehensive evaluation including a detailed echocardiogram and a significant proportion were subjected to CMR and none exhibited any evidence suggesting structural cardiac anomalies.
Further support for the presence of BrS is underscored by the genetic yield (23%) of pathogenic SCN5A mutations, similar to existing literature.\textsuperscript{14}

In the 6 families in whom a pathogenic mutation was identified in evaluated relatives, we were unable to perform post-mortem analysis in the tissues of the victims for confirmation of the genotype since no tissue was available by the time the relatives were evaluated in our clinic. In the UK, the Human Tissue Act does not permit retention of tissue as part of a deceased patient’s record, and retention for research requires familial consent at the time of post-mortem.\textsuperscript{35} As such, in the majority of cases histological slides are prepared, reported and imaged at the time of the post-mortem examination, allowing early return of the tissue for burial or cremation.

Conclusion

The current study underscores the need for accurate interpretation of autopsy findings in cases of SCD to avoid erroneous diagnoses with potentially devastating implications for surviving relatives. Our data suggest that all SCDs with inconclusive autopsy findings should be regarded as potential SADS deaths and comprehensive evaluation of family relatives for both inherited primary arrhythmogenic syndromes and structural cardiac abnormalities should be advocated.

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Conflict of Interest Disclosures: Dr Behr receives research funds from Biotronik and Boston Scientific.

References:


Mouse model of SCN5A-linked hereditary Lenègre’s disease; Age-related conduction slowing and myocardial fibrosis. *Circulation*. 2005;111:1738-1746.


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**Table 1**: Pathological criteria for defining cardiac pathology and certainty of causal effect in sudden cardiac death autopsies

<table>
<thead>
<tr>
<th>Post-mortem findings highly suggestive of causal effect</th>
<th>Post-mortem findings of uncertain significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hypertrophic cardiomyopathy</strong></td>
<td></td>
</tr>
<tr>
<td>Macroscopic</td>
<td></td>
</tr>
<tr>
<td>Left ventricular wall thickness ≥15 mm and/or heart weight ≥500 g</td>
<td>Microscopic Myocyte hypertrophy + disarray + interstitial fibrosis +/- abnormal intra-myocardial small vessels</td>
</tr>
<tr>
<td>Arrhythmogenic right ventricular cardiomyopathy</td>
<td></td>
</tr>
<tr>
<td>Macroscopic</td>
<td></td>
</tr>
<tr>
<td>Right ventricular thinning + fatty replacement + fibrosis</td>
<td>Microscopic Fat + fibrosis of the wall of the right and/or left ventricle</td>
</tr>
<tr>
<td>Dilated cardiomyopathy</td>
<td></td>
</tr>
<tr>
<td>Macroscopic</td>
<td></td>
</tr>
<tr>
<td>Heavy heart with dilated ventricles and absence of coronary artery disease</td>
<td>Microscopic Absence of inflammatory myocardial disease</td>
</tr>
<tr>
<td>Coronary atherosclerosis</td>
<td></td>
</tr>
<tr>
<td>Macroscopic</td>
<td></td>
</tr>
<tr>
<td>Atherosclerosis with estimated luminal narrowing &gt;75%</td>
<td>Microscopic Acute or chronic infarction in the left ventricle</td>
</tr>
<tr>
<td>Myocarditis</td>
<td></td>
</tr>
<tr>
<td>Macroscopic</td>
<td></td>
</tr>
<tr>
<td>Normal or dilated ventricles</td>
<td>Microscopic Inflammation with myocyte necrosis</td>
</tr>
<tr>
<td>Mitral valve papillary muscle or chordae tendineae rupture with marked ballooning of both leaflets above the atrioventricular junction</td>
<td></td>
</tr>
<tr>
<td>Aortic stenosis with left ventricular hypertrophy</td>
<td>Isolated bicuspid aortic valve</td>
</tr>
</tbody>
</table>

Mild ventricular dilatation in the absence of significant fibrosis or myocardial inflammation

Atherosclerosis with estimated ≤50% luminal narrowing of the coronary arteries or 2mm probe patent in the absence of acute or chronic infarction

Scattered lymphocytic inflammatory foci with no fibrosis or myocyte necrosis

Floppy mitral valve with mild ballooning between chordae in one or both leaflets
Table 2: Characteristics of victims of sudden cardiac death with autopsy findings of uncertain significance. A comparison is made with victims with normal post-mortem (SADS).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Uncertain significance n=41</th>
<th>SADS deaths n=163</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age [range] (years)</td>
<td>29.9±14.4 [4-59]</td>
<td>27.6±11.1 [1-56]</td>
<td>0.267</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>80%</td>
<td>67%</td>
<td>0.128</td>
</tr>
<tr>
<td>Ethnicity (Caucasian)</td>
<td>93%</td>
<td>91%</td>
<td>1.000</td>
</tr>
<tr>
<td>Mode of death</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Asleep/At rest</td>
<td>61%</td>
<td>61%</td>
<td></td>
</tr>
<tr>
<td>During/post exertion</td>
<td>37%</td>
<td>17%</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>2%</td>
<td>22%</td>
<td></td>
</tr>
<tr>
<td>Reported antecedent cardiac symptoms*</td>
<td></td>
<td></td>
<td>0.250</td>
</tr>
<tr>
<td>Syncope</td>
<td>37%</td>
<td>27%</td>
<td></td>
</tr>
<tr>
<td>Prior family history of premature (&lt;55 years)</td>
<td></td>
<td></td>
<td>0.369</td>
</tr>
<tr>
<td>sudden cardiac death</td>
<td>25%</td>
<td>17%</td>
<td></td>
</tr>
</tbody>
</table>

* chest pain, palpitations, shortness of breath, pre-syncope, syncope

Figure Legends:

Figure 1: Flow chart of study cohort (solid arrows) including diagnostic yield

Abbreviations: SCD, sudden cardiac death; SADS, Sudden arrhythmic death syndrome; CPVT, Catecholaminergic polymorphic ventricular tachycardia.

Figure 2: Histogram depicting the diagnostic yield of familial evaluation in victims of sudden cardiac death. The x-axis represents the number of families and the y-axis the pathology identified in the deceased during post mortem evaluation of the heart. The different colours within the columns represent the diagnosis established after cardiac evaluation of surviving relatives with absolute numbers of families stated within the relevant colour.

Abbreviations: LVH, left ventricular hypertrophy.
**Figure 3:** Histopathological slides of: 1. An individual who exhibited coronary artery disease on autopsy and subsequent familial evaluation identified BrS: A. Macroscopic examination of the left anterior descending coronary artery in an otherwise normal heart shows eccentric atheroma. This can be opened with a 2mm probe (black arrow), indicating that there would have been normal blood flow during life; B. Staining with Trichrome stain (Elastin Van Gieson) confirmed eccentric atheroma. 2. An individual who exhibited isolated left ventricular hypertrophy on autopsy and subsequent familial evaluation identified Long-QT syndrome: A. Short axis slice showing a circumferentially thickened left ventricular wall measuring 2cm. The left ventricular cavity diameter is also reduced; B. Haematoxylin and eosin staining confirms idiopathic myocyte hypertrophy with enlarged box-shaped nuclei. No myocyte disarray is noted. 3. An individual who exhibited myocardial fibrosis on autopsy and subsequent familial evaluation identified BrS: x2 (A) and x10 (B) magnification of picro-sirius red staining shows extensive myocardial replacement with collagen (stained red) in the left ventricular wall from epicardium into mid-myocardium. There is also fine interstitial collagen surrounding individual myocytes (yellow). Mild fatty infiltration is also noted within the collagen areas. 4. An individual who exhibited right ventricular fatty infiltration on autopsy and subsequent familial evaluation identified BrS: x4 (A) and x20 (B) magnification of haematoxylin and eosin stain of the right ventricular wall showing significant fatty infiltration in the outer third of the myocardium (stained red). There is no fibrous tissue.

**Figure 4:** Pie charts depicting the results of familial evaluation in the autopsy findings of uncertain significance and the SADS cohorts.
SCD victims whose families were referred for cardiac evaluation n=368

Complete post-mortem, Negative toxicology, No pre-morbid cardiac history n=340

Group 1
Definite cardiac pathology

Group 2
Normal autopsy (SADS)

Group 3
Autopsy of uncertain significance n=41 (12%)

Positive family screening n=21
Cardiomyopathy n=2
Brugada syndrome n=14

Arrhythmogenic syndrome n=19
Long QT syndrome N=4
CPVT n=1

Negative family screening n=20
LQTS
CPVT
BrS
Familial AF
ARVC
HCM

Uncertain Significance

SADS

Circulation Arrhythmia and Electrophysiology
JOURNAL OF THE AMERICAN HEART ASSOCIATION
Sudden Cardiac Death with Autopsy Findings of Uncertain Significance: Potential for Erroneous Interpretation

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SUPPLEMENTAL MATERIAL
**Supplemental Table:** Detailed presentation of the characteristics of victims of sudden cardiac death and relatives diagnosed with a condition in families where the presence of an inherited arrhythmogenic syndrome was confirmed by the presence of a pathogenic mutation in the relatives.

<table>
<thead>
<tr>
<th>Victims age, gender, mode of death</th>
<th>Post-mortem findings</th>
<th>Clinical phenotype</th>
<th>Pathogenic mutation identified in relatives</th>
<th>Clinical findings in relatives diagnosed with a hereditary arrhythmogenic syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>27 years Female At rest</td>
<td>Septal subendocardial fibrosis</td>
<td>LQTS SCN5A:p.G840R</td>
<td>QTc (Bazett) of 479ms(^{1/2}) (65bpm) and non-sustained VT in father, in context of family history (Schwartz score 4.5). Brother has QTc of 460ms(^{1/2}) (84bpm) with prolongation late in recovery post-exercise (Schwartz score 3).</td>
<td></td>
</tr>
<tr>
<td>34 years Male At rest</td>
<td>Lipomatous hypertrophy of atrial septum</td>
<td>LQTS SCN5A:p.E1784K</td>
<td>Resting QT prolongation in 2 daughters (Bazett QTc 497ms(^{1/2}) at 70bpm and 490ms(^{1/2}) at 71bpm) with syncope in one consistent with Schwartz score 4 in both.</td>
<td></td>
</tr>
<tr>
<td>17 years Male</td>
<td>Marked right ventricular dilatation</td>
<td>CPVT RYR2:p.A4556T</td>
<td>Exertional polymorphic non-sustained ventricular tachycardia in mother; bidirectional ventricular tachycardia</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>Gender</td>
<td>Activity</td>
<td>Findings</td>
<td>Genotype</td>
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<tr>
<td>39 years</td>
<td>Male</td>
<td>Cycling</td>
<td>Mitral valve prolapse (floppy mitral valve with mild ballooning)</td>
<td>BrS SCN5A:p.H558fs</td>
</tr>
<tr>
<td>30 years</td>
<td>Male</td>
<td>At rest</td>
<td>Left ventricular Hypertrophy. Maximal wall thickness 18mm No significant disarray</td>
<td>BrS SCN5A:p.R376H</td>
</tr>
<tr>
<td>37 years</td>
<td>Male</td>
<td>Asleep</td>
<td>Calcified atrial mass</td>
<td>BrS SCN5A:p.A1680T</td>
</tr>
</tbody>
</table>
Early Repolarisation Patterns in Relatives of Individuals with Autopsy Findings of Uncertain Significance

All ECG recordings were reviewed retrospectively for the presence of early repolarization (ER), based on the results of recent studies which indicate that early repolarization in the inferior and lateral leads may represent a potentially heritable marker of malignant arrhythmias,\textsuperscript{1} particularly in the context of SADS.\textsuperscript{2} Inferior (II, III, AVF) and lateral (I, AVL, V4-V6) leads were assessed for the presence of J-point elevation defined as $\geq 0.1$ mV in $\geq 2$ leads in the same territory. The ER pattern was further classified according to the morphology of the terminal QRS in the majority of the leads as notched, slurred or indeterminate. In the presence of ER, the ST-segments were assessed for the presence of ST-segment elevation and a distinct ST-segment morphology (ascending or horizontal/descending).\textsuperscript{1}

The prevalence of ER pattern in the inferior and/or lateral leads in our cohort was lower than that observed by Nunn et al., and comparable to the prevalence reported in healthy controls.\textsuperscript{2} Of the 157 relatives evaluated, 10.2\% (n=16) exhibited the ER pattern in the inferior and/or lateral leads and less than 2\% (n=3) demonstrated a slurred terminal QRS complex with a descending ST-segment, which is the ER pattern predominantly linked to the risk of sudden death in the general population.\textsuperscript{1} Although the prevalence of ER was higher in relatives without a diagnosis of arrhythmogenic syndrome or cardiomyopathy compared to relatives with a diagnosis, the difference did not achieve statistical significance (11.6\% versus 5.6\%, $p=0.366$). Only one family
consisted of ≥2 relatives who exhibited ER offering little support to the theory of a
potentially inheritable pro-arrhythmic trait. In that particular family the deceased’s post-
mortem revealed mitral valve prolapse and no diagnosis was established after
comprehensive cardiac evaluation of blood relatives.

References

1. Tikkanen JT, Juhani Junttila M, Anttonen O, Aro AL, Luttinen S, Kerola T, Sager SJ,
   Rissanen HA, Myerburg RJ, Reunanen A, Huikuri HV. Early Repolarization.
   Electrocardiographic phenotypes associated with favourable long-term outcome.

2. Nunn LM, Bhar-Amato J, Lowe MD, Macfarlane PW, Rogers P, McKenna WJ, Elliott
   PM, Lambiase PD. Prevalence of J-point elevation in sudden arrhythmic death syndrome