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CLINICAL RESEARCH
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Insights into sudden cardiac death: exploring the potential relevance of non-diagnostic autopsy findings

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Aims Unexplained sudden cardiac death (SCD) may be attributable to cardiogenetic disease. Presence or absence of autopsy anomalies detected following premature sudden death direct appropriate clinical evaluation of at-risk relatives towards inherited cardiomyopathies or primary arrhythmia syndromes, respectively. We investigated the relevance of non-diagnostic pathological abnormalities of indeterminate causality (*uncertain*) such as myocardial hypertrophy, fibrosis, or inflammatory infiltrates to SCD.

Methods and results At-risk relatives of unexplained SCD cases aged 1–64 years without prior cardiac disease ($n = 98$) with either normal and negative (40%, true sudden arrhythmic death syndrome; SADS) or isolated non-diagnostic (60%, uncertain sudden unexplained death; SUD) cardiac histological autopsy findings at a central forensic pathology unit were referred to the regional unexplained SCD clinic for clinical cardiac phenotyping. Uncertain SUD were older than true SADS cases (31.8 years vs. 21.1 years, $P < 0.001$). A cardiogenetic diagnosis was established in 24 families (24.5%) following investigation of 346 referred relatives. The proportions of uncertain SUD and true SADS explained by familial cardiogenetic diagnoses were similar (20% vs. 31%, $P = 0.34$, respectively), with primary arrhythmia syndromes predominating. Unexplained SCD cases were more likely than matched non-cardiac premature death controls to demonstrate at least one uncertain autopsy finding ($P < 0.001$).

Conclusion Primary arrhythmia syndromes predominate as familial cardiogenetic diagnoses amongst both uncertain SUD and true SADS cases. Non-diagnostic or uncertain histological findings associate with SUD, though cannot be attributed a causative status. At-risk relatives of uncertain SUD cases should be evaluated for phenotypic evidence of both ion channel disorders and cardiomyopathies.

Keywords Sudden arrhythmic death syndrome • Sudden unexplained death • Inherited cardiac conditions • Ion channel disorders

Introduction

A proportion of sudden cardiac deaths (SCDs) are unexpected and unexplained; they may remain unexplained despite comprehensive autopsy.[1–4] Unexplained SCD make up the majority of SCD in those aged under 35 years.[5] Unexplained SCD cases aged between 1 and 64 years with negative autopsy, negative toxicological analysis, and no prior known cardiac history are classified as sudden arrhythmic death

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syndrome (SADS).[3] Around one-third of SADS cases are related to inherited primary arrhythmia syndromes such as long QT syndrome (LQT) or Brugada syndrome (BrS) that may be diagnosed by evaluating surviving blood relatives, who remain at risk, and by direct mutation analysis, although variable diagnostic yields have been reported.[2,4,5] Genetic cardiomyopathies, such as hypertrophic or arrhythmogenic cardiomyopathies, have a specific phenotype and may also be first identified, following premature SCD, by autopsy.[2] Where genetic disease is suspected as the primary cause for SCD, evaluation of at-risk relatives is recommended to identify additional family members who may be at risk.[3,6]

Detailed cardiac pathological and histological post-mortem examination may identify abnormalities of gross cardiac or myocardial structure, which are insufficient to meet diagnostic criteria for known phenotypes or pathologies. These may be insufficient to accord as causative for SCD, but beyond what a pathologist would accept as the spectrum of normal.[7] These cases often remain certified as 'unascertained' cause of death. These minor, but non-diagnostic post-mortem findings have unknown clinical significance and no accepted classification nomenclature. For the purpose of this article, these have been referred to as uncertain, as pertains to the cause of death. The histological or structural changes described are not uncertain *per se*. Examples of such 'uncertain' pathological abnormalities found in the absence of associated features of cardiomyopathy, myocarditis, and/or myocardial infarction include: (i) left ventricular hypertrophy; (ii) myocardial inflammatory infiltrates; (iii) non-critical coronary artery disease; (iv) idiopathic ventricular myocardial fibrosis; and (v) cardiomegaly. The isolated appearance of any of these features at autopsy creates diagnostic grey zones.[7,8]

Existing hypotheses suggest that these uncertain findings may represent either an innocent bystander of no significance or a risk factor for SCD.[7] It is plausible that they are: an unrecognized primary cause of SCD; an arrhythmic trigger that co-exists with an (undiagnosed) primary arrhythmia syndrome; a subtle morphological expression of cardiac ion channel disease; or an early or mild expression of a cardiomyopathy. However, little evidence to support or refute these theories exist. Currently, the true aetiological impact of cardiac autopsy findings of uncertain significance to the death remains to be elucidated. We aimed to study this aspect of SCD by comparing demographic and histopathological characteristics of uncertain cardiac autopsy findings amongst unexplained SCD cases and matched non-cardiac premature deaths.

Methods

Setting

Unexpected deaths in the state of Victoria (Australia) are referred to the Victorian Coroner under the Coroners Act (2010). The coroner directs a pathologist from the Victoria Institute of Forensic Medicine (VIFM) to perform an autopsy evaluation. Post-mortem reports are released only after verification of findings by a second pathologist in cases with an unascertained cause of death. The Royal Melbourne Hospital (RMH) has provided a dedicated unexplained SCD familial cardiological and genetic evaluation clinical service since 2007. Thus, local surviving first degree at-risk relatives of unexplained SCD cases in Victoria have been referred directly by VIFM pathologists to the RMH specialist service since 2007.[4,9] In keeping with a referral pattern that has largely excluded premature sudden death cases with ante-mortem diagnoses of schizophrenia or Type I diabetes mellitus, these were not included in the study sample, to avoid potential introduction of bias.

Cohorts

A total of 110 unexplained SCD cases aged 1–64 years who underwent VIFM autopsy between May 2007 and August 2016 resulting in referral to RMH and acceptance for familial cardiogenetic evaluation were identified. *Figure 1* summarizes the study cohort and investigations undertaken. Cases referred following proband autopsy elsewhere were excluded. Twelve cases were excluded due to diagnosis of sudden unexplained death in epilepsy (SUDEP) based on ante-mortem clinical diagnosis of epilepsy supported by response to antiepileptics, imaging, or electroencephalogram. Thus, 98 unexplained SCD cases were included in this study. All unexplained SCD cases fulfilled definition for SADS[1,3] having witnessed sudden death or been seen well within 24 h of death with no ante-mortem cardiac diagnosis, negative non-cardiac autopsy, and negative toxicological analysis. These were then divided into two subgroups: the uncertain sudden unexplained death (SUD) group ($n = 59$, with uncertain histopathological findings) and the true SADS group ($n = 39$, with completely normal cardiac post-mortem examinations).

The control cohort was matched from non-cardiac premature deaths (road traffic accidents and witnessed drownings) from autopsies performed at the VIFM. Matching by risk-set sampling was performed by: age at time of death; gender; family-reported ethnicity; year of autopsy; and body mass index (BMI). In accordance with exclusion of cases, controls with ante-mortem diagnoses of schizophrenia, epilepsy or Type I diabetes mellitus were excluded.

Post-mortem evaluation

All unexplained SCD and controls underwent post-mortem evaluation as specified by VIFM Standard Operating Procedure Standards for Medicolegal Death Investigation.[8,10,11] The autopsies were therefore performed in a standard manner and included: external examination including height and weight; examination of major organs including weight; distribution and stenoses in major arteries. The cardiac autopsies on all cases and controls specifically included measurements of heart weight, valve circumference and ventricular wall thicknesses in addition to description of pericardium, myocardium, cardiac chambers, cardiac valves; distribution, course and severity of stenoses in coronary arteries were additionally assessed. Multiple sections from myocardium, lung, kidney, liver, and brain were stained with haematoxylin and eosin for histological examination. Myocardial sections were taken and examined from multiple sections in the left ventricle (mid-ventricular anterior, lateral, posterior, and septum), from inflow and outflow tracts and lateral wall of the right ventricle and from each major epicardial coronary artery. Unexplained SCD cases underwent histological assessment of AV nodal (conducting system) tissue, which was not routinely undertaken in controls. Twenty (20%) unexplained SCD cases were randomly selected for additional review by a cardiovascular pathologist (S.P.) to assess interobserver agreement in a blinded fashion. Routine toxicology included analysis of blood and urine for therapeutic and illicit substances.

Uncertain histopathological findings

Presence of uncertain findings at cardiac autopsy was determined as per Supplementary material online, *Table S1* , in line with international consensus.[8] Specifically, the following findings were considered uncertain: minor mitral valve or chordal abnormalities; aortic coarctation without fibrosis, hypertrophy or dissection; moderate coronary disease without infarction; intramyocardial coronary arteries; fibromuscular dysplasia of AV nodal or coronary arteries; right ventricular invasive fat and/or minor

Figure 1 Flowchart illustrating numbers of cases evaluated at each stage of study. SADS, sudden arrhythmic death syndrome; SCD, sudden cardiac death; SUD, sudden unexplained death; SUDEP, sudden unexplained death in epilepsy; RMH, Royal Melbourne Hospital; VIFM, Victorian Institute of Forensic Medicine. Key: *Includes five cases without coronial permission to refer.

fibrosis[12]; dilated left ventricle; cardiomegaly (by heart weight[13]); isolated myocyte disarray, hypertrophy, or fibrosis; and minor myocardial inflammatory infiltrates.[8]

Diagnosis of genetic disease

Clinical cardiological evaluation

All referred first degree at-risk relatives of unexplained SCD cases were clinically evaluated (including family history and pedigree) by electrocardiogram (ECG), echocardiography and exercise tolerance test. Following initial negative evaluation and where clinical suspicion of primary arrhythmia syndrome and/or cardiomyopathy existed, additional investigations included: flecainide (2007–2014) or ajmaline (2014–2016) provocation testing including 'high right precordial lead' ECG recording[14]; epinephrine provocation testing (Mayo Clinic protocol[15]); signal-averaged ECG; ambulatory Holter ECG; and further structural imaging or coronary angiography.[4] Investigations were retrospectively and independently reviewed by two investigators (J.V. and H.R.) to establish possible or probable diagnosis of genetic cardiac disease in family members based on current published diagnostic criteria.[3,6,12,16]

Genetic testing

Mutation analysis of common risk genes was offered following any probable or definite clinical cardiological diagnosis of inherited disease made in an at-risk relative, with appropriate counselling. This was undertaken on retained post-mortem DNA from the SUD proband, where available, or else on phenotypically affected surviving at-risk relatives' DNA. Methodology changed during the study period from selected risk-genes with conventional PCR and Sanger sequencing to cardiac risk panel by next generation sequencing, including exome sequencing.

Research ethics

The study was approved by RMH and VIFM Research Ethics Committees (QA2016109 and RAC020/16, respectively).

Statistical analysis

Data analysis was undertaken using R version 3.3.2 (R Development Core Team). Data are expressed as mean ± standard deviation. Population means and proportions were compared with *t*-test and Fisher's exact test, respectively.

Results

Demographics

A total of 98 unexplained SCD cases (60% male; 79% Caucasian; age 27.5 ± 15.5 years) were included. At least one uncertain autopsy finding was identified in 59 (60%) cases (uncertain SUD cases), with 39 cases (true SADS cases) having entirely normal cardiac autopsies. Interobserver agreement on autopsy classification was 100% in the 20 randomly selected SUD cases. Uncertain SUD cases were significantly older (31.8 years vs. 21.1 years, $P < 0.001$) than true SADS,

with no difference in gender proportions (61% vs. 59% male, $P = 1.0$). Age distribution of uncertain SUD cases by gender is illustrated in Supplementary material online, *Figure S1*. The age distribution of male cases was skewed to the right, with the modal age between 20 and 30 years. In contrast, the female distribution was bimodal, with peak frequencies at teenage years and between 40 and 60 years of age.

Clinical characteristics

No significant differences between uncertain SUD and true SADS cases were seen in proportions of cases who were obese (29% vs. 15%, $P = 0.15$, respectively), or had therapeutic levels of antidepressants detected on toxicology (10% vs. 10%, $P = 1.0$, respectively). Additionally, when comparing uncertain SUD with true SADS cases, there was no statistically significant difference in proportions who died during sleep (36% vs. 56%, $P = 0.06$, respectively) or during presumed parasympathetic predominance, i.e. combination of deaths during rest or sleep (83% vs. 90%, $P = 0.40$, respectively).

Prevalence of uncertain cardiac autopsy findings

The uncertain cardiac autopsy findings identified in unexplained SCD cases ($n = 98$) and controls ($n = 28$) are illustrated in *Figure 2*. The most prevalent uncertain findings identified amongst unexplained SCD cases were: hypertrophy of the left ventricle ($n = 14$); cardiomegaly ($n = 13$) (based on a heart weight >95% confidence limit for age, gender, and body size in large population study[13]); inflammation ($n = 13$); and left ventricular fibrosis ($n = 12$). Left ventricular fibrosis ($n = 6$), inflammation ($n = 4$), and minor coronary artery disease ($n = 6$) were most common in controls, although their prevalences were all numerically lower than those seen in matched unexplained SCD cases. Unexplained SCD cases were seen to be significantly more likely than controls to demonstrate at least one uncertain autopsy finding ($P < 0.001$, *Figure 3*).

Evaluation of at-risk relatives

Clinical evaluation

A total of 346 at-risk relatives (mean 3.5 ± 1.7 per unexplained SCD proband) were evaluated cardiologically resulting in a probable or definite diagnosis in 35 (10.1%) surviving relatives. This represents a potential cardiogenetic diagnosis for the aetiology of unexplained SCD in 24 probands (24.5%); the majority of the studied SCD cases thus remained unexplained following cardiac evaluation of at-risk relatives. There was no difference in number of relatives evaluated per proband between uncertain SUD and true SADS cases (3.6 vs. 3.5, $P = 0.85$, respectively).

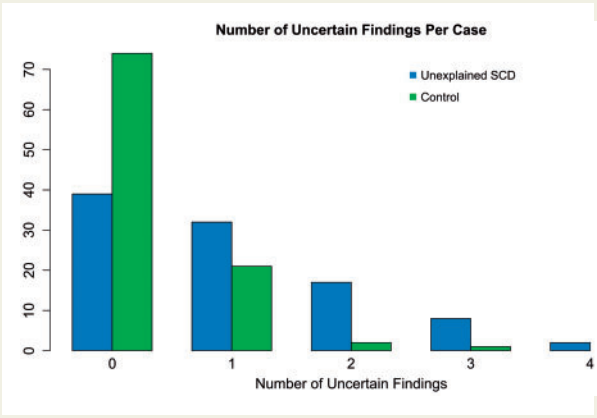


Figure 3 Histogram of number of uncertain cardiac autopsy findings per case identified in unexplained sudden cardiac death (SCD, blue) probands and non-cardiac premature deaths (control, green). Significantly more unexplained SCD cases with ≥1 uncertain finding ($P < 0.001$).

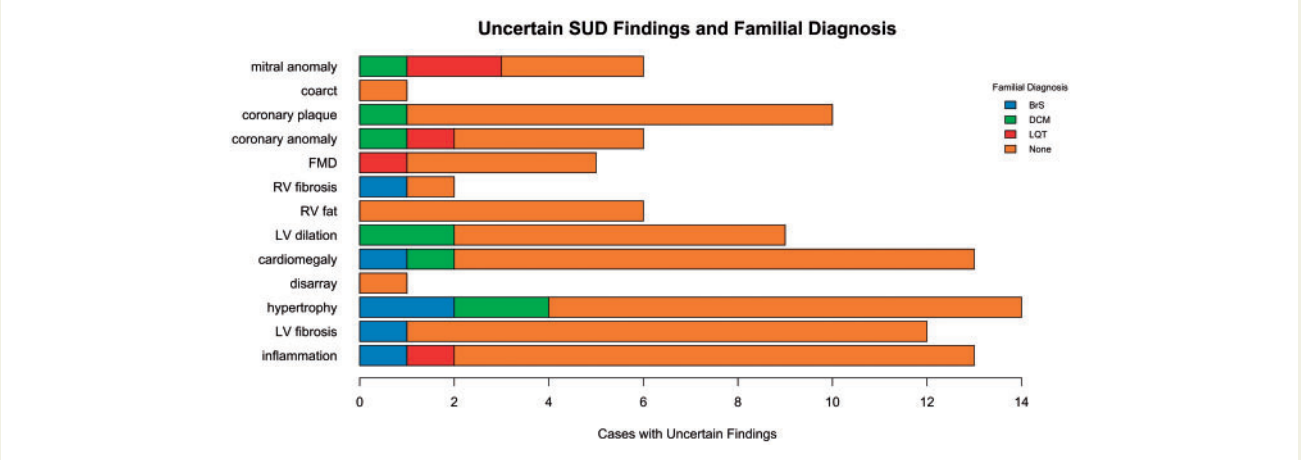


Figure 2 Uncertain autopsy findings identified following unexplained sudden cardiac death and associated cardiogenetic clinical phenotypes identified in blood relatives. BrS, Brugada syndrome; DCM, dilated cardiomyopathy; FMD, fibromuscular dysplasia of coronary/AV nodal arteries; LV, left ventricular; LQT, long QT syndrome; SUD, sudden unexplained death; RV, right ventricular.

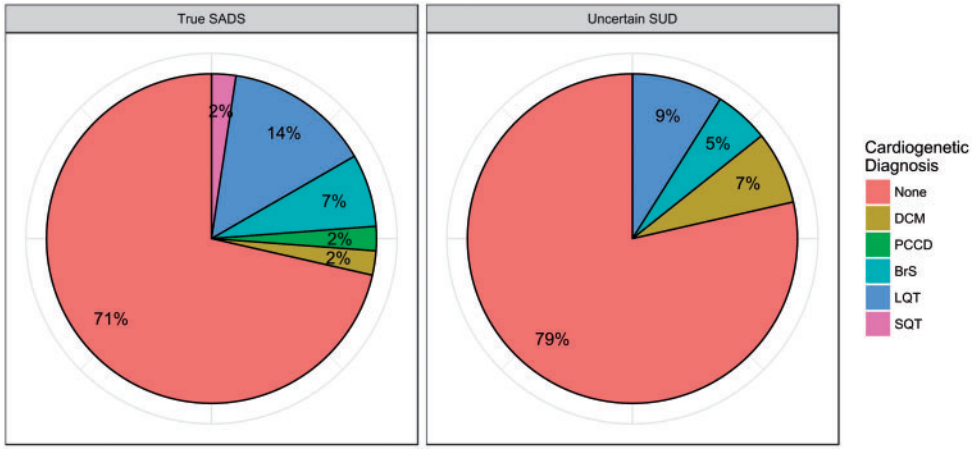


Figure 4 Diagnostic yield of cardiogenetic disease following cardiological evaluation of blood relatives of unexplained sudden cardiac death probands with normal (left; true SADS) and non-diagnostic (right; uncertain SUD) cardiac autopsy findings. No significant difference between proportions identified with cardiogenetic disease ($P = 0.34$). BrS, Brugada syndrome; DCM, dilated cardiomyopathy; LQT, long QT syndrome; PCCD, progressive cardiac conduction disease; SADS, sudden arrhythmic death syndrome; SQT, short QT syndrome; SUD, sudden unexplained death.

Clinical diagnoses made

Twelve unexplained SCD probands with a familial cardiac diagnosis had uncertain autopsy findings (i.e. were uncertain SUD cases). Amongst uncertain SUD cases with a familial diagnosis, primary arrhythmia syndromes (LQT and BrS) predominated (*Figure 4*). Three of these familial diagnoses relied upon drug provocation to unmask concealed phenotypes in surviving relatives (1 LQT and 2 BrS). Non-ischaemic dilated cardiomyopathy was retrospectively diagnosed in a significant minority of uncertain SUD cases who received a diagnosis following familial evaluation (33%, $n = 4/12$, *Figure 4*); in comparison, cardiomyopathy constituted a non-significantly smaller minority ($P = 0.32$) of familial diagnoses amongst true SADS cases (8%, $n = 1/12$, *Figure 4*). Notably, of the four uncertain SUD cases with subsequent familial diagnosis of cardiomyopathy, two families had more than one affected surviving relative. Two uncertain SUD cases were diagnosed on the basis of a single affected relative, although one of these families had an extensive maternal family history of premature sudden death in the context of the uncertain SUD case's mother being diagnosed with dilated cardiomyopathy. The uncertain SUD autopsies in these cases identified at least one of hypertrophy, cardiomegaly or chamber dilatation, but no fibrosis. No genetic structural cardiac disease was diagnosed in any relatives.

Clinical yield and management

The yields of familial cardiogenetic diagnoses following uncertain SUD and true SADS were similar (20% vs. 31%, $P = 0.34$, *Figure 4*, respectively). Appropriate management, including risk modification treatment if applicable, was offered to any at-risk relatives diagnosed with cardiac disease, in line with contemporaneous clinical guidance.[6]

Mutation analysis

Mutation analysis was offered to 24 families following unexplained SCD with phenotypic diagnosis of inherited disease relevant to the probands' death. Two families declined consent. Two DNA samples failed extraction due to inadequate quantity or quality. Thus, 20 unexplained SCD probands (10 true SADS and 10 uncertain SUD) underwent mutation analysis. Of these, eight probands were determined to carry possible or probable disease-associated (pathogenic) mutations. Genetic yield was thus 40% ($n = 8/20$): BrS (*SCN5A*-c.5698C>T, *SCN5A*-c.3352C>T); LQT (*KCNH2*-c.3355G>T, *SCN5A*-c.1232G>A, *KCNH2*-c.233del); dilated cardiomyopathy (*ACTC1*-c.527C>T, *MYH6*-c.3289G>A, *SCN5A*-c.659C>T). No significant difference was seen in molecular genetic yield of uncertain SUD ($n = 5/10$; LQT2, LQT3, BrS, 2 dilated cardiomyopathy) vs. true SADS ($n = 3/10$; LQT2, BrS, dilated cardiomyopathy) cases ($P = 0.65$).

Correlation between uncertain cardiac autopsy findings and diagnosis in relatives

Uncertain histological findings identified

There were no definite associations between specific uncertain pathological abnormalities and familial genetic diseases. Examples of uncertain histological findings in SUD cases are illustrated in *Figure 5*. *Figure 5A* shows ventricular myocardial hypertrophy and idiopathic fibrosis of a SUD proband in the context of a prior family history of premature SCD. Despite comprehensive evaluation of two surviving at-risk relatives, no cardiogenetic diagnosis was made. In fact, the majority (80%, $n = 47/59$) of uncertain SUD cases remained unexplained following familial cardiological evaluation.

Histological findings potentially suggestive of cardiomyopathy

Other borderline diagnostic criteria for cardiomyopathic processes were also seen in uncertain SUD cases where a cardiogenetic diagnosis was made in at-risk relatives. For example, fibro-fatty replacement of ventricular myocardium insufficient to meet minor histological criteria for the diagnosis of arrhythmogenic right ventricular

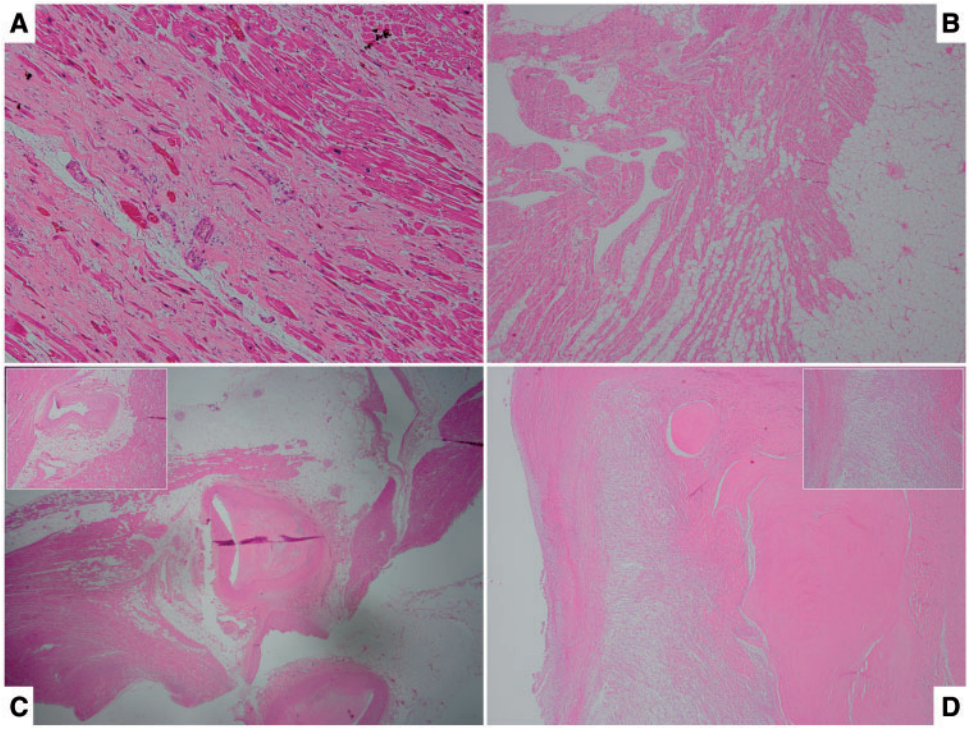
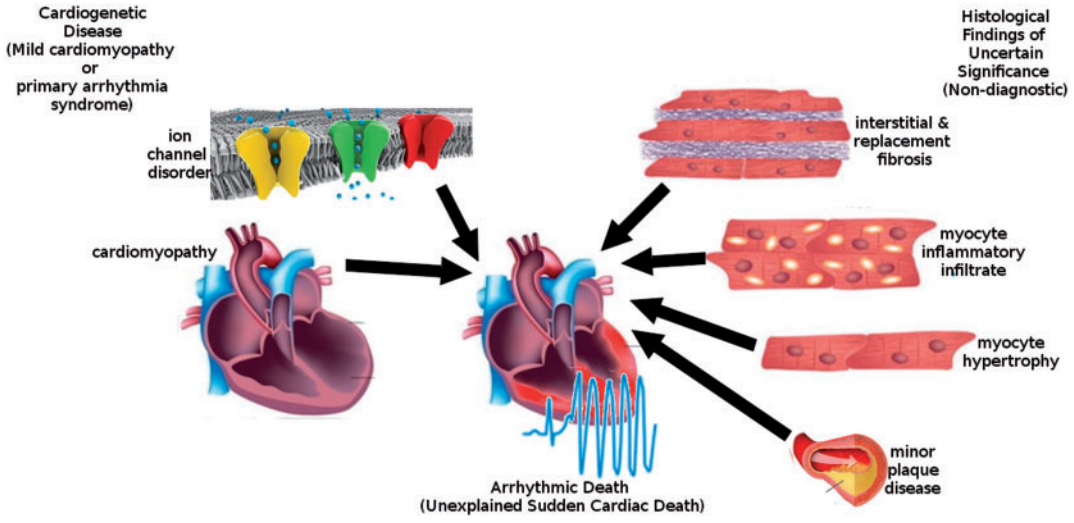


Figure 5 Examples of uncertain histological findings identified in sudden unexplained death (SUD) cases (haematoxylin and eosin stained sections): (*A*) myocardial hypertrophy and pericellular fibrosis in the septum centrally (×10 magnification), identified in a family with no definite cardiogenetic disease; (*B*) fat infiltration and replacement fibrosis in the right ventricle (×2 magnification) insufficient to meet minor criteria for arrhythmogenic cardiomyopathy diagnosis, identified in a family with Brugada syndrome; (*C*) moderate left anterior descending coronary artery stenosis (assessed as 60% stenosis in a collapsed artery that was macroscopically patent prior to histological slide creation) due to soft plaque (main image ×2 magnification) in a 35-year-old, also associated with a distal intramyocardial course (inset image ×10 magnification), identified in a family with dilated cardiomyopathy; (*D*) myxoid mitral valve degeneration (main image ×2 and inset image ×10 magnification), identified in a family with long QT syndrome.



Take home figure Risk factors for unexplained sudden cardiac death: cardiogenetic diseases and uncertain cardiac histological characteristics are both implicated.

cardiomyopathy (ARVC) was present in a proband where family was diagnosed with BrS (*Figure 5B*); this case also demonstrated associated left ventricular fibrosis. Incidental pathological findings of uncertain significance, apparently unrelated to the underlying familial cardiac disease were also identified in some cases. For instance, a 35-year-old man with uncertain SUD autopsy had moderate (estimated 50–70% luminal stenosis) in his mid-left anterior descending artery caused by soft plaque and a 10 mm intramyocardial course at 5 mm depth more distally in the same vessel (*Figure 5C*); dilated cardiomyopathy was identified in his mother. Myxoid mitral valve degeneration was identified in a 51-year-old woman (*Figure 5D*) at autopsy; a diagnosis of LQT was subsequently made following cardiological evaluation of at-risk relatives.

Discussion

We describe consecutive cases of unexplained SCD and the likely causative cardiogenetic disorders responsible, where at-risk relatives were evaluated at The Royal Melbourne Hospital following referral from the Victorian Institute of Forensic Medicine. Cardiac post-mortem findings of uncertain significance have been analysed in a systematic way with a uniform autopsy and cascade familial evaluation protocol. This study is strengthened by the availability of age, gender, and BMI-matched non-cardiac premature death controls, with all cases and controls undergoing comparable autopsy evaluation at a single institution. To our knowledge, only one prior uncertain autopsy in unexplained SCD series has been reported[7]; Supplementary material online, *Table S2* details comparative strengths of this study.

Characteristics of uncertain sudden unexplained death cases

The demographic and clinical characteristics of our uncertain SUD cases are in keeping with other contemporary premature SCD series, with: predominance of male cases; death at rest or during sleep.[2,4,17,18]

Acquired risk factors for sudden unexplained death

Uncertain autopsy findings were identified more commonly in older unexplained SCD cases. This raises the possibility that sudden death in these cases may be related to an acquired risk, which is more likely to manifest later in life. However, we have been unable to demonstrate that uncertain cardiac histopathological findings associate with other potential contributors to premature SCD. Specifically, although a numerically greater proportion of obese SUD cases had uncertain pathology, this was not statistically significant. The mechanism of increased SCD and mortality associated with obesity remains postulated but uncorroborated.[19] Less surprisingly, antidepressant use did not associate with uncertain pathological findings. Some antidepressants are known to prolong QT interval or unmask concealed BrS. These phenomena would not be expected to result in histological findings at autopsy, although may still be an acquired risk factor for premature SCD in the presence of underlying cardiogenetic risk.

Proportion of uncertain autopsy sudden unexplained death cases

Papadakis *et al.*[7] reported 20% ($n = 41/204$) of SUD cases as 'uncertain', while we identified 60% ($n = 59/98$). All our unexplained SCD cases and controls underwent specialist macroscopic and microscopic post-mortem cardiac examination, which contrasts with the prior report which relied predominantly upon autopsy reports, with only a minority receiving a cardiac autopsy to specialist standards. The difference in proportion of uncertain SUD cases may be explained by the more comprehensive cardiac pathological examination and routine study of histological sections from multiple bilateral ventricular myocardial sites in this series. Moreover, detailed cardiac post-mortem examination allowed inclusion of cardiomegaly (as defined by heart weight) as an uncertain finding, independent of cellular hypertrophy and chamber dilatation. It is also important to entertain the possibility that some autopsy reports may erroneously diagnose uncertain findings as definite cardiac pathology, obfuscating the true proportion of uncertain SUD cases in the absence of routine specialist examination.[20]

Yield of cardiogenetic diagnoses

Papadakis *et al.*[7] demonstrated similar diagnostic cardiological yields in at-risk relatives following uncertain SUD autopsy findings as seen in true SADS cases in their UK clinic; ion channel disease predominated amongst their cardiogenetic diagnoses. This was despite idiopathic hypertrophy and fibrosis representing approximately half of all uncertain cases studied. Our yield of cardiogenetic diagnoses was lower than that seen by Papadakis *et al.*[7] for both true SADS and uncertain SUD cases. Ethnic differences in local populations may further contribute. Additionally, their use of ajmaline provocation in all cases may explain a higher diagnostic yield of BrS,[14,21,22] in comparison to our initial protocol using flecainide until 2014.

We identified myocardial hypertrophy, inflammatory cell myocardial infiltration, and ventricular fibrosis as the most prevalent uncertain findings in unexplained SCD cases, followed by non-obstructive coronary disease and cardiomegaly. Four of these uncertain findings would traditionally be associated with a forme fruste of a cardiomyopathic process. Our data supports that from Papadakis *et al.*[7] identifying primary arrhythmia syndromes as the most commonly identified cardiogenetic aetiology in these uncertain SUD cases. Therefore, we propose that uncertain SUD be considered part of the spectrum of SADS with regard to the necessity for comprehensive cardiological evaluation of at-risk relatives beyond assessment for cardiomyopathies.

Relevance of uncertain autopsy findings

In accordance with the report by Papadakis *et al.*,[7] the majority of uncertain SUD cases remained unexplained despite comprehensive non-invasive cardiological evaluation of all surviving first-degree at-risk relatives. Although overlap syndromes are recognised and can cause diagnostic dilemmas, no clear pattern of potential for erroneous diagnoses was seen.

Brugada syndrome

Fibro-fatty replacement of ventricular myocardium insufficient to meet minor histological criteria for the diagnosis of ARVC was seen in a proband where family was diagnosed with BrS (*Figure 5B*). Notably, this case also had left ventricular fibrosis present. An

association between both post-mortem and *in vivo* myocardial fibrosis and familial or personal diagnosis of BrS, respectively have been previously reported.[23] A unifying pathophysiological mechanism for the phenotypic overlap between sodium channelopathy and desmosomal disease has been postulated.[24] Specifically, research supports an interaction between these molecular structures at the intercalated disc, with cellular experiments demonstrating desmosomal protein-related sodium channel dysfunction.[24] Although controversial, it is therefore plausible that ARVC and BrS are disorders of the same disease spectrum. Thus, the apparent pathological finding of uncertain significance in this unexplained SCD case may be a diagnostic feature of the familial condition detected.

Idiopathic left ventricular fibrosis

One of the most prevalent uncertain autopsy findings identified in unexplained SCD was myocardial fibrosis (12%). This replicates prior European autopsy series in premature SCD, with up to 25% prevalence of fibrosis seen in adrenergic or sporting deaths.[25,26] The underlying aetiology for fibrosis may be healed myocarditis or mild cardiomyopathy, although the possibility of contribution from repetitive insult from strenuous exercise in lifelong athletes has also been postulated.[25,27] Recently, particular attention has been placed on the association with right ventricular pathology in individuals demonstrating left ventricular fibrosis and the overlap with arrhythmogenic cardiomyopathy.[26]

Dilated cardiomyopathy

Approximately 25% of non-ischaemic cardiomyopathy is genetic in aetiology.[28] In the context of a first-degree family history of unexplained SCD, this is generally considered likely to be the cause of the death. Two of the four uncertain SUD cases diagnosed with dilated cardiomyopathy following familial cardiac evaluation in this study were on the basis of more than one affected relative, which is strongly supportive of a genetic aetiology. The significant minority of diagnoses of cardiomyopathy amongst uncertain SUD cases may also be explained by the difficulty in securing a diagnosis on the basis of autopsy alone. The hallmarks of dilated cardiomyopathy are chamber dilatation and/or cardiomegaly; in the absence of fibrosis or antemortem diagnosis, unambiguously ascribing this as diagnostic of cardiomyopathy is unviable and would result in frequent overdiagnosis. Therefore, this genetic diagnosis may reasonably be over-represented amongst uncertain SUD cases, particularly those with evidence of chamber dilatation and/or cardiomegaly without fibrosis, consistent with our data.

Impact on diagnosis

The detection of other uncertain findings at autopsy that may lead to a presumptive but erroneous cause for sudden death warrants attention. For example, the appearance of moderate plaque disease and intramyocardial course of the left anterior descending artery prompted the initial clinical impression of possible coronary spasm; the SUD proband's mother was subsequently diagnosed with dilated cardiomyopathy. Moreover, detailed history revealed an extensive third-degree family history of premature sudden death amongst male members of his maternal at-risk relatives which had not previously been investigated.

Role in sudden death aetiology

Our clinical cardiogenetic yield was similar between uncertain SUD and true SADS cases. However, there was also a statistically significant excess of uncertain findings amongst unexplained SCD cases in comparison with non-cardiac death controls. Therefore, it is incongruent to view these uncertain findings as innocent bystanders in the apparently unexplained SCD; uncertain findings associate with predisposition to sudden death. Additionally, where a familial diagnosis was made, many of the uncertain autopsy findings appeared not to relate to the pathophysiology of the underlying cardiogenetic disorder. In these cases, it appears that the uncertain finding may contribute to, but not primarily cause, the SCD. Therefore, whether this represents a combination of underlying genetic predisposition with contributory acquired risk of 'uncertain' findings remains to be fully determined. Nonetheless, the combination of these findings seen in our study provides compelling evidence to support this premise.

Limitations

We were unable to associate non-histopathological findings, such as obesity, with SCD risk due to the use of BMI-matching for controls. Moreover, the use of alcohol and antidepressants in our control cases may not be representative of the underlying population, precluding analysis of these as risk factors for SCD outside cases with uncertain histological findings. Molecular autopsy was only undertaken following establishment of a familial cardiogenetic phenotype and next-generation sequencing was not employed routinely.

Conclusions

Isolated uncertain histological findings identified at specialist cardiac autopsy associate with unexplained SCD and should therefore be considered potentially relevant to the death. Comprehensive non-invasive cardiac evaluation of at-risk relatives following uncertain SUD identifies similar yield of cardiogenetic disease to true SADS cases, with primary arrhythmia syndromes predominating over myocardial diseases. We propose that at-risk relatives should be referred for cardiac evaluation focusing on phenotypic assessment for both ion channel disorders and cardiomyopathies following identification of uncertain cardiac findings at post-mortem examination after premature sudden death. The exact mechanism(s) whereby these uncertain post-mortem findings result in or contribute to death remains unclear, though mechanistically suggestive of substrate more at risk for a ventricular arrhythmic event.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

Conflict of interest: none declared.

References

1. Behr E, Wood DA, Wright M, Syrris P, Sheppard MN, Casey A, Davies MJ, McKenna W; Sudden Arrhythmic Death Syndrome Steering Group. Cardiological assessment of first-degree relatives in sudden arrhythmic death syndrome. *Lancet* 2003;**362**:1457–1459.
2. Miles CJ, Behr ER. The role of genetic testing in unexplained sudden death. *Transl Res J Lab Clin Med* 2016;**168**:59–73.

3. Priori SG, Wilde AA, Horie M, Cho Y, Behr ER, Berul C, Blom N, Brugada J, Chiang C-E, Huikuri H, Kannankeril P, Krahn A, Leenhardt A, Moss A, Schwartz PJ, Shimizu W, Tomaselli G, Tracy C, Ackerman M, Belhassen B, Estes NAM, Fatkin D, Kalman J, Kaufman E, Kirchhof P, Schulze-Bahr E, Wolpert C, Vohra J, Refaat M, Etheridge SP, Campbell RM, Martin ET, Quek SC. Executive summary: HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes. *Europace* 2013; **15**:1389–1406.
4. Kumar S, Peters S, Thompson T, Morgan N, Maccicoca I, Trainer A, Zentner D, Kalman JM, Winship I, Vohra JK. Familial cardiological and targeted genetic evaluation: low yield in sudden unexplained death and high yield in unexplained cardiac arrest syndromes. *Heart Rhythm* 2013;**10**:1653–1660.
5. Bagnall RD, Weintraub RG, Ingles J, Duflou J, Yeates L, Lam L, Davis AM, Thompson T, Connell V, Wallace J, Naylor C, Crawford J, Love DR, Hallam L, White J, Lawrence C, Lynch M, Morgan N, James P, Sart D, Du Puranik R, Langlois N, Vohra J, Winship I, Atherton J, McGaughran J, Skinner JR, Semsarian C. A prospective study of sudden cardiac death among children and young adults. *N Engl J Med* 2016;**374**:2441–2452.
6. Priori SG, Blomström-Lundqvist C, Mazzanti A, Blom N, Borggrefe M, Camm J, Elliott PM, Fitzsimons D, Hatala R, Hindricks G, Kirchhof P, Kjeldsen K, Kuck K-H, Hernandez-Madrid A, Nikolaou N, Norekvål TM, Spaulding C, Van Veldhuisen DJ. ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: The Task Force for the Management of Patients with Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death of the European Society of Cardiology (ESC). Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC). *Eur Heart J* 2015;**36**:2793–2867.
7. Papadakis M, Raju H, Behr ER, De Noronha SV, Spath N, Kouloubinis A, Sheppard MN, Sharma S. Sudden cardiac death with autopsy findings of uncertain significance: potential for erroneous interpretation. *Circ Arrhythm Electrophysiol* 2013;**6**:588–596.
8. Basso C, Aguilera B, Banner J, Cohle S, Amati G, d' Gouveia RH, de Gioia C, di Fabre A, Gallagher PJ, Leone O, Lucena J, Mitrofanova L, Molina P, Parsons S, Rizzo S, Sheppard MN, Mier MPS, Kim Suvarna S, Thiene G, Wal A, van der Vink A, Michaud K; Association for European Cardiovascular Pathology. Guidelines for autopsy investigation of sudden cardiac death: 2017 update from the Association for European Cardiovascular Pathology. *Virchows Arch* 2017;**471**:691–705.
9. Thompson T, Morgan N, Connell V, Zentner D, Davis A, Pflaumer A, Woodford NW, Winship I. The role of specialist nurses in cardiac genetics—the Victorian experience: supporting partnerships in care. *Aust J Adv Nurs* 2015;**33**:53–57.
10. Basso C, Burke M, Fornes P, Gallagher PJ, Gouveia RH, de Sheppard M, Thiene G, Wal van der; A. Association for European Cardiovascular Pathology. Guidelines for autopsy investigation of sudden cardiac death. *Virchows Arch* 2008;**452**:11–18.
11. Skinner JR, Duflou JA, Semsarian C. Reducing sudden death in young people in Australia and New Zealand: the TRAGADY initiative. *Med J Aust* 2008;**189**:539–540.
12. Marcus FI, McKenna WJ, Sherrill D, Basso C, Bauce B, Bluemke DA, Calkins H, Corrado D, Cox MGPJ, Daubert JP, Fontaine G, Gear K, Hauer R, Nava A, Picard MH, Protonotarios N, Saffitz JE, Sanborn DMY, Steinberg JS, Tandri H, Thiene G, Towbin JA, Tsatsopoulou A, Wichter T, Zareba W. Diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia: proposed modification of the Task Force Criteria. *Eur Heart J* 2010;**31**:806–814.
13. Wingren CJ, Ottosson A. Postmortem heart weight modelled using piecewise linear regression in 27,645 medicolegal autopsy cases. *Forensic Sci Int* 2015;**252**:157–162.
14. Govindan M, Batchvarov VN, Raju H, Shanmugam N, Bizrah M, Bastiaenen R, Kiotsekoglou A, Camm J, Behr ER. Utility of high and standard right precordial leads during ajmaline testing for the diagnosis of Brugada syndrome. *Heart* 2010; **96**:1904–1908.
15. Vyas H, Hejlik J, Ackerman MJ. Epinephrine QT stress testing in the evaluation of congenital long-QT syndrome: diagnostic accuracy of the paradoxical QT response. *Circulation* 2006;**113**:1385–1392.
16. Elliott P, Andersson B, Arbustini E, Bilinska Z, Cecchi F, Charron P, Dubourg O, Kühl U, Maisch B, McKenna WJ, Monserrat L, Pankuweit S, Rapezzi C, Seferovic P, Tavazzi L, Keren A. Classification of the cardiomyopathies: a position statement from the European Society Of Cardiology Working Group on Myocardial and Pericardial Diseases. *Eur Heart J* 2007;**29**:270–276.
17. Mellor G, Raju H, Noronha SV, de Papadakis M, Sharma S, Behr ER, Sheppard MN. Clinical characteristics and circumstances of death in the sudden arrhythmic death syndrome. *Circ Arrhythm Electrophysiol* 2014;**7**:1078–1083.
18. Lahrouchi N, Raju H, Lodder EM, Papatheodorou E, Ware JS, Papadakis M, Tadros R, Cole D, Skinner JR, Crawford J, Love DR, Pua CJ, Soh BY, Bhalshankar JD, Govind R, Tfelt-Hansen J, Winkel BG, Werf C, van der Wijeyeratne YD, Mellor G, Till J, Cohen MC, Tome-Esteban M, Sharma S, Wilde AAM, Cook SA, Bezzina CR, Sheppard MN, Behr ER. Utility of post-mortem genetic testing in cases of sudden arrhythmic death syndrome. *J Am Coll Cardiol* 2017;**69**:2134–2145.
19. Kotsis V, Tsioufis K, Antza C, Seravalle G, Coca A, Sierra C, Lurbe E, Stabouli S, Jelakovic B, Redon J, Redon P, Nilsson PM, Jordan J, Micic D, Finer N, Leitner DR, Toplak H, Tokgozoglu L, Athyros V, Elisaf M, Filippatos TD, Grassi G. Obesity and cardiovascular risk: a call for action from the European Society of Hypertension Working Group of Obesity, Diabetes and the High-risk Patient and European Association for the Study of Obesity: part B: obesity-induced cardiovascular disease, early prevention strategies and future research directions. *J Hypertens* 2018;**36**:1441–1455.
20. Noronha SV, de Behr ER, Papadakis M, Ohta-Ogo K, Banya W, Wells J, Cox S, Cox A, Sharma S, Sheppard MN. The importance of specialist cardiac histopathological examination in the investigation of young sudden cardiac deaths. *Europace* 2014;**16**:899–907.
21. Wolpert C, Echternach C, Veltmann C, Antzelevitch C, Thomas GP, Spehl S, Streitner F, Kuschyk J, Schimpf R, Haase KK, Borggrefe M. Intravenous drug challenge using flecainide and ajmaline in patients with Brugada syndrome. *Heart Rhythm* 2005;**2**:254–260.
22. Papadakis M, Papatheodorou E, Mellor G, Raju H, Bastiaenen R, Wijeyeratne Y, Wasim S, Ensam B, Finocchiaro G, Gray B, Malhotra A, D'Silva A, Edwards N, Cole D, Attard V, Batchvarov VN, Tome-Esteban M, Homfray T, Sheppard MN, Sharma S, Behr ER. The diagnostic yield of Brugada syndrome after sudden death with normal autopsy. *J Am Coll Cardiol* 2018;**71**:1204–1214.
23. Nademanee K, Raju H, Noronha SV, de Papadakis M, Robinson L, Rothery S, Makita N, Kowase S, Boonmee N, Vitayakritsirikul V, Ratanarapee S, Sharma S, Wal AC, van der Christiansen M, Tan HL, Wilde AA, Nogami A, Sheppard MN, Veerakul G, Behr ER. Fibrosis, Connexin-43, and conduction abnormalities in the Brugada syndrome. *J Am Coll Cardiol* 2015;**66**:1976–1986.
24. Corrado D, Zorzi A, Cerrone M, Rigato I, Mongillo M, Bauce B, Delmar M. Relationship between arrhythmogenic right ventricular cardiomyopathy and Brugada syndrome: new insights from molecular biology and clinical implications. *Circ Arrhythm Electrophysiol* 2016;**9**:e003631.
25. Finocchiaro G, Papadakis M, Robertus J-L, Dhutia H, Steriotis AK, Tome M, Mellor G, Merghani A, Malhotra A, Behr E, Sharma S, Sheppard MN. Etiology of sudden death in sports: insights from a United Kingdom Regional Registry. *J Am Coll Cardiol* 2016;**67**:2108–2115.
26. Gioia CRT, di Giordano C, Cerbelli B, Pisano A, Perli E, De Dominicis E, Poscolieri B, Palmieri V, Ciallella C, Zeppilli P, Amati G. D. Nonischemic left ventricular scar and cardiac sudden death in the young. *Hum Pathol* 2016;**58**: 78–89.
27. Zorzi A, Perazzolo Marra M, Rigato I, De Lazzari M, Susana A, Niero A, Pilichou K, Migliore F, Rizzo S, Giorgi B, De Conti G, Sarto P, Serratosa L, Patrizi G, De Maria E, Pelliccia A, Basso C, Schiavon M, Bauce B, Iliceto S, Thiene G, Corrado D. Nonischemic left ventricular scar as a substrate of life-threatening ventricular arrhythmias and sudden cardiac death in competitive athletes. *Circ Arrhythm Electrophysiol* 2016;**9**:e004229.
28. Hershberger RE, Siegfried JD. Update 2011: clinical and genetic issues in familial dilated cardiomyopathy. *J Am Coll Cardiol* 2011;**57**:1641–1649.