Clinical update

Sudden cardiac death in the young: the molecular autopsy and a practical approach to surviving relatives

Christopher Semsarian1,2,3*, Jodie Ingles1,2,3, and Arthur A.M. Wilde4,5

1 Agnes Ginges Centre for Molecular Cardiology, Centenary Institute, Sydney, Australia; 2 Sydney Medical School, University of Sydney, Sydney, Australia; 3 Department of Cardiology, Royal Prince Alfred Hospital, Sydney, Australia; 4 Heart Centre, Department of Cardiology, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands; and 5 Princess Al-Jawhara Al-Brahim Centre of Excellence in Research of Hereditary Disorders, Jeddah, Kingdom of Saudi Arabia

Received 8 December 2014; revised 15 February 2015; accepted 25 February 2015; online publish-ahead-of-print 12 March 2015

The sudden death of a young, apparently fit and healthy person is amongst the most challenging scenarios in clinical medicine. Sudden cardiac death (SCD) is a devastating and tragic outcome of a number of underlying cardiovascular diseases. While coronary artery disease and acute myocardial infarction are the most common causes of SCD in older populations, genetic (inherited) cardiac disorders comprise a substantial proportion of SCD cases aged 40 years and less. This includes the primary arrhythmogenic disorders such as long QT syndromes and inherited cardiomyopathies, namely hypertrophic cardiomyopathy. In up to 30% of young SCD, no cause of death is identified at postmortem, so-called autopsy-negative or sudden arrhythmic death syndrome (SADS). Management of families following SCD begins with a concerted effort to identify the cause of death in the decedent, based on either premorbid clinical details or the pathological findings at postmortem. Where no cause of death is identified, genetic testing of deoxyribonucleic acid extracted from postmortem blood (the molecular autopsy) may identify a cause of death in up to 30% of SADS cases. Irrespective of the genetic testing considerations, all families in which a sudden unexplained death has occurred require targeted and standardized clinical testing in an attempt to identify relatives who may be at-risk of having the same inherited heart disease and therefore also predisposed to an increased risk of SCD. Optimal care of SCD families therefore requires dedicated and appropriately trained staff in the setting of a specialized multidisciplinary cardiac genetic clinic.

Keywords
Sudden cardiac death • Postmortem • Molecular autopsy • Genetics • Specialized clinic • Multidisciplinary care

Introduction

Sudden cardiac death (SCD) is a tragic complication of a number of cardiovascular diseases and affects all ages.1–3 The death is often unexpected, and has a devastating impact on both the surviving family and the community. Sudden cardiac death is defined as a death occurring usually within an hour of the onset of symptoms, due to an underlying cardiac disease. The prevalence of SCD is significant, with up to 350 000 cases each year in the USA,4 translating to ∼950 deaths per day or one death every 1.5 min. Furthermore, the impact and public health burden in terms of life-years lost due to SCD in the young is greater than all individual cancers and most other leading causes of death.5

The incidence of SCD specifically in the young varies depending on the population studied and the methodologies used. The best estimate of the incidence of SCD in the general population aged 20–75 years is 1 in 1000 individuals that accounts for 18.5% of all deaths.6 In the 1–40 years age group, the incidence is 1.3–8.5 per 100 000 person-years, and up to 4.5 per 100 000 per annum amongst male US military recruits.7–11 Sudden cardiac death in children is at the lower end of incidence rates (1.3 per 100 000) while the higher incidence of SCD (8.5 per 100 000) is often amongst the older age groups closer to age 40 years, where outcomes are influenced by early coronary artery disease. Although relatively uncommon in terms of numbers, SCD of a young person is a tragedy and often perpetuated by both the sudden and unexpected nature of the event, and the victims often being apparently fit and healthy individuals with no history of symptoms or disease.

* Corresponding author: Tel: +61 2 9565 6195, Email: c.semsarian@centenary.org.au

Published on behalf of the European Society of Cardiology. All rights reserved. © The Author 2015. For permissions please email: journals.permissions@oup.com.
Causes of sudden cardiac death in the young

Sudden cardiac death can occur at all ages and, overall, coronary artery disease and acute myocardial infarction account for over 90% of cases. This distribution of cases is modified when considering SCD in the young, defined as those aged ≤40 years. Sudden cardiac death in the young is caused by a variety of disorders that can broadly be categorized into structural and purely arrhythmogenic causes (Figure 1). Structural causes of SCD include the inherited cardiomyopathies, such as hypertrophic cardiomyopathy (HCM), dilated and restrictive cardiomyopathies, arrhythmogenic right ventricular cardiomyopathy (ARVC, more recently AC), and left ventricular non-compaction. Other structural causes of SCD in the young include myocarditis, congenital heart diseases, and coronary artery disease. Hypertrophic cardiomyopathy remains the most common structural cause of SCD in the young, including competitive athletes, although in some regions of the world, ARVC may be the cause of SCD in up to 25%. Importantly, in all structural causes of SCD in the young, the postmortem examination has a high probability of identifying the cause of death.

In contrast, purely arrhythmogenic causes of SCD in the young are more difficult to identify as a cause of death at postmortem. These primary arrhythmogenic disorders include familial long-QT syndrome (LQTS), catecholaminergic polymorphic ventricular tachycardia (CPVT), Brugada syndrome (BrS), idiopathic ventricular fibrillation (IVF), and short-QT syndrome. Since these disorders rarely cause any structural change to the heart, postmortem is often ‘negative’, i.e. no cause of death is identified at postmortem, including normal histopathology and normal toxicology analysis (Table 1). Such cases in which no abnormalities are found at postmortem are often classified as ‘unascertained’ and this occurs in up to 30% of SCD cases in the young, strongly suggesting an underlying arrhythmogenic cause (Figure 1). The true incidence of SCD is likely to be an underestimate since primary arrhythmogenic disorders can predispose to more overt causes of death. For example, young deaths attributed to events such as ‘drowning’ and ‘motor vehicle accidents’ may have been directly precipitated by a ventricular arrhythmia, and is further illustrated by the association between swimming and development of ventricular arrhythmias in patients with familial LQTS and CPVT.
Sudden unexplained death cases have been given a number of different terms, including sudden unexplained/unexpected death (SUD), sudden unexplained/unexpected death syndrome (SUDS), sudden unexpected nocturnal death syndrome (SUNDS), and sudden unexplained death in the young (SUDY). For the purposes of this review, this group will be referred to as sudden arrhythmic death syndrome (SADS) with ‘young’ defined as age 1–40 years, and deaths in infancy at age 0–1 years referred to as sudden infant death syndrome (SIDS).23

Genetic testing in sudden death: the ‘molecular autopsy’

The identification of the cause of SCD in a young person is a critical step in determining whether there is an underlying genetic aetiology and if so, how this impacts on the clinical and genetic evaluation of surviving at-risk family members. This is particularly important in the evaluation of SADS cases where no cause of death is identified at post-mortem, i.e. SADS cases. The role of genetic testing in the setting of SADS cases was initiated over a decade ago.24 In SADS, where no cause of death is identified after comprehensive postmortem examination, genetic testing of the decedent’s blood sample (collected at postmortem) may identify an underlying cause. This process has been termed the ‘molecular autopsy’, and involves deoxyribonucleic acid (DNA) extraction from postmortem blood, followed by DNA analysis of selected candidate genes responsible for the main primary arrhythmogenic diseases (Figure 2).25

Most molecular autopsy studies performed to date are based on the current international Heart Rhythm Society USA/European Heart Rhythm Association (HRS/EHRA) guidelines, which recommend comprehensive, or targeted, postmortem genetic testing in SADS cases, i.e. a molecular autopsy, if circumstantial evidence points towards a clinical diagnosis of LQTS or CPVT.26 The molecular autopsy has focused on direct DNA sequencing of the protein coding exons of four genes, i.e. the three major LQTS genes (KCNQ1, KCNH2, SCN5A) and the CPVT gene (RYR2; Table 3)26–28
In addition, mutations in the SCN5A gene also cause BrS. While initial studies in selected SADS populations reported detection rates for a causative (pathogenic) mutation of up to 34%,24,29 more recent population-based studies suggest the detection rate with the four-gene molecular autopsy is more likely to be up to 15–20%.30 Indeed there has been a great variation in the diagnostic yield of the molecular autopsy, ranging from 0 to 35%.24,30–37 This largely reflects a range of clinical and methodological issues relating to the type of DNA obtained (blood vs. paraffin-embedded tissue), selection bias of the populations studied, the definition of sudden death (< 1 h to < 24 h of symptoms), different regulations regarding autopsy procedures, and variation in stringency and interpretation of DNA variants in terms of pathogenicity.

Important research into the clinical and genetic basis in SIDS amongst infants aged < 1 year has identified similar findings suggesting a proportion of deaths are due to underlying primary arrhythmogenic disorders.38–40 A number of studies suggest up to 10% of SIDS cases may have an underlying pathogenic mutation in the main LQTS genes,31 as well as some SIDS cases demonstrating potential pathogenic variants in other genes responsible for CPVT (RYR2) and Brugada syndrome (SCN5A).42,43 These findings have significant clinical implications both in terms of diagnosis and screening of family members, as well as highlighting the importance of a detailed family history including a history of SIDS cases.
Management of families with sudden cardiac death in the young

The diagnosis, management, and ongoing care of families in which SCD has occurred in a young relative are amongst the most challenging scenarios in clinical medicine. The death is by definition sudden, always unexpected, and typically occurs in an apparently well young person. Families, particularly parents, are confronted with many questions at the time of death. The two most commonly asked questions are ‘why did my son/daughter/sibling/partner die suddenly?’ and ‘how can we prevent this happening to any other members of our family?’. Indeed, it is on this basis that much of the management of these families is centred. In general terms, clinical management is guided by the goal to establish a cause of death (focusing on the victim), and the clinical screening and management of the surviving family members (focusing on the family).

Investigation of the victim (the decedent)

Gathering as much information about the decedent is a critical aspect of the initial investigation in establishing a cause of death, especially in cases where the sudden death is unexplained. Such an investigation should include obtaining a premorbid medical history such as syncopal episodes, exertional symptoms, intercurrent illnesses, recent pharmacological therapies, previous ECGs, or other relevant studies. This investigation should also include a comprehensive third-generation family pedigree focused on identifying a family history of cardiac disease, premature sudden death, or suspicious deaths, e.g. SIDS cases, drowning in experienced swimmers in shallow waters, and single car motor vehicle accidents. Other relevant family history information can include family members with epilepsy, identifying ‘fainters’, and any other unusual symptoms or clinical presentations. In addition, it is important to investigate the circumstances of SCD, including activity at the time of death, the level of physical activity, and symptoms immediately preceding the death. This often relies on obtaining information from available ambulance and police reports.

Ideally, a full postmortem examination by a trained cardiac pathologist should be undertaken in all cases of sudden death in the young. This includes detailed macroscopic and histological evaluation of the heart, as well as other key organs such as the brain, with the purpose to identify any non-cardiac causes of death, e.g. pulmonary embolism, before focusing on specific cardiac pathologies. Postmortem evaluation of the heart and specifically measurements of cardiac dimensions and wall thickness should be compared with appropriate autopsy-based reference values rather than echocardiography-based values in living subjects. Obtaining a postmortem blood sample in young SCD cases is now recommended by the HRS/EHRA guidelines and is mandated in several countries, including Australia and New Zealand. As indicated previously, the availability of the postmortem blood samples provides the opportunity to perform genetic studies that may provide valuable information to the family.

Despite our best efforts, the cause of SCD in a young individual is sometimes not established. Frequently the event is the first presentation of the disease or in some cases there may have been nonspecific symptoms that were never formally evaluated. Furthermore, the deaths are frequently un witnessed, and the ambulance and police reports shed little light on the circumstances at the time of death. The use of standardized questions for police and other authorities to use have been established in some countries and may assist in gathering more precise premorbid details. Following postmortem investigation, no cause is identified in up to 30%. Importantly, whether a cause of death is established or not, the possibility of an underlying inherited cardiac disorder remains in many cases, including the possibility of a primary arrhythmogenic disease in all SADS cases and their families. It should also be noted that recent studies suggest that in addition to true SADS cases, postmortem examinations may reveal some non-specific changes of uncertain clinical significance, such as unclassified ‘cardiomegaly’ or minor cardiac histopathological changes. Importantly, the chance of finding an underlying primary arrhythmogenic syndrome in these uncertain ‘borderline’ cases is as high as in those with true SADS.

Management of the surviving family

In SADS cases, underpinning the clinical evaluation and screening of family members is the presumption that the underlying cause was a primary inherited arrhythmogenic disorder such as LQTS, CPVT, or BrS. By definition these sudden death cases are unexplained at postmortem (‘autopsy negative’), and there is often no clue as to whether an arrhythmogenic disorder exists and if so, which disease. Importantly however, over 95% of cardiac genetic disorders in the general population are inherited as an autosomal-dominant trait such that first-degree relatives have a one in two (50%) chance of inheriting the same gene mutation. Therefore, standard approaches to clinical evaluation of first-degree relatives in the first instance is important, and may reveal the disease in the family.

The clinical investigation of the family, including first-degree relatives, obligate carriers, and symptomatic relatives, is summarized in Figure 3, and is based largely on the recent HRS/EHRA consensus document for inherited heart diseases. Clinical investigation involves two tiers of evaluation. All relatives should have a comprehensive medical and family history, physical examination, resting and exercise ECGs, and a standard transthoracic echocardiogram. Depending on the clinical situation, further second tier investigations may include cardiac magnetic resonance (CMR) imaging (e.g. suspected

### Table 3 Current 4-gene molecular autopsy

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Encoded protein</th>
<th>Disease</th>
<th>% of disease</th>
<th>% of SADS*</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCNQ1</td>
<td>I_S, K⁺ channel α-subunit</td>
<td>LQTS1</td>
<td>35–40</td>
<td>10–15</td>
</tr>
<tr>
<td>KCNH2</td>
<td>I_S, K⁺ channel α-subunit</td>
<td>LQTS2</td>
<td>30–35</td>
<td>1–5</td>
</tr>
<tr>
<td>SCNSA</td>
<td>I_Na, Na⁺ channel α-subunit</td>
<td>LQTS3, BrS</td>
<td>5–10</td>
<td>&lt;1</td>
</tr>
<tr>
<td>RYR2</td>
<td>Ryanodine receptor</td>
<td>CPVT1</td>
<td>60–65</td>
<td>10–15</td>
</tr>
</tbody>
</table>

LQTS, long-QT syndrome; BrS, Brugada syndrome; CPVT1, catecholaminergic polymorphic ventricular tachycardia type 1; SADS, sudden arrhythmic death syndrome (normal postmortem).

*Pick-up rate for molecular autopsy.
ARVC), 24 h ECG monitoring and signal-averaged ECG, and pharmacological challenge tests, such as a flecainide/ajmaline challenge in suspected BrS patients. Clinical evaluation alone in families with sudden unexplained death may identify an underlying cause in up to 50% of selected and comprehensively evaluated families. Concurrent with the clinical evaluation of the family, a molecular autopsy (i.e. postmortem genetic testing) may help to elucidate the cause of death in the deceased. In cases where a genetic diagnosis is made in the decedent, initial genetic testing should focus on the parents to determine whether the gene mutation is inherited, or arose de novo in the decedent. In sudden death cases where the identified mutation in the decedent did not arise de novo, the major utility of the genetic result is in cascade screening of at-risk family members. Offering cascade screening to asymptomatic relatives should always be performed in conjunction with clinical evaluation and only alongside comprehensive pre- and post-test genetic counselling. Importantly, in SADS cases where the postmortem was negative and no blood/DNA was collected, genetic testing should not be initiated in any living family relatives unless a clear disease phenotype is present. Using both clinical and genetic evaluation of the family, if an underlying diagnosis is made, then subsequent management and follow-up depends on the disease in question, and will often trigger more specific family cascade clinical screening and, if available, genetic testing. If no diagnosis is made after comprehensive clinical (+/- genetic analysis), then asymptomatic adult relatives are generally followed up till age 40 years (by which time most genetic heart diseases have expressed phenotypically), and can be discharged from care on the proviso that new symptoms or family information should be reported immediately. More commonly, the relative being screened is a child, in which case regular follow-up is indicated until adulthood (up to age 40 years), with the knowledge that many genetic heart diseases most commonly manifest clinical disease in the second decade of life, such as the inherited cardiomyopathies.

**Key role of specialized multidisciplinary cardiac genetic services**

Family management in the setting of SCD of a young person is complex and ideally suited to a multidisciplinary specialized approach. The range of management issues is diverse, including clinical cardiovascular care, genetic evaluation such as ordering genetic tests, interpretation of results and conveying the information to the surviving family, and managing the ongoing psychosocial wellbeing of the families. The specialized multidisciplinary cardiac genetic clinic is a model used globally, and is dedicated to cardiac genetics with appropriately trained staff. The multidisciplinary clinic provides expertise not only in the clinical and genetic aspects of disease, but also in the integration of key links with other critical members of the team in addition to the cardiologist, including genetic counsellors, geneticists, forensic pathologists, nurses, clinical psychologists, primary care physicians, and patient support groups (Figure 4).

**Emerging concepts in the investigation of sudden cardiac death**

Most recently, newer approaches to the investigation of SCD in the young, both from a clinical and genetic perspective, have emerged.
These advances provide opportunities to both improve the ability to identify the cause of death, as well as assist in further enhancing the opportunities to identify at-risk family members and thereby facilitate the initiation of appropriate prevention strategies. Three interesting recent advances have shown some early promise in contributing to the model of care in SCD families.

### The ‘exome-wide molecular autopsy’

Until recently, the molecular autopsy has been confined to 4 targeted genes focused on the common causes of LQTS, BrS and CPVT. Recent advances in next generation sequencing technologies have allowed ever expanding panels of genes (cardiac gene panels with up to 200 genes) to be resequenced from comparatively small quantities of DNA, with unprecedented throughput capabilities, and in a cost-efficient manner. This includes sequencing the protein coding exons of all $\approx 22,000$ genes, i.e. the exome, or the entire ‘genome’. Next generation sequencing technologies thus tantalizingly offer the technology for an ‘exome-wide molecular autopsy’, and potentially allow genetic testing of all major disease-associated genes, as well as genes less frequently involved in any given disease.

We recently adopted this exome-wide approach in a series of SADS cases. Using stringent pathogenicity criteria, a likely pathogenic variant was identified in 32% of cases. Moreover, the exome data provided a number of gene variants currently being evaluated for novel disease associations, as well as an archive of genotypes to be mined in the future as new SADS-associated genes are characterized. The exome-wide molecular autopsy was also reported in a single sudden death case, in which a myosin heavy chain ($\text{MYH7}$) gene mutation was identified in a female aged 16 years, further highlighting the possibilities of expanding the genetic basis of the molecular autopsy.

Such excitement has to be balanced with the challenges which are inevitable in trying to decipher extensive amounts of genetic data, determining which DNA changes are more likely to be pathogenic particularly given the decedent by definition has no phenotype, the emerging understanding of multigenic models of disease where several variants may cumulatively contribute to disease, and the inevitable clinical implications of incidental and secondary findings. There are also issues related to the level of consent, the often grey zone of research vs. clinical testing, and how genetic results should be reported. Thus, the use of whole-exome and genome approaches to the investigation of SADS needs to be approached with caution, rigorously evaluated in the research setting, so that we can fully understand the true trade-off between a potential increase in pick-up rate from the molecular autopsy, and identification of DNA variants which may play no role in the cause of death but be misinterpreted in the clinical setting as a causative gene mutation. These ‘variants of uncertain significance’ (VUS) should never be used in cascade genetic testing as their pathogenicity is unknown and may be reclassified over time as either pathogenic, but also benign.

### Role of postmortem computer tomography and cardiac magnetic resonance imaging

While the conventional postmortem remains the cornerstone of investigation of young sudden death cases, there is emerging evidence that other imaging modalities may be helpful in improving diagnostic accuracy in a non-invasive fashion, and may overcome some of the reservations by families to proceed with the postmortem for religious, logistic, personal, or cultural reasons. These modalities include computer tomography (CT) scanning and CMR imaging. Using postmortem imaging as an alternate, ‘minimally invasive’ autopsy has been proposed. The majority of studies to date examining the accuracy of postmortem CMR imaging examination have focused on foetal and neonatal deaths. Two recent studies have shown great promise in terms of utilizing CT and CMR imaging in determining the cause of sudden death in adults, including young adults with a range of genetic heart diseases including HCM and ARVC. While the studies represent small numbers of young sudden death cases, the possibility of additional diagnostic tools to elucidate the cause of SCD is an exciting development.
Providing psychological care for sudden cardiac death families

The clinical and genetic aspects of family management while important should not be the only factors to be considered. It is increasingly recognized that surviving family members, and in particular the mothers of the decedent, suffer significant and ongoing psychological issues after the death.42,63 In a recent study, more than half of mothers reported survey scores suggestive of probable clinical anxiety on average 4 years after the death.43 The profound grief of losing a family member so suddenly is something that the family never comes to terms with, particularly in cases where the cause of death remains undetermined. In an ideal setting, the ability to draw on the expertise of a psychologist within the multidisciplinary specialized clinic team would ensure timely psychological care could be delivered. In reality, this is difficult to implement and so providing referral to an external psychologist may be an effective alternative. The role of the cardiac genetic counsellor in providing education, normalizing grief responses and developing a strong bond with the family as a support person should not be underestimated and may provide some psychological benefit.64

Conclusions

Sudden cardiac death is a rare but tragic outcome of a number of cardiovascular diseases. In the young, genetic heart diseases are an important cause of SCD, and have major implications for surviving at-risk family members. Detailed evaluation of the premorbid history, a third-generation family pedigree, comprehensive and expert postmortem examination is essential. In SADS cases where no cause of death is identified at postmortem, timely and targeted clinical evaluation, coupled with genetic testing of postmortem blood in a specialized multidisciplinary clinic setting, are key. The ultimate goal is to prevent future adverse clinical outcomes and SCD events in surviving relatives. Newer genetic and imaging developments may further refine our diagnostic and management strategies for SCD families.

Funding

We acknowledge the support from the Netherlands CardioVascular Research Initiative: the Dutch Heart Foundation, Dutch Federation of University Medical Centres, the Netherlands Organisation for Health Research and Development, and the Royal Netherlands Academy of Sciences (CVON-Predict, AAMW). C.S. is the recipient of an National Research and Development, and the Royal Netherlands Academy of Sciences (CVON-Predict, AAMW) grant. C.S. is the recipient of an National Heart, Lung, and Blood Institute (U01 HL111997) grant. C.S. is the recipient of an National Health and Medical Research Council (NHMRC) Practitioner Fellowship (#1059156). J.I. is the recipient of an NHMRC and National Heart Foundation of Australia Early Career Fellowship (#1036756).

Conflict of interest: none declared.

References

genetic testing for the channelopathies and cardiomyopathies this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). Heart Rhythm 2011;8:1308–1339.


