Molecular autopsy in sudden cardiac death – ethical issues and clinical implication for relatives

Miruna Mihaela Micheu1,*, Ionuț Popescu2,3, Corneliu Octavian Căpățiñă1, Ligia Elena Barbarii4, Maria Dorobanțu1

Abstract: Sudden cardiac death is a devastating and tragic event, especially in young, fit and healthy individuals with no prior symptoms or medical conditions. In these cases, death is caused mainly by a variety of inherited cardiac disorders, either with structural abnormalities or purely electrical heart diseases. Although in most cases conventional autopsy is able to establish the cause of death, there are cases when standard post-mortem examination fails to reveal the underlying cause of decease. Molecular autopsy (post-mortem genetic testing) is a requisite for the accurate diagnosis and also a standard of care along with detailed cardiologic assessment and informed genetic counselling of surviving relatives. This multidisciplinary approach could prevent future tragic events and facilitate development of preventive strategies.

The goal of this review is to summarize the current state of knowledge regarding various genetic causes of sudden cardiac death emphasizing the requisite of post-mortem genetic testing for identifying surviving relatives at risk for future lethal events.

Key Words: molecular autopsy, sudden cardiac death, inherited cardiomyopathies, channelopathies.

When witnessed, sudden cardiac death (SCD) is defined as a natural unexpected death without an obvious non-cardiac cause occurring within 1 hour of symptom onset, while in unwitnessed settings the natural unexpected death occurs within 24 hours of last being observed in normal health [1, 2].

The incidence of SCD across Europe and United States is significant, but widely variable, existing studies reporting numbers ranging from 18.6 to 128 cases/100000 inhabitants/year depending on the studied population and the methodologies used [3–6]. Nevertheless, exact numbers are absent for many regions of the world both in general population and in young people [7].

Existing studies reported that SCD is relatively rare under the age of 40 years, with an incidence between 1.3–8.5/100000 persons/year [8, 9], but it might be undervalued since not all possible cases of SCD - such as drowning or traffic accidents - were included [10]. By its unexpected nature, SCD is a devastating and tragic event, especially in young, fit and healthy individuals with no prior symptoms or medical conditions [6]. In these cases, SCD is caused mainly by a variety of inherited cardiac disorders, either with structural abnormalities or purely electrical heart diseases. Although in most cases conventional autopsy is able to establish the cause of death, there are cases when standard post-mortem

1) Clinical Emergency Hospital of Bucharest, Department of Cardiology, Bucharest, Romania

* Corresponding author: Clinical Emergency Hospital of Bucharest, 8th Floreasca way, 1st District, 14461, Bucharest, Romania, Email: mirunamicheu@yahoo.com

2) “Carol Davila” University of Medicine and Pharmacy, Department of Legal Medicine and Bioethics, Bucharest, Romania

3) "Mina Minovici" National Institute of Legal Medicine, Dept. of Legal Medicine, Bucharest, Romania

4) "Mina Minovici” National Institute of Legal Medicine, Dept. of Forensic Genetics, Bucharest, Romania
examination fails to reveal the underlying cause of decease, particularly in case of children and young adults. Studies have shown that in previously healthy children, adolescents and young adults 3% to 5% of sudden deaths are classified as autopsy-negative sudden unexplained death (SUD), with no detectable anatomic anomalies [8-10]. In Europe, about 5-10% of sudden death are SUD, usually supposed to be the consequence of sudden arrhythmic death syndrome [11].

Post-mortem genetic testing is recommended in such cases, as a critical step in determining whether there is an underlying genetic aetiology of death. Post-mortem genetic testing – or molecular autopsy - comprises deoxyribonucleic acid (DNA) extraction from post-mortem blood or tissues (if possible fresh or frozen), followed by DNA analysis of genes known to be responsible for the inherited cardiac disorders. This additional analysis added to the standard autopsy could explain up to 15–25% of SUD.

A precise diagnosis regarding the cause of death have huge implications on clinical and genetic evaluation of surviving at-risk family members, taking into account that a family history of cardiac arrest in a first-degree relative has been proved to be associated with a two-fold increase in risk of fatal events [12]. The management of surviving relatives is centred on post-mortem findings in victim, which triggers explicit genetic counselling, extended screening and targeted strategies in order to prevent future tragedies [6, 13].

In spite of clinical recommendations on genetic testing, the management of SCD victims and their families is still a challenging issue [14].

A survey of current forensic autopsy practices in SCD victims conducted in 2011 revealed that molecular autopsy was performed in only a few academic centers. An on-line questionnaire addressing routine procedures employed in cases of SCD (e.g. autopsy ordering, cardiac examination, sampling and storage of material for genetic analyses, molecular autopsy) was sent by e-mail to members of different forensic medical associations. More than half of respondents (60% out of 97) testified the lack of required resources to perform genetic post-mortem testing, while 40% reported not even to collect adequate biological samples for subsequent analysis, despite working at university hospitals [14].

These data highlights the importance in establishing guidelines for genetic testing in legal medicine.

As a result, recent guidelines concerning forensic examination in SUD recommend genetic testing as standard of care, especially in young victims. The “2015 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death” presents DNA testing as a major component of post-mortem examination, molecular analysis of biological samples being a Class I recommendation in all victims of SUD. The guidelines also acknowledge the correct aetiological diagnosis as a key step towards efficient management of patients and the prevention of SCD. Consequently, cascade screening should always be performed in families with a history of SCD in young people in order to enable precocious diagnosis and better prognosis through lifestyle modifications and targeted therapies [15]. According to ESC recommendations, “a properly conducted autopsy should provide answers to the following issues: (I) whether the death is attributable to a cardiac disease, (II) the nature of the cardiac disease (if present), (III) whether the mechanism of death was arrhythmic, (IV) whether there is evidence of a cardiac disease that may be inherited and thus requires screening and counselling of relatives and (V) the possibility of toxic or illicit drug use or other causes of unnatural deaths” [16].

Similar to violent death, SCD has been recognised as a source of extreme distress in loved ones left behind, with increased morbidity and mortality in the mourning period [17]. Often, family members experience post-traumatic stress disorder, complicated grief or depression. In circumstance of an inherited heart condition, the bereaved families face additional challenges, struggling with the uncertainty as regards their own future risk for tragic events. In this regard, establishing the actual cause of decease is a key element in understanding and accepting the unexpected tragedy, as well as in understanding whether the risk of life-threatening events may extend to other family members.

Frequent inherited cardiac disorders as underlying cause of SCD

Recently, the importance of genetic disorders affecting either the integrity of the heart’s muscle or its electrical function as underlying cause of SCD has been widely acknowledged.

Two types of monogenic heart disease predispose to SCD: familial cardiomyopathies and disorders of heart rhythm. There is mounting evidence that in some families coexist more than one disease-causing mutation, in either different alleles of the same gene, or in different genes [18, 19].

Cardiomyopathies are defined by both structural and functional abnormalities of the ventricular myocardium that are unexplained by flow limiting coronary artery disease or abnormal loading conditions [20]. Due to particular macroscopic findings, the autopsy has a high probability of identifying the cause of death.

Inherited cardiomyopathies encompass hypertrophic cardiomyopathy (HCM), dilated and restrictive cardiomyopathies, arrhythmogenic right ventricular cardiomyopathy (ARVC), and left ventricular non-compaction.

Hypertrophic cardiomyopathy is primarily characterized by increased LV wall thickness that is not
Mutations in the genes encoding beta-myosin heavy chain (MYH7) and myosin-binding protein C (MYBPC3) explain 75% of inherited HCMs. Other sarcomeric genes such as cardiac troponin I and T (TNNT2, TNNI3), tropomyosin alpha-1 chain (TPM1) and myosin light chain 3 (MYL3), as well as non-sarcomeric genes (e.g. genes encoding plasma membrane and mitochondrial proteins), can be affected [21, 26].

It has been shown that about 5% of patients with familial HCM have multiple sarcomeric protein mutations, with up to three different mutations; these heterozygous status have been associated with a more severe phenotype and a poorer prognosis [18, 27].

Recently, Hartmannova and colleagues brought into question the X-linked inheritance of HCM caused by a mutation in the gene encoding the four-and-a-half LIM domain 1 (FHL1) [28].

Also, sporadic HCM cases due to de novo mutations have been described [26].

Dilated cardiomyopathy (DCM) is characterized by left ventricle dilatation and systolic dysfunction in the absence of abnormal loading conditions or coronary artery disease sufficient to cause global systolic impairment [20].

Mutations in more than 50 genes have been found to cause familial DCM, the majority prompting the disease in an autosomal dominant manner, with reduced penetrance and variable expressivity. Also, autosomal-recessive mutations and X-linked recessive inheritance are associated with inherited DCM.

Among the most common affected genes are those encoding sarcomere and desmosomal proteins, approximately 20-25% of cases being attributed to protein-truncating mutations in titin (TTN) [29]. However, in patients with conduction diseases, mutations in lamin A/C (LMNA) and desmin have been described quite frequently [30].

Restrictive cardiomyopathy is the rarest form of all the cardiomyopathies; it is defined by impaired muscle relaxation, normal or reduced diastolic volumes of one or both ventricles, normal or reduced systolic volumes and normal ventricular wall thickness [16].

Mutations in sarcomeric protein genes such as TNNI3 and cardiac myosin binding protein C (MYBPC3) have been linked to familial form [31].

Arrhythmogenic right ventricular cardiomyopathy is characterized by progressive replacement of the myocardium by adipose and fibrous tissue [32, 33]; the right ventricle is primarily affected, but left ventricle involvement have been described in over 50% of cases [34].

Existing data estimate the prevalence of ARVC between 1 in 1000 to 1 in 5000 cases in general population; moreover, this inherited condition have been proved to be an important cause of SCD in young adults, accounting for up to 25% of unexpected deaths in children and adolescents between 1 and 19 years of age [35]. Since arrhythmias are often triggered by exercise, is not surprising that ARVC has been reported as the main cause of SCD in the young athletes [36].

ARVC is usually inherited in an autosomal dominant manner, produced by mutations in genes encoding desmosomal proteins. In this regard, ARVC can result from mutations in at least eight genes: TGFBR3 (locus: ARVD1), RYR2 (ARVD2), TMEM43 (ARVD5), DSP (ARVD8), PKP2 (ARVD9), DS1G2 (ARVD10), DSC2 (ARVD11), and JUP (ARVD12), PKP2 mutations being the most common. Four additional genes associated with ARVC have been mapped but not yet identified, namely locus ARVD3, ARVD4, ARVD6, and ARVD7 respectively [37].

Mutations in non-desmosomal genes and rare recessive forms (e.g. Carvajal syndrome and Naxos disease) have been also described [38].

Left-ventricular non-compaction is a genetically heterogeneous disorder with a sporadic and familial form, characterized by prominent ventricular trabeculations and deep intertrabecular recesses in the left and/or right ventricle, which are often associated with a thin compacted epicardial myocardial layer [16, 39].

Familial recurrence varies between 18 and 50% of the cases, commonly with an autosomal dominant pattern of inheritance, but autosomal recessive and X-linked inheritance were witnessed too [40]. Various mutations in genes encoding sarcomeric, cytoskeletal and nuclear membrane proteins have been linked to familial form [41].

Inherited primary arrhythmia syndromes recognized to predispose to SCD in children and young adults include long QT syndrome (LQTS), short QT syndrome (SQTS), Brugada syndrome (BrS) and catecholaminergic polymorphic ventricular tachycardia (CPVT).

Congenital arrhythmia syndromes are primarily caused by mutations in genes encoding either ion channel subunits or proteins that interact with ion channels.

Studies conducted in the field identified three mechanisms responsible for arrhythmia predisposition: abnormal repolarization (LQTS, SQTS, BrS), slow ventricular conduction (BrS), and aberrant intracellular Ca²⁺ homeostasis (CPVT) [42].

Long QT syndrome. The inherited form occurs in approximately 1 in 2500–3500 individuals [43]. It is
characterized by ventricular repolarization anomalies (QT interval prolongation and T wave abnormalities) and increased risk of potentially fatal ventricular arrhythmias in apparently healthy young adults and children [42]. Epidemiological studies estimate the annual rate of SCD in patients with untreated LQTS between 0.33 and 0.9%, while syncope is estimated to be present in about 5% of cases [16, 44, 45].

From a genetic point of view, LQTS is quite heterogeneous; up to date, mutations in 13 genes encoding voltage-gated K+ channel subunits (KCNQ1, KCNH2, KCNE1, KCNE2) [46, 47], voltage-gated Na+ channel subunits (SCN5A, SCN4B) [48, 49], an L-type Ca2+ channel (CACNA1C) [50], inwardly rectifying K+ channels (KCNJ2, KCNJ5) [51, 52], and various channel-interacting proteins (ANK2, CAV3, AKAP9, SNTA1) [53-56] have been identified [42]. Based on the underlying monogenic defect, over ten different types of LQTS have been described.

The most common genetic subtype is LQT1, caused by mutations in KCNQ1 gene [47]. Alteration in KCNQ1, KCNH2 and SCN5A genes explain approximately 90% of positively genotyped cases [38, 46, 57].

The syndrome is usually transmitted in an autosomal-dominant fashion as isolated prolongation of the QT interval (Romano-Ward syndrome) or accompanied by extracardiac manifestation (Andersen–Tawil and Timothy syndrome) [50, 51, 58], and less commonly as an autosomal recessive disease combined with congenital deafness (Jervell and Lange-Nielsen syndrome) [59].

Short QT syndrome is characterized by a reduced duration of cardiac repolarization and an increased risk of atrial and ventricular arrhythmias. The condition is associated with a high incidence of SCD in newborns and young patients, the likelihood of a first cardiac arrest by the age of 40 years exceeding 40% [60].

Six genes have been linked to SQTS, encoding either K+ channel (KCNQ1, KCNH2, KCNJ2) [61] or Ca2+ channel components (CACNA1C, CACNB2, CACNA2D1) [62, 63].

Brugada syndrome is characterized by specific baseline or induced ECG pattern (i.e. ST-segment elevation in leads V1–V3 with complete or incomplete right bundle branch block and normal QT intervals) and life-threatening ventricular arrhythmias usually occurring during sleep, in the absence of myocardial ischemia, electrolyte abnormalities or structural heart disease [64].

The ECG abnormalities may not be observable until exposed by administration of Na+ channel blocking agents (flecainide, ajmaline or procainamide) [65], fever [66], excessive alcohol intake or large meals.

The prevalence varies from 1 in 1000 to 1 in 10000 with higher occurrence in individuals from Southeast Asian countries [67]. Inheritance is autosomal dominant with incomplete age and sex-related penetrance. Characteristically, symptoms (syncope and life-threatening ventricular arrhythmias) occur during sleep, in men in the fourth decade [68].

Mutations in 16 genes have been associated with Brugada syndrome (SCN5A, SCN1B, SCN2B, SCN3B, GPD1L, CACNA1C, CACNB2, CACNA2D1, KCND3, KCNE3, KCNE1L (KCNE5), KCNJ8, HCN4, RANGERF, SLMAP, and TRPM4), SCN5A being the most frequently affected [69].

Catecholaminergic polymorphic ventricular tachycardia. According to “2015 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death”, CPVT is characterized by a structurally normal heart, normal resting ECG and exercise- or emotion-induced bidirectional or polymorphic ventricular tachycardia.

The condition has an estimated prevalence of 1 in 10000 [70]. It is typically diagnosed during childhood - the average age of onset of symptoms being between age seven and twelve years old [71]. Without appropriate treatment, the disease is highly fatal, since in approximately 30% of cases sudden cardiac arrest is the first manifestation [72].

CPVT is transmitted in families as an autosomal dominant trait (mutations in RYR2 encoding the cardiac ryanodine receptor/Ca2+ release channel) [73] or less commonly as an autosomal recessive disease (mutations in CASQ2, encoding the Ca2+-binding protein calsequestrin) [38, 74].

Mutations in other four genes (KCNJ2, Ank2, TRDN and CALM1) have been linked to CPVT or related phenotypes of adrenergically induced life-threatening arrhythmias [75].

**SCD in selected populations - athletes**

Unexpected death in young competitive athletes is a sporadic yet highly visible and devastating event that generates extensive publicity. In recent years, numerous studies provided valuable insights as regards the challenges faced when assessing underlying cause of SCD in athletes [22, 76, 77].

The precise incidence of SCD in young athletes is not known, existing data being quite heterogeneous in terms of studied population and reported numbers. Conducted studies revealed incidences ranging between 1 per 917000 and 1 per 3000 depending on population, geographic region and methodology [77, 78].

As regarding the aetiology, current data are limited mainly by the lack of a comprehensive forensic examination concluded by an experienced cardiac pathologist. A study conducted by de Noronha and colleagues comparing the interpretation of postmortem conclusions between a referring pathologist and a specialist cardiac pathologist demonstrated a 40% discrepancy with respect to the real cause of death [79].
CONCLUSIONS

Inherited cardiac diseases are an important cause of SCD, especially in the young. Molecular autopsy is a requisite for the accurate diagnosis and also a standard of care along with detailed cardiologic assessment and informed genetic counselling of surviving relatives. This multidisciplinary approach could prevent future tragic events and facilitate development of preventive strategies.

Conflict of interest. The authors declare that they have no conflict of interest concerning this article.

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Micheu MM et al. Molecular autopsy in sudden cardiac death – ethical issues and clinical implication for relatives
