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Genetic analysis of sudden unexplained death: A multidisciplinary approach

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ABSTRACT

Each year infants, children and young adults die suddenly and unexpectedly. In many cases the cause of death can be elucidated by medico-legal autopsy, however, a significant number of these cases remain unexplained despite a detailed postmortem investigation and are labeled as sudden unexplained death (SUD). Post-mortem genetic testing, so called molecular autopsy, revealed that primary arrhythmogenic disorders including long QT syndrome and catecholaminergic polymorphic ventricular tachycardia (CPVT) may account for a certain number of these cases. Because of the inheritance of these diseases, close relatives of the deceased may also at potential risk of carrying fatal cardiac disorders. Therefore, advanced diagnostic analyses, genetic counseling and interdisciplinary collaboration should be integral parts of clinical and forensic practice. In the present study, we performed mutation analyses of the major genes causing cardiac channelopathies in 15 SUD cases. In four cases we found putative pathogenic mutations in cardiac ion channel genes. Clinical and genetic examination of family members of SUD victims was also performed and affected family members were identified. This study demonstrates that molecular genetic screening needs to become an inherent part of the postmortem examination. This will enhance the ability of screening family members of SUD victims who may be at risk. The present data also illustrate that detection and follow up of familial cases of sudden death is challenging and requires a close multidisciplinary collaboration between different medical disciplines, with great responsibility for the forensic pathologist.

1. Introduction

Sudden cardiac death (SCD) is an unexpected death due to heart problems, which occurs within a short period of time from the onset of any heart related symptoms. As a leading cause of death in developed countries, coronary heart diseases account for the majority of these death cases [1].

A significant number of sudden deaths in young people (<35 years) remain unexplained even after comprehensive postmortem investigation, including autopsy, and are labeled as autopsy-negative sudden unexplained death (SUD) [2–4]. Nearly half of the young victims had been in good health without any warning signs. Their death often occurs as the sentinel event, thus, challenging the forensic pathologist [5]. Based on impressive advances in molecular biology, it is assumed that the majority of cardiac disorders leading to sudden death in young people are due to genetic abnormalities [6].

Genetic heart diseases can be broadly categorized into those in which structural changes are predominant, such as hypertrophic cardiomyopathy (HCM), arrhythmogenic right ventricular cardiomyopathy (ARVC) or diseases with primary arrhythmogenic abnormality, which includes long QT syndrome (LQTS), Brugada syndrome, catecholaminergic polymorphic ventricular tachycardia (CPVT) and short QT syndrome [7]. These primary electrical diseases are caused mainly by mutations in genes encoding cardiac ion channels and receptors. Most mutations leading to ion channel dysfunctions alter the electrical activity in the heart and predispose individuals to fatal cardiac arrhythmia, without morphological changes of the cardiac tissue. In population-based studies at least one third of SUD in young were found to have a

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genetic origin, such as mutations in the gene of the cardiac ryanodine receptor (*RyR2*) which accounts for nearly 14% of these cases [8]. Behr et al. [2] performed a detailed cardiovascular evaluation of relatives in 32 cases of SUD and found that in 22% of these families signs of an inherited cardiac disease, most likely long QT syndrome were diagnosed. Tan et al. [9] observed that in 28% of such families cardiac channelopathies were present following a clinical assessment of first-degree relatives of young SUD victims, thus, pointing to the importance of extensive testing of SUD victims and/or family members by clinical and genetic evaluation [10,11]. Considering that clinical and postmortem genetic analyses suggest that approximately one-third of SUD cases after the first year of life may be the result of an inherited lethal channelopathy, postmortem genetic screening has an important role in elucidating the cause of death and should be carried out as a standard care for SUD [12,13].

In this study we present a multidisciplinary collaboration with the aim of proper management of families where there had been SUD in young. This multifaceted approach is crucial for the management of the clinical, genetic, psychological and social complexities observed in surviving family members.

2. Material and methods

2.1. Study subjects

For the present genetic study, fifteen forensic cases of SUD were selected. The cases were selected on the basis of the clinical history, circumstances of death and autopsy findings. The deceased were 1–40 years old, had been seen alive 24 h before death and the death was unexplained, because no relevant medical history was known. Only in one case a Brugada syndrome had been diagnosed. In no case abnormalities had been detected by medico-legal autopsy, including toxicological investigations and histological examinations of heart tissue. Medico-legal investigations were done according to [14]. Most of the histological examinations were performed according to the guidelines for autopsy investigation of sudden cardiac death [15], but using different staining procedures, i.e. hematoxylin–eosin, Azan and PAS stainings of the myocardium, in some special cases an additional staining with Prussian blue was performed. The study was approved by the local ethics committee.

2.2. Genetic analysis

Genetic analysis was carried out, when the cause of death was not elucidated by autopsy. DNA was extracted from blood and tissue samples using standard phenol–chloroform procedures. Targeted mutational analysis of the major Ion-channel genes *SCN5A* (NC_008934), *KCNH2* (NC_008916), *KCNQ1* (NG_008935), *KCNE1* (NC_009091), *KCNE2* (NC_008804), *KCNJ2* (NC_000017), *SCN4B* (NG_011710), *ANK2* (NG_009006.2), *CASQ2* (NG_008802.1) and of the cardiac ryanodine receptor gene *RyR2* (NG_008799) including 20 exons (3, 8, 14, 15, 44–47, 49, 83, 88, 90, 93, 96, 97, 100–103, 105) was performed using polymerase chain reaction (PCR) with published [16–18] and redesigned primers (primer sequences upon request). These exons composing the most affected regions related to LQTS, SQTS, CPVT and the Brugada syndrome. For instance, nearly 90% of *RyR2*-mutation positive cases would be discovered by selective analysis of these exons [19]. Genes with a rare frequency, are not completely screened so far. Direct sequencing of the amplicons was performed as described previously [17]. A control group of 200 healthy and unrelated subjects (400 alleles) was screened by denaturing high-performance liquid chromatography (DHPLC) to exclude DNA polymorphisms.

2.3. Assessment of relatives

The second aim of the study was to include first-degree relatives of the victim with potentially pathogenic mutations into the genetic screening. The family of the deceased was informed with the permission of the investigating prosecutor about the results of the genetic analysis. All family members were encouraged to seek genetic counseling. After obtaining informed consent from the relatives, blood samples were collected for genetic analysis. In one case (case 15) genetic screening of the first-degree relatives of the deceased was requested by the family, since no postmortem genetic screening had been performed after autopsy ten years ago. The results of the genetic analysis were transmitted to the relatives either by the cardiologist and/or the genetic counselor. If a mutation in the genes of the SUD victim was identified, the first-degree relatives underwent cardiological assessment that included a detailed clinical, family history and physical examination, a 12-lead electrocardiogram, exercise-testing, echocardiogram, antiarrhythmic drug provoking test, 24-hour-holter monitoring and possibly invasive electrophysiological diagnostic. Standard criteria for the diagnosis of LQTS [20], Brugada syndrome [21,22] and CPVT [1,23] were used.

3. Results

A multidisciplinary collaboration was established between the departments of medical genetics, forensic medicine and a cardiology center. 15 SUD cases were identified where morphological changes of the heart were not detected by autopsy and an arrhythmic syndrome had been assumed. If a mutation was detected by genetic testing, the investigating prosecutor was informed. With his permission the forensic pathologist was able to approach the family suggesting genetic counseling, genetic analysis and cardiological assessment (Fig. 1).

Table 1 summarizes the characteristics of the 15 SUD cases. The mean age of the deceased was 24.6 years ± 9.7 (mean ± SD). A family history of SUD was reported in three cases (case 11, 13, 15) and medical history of seizures or syncopes before SUD in three cases as well (case 5, 13, 15), whereas in 12 cases (80%) sudden death was the sentinel event. However, in one case (case 6) a cardiac channelopathy had been suspected, but no risk evaluation or treatment had been performed. Most of the deaths occurred during sleep (10 cases).

3.1. Postmortem genetic testing

We found that four of the fifteen deceased exhibited rare sequence variations in the *SCN5A*-, *KCNJ2*- and *RyR2* genes, which are potentially pathogenic. All mutations are affecting highly conserved amino acid residues and were absent in 200 healthy control samples (Fig. 2). Additionally two sequence variations were identified leading to an amino acid replacement, but with predicted benign properties. All variants where screened in gene specific databases and in the exome sequencing databases 1000 genome (http://www.1000genomes.org) and the Exome sequencing project (http://evs.gs.washington.edu/EVS). Predictions of the possible impact of these variations on structure and function of the protein involved were performed using PolyPhen2 (http://genetics.bwh.harvard.edu/pph2). The presumed pathogenic and benign mutations are listed in Table 2.

3.2. Identification of disease-carrying relatives

First-degree relatives from three of the four mutation-positive deceased were tested. One family declined genetic screening.

In all families the detected mutations were found to be inherited in an autosomal-dominant trait (Fig. 3). Although incomplete penetrance and variable expression was the norm,

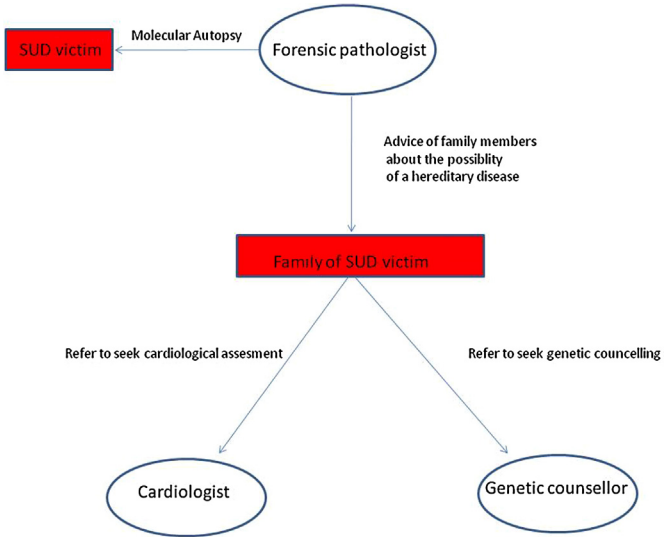

Fig. 1. Flow chart of a multidisciplinary cooperation in cases of SUD.