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# Relevance of molecular testing in patients with a family history of sudden death

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ABSTRACT

Sudden cardiac death (SCD) is a major cause of death in industrial countries. Although SCD occurs mainly in adults, it may also affect young persons, where genetic cardiac disorders comprise at least half of these cases. This includes primary arrhythmogenic disorders such as long QT syndrome and inherited cardiomyopathies. However, in many cases, postmortem examinations provide no conclusive results explaining the cause of death. Since family members of the deceased may eventually have inherited the same disease, they are at risk of SCD.

In the present study, 28 patients with a family history of sudden unexplained death (SUD), of survived cardiac arrest and with a clinical diagnosis of an inherited cardiac disease were screened using phenotype-guided molecular analysis of genes associated with arrhythmogenic cardiac diseases. In 64% of the cases, gene variants with potentially pathogenic cardiac effects were detected suggesting that an arrhythmia syndrome may have caused the death of the deceased family member. Therefore, we recommend that relatives of SUD victims should undergo extended cardiac examination and, depending on the clinical diagnosis, a targeted genetic analysis should follow, which is crucial to identify family members at risk.



## 1. Introduction

Sudden cardiac death (SCD) as defined by the World Health Organization, takes place during the first hour after the onset of symptoms in patients with known or unknown cardiac diseases [1]. SCD is a leading cause of death in industrial countries with an incidence of 18.6–126/100,000 per year. For the German State Lower Saxonia, an incidence rate of 81/100,000 was recently established [2]. It is generally accepted that coronary artery disease and myocardial infarction are the most common cardiac pathologies underlying SCD in elderly people, whereas in the younger population, a considerable proportion of SCD can be attributed to hereditary structural cardiac abnormalities such as hypertrophic/dilated cardiomyopathy (HCM/DCM) and arrhythmogenic right ventricular cardiomyopathy (ARVC). Besides these structural abnormalities, a certain number of sudden deaths, particularly in young people, remain unexplained even after detailed postmortem investigations. Several studies including cardiological assessment of first-degree relatives and postmortem genetic analysis have shown that primary cardiac arrhythmogenic diseases such as long QT-syndrome (LQTS), catecholaminergic polymorphic ventricular tachycardia (CPVT), or Brugada syndrome (BrS) account for a significant number of sudden unexplained death (SUD) cases [3–6]. A wide spectrum of pheno- and genotypical expressions, ranging from asymptomatic patients with a normal resting ECG to patients with distinct QT prolongation, eventually may cause sudden death [7].

Since these cardiac diseases are often inherited, close relatives of the deceased may also be at risk of exhibiting potentially fatal cardiac disorders. Therefore, extensive evaluation, including medical history, clinical parameters and genetic examination of SUD victims and in particular of their family members should be an integral part of clinical and forensic practice [8–11].

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The aim of the present study was to determine the value of clinical and genetic investigations in families with a history of SUD to underscore the importance of postmortem genetic testing as a prospective life saving clue. Adequate management of such cases requires a multidisciplinary approach including the general practitioner who certified death, the forensic pathologist performing the autopsy and collecting the samples, the case manager to inform the family, geneticist and cardiologist for clinical as well as genetic investigations of family members to evaluate their risk of sudden death.

## 2. Methods

### 2.1. Study group

Patients (n = 28) from the implantable cardioverter defibrillator (ICD) outpatient clinic were evaluated. Inclusion criteria were: survived cardiac arrest, absence of structural cardiac abnormalities, except in the ARVC group, a family history of SUD and the presumed diagnosis of an inherited cardiac disease based on clinical findings. Patients with the diagnosis HCM/DCM patients were not available to include in this study.

In most of the cases, no autopsy was performed or no detailed information about the postmortem investigations was available.

### 2.2. Clinical examination of affected family members with a family history of SUD

Before implantation of the ICD, patients underwent complete cardiac examination including detailed clinical history evaluation, 12-lead electrocardiogram, exercise-testing, 24-h-holter monitoring, coronary angiography, transthoracic echocardiography, and eventually electrophysiological tests. If the diagnosis of ARVC was assumed, cardiac magnetic resonance (CMR) imaging was performed. Standard criteria for the diagnosis of LQTS, Brugada syndrome [12], ARVC [13] and CPVT [12] were used as described. If type 2 or 3 of the Brugada-ECG had been noticed, a provoking test with the sodium channel blocker ajmaline was carried out with respect to the ability to unmask a possibly latent diagnostic Brugada-ECG. In three families, extended examination including first degree relatives was performed.

### 2.3. Genetic screening

This study was approved by the local ethical committee. All patients were encouraged to seek genetic counseling. After obtaining informed consent, blood samples were taken for genetic analysis. Genetic screening included the major LQTS, BrS as well CPVT related genes *SCN5A* (NM_198056), *KCNH2* (NM_000238), *KCNQ1* (NM_000218), *KCNE1* (NM_000219), *CASQ2* (NM_008802.1), 20 exons (3, 8, 14, 15, 44–47, 49, 83, 88, 90, 93, 96, 97, 100–103, 105) of the cardiac ryanodine receptor gene *RyR2* (NM_001035.2) and the major ARVC genes *PKP2* (NM_001005242.2), *DSG2* (NM_001943.3), *DSC2* (NM_024422.4) and *DSP* (NM_004415.2). Coding regions of these genes exhibit most of the known mutations related to LQTS, SQTS, BrS, CPVT and ARVC. Polymerase chainreaction (PCR) was performed using published [14,15,16] and redesigned primers (primer sequences upon request). Nucleotide sequence analysis was carried out using the ABI 3130 automated sequencer (Applied Biosystems, Germany). The data were analyzed applying the SeqScape v2.5 software (Applied Biosystems, Germany).

The sequence variations identified were compared with the corresponding DNA sequence from 200 healthy individuals and screened against the Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/index.php) as well as the Exome Variant Server (EVS, http://evs.gs.washington.edu/EVS/).

PredictSNP and Meta-SNP (http://snps.biofold.org/cgi-bin/job.cgi?njob=15602&wdir=Meta-SNP-132391) were used to evaluate the impact of the sequence abnormalities on protein structure and function.

## 3. Results

### 3.1. Evaluation results

Twenty-eight patients of the ICD outpatient clinic were included in this study (17 males, 11 females, mean age 41 years). All had been successfully resuscitated after a cardiac arrest. In each case, a family history of SUD was reported. In three cases, clinical examination and genetic extended screening of first degree relatives of the deceased were carried out. Due to the lack of samples, post mortem genetic screening of the deceased could not be performed.

Fig. 1 shows the results of the patients' clinical diagnosis. In 68% (19/28) of the patients, the diagnosis was established by clinical examination, whereas in 32% (9/28) only a possible diagnosis of the underlying disease could be determined. Among the cases of confirmed arrhythmia syndromes, ARVC was most frequently diagnosed (36%), followed by LQTS and BrS accounting for 18% and 11%, respectively, while CPVT was detected in only 3% of the patients.

### 3.2. Molecular genetic investigations

In 90% of the patients with a confirmed or possible clinical diagnosis of a heart disease (Fig. 2), potentially pathogenic sequence variations were mainly found in ARVC associated genes (Table 1). In four patients exhibiting clinical LQTS symptoms, sequence variations in the genes *KCNQ1, KCNH2, SCN5A* and *KCNE1* were detected, whereas in one patient with LQTS phenotype, one sequence variation in the *RYR2* gene was found. In two patients with clinically confirmed BrS, mutations in the *SCN5A* and *KCNH2* genes were identified. In one patient with clinically diagnosed BrS, no sequence variation was found. Nine patients, who had been diagnosed with ARVC, presented five sequence variations in the *PKP2*, one in the *DSP*, one in the *DSC2* and four in the *DSG2* gene, respectively. In one patient, variants in three different genes were

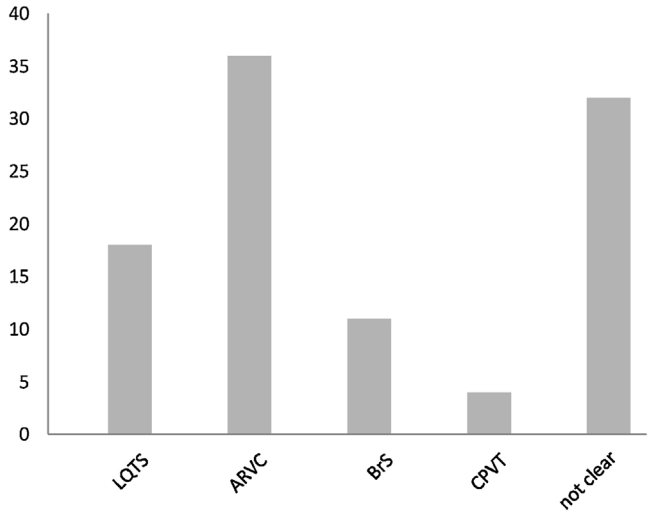


**Fig. 1.** Clinical cardiac diagnosis of patients resuscitated after cardiac arrest and exhibiting a family history of sudden death (in %).