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Genotype-phenotype dilemma in a case of sudden cardiac death with the E1053K mutation and a deletion in the *SCN5A* gene

T. Jenewein[a,1], B.M. Beckmann[b,c,1], S. Rose[a], H.H. Osterhues[d], U. Schmidt[e], C. Wolpert[f], P. Miny[g], C. Marschall[h], M. Alders[i], C.R. Bezzina[j], A.A.M. Wilde[j,k], S. Kääb[b,c], S. Kauferstein[a,*]

[a] *Institute of Legal Medicine, University of Frankfurt, Frankfurt am Main, Germany*
[b] *University Hospital Munich, Department of Medicine I, Ludwig Maximilians University, Munich, Germany*
[c] *German Cardiovascular Research Center (DZHK), Partner Site: Munich Heart Alliance, Munich, Germany*
[d] *District Hospital Loerrach, Medical Clinic, Loerrach, Germany*
[e] *Institute of Legal Medicine, University Hospital of Freiburg, Freiburg, Germany*
[f] *Klinik für Innere Medizin, Cardiology Klinikum Ludwigsburg, Ludwigsburg, Germany*
[g] *Medical Genetics, University Hospital Basel, Basel, Switzerland*
[h] *Center of Human Genetics and Laboratory Diagnostics, Martinsried, Germany*
[i] *Department of Clinical Genetics, Amsterdam Medical Center, Amsterdam, The Netherlands*
[j] *Heart Centre, Department of Clinical and Experimental Cardiology, Amsterdam Medical Center, University of Amsterdam, Amsterdam, The Netherlands*
[k] *Princess Al-Jawhara Al-Brahim Centre of Excellence in Research of Hereditary Disorders, Jeddah, Kingdom of Saudi Arabia*

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ABSTRACT

Mutations in the cardiac sodium channel gene *SCN5A* may result in various arrhythmia syndromes such as long QT syndrome type 3 (LQTS), Brugada syndrome (BrS), sick sinus syndrome (SSS), cardiac conduction diseases (CCD) and possibly dilated cardiomyopathy (DCM). In most of these inherited cardiac arrhythmia syndromes the phenotypical expression may range from asymptomatic phenotypes to sudden cardiac death (SCD).

A 16-year-old female died during sleep. Autopsy did not reveal any explanation for her death and a genetic analysis was performed. A variant in the *SCN5A* gene (E1053K) that was previously described as disease causing was detected. Family members are carriers of the same E1053K variant, some even in a homozygous state, but surprisingly did not exhibit any pathological cardiac phenotype. Due to the lack of genotype-phenotype correlation further genetic studies were performed. A novel deletion in the promoter region of *SCN5A* was identified in the sudden death victim but was absent in other family members.

These findings demonstrate the difficulties in interpreting the results of a family-based genetic screening and underline the phenotypic variability of *SCN5A* mutations.

1. Introduction

Mutations in the *SCN5A* gene, which encodes the α-subunit of the cardiac sodium channel $Na_v1.5$, may cause either a gain or a loss of channel function. Gain-of-function mutations may lead to the long QT syndrome type 3 (LQTS) [1] whereas loss-of-function mutations are associated with a spectrum of arrhythmia phenotypes including Brugada syndrome (BrS) [2], sick sinus syndrome (SSS) [3], cardiac conduction diseases (CCD) [4] and possibly dilated cardiomyopathy (DCM) [5]. Most of the inherited cardiac arrhythmia syndromes show variable phenotypic severity ranging from absence of any symptoms to sudden cardiac death (SCD). Mixed phenotypes (overlap syndromes, e.g. presentation of ECG features of Brugada syndrome, conduction disorders and long QT syndrome type 3) have also been described [6,7].

The Brugada syndrome is an inherited autosomal-dominant arrhythmogenic disorder and is associated with an increased risk for SCD due to polymorphic ventricular tachycardia and/or ventricular fibrillation [8,9]. At present, seventeen genes have been putatively linked to BrS, but mutations in these genes are found only in about 25–35% of BrS cases [10]. Most rare variants are

* Corresponding author.
E-mail address: kauferstein@em.uni-frankfurt.de (S. Kauferstein).
[1] These authors contributed equally to this work.

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found in *SCN5A* and are present in 11–28% of the cases [11]. The clinical phenotype of a particular variant in the *SCN5A* gene may show a variable penetrance among individuals even within the same family. In some BrS-affected families, Probst et al. [12] found that *SCN5A* gene mutations are not solely causal for the manifestation of BrS and suggested that other factors beyond mutant sodium channels (e.g. genetic variation in other genes, alteration in transcription/translation, RNA processing and protein degradation) may contribute to different BrS phenotypes. More recent data present evidence that BrS might actually have an oligogenic basis [13].

The phenotypic variability associated with *SCN5A* mutations can also be partly explained by the co-inheritance of additional genetic variants in that gene, which include the presence of the common *SCN5A* polymorphism H558R and the presence of *SCN5A* regulatory variants, which cause variable transcription of the cardiac sodium channel gene [13–21].

Here we describe the difficulties in interpreting results of a postmortem genetic analysis in a case of sudden cardiac death. Besides a variant in the coding area of the *SCN5A* gene, a deletion in the promotor region in that gene was detected. Genetic screening of family members of the deceased revealed the presence of the variant, but not of the deletion; none of them showed clinically cardiac symptoms.

2. Material and methods

2.1. Clinical studies/study subjects

Following the sudden cardiac death (SCD) of a 16 years old female medico-legal autopsy was performed. The girl was found dead in her bed in the morning. During autopsy, acute cardiac ischemia was detected along with pronounced congestion and edema of the lungs, mild cerebral edema and congestion of internal organs. Additionally, a small bundle of myocardial fibers bridging the *ramus interventricularis anterior* was found. Histological examination revealed focal necrosis of myocardial cells, but no signs of inflammation or any other pathological condition. Toxicological analysis of heart and femoral blood samples, urine and stomach contents revealed no abnormalities. Except from occasional headaches, the 16 years old girl had never displayed any relevant symptoms, in particular, seizure-like activity or syncopes before SCD. No family history of SCD was reported. Since the cardiac ischemia could not sufficiently be explained by the morphological, histological, and toxicological findings, the deceased girl's parents consented in a postmortem genetic screening. The genetic analysis was carried out in the context of the research project "molecular basis of diagnosis and etiology of genetic cardiovascular diseases as cause for sudden cardiac death" of the Institute of Forensic Medicine in Frankfurt. As this analysis revealed a potentially pathogenic variant, previously described as probably disease causing, the first-degree relatives were encouraged to seek genetic counseling. After obtaining informed consent of six family members, blood samples were collected and analyzed. It was recommended for all family members to undergo cardiological assessment including resting and exercise ECG and Ajmaline testing.

2.2. Genetic screening

Genomic DNA was extracted from blood samples using standard phenol chloroform procedures. Genetic screening including the major LQTS, BrS and CPVT related genes *KCNQ1* (NG_008935), *KCNH2* (NG_008916), *SCN5A* (NG_008934), *KCNE1* (NG_009091), *KCNE2* (NG_008804), *KCNJ2* (NG_008798), *SCN4B* (NG_011710), *HCN4* (NG_009063) and 20 exons (3, 8, 14, 15, 44–47, 49, 83, 88, 90, 93, 96, 97, 100–103, 105) of *RyR2* (NG008799) as well as the major ARVC genes *PKP2* (NG_009000), *DSG2* (NG_007072), *DSC2* (NG_008208) and *DSP* (NG_008803) was performed using polymerase chain reaction (PCR) with published [22–25] and redesigned primers (primer sequences upon request). Direct sequencing of the amplicons was performed in the presence of fluorescence–labeled dideoxynucleotides (BigDye® Terminator Cycle Sequencing Kit, Life Technologies, Germany) and primer in both sense and antisense direction. Sequencing products were examined with a 3130xl Genetic Analyzer (Applied Biosystems, Germany). Data analysis was done with SeqScape v2.5 (Applied Biosystems, Germany) and the NCBI (National Center of Biotechnology) reference sequence NC_008934. In case of the homozygous carrier of the variant, the exon containing the sequence variant was amplified with a second set of primers to rule out the possibility of a primer binding site mutation in one allele. Variant filtering was performed with the ExAC database (Exome Aggregation Consortium) and a minor allele frequency (MAF) cut-off of $<0.1\%$ was used.

2.3. Multiplex ligation-dependent probe amplification (MLPA)

MLPA is a semi-quantitative technique for detecting large deletions and duplications in a DNA sample. MLPA was performed on genomic DNA using the SALSA P108 *SCN5A* kit (MRC-Holland, Amsterdam, the Netherlands) according to the manufacturer's protocol. Three different control samples were included in each MLPA experiment. The SALSA P108 MLPA kit contains probes for each exon of the *SCN5A* gene (29 exons, including non-coding exon 1) and one probe upstream of exon 1. The amplification products were separated and identified by capillary electrophoresis using a 3130xl Genetic Analyzer (Applied Biosystems, Germany). Data analysis of the electropherograms was done with the Coffalyser. Net software (www.mlpa.com) performing two normalization steps of the MLPA peak pattern. Each probe peak of each sample was compared with the peaks of the reference probes (intrasample normalization). By comparing the relative probe peak of the sample to all control samples final probe ratios were calculated (intersample normalization). The resulting probe ratio for a normal DNA sample is ~1.0 (0.8–1.2), for a heterozygous deletion ~0.5 (0.4–0.65).

2.4. Analyzing the SCN5A promotor region using real-time quantitative PCR

To confirm the deletion in the 5′ upstream region of the *SCN5A* gene real-time quantitative PCR (RT-qPCR) using DNA binding SYBR® GreenER™ (EXPRESS SYBR® GreenER™ qPCR SuperMixes and Two-Step qRT-PCR Kit, Invitrogen) and PCR primer flanking the suspected boundaries of the deletion was performed. The method included amplification of three segments in the 5′ upstream sequence of *SCN5A* and two reference loci on chromosome 3 with normal copy number (*GPR15* and *ZNF80* [26]) for normalization. Amplification mixtures (20 μl) contained EXPRESS SYBR® GreenER™ master mix, 200 nM of each forward and reverse primer, 500 nM ROX reference dye and 1,25 ng template DNA. All reactions were performed in triplicates. The Applied Biosystems StepOnePlus™ Real-Time PCR System was used for the PCR reaction and the cycling conditions were as follows: 95 °C for 2 min, 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Melting curve analysis was generated for every amplification product to ensure the specificity of the PCR reaction. Calculation of copy numbers of the prospected segments was done with the Data Assist™ v3.01 Software (Applied Biosystems, Germany) using the comparative C_T ($\Delta\Delta C_T$) method [27]. Using *GPR15* (G Protein-coupled Receptor 15) and *ZNF80* (Zinc Finger Protein 80) as reference genes with normal copy number,