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Abstract

Sudden cardiac death (SCD) is a common cause of death in young adults. In up to 80% of cases a genetic cause is suspected. Next-generation sequencing of candidate genes can reveal the cause of SCD, provide prognostic management, and facilitate pre-symptomatic testing and prevention in relatives. Here we present a proband who experienced SCD in his sleep for which molecular autopsy was performed.

We performed a post-mortem genetic analysis of a 49-year-old male who died during sleep after competitive kayaking, using a Cardiomyopathy and Primary Arrhythmia next-generation sequencing panel, each containing 51 candidate genes. Autopsy was not performed.

Genetic testing of the proband resulted in missense variants in *KCNQ1* (c.1449C>A; p.(Asn483Lys)) and *DSG2* (c.2979G>T; p.(Gln993His)), both absent from the gnomAD database. Familial segregation analysis showed *de novo* occurrence of the *DSG2* variant and presence of the *KCNQ1* variant in the proband's mother and daughter. *KCNQ1* p.(Asn483Lys) was predicted to be pathogenic by MutationTaster. However, none of the *KCNQ1* variant carrying family members showed long QTc on ECG or Holter. We further functionally analysed this variant using patch-clamp in a heterologous expression system (Chinese Hamster Ovary (CHO) cells) expressing the *KCNQ1* mutant in combination with *KCNE1* wild type protein and showed no significant changes in electrophysiological function of Kv7.1.

Based on the above evidence, we concluded that the *DSG2* p.(Gln993His) variant is the most likely cause of SCD in the presented case, and that there is insufficient evidence that the identified *KCNQ1* p.(Asn483Lys) variant would confer risk for SCD in his mother and daughter. Fortunately, the *DSG2* variant was not inherited by the proband's two children. This case report indicates the added value of molecular autopsy and the importance of subsequent functional study of variants to inform patients and family members about the risk of variants they might carry.

Key words: arrhythmogenic right ventricular cardiomyopathy, molecular autopsy, Sudden Cardiac Death, inherited cardiac arrhythmia, clinical report

Introduction

Sudden cardiac death (SCD) has an estimated annual incidence of 1:1000. In the young (<50 years) in up to 80% of the SCD cases a genetic cause is suspected, with inherited cardiac arrhythmia or cardiomyopathy among the main disease categories (1). Next-generation sequencing (NGS) of candidate genes can reveal the cause of SCD, provide prognostic management, and facilitate pre-symptomatic testing and prevention in relatives. Generally speaking, mutations affecting ion channels involved in generation and conduction of action potentials in the heart's electrical system are underlying cardiac arrhythmias, while cardiomyopathies are caused by mutations affecting structural and/or cell-cell adhesion proteins. Several reports have been published regarding the screening of genes related to inherited cardiac arrhythmias and cardiomyopathies in SCD cases, both in larger cohorts as well as in smaller groups (2-10). In a recent study with 70 individuals, who were tested for 100 arrhythmia and cardiomyopathy related genes, 16% of SCD cases carried pathogenic or likely pathogenic variants (2). In another SCD study where 100 genes related to inherited cardiac diseases were tested in 61 individuals, 21 individuals (34%) carried a variant with a likely functional effect (3). A larger study with 302 SCD cases revealed a yield of 13% after testing 77 genes (4). Functional testing of variants can help with the interpretation of the pathogenicity of the variant, both in variants located in ion channel genes as well as in structural genes expressed in the heart (11, 12).

Here we present a male proband who experienced SCD in his sleep for which molecular autopsy was performed to enable the identification of a causative mutation and allow family screening and counselling.

Clinical description

The proband is a 49-year-old man who died suddenly in his sleep. The day before, he participated in a kayaking competition and finished the race without problems. The sudden cardiac death happened abroad, and no additional evaluation was carried out. An autopsy could not be performed due to the embalming of the body. His past medical history was uneventful and two previous pre-participation screening ECGs at rest and during exercise (rowing exercise) showed no abnormalities. His family history did not reveal any instances of unexplained sudden cardiac death in first or second degree relatives. His two children (10 years old son and 13 years old daughter), both competitive sporters as well, underwent cardiac evaluation by ECG, echocardiography, SA-ECG, 24-hours holter monitoring and cyclo-ergometry, but no abnormal findings were revealed.

From a recent occupational health evaluation two serum tubes were available from the deceased proband. After inverse centrifuging the serum tubes, we were able to recover white blood cells and obtained sufficient quality and amount of DNA. After informed consent and permission of his spouse post-mortem genetic analysis was performed on this sample using next-generation sequencing panels of known cardiomyopathy and primary arrhythmia genes (each n=51 genes) (13).

The molecular screening revealed two variants of uncertain significance: c.1449C>A (p.(Asn483Lys)) in *KCNQ1* (NM_001943.4, ENST00000155840) encoding the potassium channel Kv7.1 and c.2979G>T (p.(Gln993His)) in *DSG2* (NM_000218.2, ENST00000261590) encoding the cell-cell contact protein desmoglein 2. *KCNQ1* p.(Asn483Lys) was absent in the gnomAD v2.1.1. database (https://gnomad.broadinstitute.org/) and predicted to be damaging by MutationTaster and benign by SIFT and Polyphen-2. *DSG2* p.(Gln993His) was also absent from gnomAD v2.1.1 and only Polyphen-2 scored the variant as probably damaging.

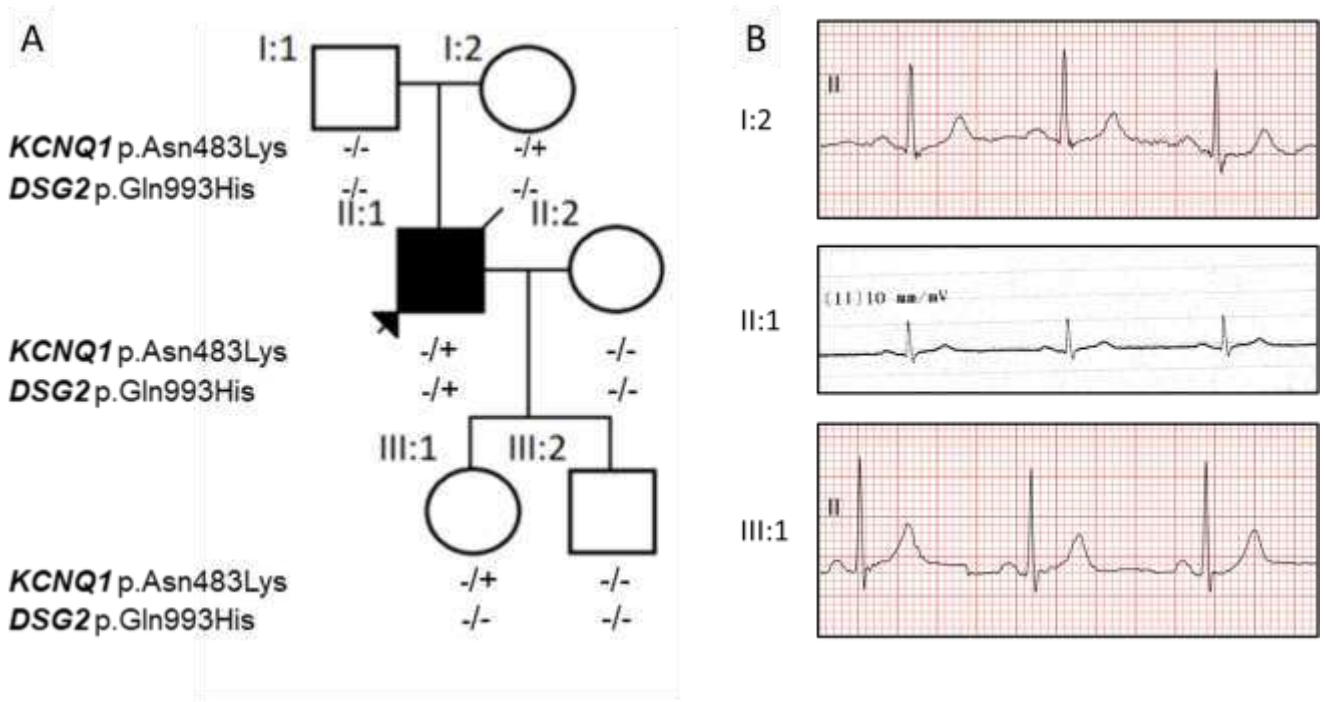

Figure 1: A. Pedigree of the family. Proband is identified with an arrow and a full symbol indicates an affected individual. B. Lead II ECG traces from three individuals (I:2; II:1 and III:1) with the KCNQ1 variant.

Segregation analysis showed that the *KCNQ1* variant was inherited from the proband's mother and transmitted to the proband's daughter. The *DSG2* variant occurred *de novo* (with proven paternity) and was not inherited by his children (**Fout! Verwijzingsbron niet gevonden.**A). The mother and daughter showed normal ECG at rest (Figure 1B) and during cyclo-ergometry, as well as a 24-hours holter registration with normal QTc intervals. Because of the daughter's sport activities, it was decided to put her on beta-blockers and limit competitive sporting as long as the pathogenicity of the *KCNQ1* variant was not excluded, as intensive sporting is known to be a trigger for arrhythmias in Long QT syndrome type 1 (LQT1) (14).

In order to further investigate the pathogenic nature of the *KCNQ1* variant, we tested the variant electrophysiologically in Chinese Hamster Ovarian cells (CHO). Both wild type (WT, Kv7.1) and mutant (MUT, Kv7.1-Asn483Lys) were tested on their own as well as in combination, and we always co-expressed the auxiliary Mink protein encoded by the *KCNE1* gene, that is necessary for proper

functioning of the ion channel. Ionic currents were recorded during the following pulse protocol: starting from a -80mV holding potential, a 6s depolarizing pulse from -60 to +80mV in 10mV steps was forced to the cells after which they were repolarized to -40mV to measure the tail current. Whole cell current recordings show no significant differences in current density between the different combinations (WT, MUT, WT/MUT). Normalized tail current amplitudes (I/Imax) were plotted as a function of the depolarizing potential. The resulting voltage dependence of channel activation was not impaired by the variant and also both activation and inactivation kinetics were the same in the different combinations (WT, MUT, WT/MUT) (Figure 2).

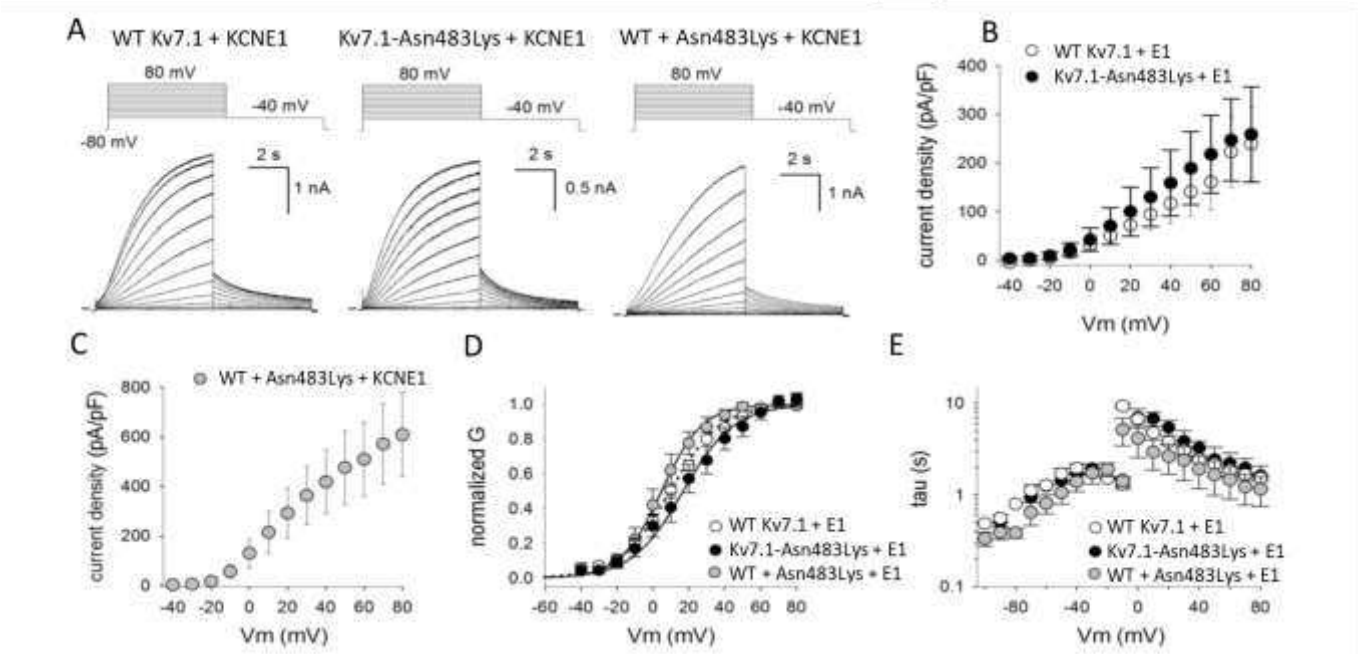

Figure 2: Electrophysiological properties of WT Kv7.1 and Kv7.1-Asn483Lys co-expressed with KCNE1 (representing in vivo I_{Ks} current).

A) Displayed from left to right are representative whole cell ionic current recordings of CHO cells expressing WT Kv7.1 + KCNE1, Kv7.1-Asn483Lys + KCNE1 and the co-expression of WT Kv7.1 with Kv7.1-Asn483Lys + KCNE1. The K+ selective currents were elicited with the pulse protocol shown on top. Horizontal bar at the start indicates the zero current level. B) Current density of WT Kv7.1 + KCNE1

(white circles, n = 5) and Kv7.1-Asn483Lys + KCNE1 (black circles, n = 7) obtained by normalizing the peak current amplitudes after 6 seconds depolarization, from recordings shown in panel b, to the cell capacitance. C) Current density of the co-expression of WT Kv7.1 with Kv7.1-Asn483Lys + KCNE1 (n = 5). In this condition the amount of Kv7.1 α-subunit cDNA doubled compared to the expression of WT Kv7.1 and Kv7.1-Asn483Lys alone. Accordingly, the current density increased a 2-fold compared to the data displayed in panel c. The data from panel c and d indicate that the Asn483Lys mutation does not affect current expression. D) Voltage dependence of channel activation obtained by plotting the normalized amplitudes of the tail currents at -40 mV, elicited after 6 seconds depolarization as shown in panel b, as a function of the depolarizing potential. E) Voltage-dependent kinetics of channel activation and deactivation. Shown values are the means ± S.E.M. with n the number of cells analyzed.

Discussion and Conclusions

Genetic analysis of the proband's DNA revealed two possible causal variants for his SCD, namely p.(Asn483Lys) in *KCNQ1* and p.(Gln993His) in *DSG2.* The Kv7.1 potassium channel is responsible for the delayed rectifier current I_{Ks}, important in the repolarization of the cardiac action potential. Mutations in *KCNQ1* are a known cause of LQT1 which can cause ventricular fibrillations and lead to SCD (15). Pathogenic mutations are mostly located in the transmembrane part of the channel as well as the C-terminal domain of the protein (16), where our variant is located. Many variants have been modelled in heterologous expression systems, often showing a loss-of-function or a dominant negative effect on the functioning of Kv7.1 (17-20). Although sometimes, a variant is reported that does not have an effect on the functioning of the channel (21). Wedekind et al. found two variants (p.(Val254Met) and p.(Val417Met)) in a family that were located in cis on the same *KCNQ1* allele and electrophysiological data showed that only p.(Val254Met) had an effect on the function of Kv7.1 (21).

The electrophysiological data we generated in a heterologous expression system indicates that the function of the Kv7.1 protein is not altered by the p.(Asn483Lys) variant, which is consistent with the

normal results of the QTc measurements in the proband's mother and daughter. Based on these results, we concluded that there is insufficient evidence that this variant would confer risk for SCD in the mother and daughter of the proband. Beta-blockade was discontinued in the daughter and she was allowed to continue her sports career, with close clinical follow-up.

Desmoglein-2 is a cadherin providing cell-cell contact in cardiac desmosomes. In 2006, Pilichou et al and Awad et al, were the first to report *DSG2* variants in family members with ARVC (22, 23). ARVC is characterized by fibro-fatty replacement, predominantly in the right ventricle, which can induce ventricular arrhythmias and eventually lead to SCD (22). The *DSG2* p.(Gln993His) variant which occurred *de novo* in this described case is located in the intracellular repeat unit domain (RUD). Previously, two variants in this RUD were reported. A p.(Val920Gly) variant was found in the father of a boy who died at age 17. Post-mortem analysis of the boy suggested left ventricular involvement in ARVC as the cause of death. The father was asymptomatic but had late potentials on signal-averaged ECG and cardiac imaging revealed hypokinesia of the anterior wall of the right ventricular outflow tract and the apical free wall (24). A functional study showed that this variant reduces cell-cell cohesion in HL-1 cardiomyocytes (25). Another p.(Tyr1047Arg) variant in the same RUD was reported in a patient with ARVC and a family member whose phenotype was not specified in the report (26). Together, these findings support the potential pathogenicity of variants located in the RUD domain of *DSG2*.

The *DSG2* p.(Gln993His) variant occurred *de novo* (with proven paternity) in a proband without family history (PS2 argument). In addition, we can assign PM2 (moderate evidence) for absence in controls and and PP2 (supportive evidence) for a missense variant in a gene that has a low rate of benign missense variation and where missense variants are a common mechanism of disease. Overall, the variant is classified as a likely pathogenic variant (according to the ACMG-rule "1 Strong (PS1–PS4) AND 1–2 Moderate (PM1–PM6)"). (27). Based on all the mentioned evidence and the fact that ARVC is a typical cause of death in competitive sportsmen, we can conclude that the *DSG2* p.(Gln993His) variant is the most likely cause of SCD in the proband. We proved that the *KCNQ1* variant did not interfere

with the function of Kv7.1. As such, the daughter could be taken off the beta-blocker treatment. But since still additional unknown genetic variants not detected by the used gene panels could have contributed to the proband's SCD, close clinical follow-up of the children remains warranted when performing competitive sports. A limitation in this study is the fact that an autopsy was not performed, which could have revealed typical ARVC-related abnormalities in the structure of the proband's heart. This case report indicates the added value of molecular autopsy and the importance of subsequent functional study of detected variants to inform patients and family members about the risk of variants they might carry.

List of abbreviations

ARVC: arrhythmogenic right ventricular cardiomyopathy

CHO: Chinese Hamster ovarian

ECG: Electrocardiogram

LQT1: Long QT syndrome type 1

NGS: Next-generation sequencing

RUD: repeat unit domain

SA-ECG: Signal-averaged electrocardiogram

SCD: Sudden cardiac death

Acknowledgments

Declarations

Ethics approval and consent to participate

This study was approved by the Ethical Committee of the Antwerp University Hospital.

Consent for publication

Written consent for publication was obtained from the proband's wife.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request. Both variants have been submitted to the Clinvar database (*KCNQ1*: VCV000927801; *DSG2*: VCV000996557).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

E.Sim., A.L., J.S., B.L. and M.A: Conceptualization, Methodology; J.S., E.Sie., E.V.C., B.L., M.D.: Investigation; E.Sim.: Writing - Original Draft; A.L., J.S., D.S., L.V.L., B.L. and M.A: Writing - Review & Editing; E.Sim, A.N: Visualization; E.Sim., A.L., A.N., B.V., M.D., D.S. and M.A.: Validation; All authors have read and approved the manuscript.

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