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ORIGINAL PAPER

Unexplained cardiac arrest: a tale of conflicting interpretations of *KCNQ1* genetic test results

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Abstract

Objective Unexplained cardiac arrest (UCA) is often the first manifestation of an inherited arrhythmogenic disease. Genetic testing in UCA is challenging due to the complexities of variant interpretation in the absence of supporting cardiac phenotype. We aimed to investigate if a *KCNQ1* variant [p.(Pro64_Pro70del)], previously reported as pathogenic, contributes to the long-QT syndrome phenotype, co-segregates with disease or affects *KCNQ1* function in vitro.

Methods DNA was extracted from peripheral blood of a 22-year-old male after resuscitation from UCA. Targeted exome sequencing was performed using the TruSight-One Sequencing Panel (Illumina). Variants in 190 clinically relevant cardiac genes with minor allele frequency < 1% were analyzed according to the guidelines of the American College of Medical Genetics. Functional characterization was performed using site-directed mutagenesis, expression in *Xenopus laevis* oocytes using the two-electrode voltage-clamp technique.

Results The 12-lead ECG, transthoracic echocardiography and coronary angiography after resuscitation showed no specific abnormalities. Two variants were identified: c.190_210del in-frame deletion in *KCNQ1* (p.Pro64_Pro70del), reported previously as pathogenic and c.2431C > A in *PKP2* (p.Arg811Ser), classified as likely benign. Two asymptomatic family members with no evident phenotype hosted the *KCNQ1* variant. Functional studies showed that the wild-type and mutant channels have no significant differences in current levels, conductance-voltage relationships, as well as activation and deactivation kinetics, in the absence and presence of the auxiliary subunit KCNE1.

Conclusions Based on our data and previous reports, available evidence is insufficient to consider the variant *KCNQ1*:c.190_210del as pathogenic. Our findings call for cautious interpretation of genetic tests in UCA in the absence of a clinical phenotype.

Keywords Ventricular fibrillation · Ion channel · Genetics · Arrhythmia · Sudden cardiac death

Introduction

Genetic testing in unexplained cardiac arrest (UCA) carries an inherent risk of misinterpretation of rare sequence variants in the absence of a supporting cardiac phenotype, and is therefore not recommended (Class III) by relevant professional societies and consensus [1]. Establishing a clear causal mutation in an UCA victim would allow targeted screening in the family, eventually leading to the initiation of preventive measures in living relatives at risk of sudden death. However, possible misinterpretation of variants carries a substantial risk of inappropriate interventions with inherent complications. The recent guidelines of the American College of Medical Genetics and Genomics (ACMG)

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provide more reliable criteria through the incorporation of multiple variant evidence lines in prediction schemes,[2], but major barriers still exist in interpreting radical mutations (in-frame or frameshift insertions / deletions or early truncations), which are usually considered as likely pathogenic, or at least, variants of uncertain significance.

In this case-based report, we illustrate the complexities in the determination of the underlying etiology of ventricular fibrillation (VF) and death in a young and apparently healthy individual with negative clinical and toxicological evaluation and controversial genetic test results. Our findings call for a more cautious approach when interpreting genetic variants in patients with sudden cardiac arrest without evident cardiac phenotype.

Clinical case

A 22-year-old man was admitted to our emergency department after experiencing sudden cardiac arrest in the street. He was a bartender and was walking back home at night. The first rhythm documented was VF. After prolonged resuscitation, sinus rhythm was restored. The 12-lead ECG after reanimation showed no specific abnormalities (Fig. 1a). Cardiac catheterization excluded acute coronary occlusion or any other coronary anomaly. A transthoracic echocardiography revealed no structural heart disease. Pulmonary embolism and potential cerebral causes were also ruled out with a computed tomography.

Family history was negative for inherited cardiac diseases. The initial laboratory tests revealed lactic acidosis—an expected finding at a post-resuscitation state, but no relevant electrolyte disorders were found. Screening for intoxication and drugs were also unrevealing. Despite the initial successful resuscitation, the patient died in the hospital three days after the admission due to profound neurological damage caused by prolonged brain ischemia.

Due to the initial presentation of VF in a previously asymptomatic young patient—a phenomenon characteristic to inherited arrhythmogenic diseases—a genetic etiology of death was suspected (reported yield of near 25%), and material of the case was sent for genetic testing. Additionally, clinical, genetic and cardiovascular examinations were performed in the first-degree relatives available: the 49-year-old father (mutation carrier), 50-year-old mother (mutation negative), and the 18-year-old brother (mutation carrier). All first degree relatives were asymptomatic, with normal rest 12 lead ECG (Fig. 1b, c), echocardiography, and stress test (QT-QTc values available in supplemental table 1).

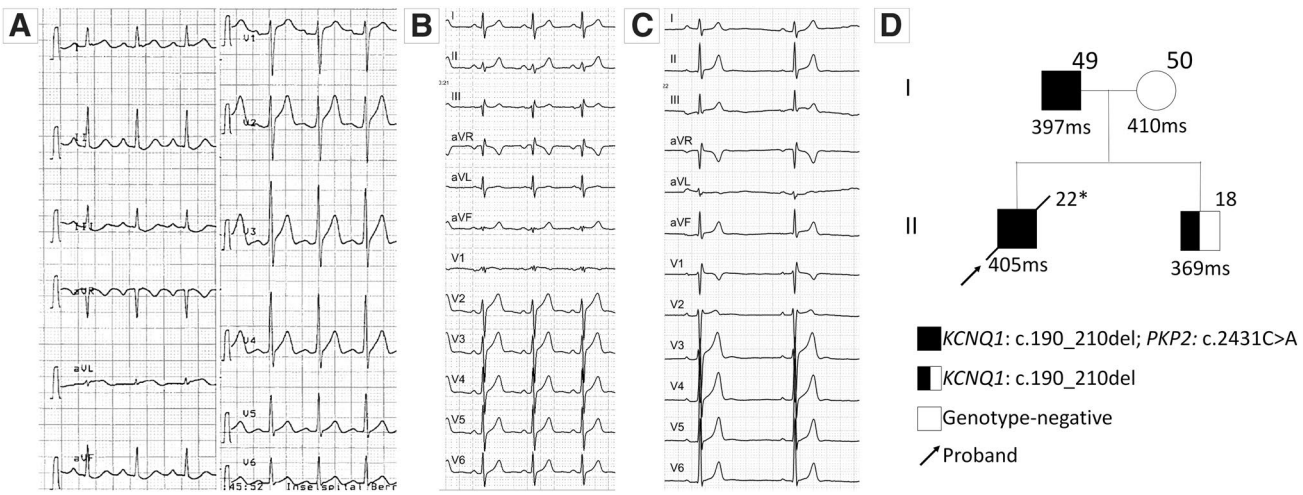

Fig. 1 The ECG and pedigree of the investigated family. **a** Resting ECG of the proband after resuscitation. Sweep speed 25 mm/s, 10 mm/ mV. Normal electrical axis (54°), RR-interval 583 ms, P 96 ms, PQ 154 ms, QT, 338 ms, QTc 405 ms. A slight concave shaped ST-segment in the inferior limb leads was noted; there were no suspicious de- or repolarization abnormalities. **b** Resting ECG of the father. Sweep speed 25 mm/s, 10 mm/ mV. Normal electrical axis (12°), RR-interval 762 ms, P 110 ms, PQ 146 ms, QRS 98 ms, QT 366 ms, QTc 397 ms. Except for the incomplete right bundle branch block, no suspicious de- or repolarization anomalies were present. **c** Resting ECG of the brother. Sweep speed 25 mm/s, 10 mm/ mV. Normal electrical axis (79°), RR-interval 1432 ms, P 96 ms, PQ 174 ms, QRS 100 ms, QT 402 ms, QTc 369 ms (Hodge). Except for the incomplete right bundle branch block, no suspicious de- or repolarization anomalies were present. **d** Pedigree. QTc values are shown below each individual. Ages at clinical evaluation are shown above each individual. *Age at sudden death

Methods

Subjects

This study was conducted in full agreement with the principles of the "Declaration of Helsinki" and laws and regulations of Switzerland. All DNA donors available for this study signed an informed consent form approved by the Cantonal Ethical Committee of Bern.

Mutational analysis

After obtaining informed consent from the family, genetic testing in the index patient was performed. DNA was extracted from peripheral blood using standard procedures. Clinical exome analysis was performed by next generation sequencing on MiSeq instrument using the TruSight One Sequencing Panel (Illumina, San Diego, CA, USA), which provides comprehensive coverage of exonic and exonic–intronic boundaries of 4813 clinically relevant genes. An in-house customized panel of 190 genes implicated in hereditary cardiovascular diseases and/or sudden cardiac death was analyzed. Read alignment and local realignment of indels were performed using CLC Workbench v7.5.1 (Qiagen, Redwood City, CA, USA). Novel, putative disease-associated sequence variants were distinguished from polymorphisms using the following filtering criteria: a change in the protein's primary structure, species conservation of the underlying amino acid, and an allele frequency below 1% based on the 1000 Genome Project database. For detailed sequence analysis and interpretation of sequence variations we used the following bioinformatic algorithms: Polyphen2 [3] SIFT [4] and Mutation Taster [5]; and the following databases: Human Gene Mutation Database (HGMD Biobase, Qiagen, Redwood City, CA, USA), 1000 Genomes,[6] and the Exome Aggregation Consortium (ExAC) browser [7]. For titin *(TTN)* we considered as putative mutations only radical mutations (i.e., nonsense, frameshift, and splice-site mutations). The published original literature was also screened to identify known disease-causing mutations in the datasets from the patient. Final interpretation of variants was performed following the guidelines established by the ACMG [2].

Molecular biology and expression of KCNQ1 channels in *Xenopus laevis* oocytes

Human KCNQ1, KCNQ1 ΔP64-P70 and KCNE1 complementary DNAs (cDNAs) cloned between HindIII and BamHI sites in a modified pCDNA3.1$^{(+)}$ vector containing 3′-*Xenopus* globin UTR and a polyadenylation signal were generated using custom gene synthesis with codon optimization for *Homo sapiens* (GeneArt, Thermo Fisher Scientific, Germany). The sequences of purified plasmid DNAs from transformed *E. coli* were verified by Sanger DNA sequencing (GATC Biotech AG, Germany), and then linearized with NotI restriction enzyme. Capped RNAs were synthesised from linearized cDNA templates using the T7 mMessage mMachine Kit (Ambion, Germany). Ovarian lobes were surgically removed from female *X. laevis* frogs anaesthetized in 0.3% tricaine (procedure approved by the Danish Veterinary and Food Administration; license number: 2014-15-0201-0031), divided into smaller clumps, and defolliculated by shaking at 200 rpm, 37 °C in OR2 (in mM: 82.5 NaCl, 2.5 KCl, 1 $MgCl_2$, 5 HEPES; pH 7.4 with NaOH) containing 1 mg/mL of Type I collagenase (Worthington Biochemical Corporation, USA). Healthy-looking stage V-VI oocytes were isolated, injected with 5–25 ng of RNA in a volume of 9–50 nL, and incubated in OR3 (50% (v/v) Leibovitz's L-15 medium (Gibco, Denmark), 1 mM glutamine, 250 µg/mL gentamicin, 15 mM HEPES; pH 7.6 with NaOH) at 18 °C, 140 rpm. We expressed wild-type (WT) and mutant (Mut) KCNQ1 channels alone to mimic homozygosity, and co-expressed WT and Mut (WT/Mut) in equal RNA amounts to mimic heterozygosity. As KCNQ1 associates with the auxiliary subunit KCNE1 to form a complex, which mediates the slow cardiac delayed rectifier potassium current (I_{Ks}), we also examined the effect of co-expressing KCNQ1 channels with KCNE1. The relative amounts of RNA for each combination are shown in Table 1.

Table 1 The relative amounts of RNA used in expressing different combinations of WT and Mut KCNQ1 in the absence and presence of KCNE1

			KCNQ1	KCNQ1 ΔP64-P70	KCNE1	H_2O
Combination	WT	− KCNE1	1	-	–	1
	Mut		–	1	–	1
	WT + Mut		1	1	–	2
	WT	+ KCNE1	1	–	1	–
	Mut		–	1	1	–
	WT + Mut		1	1	2	–

Two-electrode voltage-clamp recordings

One to two days after RNA injection, oocytes were placed one at a time in a custom-built chamber, which was continuously perfused with ND96 recording solution (in mM: 96 NaCl, 2 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$, 5 HEPES; pH 7.4 with NaOH). Current recordings were performed using a Warner OC-725C Oocyte Clamp amplifier (Warner Instrument Corp, USA). Data were acquired using the pCLAMP 10 software (Molecular Devices, USA) and a Digidata 1550 digitizer (Molecular devices), sampled at 10 kHz. Electrical powerline interference was filtered with a Hum Bug 50/60 Hz Noise Eliminator (Quest Scientific, Canada). Recording microelectrodes with resistances around 0.2–1.2 MΩ were pulled from borosilicate glass capillaries (Harvard Apparatus, USA) using a P-1000 Flaming/Brown Micropipette Puller System (Sutter Instrument, USA), and were filled with 3 M KCl. All experiments were conducted at a holding potential of − 80 mV. To determine the voltage-dependence of channel activation, depolarizing voltage pulses were applied for 2 s from − 80 to + 60 mV (− KCNE1) and 6 s from − 40 to + 90 mV (+ KCNE1) in 10 mV increments, before stepping back to − 20 mV (− KCNE1) and + 20 mV (+ KCNE1) for 1 s (Fig. 2b). To elicit channel

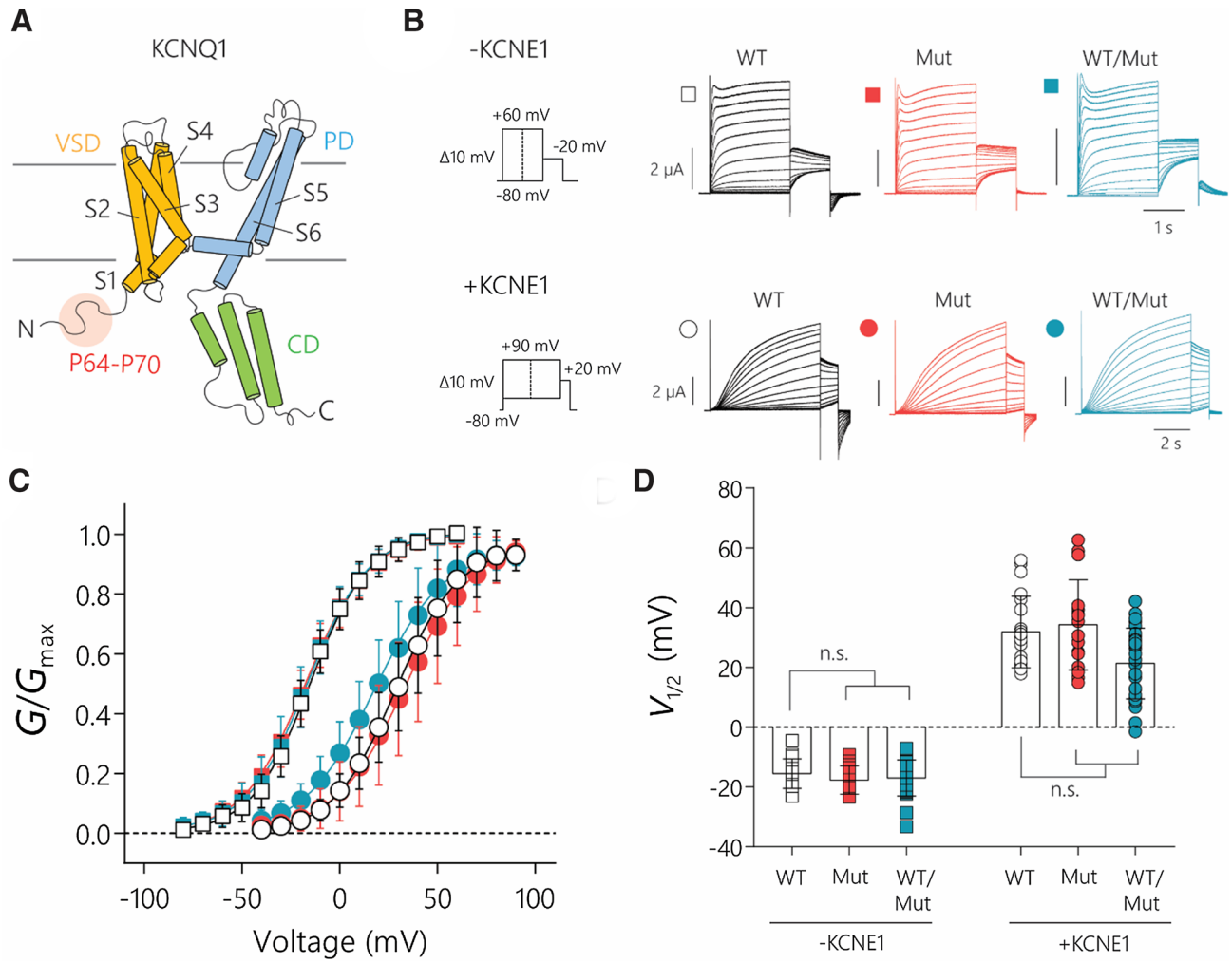

Fig. 2 G–V relationships of KCNQ1, KCNQ1 ΔP64-P70, KCNQ1+KCNQ1 ΔP64-P70 in the absence and presence of KCNE1. **a** A cartoon structure of a single KCNQ1 subunit. The S1–S4 segments form the voltage-sensing domain (VSD), whereas the S5 and S6 segment contribute to the pore domain (PD), which is connected to an intracellular cytosolic domain (CD). The deletion mutation identified in the patient (ΔP64–P70) is found in the N-terminal region (putative location indicated by the pink circle). **b** Representative current traces from *Xenopus* oocytes expressing (top panel) wild-type (WT; black), mutant (Mut; red) and WT/Mut (blue) alone (− KCNE1) or (bottom panel) in combination with KCNE1 (+ KCNE1). Voltage protocols are shown next to current traces (refer to the Methods section for further details). **c** G–V curves showing the normalized tail current amplitude (G/G_{max}) plotted against membrane voltage. **d** $V_{1/2}$ values of the G–V curves. Data are mean ± SD; For − KCNE1: WT ($n = 17$), Mut ($n = 21$), WT/Mut ($n = 26$). For + KCNE1: WT ($n = 21$), Mut ($n = 16$), WT/Mut ($n = 28$). All experiments were performed using at least three different batches of oocytes. Statistical comparison was performed using one-way ANOVA with Dunnett's test, and statistical significance was set at $p < 0.01$. n.s. not significant

deactivation, a pre-pulse of -80 to +20 mV (− KCNE1) or -80 to +60 mV (+KCNE1) was applied for 3 and 6 s, respectively, then stepped back to − 30 mV (− KCNE1) or − 20 mV (+KCNE1) for 5 s in 10 mV decrements (Fig. 4a).

Electrophysiological analysis

To determine the voltage-dependence of channel activation, tail current amplitudes at − 20 mV (− KCNE1) and +20 mV (+KCNE1) following depolarizing pulses were plotted for each cell using OriginPro (OriginLab Corporation, USA), and fitted with a Boltzmann sigmoidal function (Eq. 1), where A_1 = minimum tail current amplitude (G_{min}), A_2 = maximum tail current amplitude (G_{max}), x = voltage, x_0 = half-activation voltage ($V_{1/2}$) and dx = slope factor. The individual G–V curves were then normalised using the respective fitted G_{max} values. To illustrate the G–V relationships, averaged, normalized G/G_{max} values were plotted against voltage (Fig. 2c). Current responses elicited by depolarizing voltage steps were fitted with a double exponential function using Clampfit to obtain the fast and the slow time constants for channel activation (Fig. 3). At lower voltages, the fits were poor due to lack of channel activation, and hence time constants obtained at these voltage steps were excluded. Cells with current responses which could not be fitted with a double exponential function even at higher voltages were excluded entirely from kinetic analysis. The deactivation time constants were obtained by fitting current decays with a single exponential function (Fig. 4). Raw current traces were generally filtered at 500–800 Hz (8-pole Bessel low-pass filter) before data analysis, except for kinetic analysis. Current traces were further subjected to data reduction (substitute average by a factor of 10) for illustration. Data were presented as mean ± standard deviation (SD). For statistical comparisons, one-way ANOVA followed by the Dunnet's post hoc test (Prism 7.0, GraphPad Software, USA) was used to determine stastistical differences. A higher statistical significance threshold ($p < 0.01$) was set to minimise the contribution of experimental variability. All experiments were performed with at least three different batches of *Xenopus* oocytes.

$$y = \frac{A_1 - A_2}{1 + e^{(x-x_0)/dx}} + A_2 \quad (1)$$

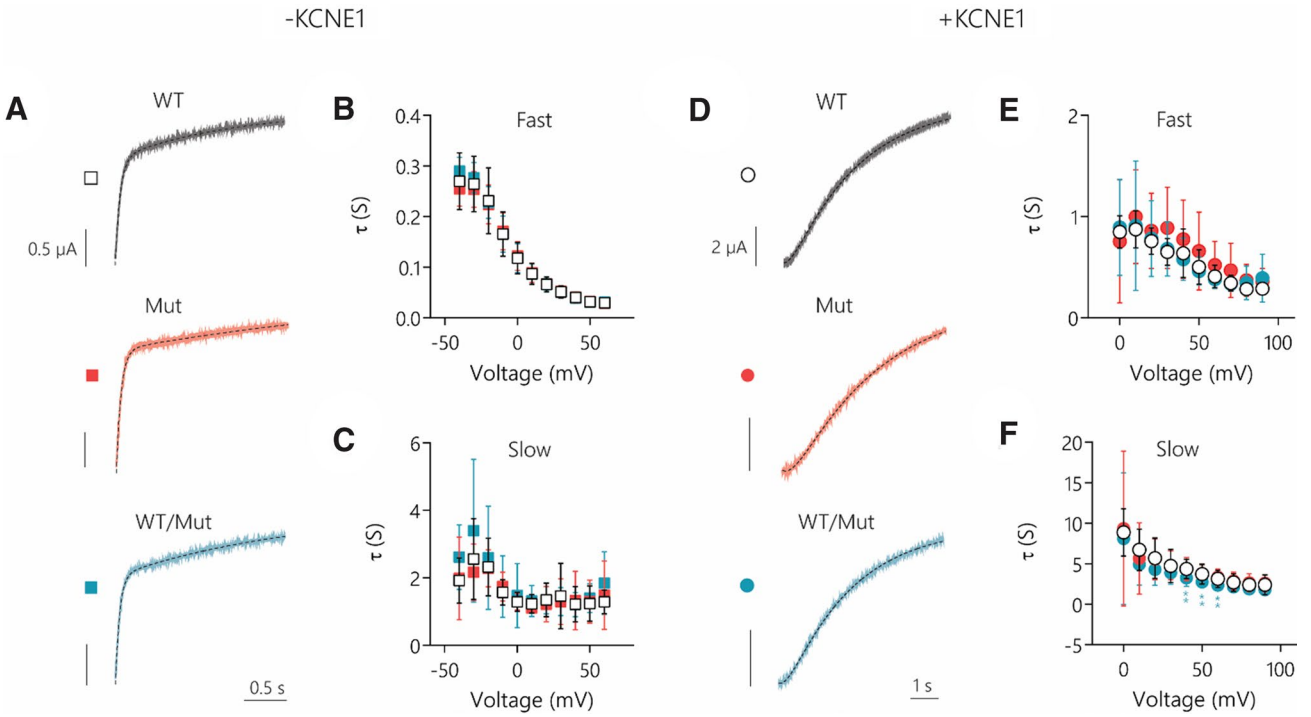

Fig. 3 Activation kinetics of KCNQ1 WT, Mut and WT/Mut channels are similar both in the absence (A–C) and presence of KCNE1 (D–F). Current traces from *Xenopus* oocytes expressing WT, Mut and WT/Mut channels **A**. alone (− KCNE1; − 80 to +60 mV) and **d** in combination with KCNE1 (+KCNE1; − 80 to +90 mV) were fitted with double exponential function (black dotted lines). Averaged fast (**b, e**) and slow (**c, f**) activation time constants derived from double exponential function fitting are plotted against tested voltage steps. Data are mean ± SD. For − KCNE1: WT ($n = 24$), Mut ($n = 26$), WT/Mut ($n = 19$). For +KCNE1: WT ($n = 18$), Mut ($n = 12$), WT/Mut ($n = 27$). All experiments were performed using at least three different batches of oocytes. Statistical comparison was performed using one-way ANOVA with Dunnett's test, and statistical significance was set at $p < 0.01$; $^{**}p < 0.01$

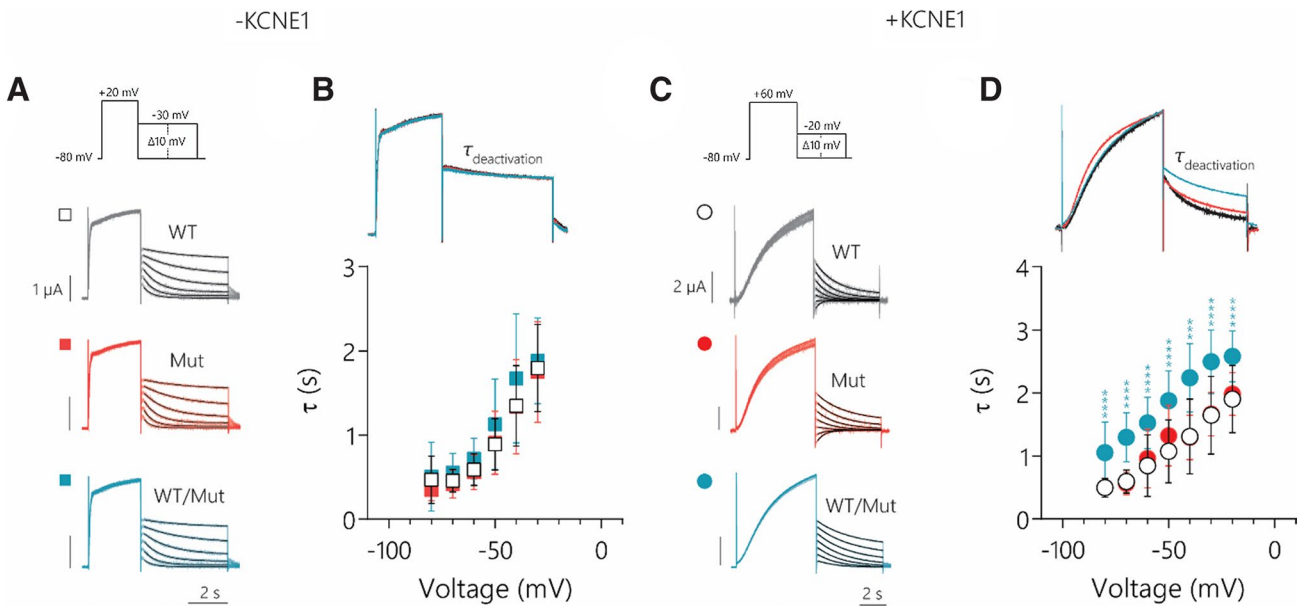

Fig. 4 Deactivation kinetics of KCNQ1 WT, Mut and WT/Mut channels in the absence (**a, b**) and presence (**c, d**) of KCNE1. To elicit channel deactivation, a pre-pulse of − 80 to +20 mV (− KCNE1) or − 80 to +60 mV (+KCNE1) was applied for 3 and 6 s, respectively, then stepped back to − 30 mV (− KCNE1) or − 20 mV (+KCNE1) for 5 s in 10 mV decrements (top panels, **a, c**). Representative current traces of WT, Mut and WT/Mut channels in response to the deactivating voltage steps are shown below the voltage protocols. Current decays were fitted with a single exponential function (indicated as black line on current traces) to obtain the deactivation time constants. Averaged deactivation time constants at all tested potentials are shown in **b, d** (bottom panels). Current traces when stepping from +20 to − 30 mV (for − KCNE1) or +60 to − 20 mV (for +KCNE1) were overlaid for comparison (top panels, **b, d**). Data are mean ± SD. For − KCNE1: WT ($n=25$), Mut ($n=28$), WT/Mut ($n=32$). For +KCNE1: WT ($n=17$), Mut ($n=11$), WT/Mut ($n=19$). Statistical comparison was performed using one-way ANOVA with Dunnett's test, and statistical significance was set at $p<0.01$. ***$p<0.001$, ****$p<0.0001$

Results

Genetic analysis

Molecular autopsy identified two heterozygous variants in the proband – a 21-nucleotide in-frame c.190_210del deletion mutation in *KCNQ1*, predicting a six-amino acid deletion (p. 64_70delPASPAAP*) in the N-terminus of the encoded KCNQ1 channel (also known as Kv7.1 or KvLQT1; Fig. 2a); and a missense c.2431C > A variant in the plakophilin-2 gene *PKP2* (p.R811S). The *KCNQ1* variant has been previously identified in three long QT syndrome (LQTS) patients and has been reported as pathogenic;[8], although neither precise phenotype nor co-segregation with the disease have been reported. However, this variant had not been functionally characterized before. Previous reports on the *PKP2*-R811S variant showed conflicting interpretations of pathogenicity. While it has been reported previously in individuals with arrhythmogenic right-ventricular cardiomyopathy (ARVC),[9, 10] this variant was also identified in healthy control individuals [11] and in 0.1% (77/66,740) of European chromosomes by the Exome Aggregation Consortium (ExAC) (http://exac.broadinstitute.org; dbSNP: rs139734328). The NHLBI Exome Sequencing Project and the 1000 Genomes Project identified the *PKP2*-R811S variant with a frequency of 0.06–0.2% in individuals of European and Mixed American ancestry, indicating it may be a rare benign variant in these populations. In silico analysis gave conflicting results with Polyphen, SIFT and Align GVGD predicting this variant to be tolerated while MutationTaster predicted this variant to be damaging. Finally, the *PKP2*-R811S variant is a semi-conservative amino acid substitution at a position that is conserved across species, and may impact secondary protein structure as these residues differ in their physicochemical properties. Therefore, the ACMG criteria predicted this variant to be 'likely benign'.

Targeted genetic screening was performed in the parents and the brother. The father tested positive for both heterozygote variants, the brother carries only the *KCNQ1* variant, and the mother carries only the WT alleles in both genes. Since the identification of *KCNQ1-p.* 64_70del-PASPAAP* was diagnostic for LQTS according to the international guidelines,[12] we initiated a beta-blocker therapy in the father and brother, who both carry the variant, but exhibited no evident phenotype, neither in the ECG at rest nor during the stress test. The pedigree is summarized in Fig. 1d.

Functional characterization of KCNQ1 ΔP64-P70

The functional properties of the mutant KCNQ1 channels expressed in *Xenopus laevis* oocytes were examined using the two-electrode voltage-clamp technique. Overall, we found no striking differences in various channel properties. (1) Current levels recorded in the absence (current ± SD when stepping from − 80 to + 60 mV; WT: 4.0 ± 1.7 µA; Mut: 3.9 ± 1.4 µA; WT/Mut: 3.1 ± 1.2 µA; *p* values ~ 0.020–0.90, One-way ANOVA with Dunnett's test) and presence of KCNE1 (current ± SD when stepping from − 80 to + 90 mV; WT: 7.6 ± 4.2 µA; Mut: 9.8 ± 6.5 µA; WT/Mut: 8.7 ± 3.6 µA; *p* values ~ 0.27–0.65; One-way ANOVA with Dunnett's test) were comparable. (2) The voltage-dependence of channel activation exhibited by Mut and WT/Mut did not differ from that of WT regardless of whether KCNE1 was present or not ($V_{1/2}$ ± SD ; − KCNE1: WT (− 16 ± 4.9 mV; $n = 17$), Mut (− 18 ± 4.8 mV; $n = 21$) and WT/Mut (− 17 ± 6.1 mV; $n = 26$); + KCNE1: WT (32 ± 12 mV; $n = 21$), Mut (34 ± 15 mV; $n = 16$) and WT/Mut (21 ± 12 mV; $n = 28$); Fig. 2c, d). (3) The activation time constants of Mut and WT/Mut channels were similar compared to WT in the absence of KCNE1 (Fig. 3a–c). When co-expressed with KCNE1, a small decrease in the slow activation time constants of the WT/Mut channels was detected at − 40, − 50 and − 60 mV ($p < 0.01$; One-way ANOVA with Dunnett's test; Fig. 3d–f). (4) In the absence of KCNE1, the ΔP64–P70 deletion mutation had no effect on the rate of channel deactivation, as evident from the overlapping deactivation time constants of WT, Mut and WT/Mut channels at all tested membrane potentials (Fig. 4a, b). Interestingly, in the presence of KCNE1, WT/Mut channels showed significantly slower deactivation time constants across the entire voltage range (− 20 to − 80 mV). The deactivation kinetics of Mut channels in combination with KCNE1 was similar to those of the WT channels (Fig. 4c, d).

Discussion

Advances in the understanding of the molecular pathophysiology of inherited arrhythmia syndromes and cardiomyopathies have revealed that 'concealed' disease forms may represent the substrate for UCA and unexplained SCD [13–16]. In the clinical case described here, we initially diagnosed a 'concealed' long QT syndrome (LQTS) in the UCA victim with a *KCNQ1*-p. 64_70delPASPAAP* variant based on previous reports and following the ACMG guidelines [2]. This was followed by targeted family screening and the initiation of beta-blocker therapy in asymptomatic relatives who carry the *KCNQ1* deletion, and were assumed to be at risk for SCD. However, mutation carriers never exhibited LQTS compatible phenotype, neither at rest nor during stress test.

KCNQ1 encodes a voltage-gated potassium channel, which in combination with the *KCNE1*-encoded auxiliary subunit, generates the slow cardiac delayed rectifier potassium current (I_{Ks}). Mutations with functional consequences (e.g. a shift in voltage sensitivity or reduced surface expression) found in the *KCNQ1* gene are frequently associated with LQTS, short QT syndrome (SQTS) and atrial fibrillation (AFib) [17]. The KCNQ1 ΔP64–P70 mutation and another deletion variant in the region immediately after P70 (KCNQ1 ΔA71–P73) have both been identified in patients with LQTS [18, 19]. These observations had prompted us to hypothesize that mutations in this region may negatively affect the expression and/or function of KCNQ1 channels, which may result in repolarization abnormalities. However, electrophysiological characterization of KCNQ1 ΔP64-P70 channels in *Xenopus laevis* oocytes revealed no obvious differences in function, except for a modest slowing of the deactivation kinetics in WT/Mut + KCNE1 channels (Fig. 4c, d). As such, there is no strong in vitro evidence supporting the previously predicted pathogenicity of this variant. Yet, it is worth noting that our study does not exclude additional factors, such as repolarization reserve, *KCNQ1*-interacting proteins and epigenetic changes, which may also determine the clinical phenotype of a mutation carrier. Under these circumstances, we believe several important points should be considered when evaluating the case.

The *KCNQ1*-p. 64_70delPASPAAP* has been previously reported as pathogenic in three patients with LQTS [8]. However, the criteria used for LQTS diagnosis, the severity of phenotype, co-segregation data and functional characterization of this N-terminal in-frame deletion were not available. These are considered reliable criteria for variant classification [2]. Our data show that carriers of the *KCNQ1*-p. 64_70delPASPAAP* do not exhibit a LQTS or SQTS phenotype, including the index case who experienced a sudden cardiac arrest. Functional studies were congruent with the clinical findings, as we did not observe functional effects consistent with a gain or loss of function of the mutant KCNQ1 channel. Therefore, we have no solid evidence in this family to consider *KCNQ1*-p. 64_70delPASPAAP* as a primary pathogenic variant. Nevertheless, a contributing role of the mutation to the sudden death of the patient cannot be definitely excluded without a detailed functional characterization of the mutant channel in a more physiologically relevant system, such as cardiomyocytes. Additionally, analyzing the phenotype in additional carriers would help to better understand this variant. Unfortunately, this information is not available in previous reports.

Mutations in *PKP2* are associated with autosomal dominant life-threatening arrhythmogenic cardiomyopathy, [20], but the proband and his father had no evidence of ARVC. The *PKP2*-R811S variant found in the index case is considered as likely benign by the current ACMG guidelines [2].

Thus, we did not consider this finding to have major implications in the cardiac arrest of this case. However, a modifying effect of this variant cannot be ruled out.

In UCA cases, in which no clinical markers of underlying disease are detected to guide the genetic testing (also referred as idiopathic VF), broad-panel genetic testing is not recommended by the international Expert Consensus Statement (class III indication) [1]. This is primarily to prevent unfounded anxiety in the relatives caused by uncertain genetic results, which may be misinterpreted leading to inappropriate interventions. Interestingly, several recent studies reported pathogenic or likely pathogenic variants in arrhythmia- and cardiomyopathy-associated genes in nearly 15% of UCA cases [13–15, 21]. We therefore believe that a carefully interpreted genetic test in this population can still provide valuable information in at least 15% of family members who, in case of being at risk, may receive preventive treatments. We thus support the concept of performing a responsible genetic test interpretation, rather than avoiding it from the outset. Further data will allow us to better interpret genetic data and will be translated into better care for the patients.

In conclusion, based on our clinical and functional data, the previously reported variant *KCNQ1*:c.190_210del ((p. 64_70delPASPAAP*)) should not be considered as primary pathogenic. Although genetic testing in sudden cardiac arrest survivors carries an inherent risk of misinterpretation, evidence supports that at least 15% of families can benefit from such a test. A careful and responsible test interpretation will provide these families with valuable information with potentially beneficial implications.

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