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Identification and characterisation of a novel KCNQ1 mutation in a family with Romano–Ward syndrome

J. Zehelein[a,*,1], D. Thomas[a,1], M. Khalil[b], A.-B. Wimmer[a], M. Koenen[c], M. Licka[a], K. Wu[a], J. Kiehn[a], K. Brockmeier[d], V.A.W. Kreye[e], C.A. Karle[a], H.A. Katus[a], H.E. Ulmer[b], W. Schoels[a]

[a]*Innere Medizin III, Universitätsklinik Heidelberg, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany*
[b]*Kinderheilkunde II, Universitätsklinik Heidelberg, Im Neuenheimer Feld 150, 69120 Heidelberg, Germany*
[c]*Abteilung Zellphysiologie, Max-Planck-Institut für medizinische Forschung, Jahnstraße 29, 69120, Heidelberg, Germany*
[d]*Abteilung Kinderkardiologie, Klinikum der Universität zu Köln, Joseph-Stelzmann-Straße 9, 50931 Köln, Germany*
[e]*Institut für Physiologie und Pathophysiologie, Universität Heidelberg, Im Neuenheimer Feld 326, 69120 Heidelberg, Germany*

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Abstract

Romano–Ward syndrome (RWS), the autosomal dominant form of the congenital long QT syndrome, is characterised by prolongation of the cardiac repolarisation process associated with ventricular tachyarrhythmias of the torsades de pointes type. Genetic studies have identified mutations in six ion channel genes, KCNQ1, KCNH2, SCN5A, KCNE1 and KCNE2 and the accessory protein Ankyrin-B gene, to be responsible for this disorder. Single-strand conformation polymorphism (SSCP) analysis and subsequent DNA sequence analysis have identified a KCNQ1 mutation in a family that were clinically conspicuous due to several syncopes and prolonged QTc intervals in the ECG. The mutant subunit was expressed and functionally characterised in the *Xenopus* oocyte expression system. A novel heterozygous missense mutation with a C to T transition at the first position of codon 343 (CCA) of the KCNQ1 gene was identified in three concerned family members (QTc intervals: 500, 510 and 530 ms, respectively). As a result, proline 343 localised within the highly conserved transmembrane segment S6 of the KCNQ1 channel is replaced by a serine. Co-expression of mutant (KCNQ1-P343S) and wild-type (KCNQ1) cRNA in *Xenopus* oocytes produced potassium currents reduced by ≈92%, while I_{Ks} reconstitution experiments with a combination of KCNQ1 mutant, wild-type and KCNE1 subunits yielded currents reduced by ≈60%. A novel mutation (P343S) identified in the KCNQ1 subunit gene of three members of a RWS family showed a dominant-negative effect on native I_{Ks} currents leading to prolongation of the heart repolarisation and possibly increases the risk of malign arrhythmias with sudden cardiac death.

Keywords: I_{Ks} current; KCNQ1 (KVLQT1); Long QT syndrome; Electrophysiology; Mutation

1. Introduction

The autosomal dominant form of the hereditary long QT syndrome [Romano–Ward syndrome (RWS)] is characterised by prolongation of the cardiac action potential, syncopal attacks, torsades de pointes arrhythmias and sudden cardiac death [1,2] with an estimated prevalence of 1 in 5000–7000 [3]. Six different genetic loci associated with RWS have been identified {KCNQ1 (KVLQT1) [LQT1]; KCNH2 (HERG) [LQT2]; SCN5A [LQT3]; Ankyrin-B [LQT4]; KCNE1 (mink) [LQT5]; KCNE2 (MiRP1) [LQT6]} [4,5]. Two of these genes, namely KCNQ1 and KCNH2, account for 42% and 45%, respectively, of the RWS mutations [4].

KCNQ1 subunits assemble with KCNE1 subunits to form I_{Ks} channels, carrying the slow activating component of the delayed rectifier K^+ current, which underlies the

* Corresponding author. Tel.: +49 6221 5638687; fax: +49 6221 564662.
E-mail address: joerg_zehelein@med.uni-heidelberg.de (J. Zehelein).
[1] Both authors contributed equally to this work.

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repolarisation phase of cardiomyocytes [6,7]. RWS patients with alterations in the KCNQ1 gene show reduced current amplitudes due to a dominant-negative suppression of KCNQ1 function [8]. Here, we describe the identification and functional characterisation of a novel KCNQ1 mutation (P343S) in three members of a RWS family. Expression of the mutant subunit resulted in a reduction of I_{Ks} currents with the mutant exhibiting a dominant-negative effect. These alterations in cellular electrophysiology are thought to underlie the clinical manifestation of the disorder.

2. Materials and methods

2.1. Clinical diagnostic

The study was based on the findings in a three-generation family with the now 18-year-old index patient suffering from recurrent syncopes and a QTc prolongation in the resting 12-lead surface electrocardiogram (ECG). The investigation conforms with the principles outlined in the Declaration of Helsinki.

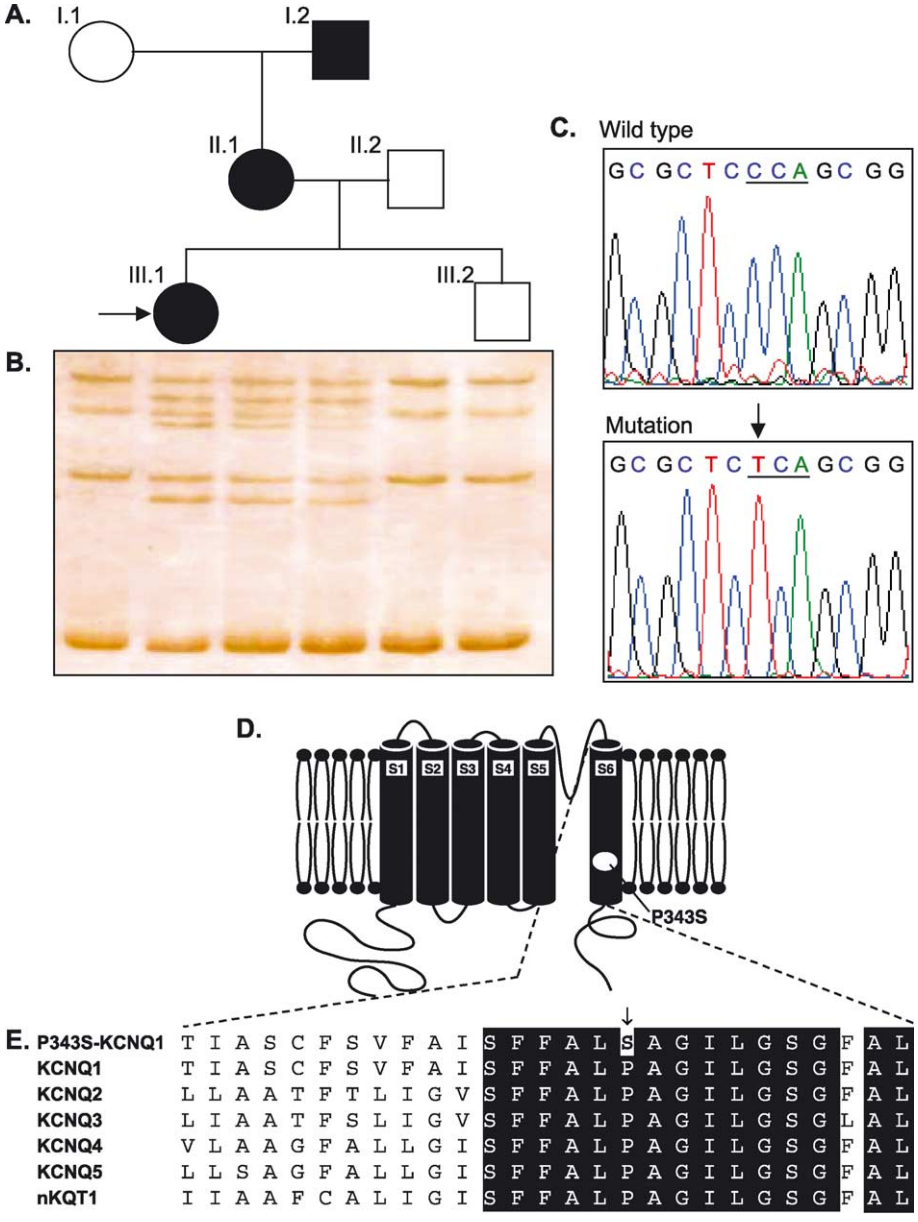

Fig. 1. Identification of the KCNQ1–P343S mutation. (A) Pedigree of the RWS family analysed. Arrow indicates the index patient. Closed symbols denote affected individuals; open symbols denote inconspicuous family members. (B) SSCP analysis of PCR products obtained from exon 6 of the KCNQ1 gene. Comparison of samples I.2, II.1 and III.1 with control samples (I.1, II.2, III.2) revealed different mobility in affected members. (C) DNA sequence of exon 6 amplified from wild type and patient III.1. A heterozygous C to T transition is observed at the first base in codon 343 of the KCNQ1 gene. (D) Hypothetical membrane folding model for the mutant KCNQ1–P343S potassium channel. (E) Alignment of S6 transmembrane domains of the mutant KCNQ1–P343S subunit, the human subunits (KCNQ1–KCNQ5) [29] and the *C. elegans* homologue (nKQT1) [17]. Identical residues are black-shaded, with the mutation marked by an arrow.

2.2. Identification of KCNQ1 mutations

Exon sequences of the KCNQ1 gene were assayed by amplification of genomic DNA extracted from blood samples of all family members (Qiagen, Hilden, Germany) followed by single-strand conformation polymorphism (SSCP) analysis as previously described [9]. Representative PCR products were obtained after 5 min at 95 °C followed by 35 cycles of 60 s at 95 °C, 60 s annealing at 58 °C and a synthesis step of 60 s at 72 °C. Amplification was finished by 10 min at 72 °C. The KCNQ1 gene primers (5′ to 3′ orientation) TGGCTGACCACT GTCCCTCT and CCCCAGGACCCCAGCTGTCCAA, previously described by Splawski et al. [10] and Neyroud et al. [11], were used to amplify exon 6 sequences in a volume of 50 μl (50 ng template DNA, 25 pM of each primer, 200 μM 4-dNTP, 1 U *Thermus aquaticus* DNA polymerase, 10 mM Tris–HCl, pH 8.3, at room temperature, 50 mM KCl, and 1.5 mM $MgCl_2$). PCR products were separated on a Multiphor II Electrophoresis System by 60 mA for 2 h at 17 °C and visualised by silver staining. Normal and aberrant PCR products were separated on 1.5% agarose gels, purified with the Qiaex gel extraction kit (Qiagen) and subcloned into the TOPO II vector (Invitrogen, Carlsbad, USA). DNA sequence was determined using an ABI Bio Systems 377 Prism Automated DNA Sequencer (Applied Biosystems).

2.3. Cloning and mutagenesis of KCNQ1

Complementary DNA encoding the human KCNQ1-Isoform 1 was cloned from a Human Heart Marathon-Ready cDNA library (Clonetech) using primers selected against published sequences (EMBL database: accession number af000571). PCR products were characterised by DNA sequence analysis and the final construct including a modified translation initiation sequence (CCACCATG) [12] was inserted into the *Bgl*II site of the *Xenopus* oocyte expression vector pSPOoD [13]. Site-directed PCR mutagenesis was performed and KCNQ1 mutants and control sequences were characterised by DNA sequence analysis of both strands.

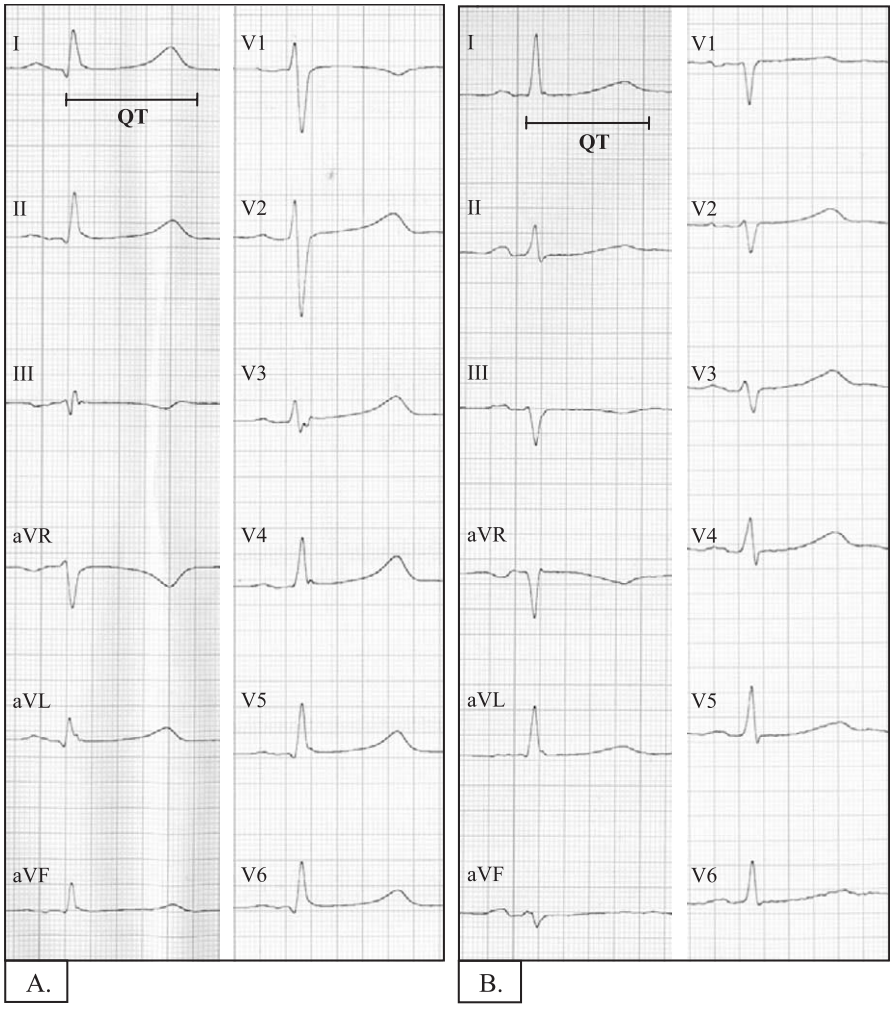

Fig. 2. Twelve-lead resting ECG of family members III.1 (A) and II.1 (B), both demonstrating a late-onset normally appearing T-wave pattern with marked QT prolongation.

2.4. Heterologous gene expression in Xenopus laevis oocytes

KCNQ1, KCNQ1-P343S and KCNE1 cRNAs were synthesised using the mMESSAGE mMACHINE kit (Ambion, Austin, USA). Stage V–VI defolliculated *Xenopus* oocytes were injected with different amounts of cRNA encoding the mutant or wild-type subunits. All protocols for animal use were in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the U.S. National Institute of Health (NIH Publication No. 85-23, revised 1996) and the European Community guidelines for the use of experimental animals.

2.5. Electrophysiology and statistics

Potassium channel expression was determined 2–3 days after cRNA injection by two-microelectrode voltage-clamp recordings from *X. laevis* oocytes [14]. Whole-cell recordings were performed using a Warner OC-725A amplifier (Warner Instruments, Hamden, USA) and pClamp software (Axon Instruments, Foster City, USA) for data acquisition and analysis. Microelectrodes had a tip resistance ranging from 1 to 5 MΩ. Voltage clamp measurements were performed on the current of mutant and control subunits in a solution containing (in mM): 5 KCl, 100 NaCl, 1.5 $CaCl_2$, 2 $MgCl_2$ and 10 HEPES (pH 7.4 with NaOH). Current and voltage electrodes were filled with 3 M KCl solution and the recording chamber was continually perfused. All experiments were carried out at room temperature (20–22 °C). No leak subtraction was done during the experiments.

All data are expressed as mean±standard deviation, unless otherwise specified. Unpaired Student's *t* tests (two-tail tests) were used to compare the statistical significance of the results. A P value <0.05 was considered statistically significant.

3. Results

3.1. Identifying and phenotyping of the RWS family

The index patient, an 18-year-old woman (Fig. 1A, III.1), presented with a history of recurrent syncopes after exercise or emotional stress. Syncopes occurred between age 3 and 13. The last event happened during swimming and resulted in cardiac arrest. Resuscitation was successful, and the patient was then treated with propranolol 3 mg kg^{-1} day^{-1} without further syncopes occurring. The resting ECG

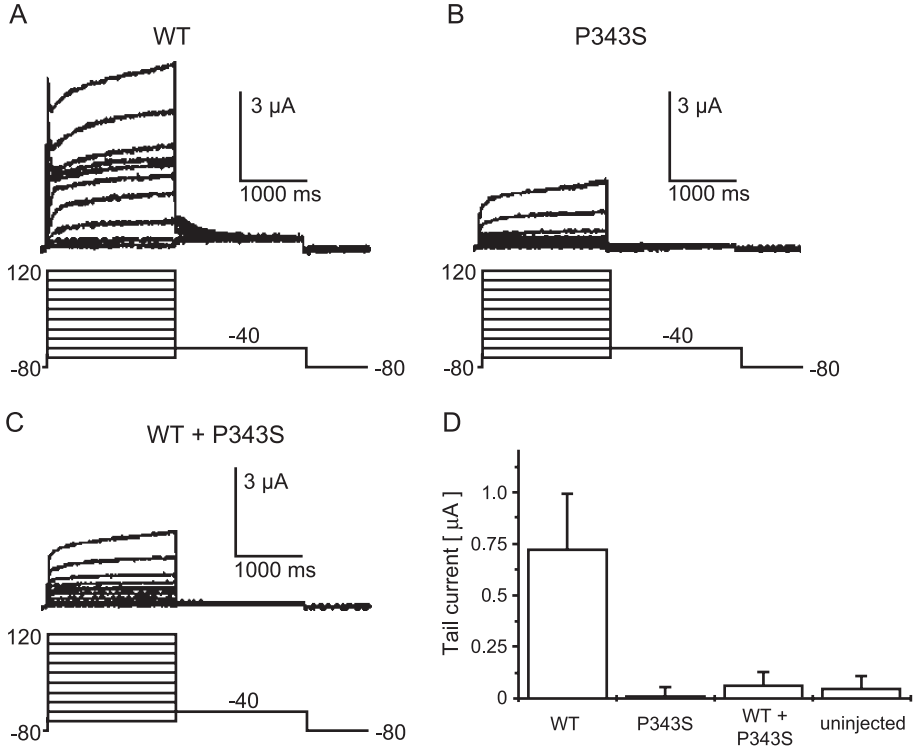

Fig. 3. Currents induced by expression of wild-type KCNQ1 and mutant KCNQ1–P343S subunits in *Xenopus* oocytes. Representative potassium current traces were recorded from *Xenopus* oocytes injected with 46 ng cRNA and expressing (A) homomultimeric wild-type or (B) mutant channels. (C) Currents induced by co-expression of equal amounts 23 ng of mutant and wild-type cRNA illustrate a dominant-negative effect of KCNQ1–P343S on wild-type K^+ currents. All experiments shown were obtained from the same batch of oocytes. (D) Mean peak tail current amplitudes obtained from two batches. The numbers of experiments performed are n=18 (wild type KCNQ1; KCNQ1–P343S) and n=20 (wild type KCNQ1+KCNQ1–P343S), respectively. The difference between KCNQ1–P343S and uninjected oocytes was not statistically significant. Voltage protocol in A–C: holding potential −80 mV, test pulse −60 to 120 mV (2 s) in 20-mV increments, return pulse constant −40 mV (2 s).

revealed a QTc interval of 530 ms (Fig. 2A). Screening of five family members indicated a prolonged QT interval in the patient's mother (age: 47, OTc: 500 ms) (Fig. 2B) and the maternal grandfather (age: 73, QTc: 510 ms) (data not shown). The resting ECGs of these three family members displayed a late-onset normally appearing T-wave pattern representing a typical configuration associated with LQT1 patients [15]. The mother suffered from recurrent syncopes before treatment with propranolol was initiated at the age of 42. The grandfather as well as his sister (from which neither ECG or blood samples could be obtained) had a history of recurrent syncopes up to the age of 35. Subsequently, no signs of cardiac disturbances appeared. ECGs and clinical history of the index patient's brother, father and maternal grandmother were inconspicuous.

3.2. Identification of the KCNQ1-P343S gene mutation

DNA samples from all family members were subjected to a mutation screening of the KCNQ1 gene by SSCP analysis. An abnormal migration pattern was identified in exon 6 of the family members I.2, II.1 and III.1 (Fig. 1B), whereas 100 unrelated controls representing 200 alleles (data not shown) displayed a pattern identical to the pattern of the unaffected family members I.1, II.2 and III.2 (Fig. 1B). DNA sequence analysis of exon 6 derived PCR products of index patients III.1, II.1 and I.2 revealed a heterozygous C to T transversion at nucleotide 1027 (Fig. 1C) corresponding to the first position of codon 343 (CCA). Accordingly, proline 343 located within a highly conserved stretch of amino acids of the transmembrane region S6 was replaced by a serine (P343S) (Fig. 1D,E).

3.3. Electrophysiological analysis of mutant KCNQ1-P343S channels

Potassium channels assembled by homomultimeric subunits were functionally characterised in *Xenopus* oocytes injected with cRNA of wild type (KCNQ1) or mutant (KCNQ1–P343S). Currents were elicited by 2-s steps to potentials ranging from −60 to 120 mV (increment 20 mV), and tail currents were recorded at −40 mV. The holding potential was −80 mV, and pulses were applied at a frequency of 0.2 Hz. KCNQ1 currents activated during membrane depolarisation with a multi-exponential time course and did not fully activate during a

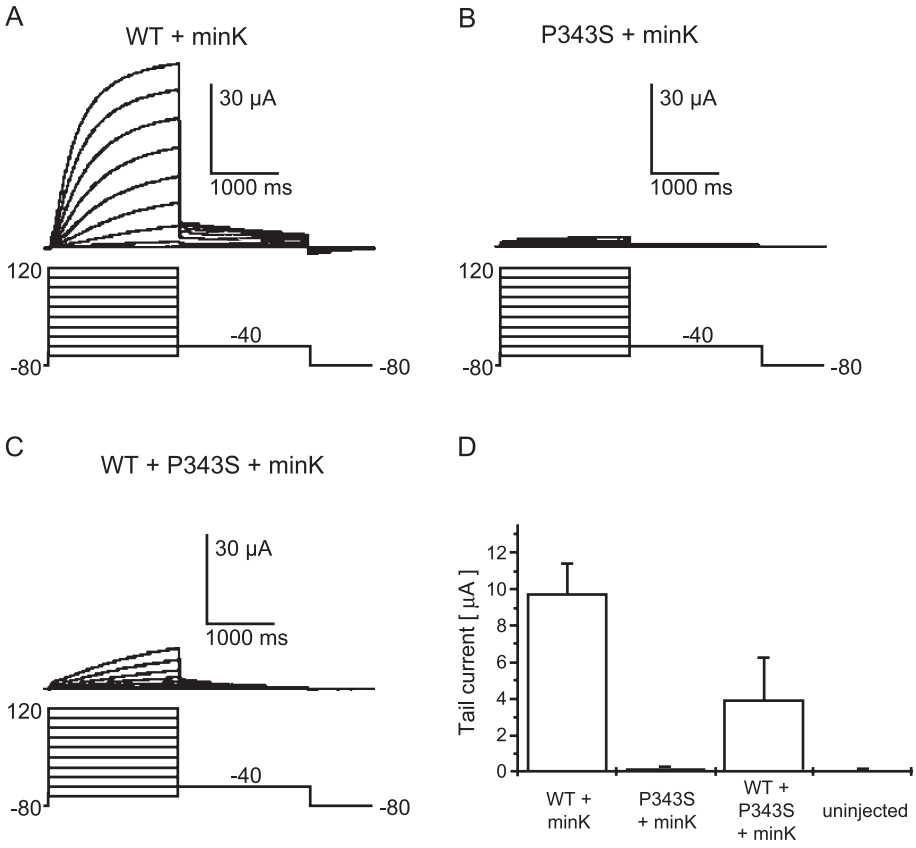

Fig. 4. Expression of I_{Ks} currents in *Xenopus* oocytes. Co-expression of wild-type KCNQ1 (11.5 ng) and KCNE1 cRNA (5.75 ng; panel A) in oocytes resulted in K^+ currents very similar to I_{Ks}. (B) Co-expression of mutant KCNQ1–P343S (11.5 ng) and KCNE1 subunits (5.75 ng). (C) I_{Ks} currents induced by co-expression of KCNE1 (5.75 ng) and equal amounts of WT (5.75 ng) and KCNQ1–P343S (5.75 ng) subunits. I_{Ks} current amplitudes were reduced by approximately 60%, indicating that KCNQ1–P343S exerted a dominant-negative effect. (D) Mean peak tail current amplitudes obtained from two batches. The numbers of experiments performed were n=18 (WT-KCNQ1+KCNE1) and n=20 (KCNQ1–P343S+KCNE1; WT-KCNQ1+KCNQ1–P343S+KCNE1), respectively. K^+ currents were elicited by 2-s voltage steps from a holding potential of −80 mV to test potentials from −60 to +120 mV in 20-mV increments.

2-s pulse (Fig. 3A). Upon membrane repolarisation at −40 mV, current deactivation was preceded by current increase due to rapid recovery from inactivation [16]. In contrast, expression of KCNQ1–P343S cRNA (Fig. 3B) resulted in mean maximum current amplitudes measured during the −40 mV pulse that were not significantly different from currents recorded from uninjected oocytes (Fig. 3D).

To determine whether mutant and wild-type subunits form heteromultimeric channels, equal amounts of wild-type and mutant cRNA were co-injected into oocytes. Only minor currents clearly different from KCNQ1 currents were observed (Fig. 3C). Thus, mutant subunits (KCNQ1–P343S) seemed to assemble with wild-type subunits. This led to current amplitudes reduced by ≈92% (Fig. 3D), indicating a dominant-negative suppression of channel function.

In human cardiac cells, the pore forming α-subunit KCNQ1 and the β-subunit KCNE1 form channels with currents identical to the slow cardiac delayed rectifier I_{Ks} [6,7].

To determine whether the mutant subunit could suppress I_{Ks}, equal amounts of either mutant (KCNQ1–P343S) or wild-type KCNQ1 cRNA were co-expressed with KCNE1 cRNA. Currents produced by KCNQ1 and KCNE1 had a much larger amplitude (Fig. 4A) than KCNQ1 currents. In contrast, a combination of KCNE1 and KCNQ1–P343S produced I_{Ks} current amplitudes strongly reduced by ≈98% (Fig. 4B and D), indicating that I_{Ks} currents were affected by the mutant subunit. Indeed, a combination of equimolar amounts of KCNQ1, KCNQ1–P343S and KCNE1 cRNA injected into *Xenopus* oocytes produced I_{Ks} currents reduced by almost 60% (Fig. 4D). Thus, co-assembling of mutant KCNQ1–P343S and wild-type subunits resulted in a dominant-negative effect on I_{Ks} currents.

4. Discussion

We have identified and characterised a novel mutation in the potassium channel subunit gene KCNQ1 in an RWS family. A heterozygous missense mutation in exon 6 replaced a proline by a serine (P343S) within a highly conserved stretch of amino acids [17]. This region, $KCNQ1_{338–353}$, is part of the transmembrane region S6 of the KCNQ channel family and represents a hot spot aligned with approximately 21% of all LQT1 mutations [4]. Particularly, KCNQ1 codons A341 and A344, flanking proline 343 (P343), are affected most frequently and cause LQT1 in at least 23 Romano–Ward families [4]. According to the crystal structure of the KcsA K^+ channel of *Streptomyces lividans* [18], the transmembrane segment S6 is part of the KCNQ1 channel pore and mutations within this region are thought to strongly influence potassium ion transport.

Family members carrying the KCNQ1–P343S mutation were sensitive to typical triggers for syncopes like exercise, swimming or emotional stress. Their ECG pattern revealed a late-onset but normally appearing T-wave pattern with marked QT prolongation, suggesting LQT1 to be the most likely candidate in this Romano–Ward family [15,19].

Hitherto, no genotype–phenotype correlation could be established that associated a specific course of the disease to mutations in distinct structural domains of the KCNQ1 channel subunit [20]. Mutants aligned to the short and highly preserved region $KCNQ1_{338–353}$ show a wide clinical spectrum ranging from asymptomatic cases to sudden death with QTc intervals varying from <440 to >550 ms [21–26].

Functional analysis of KCNQ1–P343S subunits in *Xenopus* oocytes revealed that substitution of proline 343 by a serine yielded almost inactive homopolymer channels. This observation is confirmed by other mutants (A341E, A341V, L342F, G345E) aligned to the $KCNQ1_{338–353}$ region [27,28]. Co-expression experiments with mutant and wild-type KCNQ1 subunits in *Xenopus* oocytes revealed a dominant-negative effect of the KCNQ1–P343S subunit, with current amplitudes reduced by ≈92% compared to that of wild type (KCNQ1). This is in line with previously reported experiments of KCNQ1 wild-type and mutant subunits (A341E, L342F and G345E) and supports the idea that mutant and wild-type subunits assemble to heteromultimeric but almost inactive channels [27,28]. Interestingly, a less uniform pattern appears in the presence of KCNE1 subunits and most $KCNQ1_{338–353}$ mutations affect I_{Ks} currents differently. Co-expression of KCNE1 and wild-type KCNQ1 subunits in combination with KCNQ1–P343S subunits reduces I_{Ks} currents by ≈60%. Other $KCNQ1_{338–353}$ mutants reduce I_{Ks} current amplitudes by ≈63% (KCNQ1–G345E) or ≈23% (KCNQ1–A341E), produce inactive channels (KCNQ1–L342F) or I_{Ks} currents not different from wild type (KCNQ1–A341V) [27,28].

A search for correlations between structural changes at the $KCNQ1_{338–353}$ region and implications on current density and severity of the disease yielded an inconsistent pattern and a predictable relationship has not yet been observed. KCNQ1–A341E mutants form almost inactive homomeric channels and reduce I_{Ks} currents by ≈23%. Clinical symptoms are rare. By comparison, substitution of alanine 341 by a valine instead (KCNQ1–A341V) yielded inactive homomeric channels like KCNQ1–A341E but produce I_{Ks} currents not different from wild type and, surprisingly, caused a high incidence of symptoms including sudden cardiac death [28]. The KCNQ1–P343S mutation described here produce almost inactive channels and lower I_{Ks} currents to ≈60%. The three identified carriers of the KCNQ1–P343S mutation are concerned by recurrent syncopes, but no sudden death appeared in the familial history so far.

One might assume a relationship between their effect on I_{Ks} current amplitudes and their incidence of clinical symptoms; however, $KCNQ1_{338–353}$ mutants behave differently and an increase in I_{Ks} suppression is not followed by a

growing incidence of symptoms or increasing risk of sudden cardiac death. The poor correlation between onset, course and severity of RWS and structural changes at distinct domains of the KCNQ1 subunit with their heterogeneity in functional behaviour led to the idea that epigenetic factors might play a major role in precipitating life-threatening arrhythmias in LQT1 patients.

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