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Dominant-negative I_{Ks} suppression by KCNQ1-ΔF339 potassium channels linked to Romano–Ward syndrome

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Abstract

Objective: Hereditary long QT syndrome (LQTS) is a genetically heterogeneous disease characterized by prolonged QT intervals and an increased risk for ventricular arrhythmias and sudden cardiac death. Mutations in the voltage-gated potassium channel subunit KCNQ1 induce the most common form of LQTS. KCNQ1 is associated with two different entities of LQTS, the autosomal-dominant Romano–Ward syndrome (RWS), and the autosomal-recessive Jervell and Lange–Nielsen syndrome (JLNS) characterized by bilateral deafness in addition to cardiac arrhythmias. In this study, we investigate and discuss dominant-negative I_{Ks} current reduction by a KCNQ1 deletion mutation identified in a RWS family.

Methods: Single-strand conformation polymorphism analysis and direct sequencing were used to screen LQTS genes for mutations. Mutant KCNQ1 channels were heterologously expressed in *Xenopus* oocytes, and potassium currents were recorded using the two-microelectrode voltage clamp technique.

Results: A heterozygous deletion of three nucleotides (CTT) identified in the KCNQ1 gene caused the loss of a single phenylalanine residue at position 339 (KCNQ1-ΔF339). Electrophysiological measurements in the presence and absence of the regulatory β-subunit KCNE1 revealed that mutant and wild type forms of an N-terminal truncated KCNQ1 subunit (isoform 2) caused much stronger dominant-negative current reduction than the mutant form of the full-length KCNQ1 subunit (isoform 1).

Conclusion: This study highlights the functional relevance of the truncated KCNQ1 splice variant (isoform 2) in establishment and mode of inheritance in long QT syndrome. In the RWS family presented here, the autosomal-dominant trait is caused by multiple dominant-negative effects provoked by heteromultimeric channels formed by wild type and mutant KCNQ1-isoforms in combination with KCNE1.

Keywords: Arrhythmia (mechanisms); Ion channels; K-channel; KCNQ1; Long QT syndrome

1. Introduction

Hereditary long QT syndromes (LQTS) are cardiac disorders characterized by a prolonged QT interval on the surface ECG associated with syncopal attacks and a high risk of sudden cardiac death due to ventricular tachyarrhythmias. The LQTS include the autosomal-dominant Romano–Ward syndrome (RWS) [1,2] and the autosomal-recessive Jervell and Lange–Nielsen syndrome (JLNS) [3], associated with

Abbreviations: I_{Ks}, slowly activating component of I_K; iso1, isoform 1; iso2, isoform 2; JLNS, Jervell and Lange–Nielsen syndrome; LQTS, long QT syndrome; RWS, Romano–Ward syndrome; $V_{1/2}$, half-maximal activation voltage; WT, wild type

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bilateral congenital deafness caused by abnormal homeostasis of the inner ear endolymph [4]. Seven genetic loci have been linked to hereditary long QT syndrome [5]. LQTS-1, the most frequent form of LQTS, is associated with KCNQ1 (KvLQT1) gene mutations [6]. Coexpression of the pore-forming α-subunit KCNQ1 and its regulatory β-subunit KCNE1 (minK) elicits slowly activating potassium currents with no apparent inactivation, resembling the slow component of the cardiac delayed rectifier potassium current I_{Ks} [7,8]. I_{Ks} contributes to cardiac repolarization and is a target for class III antiarrhythmic drugs such as dronedarone [9].

It is important to note that in human heart two different KCNQ1-isoforms are expressed, the normal KCNQ1 subunit (isoform 1) and an alternatively spliced subunit lacking the N-terminal 127 amino acids (isoform 2). KCNQ1-isoform 2 subunits (KCNQ1-iso2) exert a pronounced dominant-negative effect on potassium channels composed of KCNQ1-isoform 1 subunits (KCNQ1-iso1) and on I_{Ks} channels composed of KCNQ1-iso1 and KCNE1 subunits [10–12]. The balance between both isoforms of the KCNQ1 gene is thought to stabilize the slow component of the cardiac delayed rectifier potassium current I_{Ks}. Thus, mutations of the KCNQ1 gene may affect the equilibrium of functional isoform 1 and truncated isoform 2 subunits. Previous studies showed that most KCNQ1 mutants exert a dominant-negative effect on I_{Ks} channels [13,14] which could be explained by the fact that expression of mutant subunits destabilizes the balance of both isoforms, thus leading to prolonged cardiac repolarization and congenital long QT syndrome.

In the present study, we describe the electrophysiological properties of mutant KCNQ1-ΔF339 potassium channels identified in a German LQT1 family comprising three symptomatic members. Based upon our data, we discuss possible mechanisms of dominant-negative current suppression by the RWS mutant.

2. Methods

2.1. Pedigree

Our study is based on a three-generation family of German origin comprising three symptomatic individuals (Fig. 1B). The index person (III.1, now 26 years old) was brought to our attention after recurrent syncopes had occurred during physical and emotional stress (Fig. 1C). The investigation conforms to the principles outlined in the Declaration of Helsinki.

2.2. Mutation analysis

Genomic DNA was extracted from peripheral blood lymphocytes (Qiagen, Hilden, Germany), and exon sequences of the KCNQ1 gene were assayed by PCR amplification. The forward primer TGGCTGACCACTGTCCCTCT and the reverse primer CCCCAGGACCCCAGCTGTCCAA [15,16] were used to amplify exon 6 sequences [14]. PCR fragments were screened for single-stranded conformation polymorphism (SSCP) analysis and subcloned as described earlier [14].

2.3. Cloning and mutagenesis of KCNQ1 and KCNE1

Complementary DNAs encoding the human KCNQ1-isoforms 1 and 2 were cloned from the Human Heart Marathon-Ready cDNA library (Clontech, Palo Alto, USA), using primers selected against published sequences (EMBL database accession numbers af000571 and NM_181789). The coding region of human KCNE1 was amplified from genomic DNA. The final clones include a modified translation initiation sequence (CCACCATG) [17] and were generated by insertion of the PCR fragment into the pSPOoD vector [18]. Site-directed PCR-mutagenesis was performed and mutants were analysed by DNA sequence analysis of both strands as described [14].

2.4. Heterologous gene expression in Xenopus laevis oocytes

Procedures for in vitro transcription and oocyte injection have been published previously [19]. The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996). The European Community guidelines for the use of experimental animals have been adhered to.

2.5. Electrophysiology and statistics

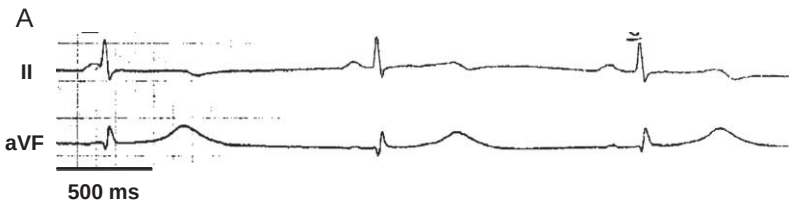
Two-microelectrode voltage-clamp recordings from *Xenopus laevis* oocytes were carried out as published previously [20].

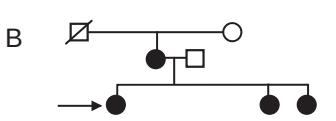
Activation curves were fit with a Boltzmann distribution: $G(V) = G_{max} / (1 - \exp[(V_{1/2} - V)/k])$, where V is the test pulse potential, $V_{1/2}$ is the half-maximal activation potential, and k is the slope of the activation curve. Data are expressed as mean ± standard error of the mean (SEM). We used paired and unpaired Student's t tests (two-tailed tests) to compare the statistical significance of the results: $p < 0.05$ was considered statistically significant. Multiple comparisons were performed using one-way ANOVA. If the hypothesis of equal means could be rejected at the 0.05-level, pairwise comparisons of groups were made and the probability values were adjusted for multiple comparisons using the Bonferroni correction.

3. Results

3.1. Clinical and genetic analyses

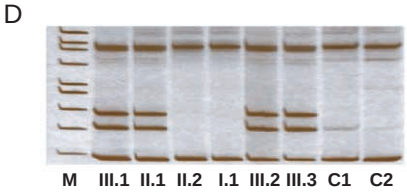
The clinical evaluation of the patients participating in this study is described in Fig. 1A–C. The index patient

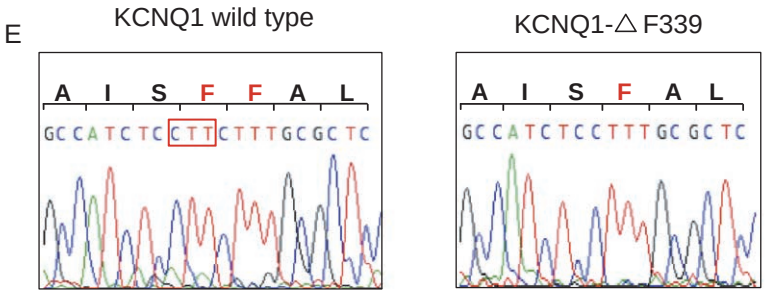




C

Patient ID	Age (years)	Sex	Heart rate (bpm)	QTc (ms)	Trigger of syncope(s)
I.1	83	F	63	400	n/a
II.1	58	F	65	470	emotion
II.2	59	M	70	410	n/a
III.1	26	F	43	580	exercise, emotion
III.2	37	F	73	470	exercise
III.3	32	F	67	460	n/a





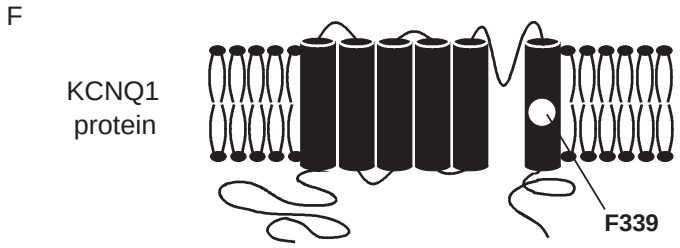


Fig. 1. Identification of the KCNQ1-ΔF339 mutation: A. Resting ECG of the index patient (III.1); B. Pedigree of the family analyzed in this study (arrow indicates the index patient). Closed symbols denote mutation carriers, open symbols indicate unaffected family members. Circles refer to women, squares indicate men; C. Clinical evaluation of LQTS-1 family members; D. SSCP analysis of PCR products obtained from exon 6 of the KCNQ1 gene. M: marker; C1, C2: control samples; E. DNA sequence of exon 6 amplified from patient III.1 and control DNA; F. Hypothetical membrane folding model for the KCNQ1-ΔF339 protein.

(III.1) presented with a history of recurrent syncopes after exercise or emotional stress. The surface ECG displays sinus bradycardia (43/min) with a junctional escape beat and pronounced QTc interval prolongation (580 ms) (Fig. 1A). Two sisters and the mother had QTc intervals of 460 ms (III.3), 470 ms (III.2), and 470 ms (II.1), respectively (Fig. 1C). The mother and one of the sisters (III.2) had a single syncope during their lifetime, whereas the second sister (III.3) was completely asymptomatic.

In order to identify the mutation leading to long QT syndrome in this family, genomic DNA from the index patient was screened for mutations in LQTS-associated genes. An abnormal migration pattern was identified in KCNQ1-exon 6 of the index patient and of family members II.1, III.2, and III.3 (Fig. 1B and D), whereas 150 unrelated controls representing 300 alleles displayed a pattern identical to the pattern of the unaffected family members I.1 and II.2. DNA sequence analysis of exon 6-derived PCR products of family members II.1, III.1, III.2, and III.3

revealed a heterozygous deletion of three nucleotides (CTT). Previously described as KCNQ1-ΔF339, this mutation deletes one of the twin phenylalanine residues at positions 339 and 340 (Fig. 1E) located in the S6 transmembrane region of the KCNQ1 channel protein (Fig. 1F).

3.2. Properties of KCNQ1-iso1 and KCNQ1-iso-ΔF339 channels

We first analyzed homomeric channels formed by wild type KCNQ1-isoform 1 subunits (KCNQ1-iso1) or mutant KCNQ1-isoform1-ΔF339 subunits (KCNQ1-iso1-ΔF339) following injection of 18.4 ng of KCNQ1-iso1 or 18.4 ng of KCNQ1-iso1-ΔF339 cRNA into *Xenopus* oocytes. Currents were activated during depolarizing steps to potentials ranging from −60 mV to +120 mV (2 s), and tail currents were recorded at −40 mV (2 s). The holding potential was −80 mV, and pulses were applied at a frequency of 0.2 Hz. This voltage protocol was used during all electrophysiological measurements. Tail currents specifically reflect KCNQ1 currents, while outward currents during the first step of the voltage protocol might be contaminated by endogenous *Xenopus* currents. Thus, only tail currents were analyzed. Expression of KCNQ1-iso1 subunits produced channels with mean tail current amplitudes of 0.55±0.07 μA (n=20; Fig. 2A and D), whereas expression of mutant KCNQ1-iso1-ΔF339 cRNA injected into oocytes from the same batches produced channels with markedly reduced tail currents (0.08±0.02 μA; n=20; Fig. 2B and D).

Since Romano–Ward syndrome is inherited in an autosomal-dominant manner, patients carry one wild type and one mutant KCNQ1 allele. Accordingly, dominant inheritance associated with a loss-of-function mutation might be explained either by haploinsufficiency or by dominant-negative effects of the mutant subunits on their wild type counterparts. To probe for dominant-negative current suppression, KCNQ1-iso1 cRNA (9.2 ng, i.e. 50% of the amount injected in Fig. 2A) and KCNQ1-iso1-ΔF339 cRNA (9.2 ng, i.e. 50% of the amount injected in Fig. 2B) were coinjected into oocytes, and the resulting currents (Fig. 2C and D) were compared with KCNQ1-iso1 currents (Fig. 2A and D). Current inhibition clearly exceeding 50% would reflect a dominant-negative effect of mutant KCNQ1 subunits, indicating that mutant and wild type subunits form heteromultimeric channels. On the other hand, current reduction to a level of 50% would indicate haploinsufficiency.

However, neither haploinsufficiency nor dominant-negative reduction of wild type KCNQ1-iso1 currents by

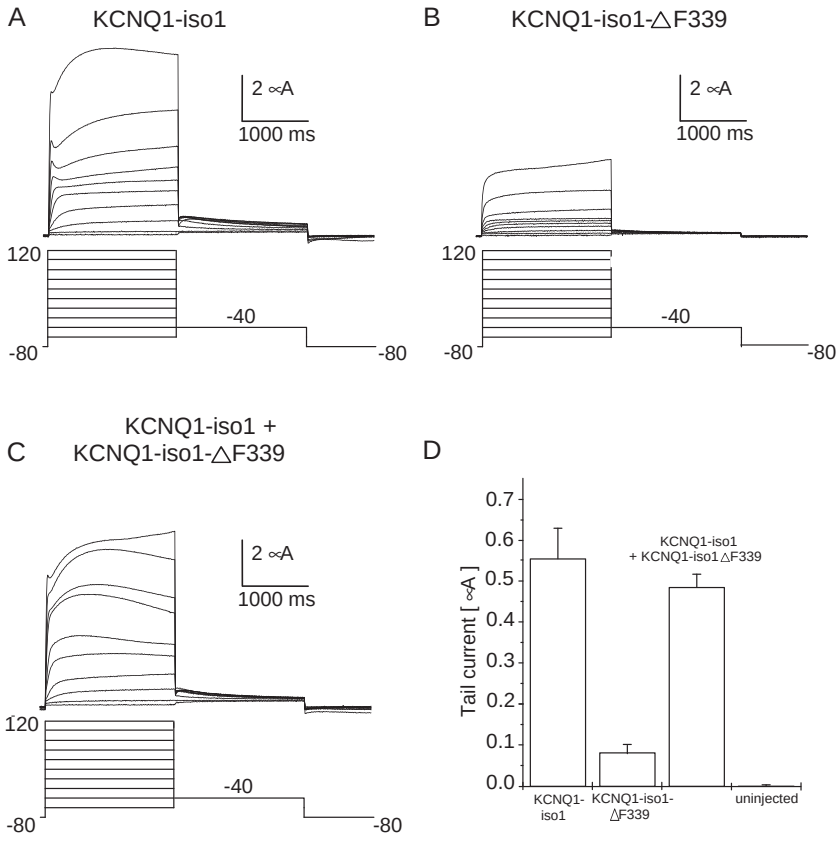


Fig. 2. Currents induced by expression of KCNQ1-iso1 or KCNQ1-iso1-ΔF339 α-subunits. Representative current traces recorded from cells expressing KCNQ1-iso1 (A) or KCNQ1-iso1-ΔF339 (B) channels are displayed. (C) Currents induced by coexpression of equal amounts of KCNQ1-iso1 subunits and KCNQ1-iso1-ΔF339 subunits. (D) Mean peak tail current amplitudes (n=20 cells per column).

mutant KCNQ1-iso1-ΔF339 subunits was observed (Fig. 2D). In contrast to other RWS mutants that reduce KCNQ1-iso1 current amplitudes at least by 50% [12,13], the mean KCNQ1-iso1 current amplitude was not significantly altered by coexpression of KCNQ1-iso1 with mutant KCNQ1-iso1-ΔF339 subunits ($n=20$).

3.3. KCNQ1-iso1-ΔF339 causes dominant-negative suppression of I_{Ks}

Outward potassium currents recorded after injection of KCNQ1-iso1 (11.5 ng) and KCNE1 (5.75 ng) cRNAs (Fig. 3A) displayed properties nearly identical to native cardiac I_{Ks}, characterized by slow activation and a linear current–voltage relationship [7,8]. In contrast, coexpression of KCNQ1-iso1-ΔF339 (11.5 ng) and KCNE1 cRNAs produced strongly reduced current amplitudes (Fig. 3B and E; $n=10$) indicating the inability of those components to assemble to form active channels.

Next, we determined whether the KCNQ1-iso1-ΔF339 mutant could exert a dominant-negative effect on I_{Ks}. Membrane currents were recorded from oocytes injected with identical amounts (5.75 ng) of KCNQ1-iso1, KCNQ1-iso1-ΔF339 and KCNE1 cRNAs, revealing that after

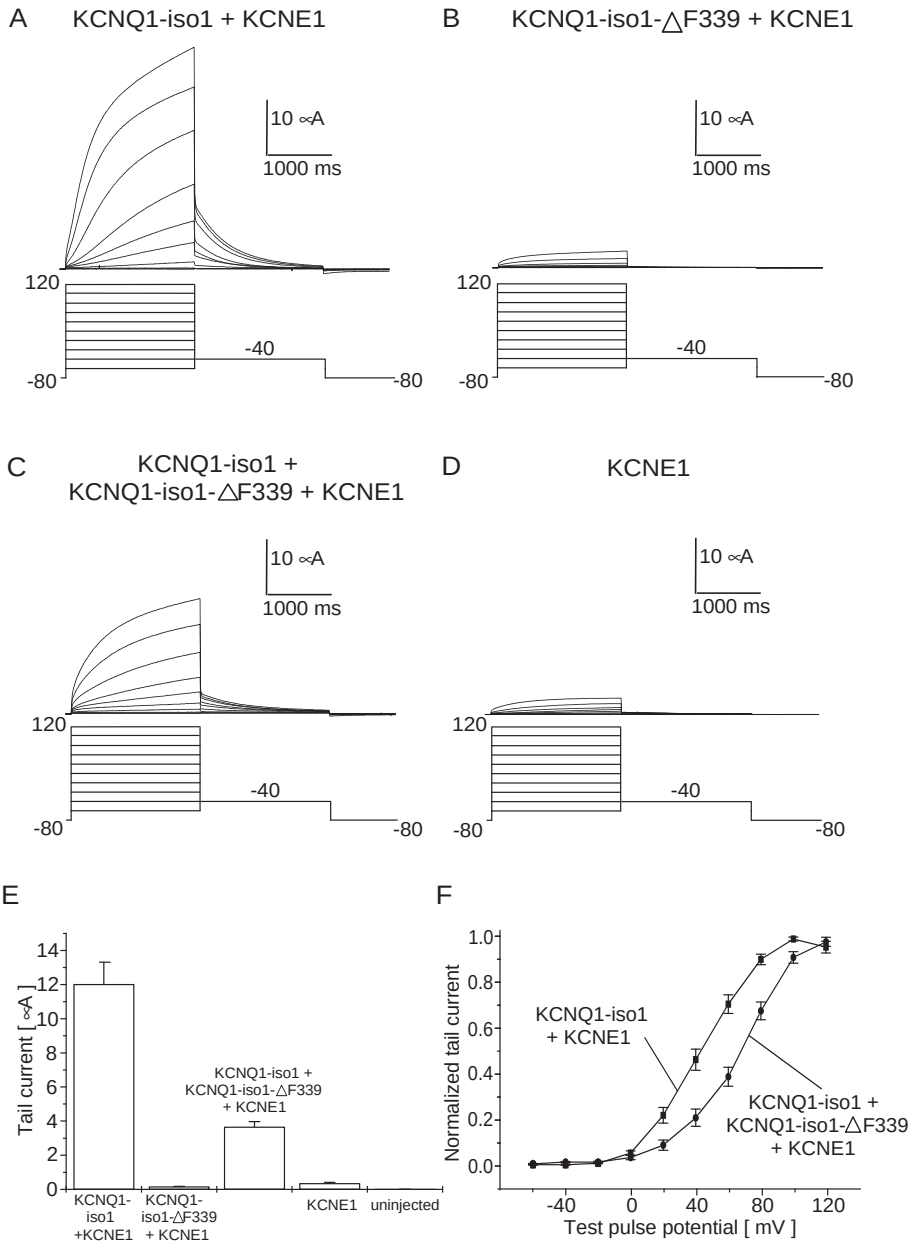


Fig. 3. Expression of I_{Ks} currents in *Xenopus* oocytes. I_{Ks} was generated by coexpression of full-length KCNQ1-iso1 (A) or mutant KCNQ1-iso1-ΔF339 (B) in combination with KCNE1 cRNA. (C) I_{Ks} currents resulting from coexpression of KCNE1 together with equal amounts of KCNQ1-iso1 and KCNQ1-iso1-ΔF339 subunits. (D) Currents induced by expression of KCNE1 β-subunits alone. (E) Mean peak tail current amplitudes ($n=10$ cells per column). (F) Normalized I-V relationship for I_{Ks} peak tail currents (mean values ± SEM are displayed; $n=10$).

coexpression of KCNQ1-iso1 and KCNQ1-iso1-ΔF339 mutant subunits, I_{Ks} currents were reduced by 69.5% (Fig. 3C and E; $n=10$). These data suggest that mutant and wild type KCNQ1-iso1 subunits coassemble with KCNE1 subunits and that resulting currents are suppressed by mutant KCNQ1-iso1-ΔF339 subunits in a dominant-negative manner. Furthermore, the corresponding activation current–voltage relationship for KCNQ1-iso1/KCNE1 currents was shifted by 25.0±4.6 mV ($n=10$) towards more positive potentials (KCNQ1-iso1+KCNE1: $V_{1/2}=42.7\pm3.6$ mV; KCNQ1-iso1+KCNQ1-iso1-ΔF339+KNCE1: $V_{1/2}=67.7\pm3.0$ mV; $n=10$) (Fig. 3F) which provides a biophysical explanation for reduced current amplitudes. Note that in all other coexpression experiments of this study, reasonable analysis of activation curves was prevented by very low I_{Ks} tail current amplitudes.

3.4. Functional effects of KCNQ1-iso2 and KCNQ1-iso2-ΔF339 subunits on KCNQ1-iso1 channels

We determined the ability of wild type and mutant KCNQ1-iso2 subunits to influence KCNQ1-iso1 currents. Expression of wild type or mutant KCNQ1-iso2 proteins (injection of 3.22 ng cRNA per oocyte) into *Xenopus* oocytes produced little or no KCNQ1 currents, while oocytes injected with the same amount of KCNQ1-iso1 cRNA produced characteristic potassium currents (Fig. 4A; $n=20$).

Previous studies on human hearts have shown that KCNQ1-iso1 and KCNQ1-iso2 may be equally expressed and that KCNQ1-iso2 exerts a dominant-negative effect on KCNQ1-iso1 currents [10,12]. We therefore mimicked the composition of subunits present in healthy subjects by injecting equimolar amounts of KCNQ1-iso1 (3.22 ng) and KCNQ1-iso2 (3.22 ng) cRNA. Under the given experimental conditions, KCNQ1-iso1 currents (Fig. 4A) were reduced by 90% ($n=20$), indicating that KCNQ1-iso2 subunits have a pronounced dominant-negative effect on KCNQ1-iso1 currents via coassembly of the respective subunits.

Romano–Ward syndrome-associated KCNQ1-iso2 mutations (in contrast to JLNS-linked KCNQ1-iso2 mutations) have been shown to exert dominant-negative effects on KCNQ1-iso1 currents as well, providing a possible explanation for the autosomal-dominant mode of inheritance of Romano–Ward syndrome [12]. Equal amounts (3.22 ng) of KCNQ1-iso1 and KCNQ1-iso2-ΔF339 cRNAs were coexpressed in oocytes, resulting in KCNQ1-iso1 current suppression by 94.5% ($n=20$).

3.5. Effects of wild type and mutant KCNQ-iso2 proteins on I_{Ks} currents

In order to examine whether mutant and wild type isoform 2 subunits exert dominant-negative effects on I_{Ks} currents, we performed a series of experiments under the conditions described above. Oocytes were injected with equal amounts (3.22 ng) of KCNQ1-iso1 or KCNQ1-iso2 cRNA together with 1.61 ng KCNE1 cRNA (Fig. 4B).

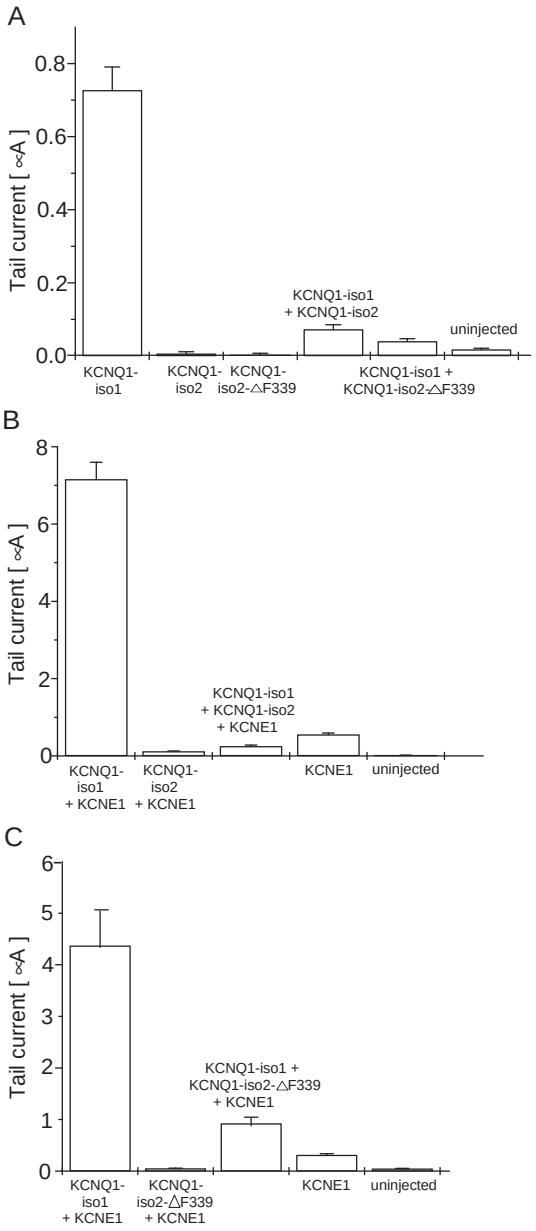


Fig. 4. A. Effects of a truncated KCNQ1 splice variant (isoform 2, wild type and mutant) on KCNQ1-iso1 currents; B. dominant-negative suppression of KCNQ1-iso1 currents by KCNQ1-iso2 in the presence of KCNE1; C. dominant-negative effects of KCNQ1-iso2-ΔF339 proteins on I_{Ks}. Data are given as mean peak tail current amplitudes ($n=20$ oocytes per column).

In contrast to I_{Ks} currents produced by channels composed of KCNQ1-iso1 and KCNE1 subunits, coexpresion of KCNQ1-iso2 and KCNE1 subunits displayed very low I_{Ks} amplitudes (Fig. 4B; $n=20$). Small I_{Ks} currents could be explained by endogenous *Xenopus* KCNQ1 channels interacting with heterologously expressed KCNE1 subunits (Fig. 4B) [8]. Consistent with the observed effects of KCNQ1-iso2 on KCNQ1-iso1 subunits, KCNQ1-iso2

subunits exerted a strong dominant-negative effect on I_{Ks} currents (Fig. 4B) with a current reduction of 96.5% ($n=20$). The presence of currents smaller than KCNE1-produced currents (Fig. 4B; $n=20$) suggests that endogenous *Xenopus* KCNQ1-iso1 subunits coassemble with injected KCNQ1-iso2 subunits.

In additional experiments we assessed the effects of mutant KCNQ1-iso2-ΔF339 subunits on I_{Ks} currents. KCNQ1-iso2-ΔF339 subunits coexpressed with KCNE1 produced virtually no I_{Ks} current (Fig. 4C; $n=20$). In the presence of KCNQ1-iso1 and KCNE1 subunits, I_{Ks} currents were suppressed in a dominant-negative manner by 78.9% ($n=20$). Thus, KCNQ1-iso2 as well as KCNQ1-iso2-ΔF339 subunits exerted dominant-negative effects on I_{Ks} by coassembly with KCNQ1-iso1. However, mutant isoform 2 (KCNQ1-iso2-ΔF339) subunits induced weaker suppression of I_{Ks} than wild type isoform 2 protein (KCNQ1-iso2).

3.6. Analysis of I_{Ks} currents present in healthy or symptomatic individuals

In the hearts of healthy subjects, KCNQ1-iso1 and KCNQ1-iso2 subunits are expressed on similar levels [10], while the presented RWS patients express the following subunits in their heart: KCNQ1-iso1, KCNQ1-iso1-ΔF339, KCNQ1-iso2, and KCNQ1-iso2-ΔF339 subunits. To mimic the condition of healthy individuals, oocytes were injected with a combination of KCNQ1-iso1 (3.22 ng), KCNQ1-iso2 (3.22 ng) and KCNE1 (1.61 ng) cRNAs. Similarly, oocytes were injected with a combination of KCNQ1-iso1 (1.61 ng), KCNQ1-iso1-ΔF339 (1.69 ng), KCNQ1-iso2 (1.61 ng), KCNQ1-iso2-ΔF339 (1.61 ng) and KCNE1 cRNAs to produce channels that appear in symptomatic individuals.

Current recordings revealed that the subunit combination of healthy individuals produced currents very similar to I_{Ks} (Fig. 5A), while the combination reflecting the subunit composition of symptomatic individuals showed a current suppression of 93% (Fig. 5B and C), indicating that the presented RWS mutation (KCNQ1-ΔF339) affects the balance of KCNQ1-isoform subunits (iso1 and iso2) and thereby causes prolongation of cardiac repolarization.

4. Discussion

4.1. KCNQ1-ΔF339 causes hereditary long QT syndrome 1

We report a German Romano–Ward syndrome (LQTS-1) family genotyped for the amino acid deletion mutation KCNQ1-ΔF339. Although described previously [6,21], the cellular electrophysiological mechanisms leading to long

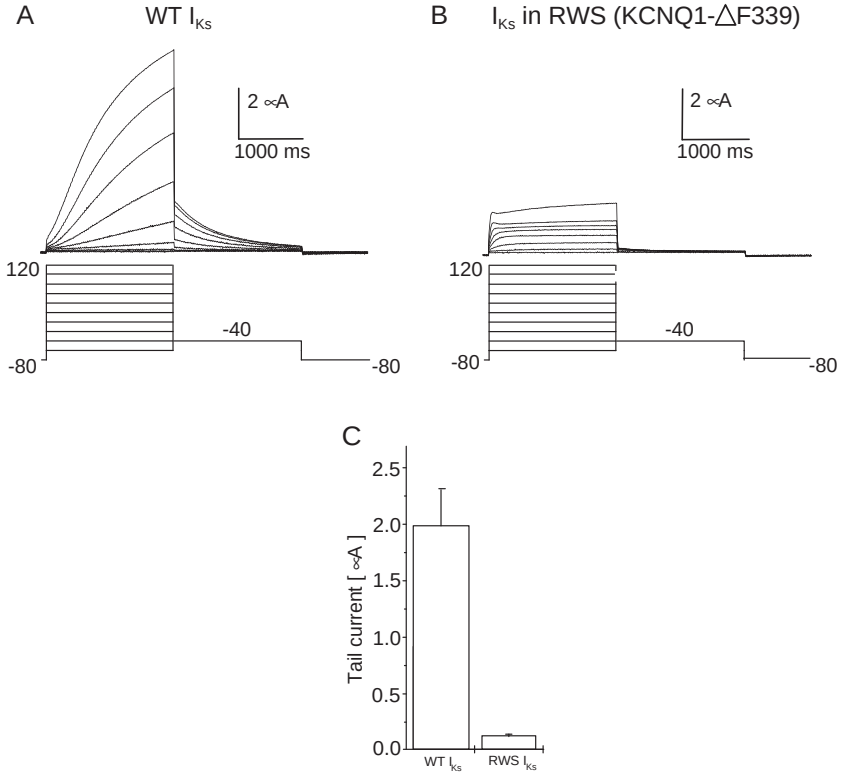


Fig. 5. Expression of I_{Ks} present in healthy individuals (wild type) or RWS (KCNQ1-ΔF339) patients. Currents produced by coexpression of KCNQ1-iso1 and KCNQ1-iso2 with KCNE1 subunits (WT, A) or KCNQ1-iso1, KCNQ1-iso1-ΔF339, KCNQ1-iso2, KCNQ1-iso2-ΔF339 (RWS, B) revealed a reduction of I_{Ks} current by 93.9% in KCNQ1-ΔF339-associated RWS patients. C. Mean peak tail current amplitudes ($n=30$ cells per column).

QT syndrome have not yet been examined. Here, we provide evidence that expression of mutant (KCNQ1-iso1-ΔF339) and wild type (KCNQ1-iso1) subunits in combination with wild type (KCNQ1-iso2) and mutant (KCNQ1-iso2-ΔF339) subunits of an N-terminal truncated splice variant cause I_{Ks} current reduction and determine the dominant mode of inheritance in heterozygous KCNQ1-ΔF339 gene carriers.

4.2. Dominant-negative I_{Ks} current reduction by mutant KCNQ1 α-subunits

Expression of homomeric wild type (KCNQ1-iso1) or mutant (KCNQ1-iso1ΔF339) channels revealed that the RWS mutant ΔF339 induced little potassium currents. In coexpression experiments, mutant KCNQ1-iso1-ΔF339 subunits did not affect wild type KCNQ1-iso1 currents (Fig. 2C). However, in the presence of KCNE1 and KCNQ1-iso1 subunits, the mutant isoform 1 suppressed I_{Ks} currents in a dominant-negative manner by 69.5% (Fig. 3C). It has been demonstrated earlier that a single residue in KCNE1 (T58) is responsible for KCNE-induced modification of KCNQ1 activation gating through specific interaction with KCNQ1 residues 338–340 [22]. In particular, mutation of F340 to cysteine abolished typical features of wild type KCNQ1/KCNE1 currents. It is tempting to speculate that deletion of F339 modifies the KCNE1 interaction site in KCNQ1, providing a molecular mechanism of dominant-negative I_{Ks} suppression associated with this RWS mutation. This hypothesis is further supported by data indicating that mutation of F339 to alanine markedly reduces the channel's affinity for block by the benzodiazepine L-7 [23]. However, it is of note that the KCNQ1 C-terminus may also be involved in KCNE1 binding and regulation of KCNQ1 activity [24]. Finally, defective KCNQ1 trafficking as underlying molecular mechanism may be ruled out, since coexpression of wild type and mutant KCNQ1 subunits in the presence of KCNE1 led to a shift of the current activation curve by 25.0 mV towards more positive potentials, indicating that wild type and mutant subunits may reach the cell membrane after correct folding and trafficking, where they produce potassium channels with reduced function and altered biophysical properties.

4.3. Electrophysiological effects of a truncated KCNQ1 splice variant

In contrast to patients with autosomal-dominant RWS, carriers of recessive JLNS mutations exhibit a relatively mild or even normal phenotype. Expression of mutant KCNQ1-iso1 subunits that either cause JLNS or RWS produced no significant potassium currents, although their cardiac phenotypes show strong differences [13]. Recent studies have shown that an N-terminal-truncated KCNQ1 variant (isoform 2) which is expressed in human heart might contribute to I_{Ks} currents [10–12]. This non-conducting splice variant lacks the N-terminal cytoplasmic domain and the initial one-third of first transmembrane domain of KCNQ1.

In a human ventricle obtained from a patient who underwent heart transplantation, Demolombe et al. measured a ratio of KCNQ1-isoform 1 and isoform 2 mRNA of approximately 5:2, and similar ratios were detected in six preparations from human atrium sampled during cardiac surgery [11]. In coexpression experiments, Escande and coworkers injected a 5:2 ratio of KCNQ1-iso1:KCNQ1-iso2 plasmids [11,12] into COS-7 cells, while Tseng and colleagues injected isoform cRNA ratios ranging from 1:4 up to 1:10 into *Xenopus* oocytes to investigate dominant-negative effects of isoform 2 [10]. RT-PCR experiments suggest an equivalent transcription of both isoforms in human heart [10]. In the present study we used RNA ratios of 1:1 to mimic transcription and translation of the respective alleles in human heart. Individual expression of KCNQ1-iso2 subunits resulted in inactive KCNQ1 channels, while coexpression with KCNQ1-iso1 subunits revealed a dominant-negative effect of the truncated variant on KCNQ1 channels (Fig. 4A). Similarly, I_{Ks} currents were strongly suppressed by KCNQ1-iso2 subunits (Fig. 4B). These findings support the notion that both KCNQ1-isoforms interact to form heteromultimeric channels and imply that the N-terminal 127 amino acids are not essential for protein trafficking and subunit assembly.

Heterozygous carriers of KCNQ1 mutations carry wild type and mutant channel subunits of both isoforms. Therefore it is important to distinguish between RWS and JLNS mutations, since mutant and wild type KCNQ1-iso2 subunits of RWS patients cause dominant-negative effects on I_{Ks} currents (Figs. 4A, C and 6B) [12], whereas JLNS mutations lack the dominant-negative effect of mutant KCNQ1-iso2 subunits on I_{Ks} (Fig. 6C) [12]. Thus, the appearance of different cardiac phenotypes among heterozygous carriers of RWS and JLNS mutations might be explained by the following mechanism: I_{Ks} currents in heterozygous carriers of RWS mutations are suppressed through dominant-negative effects of wild type and mutant KCNQ1-iso2 subunits and mutant KCNQ1-iso1 subunits. In contrast, in JLNS gene carriers, I_{Ks} is influenced only by the dominant-negative effect of KCNQ1-iso2 subunits. Furthermore, RWS-associated, but not JLNS-associated mutant KCNQ1 isoform 1 proteins (KCNQ1-iso1) exert dominant-negative effects on I_{Ks} (Fig. 6B and C) [12,13].

Interestingly, the I_{Ks}-suppressing dominant-negative effects of KCNQ1-iso2 (96.5%) or the RWS-associated mutant KCNQ1-iso2-ΔF339 (78.9%) are more pronounced compared to dominant-negative effects of KCNQ1-iso1-ΔF339 on I_{Ks} (69.5%) (Fig. 6B). Thus, in heterozygous carriers, the amplitude of I_{Ks} currents appears to be strongly affected by the dominant-negative properties of

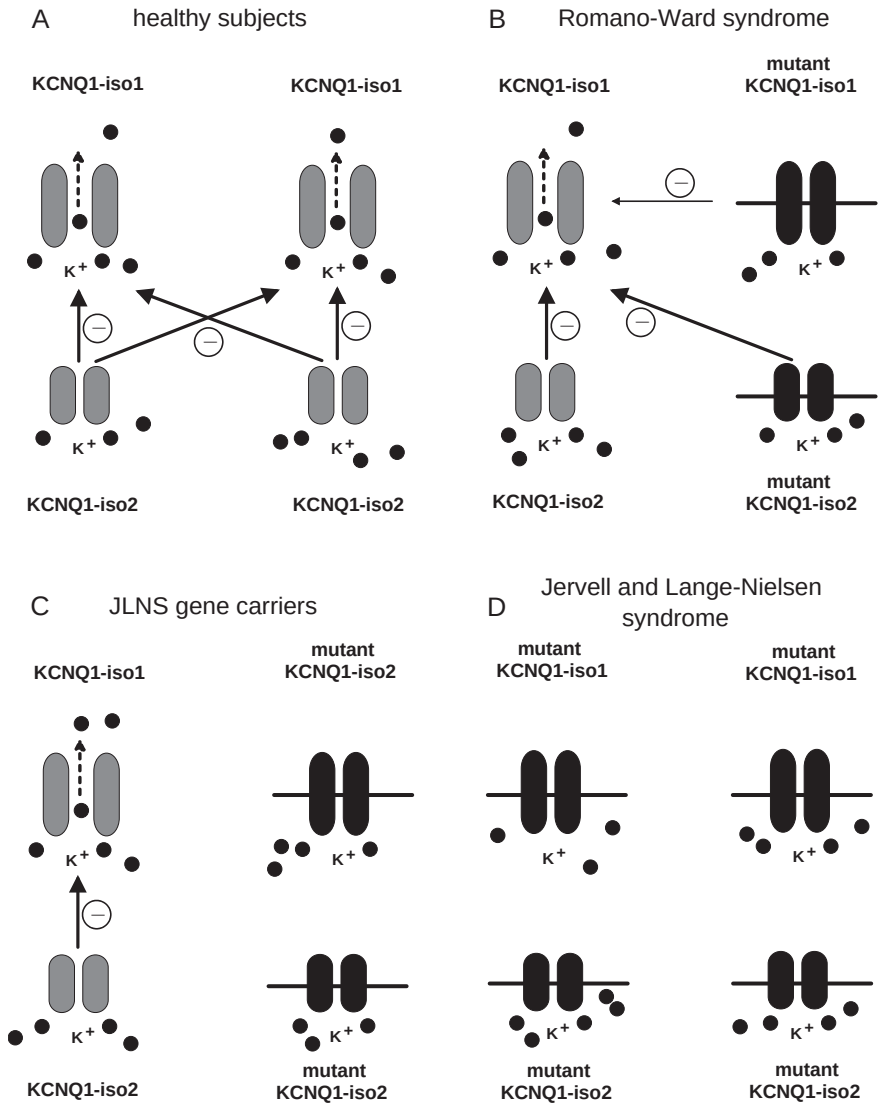


Fig. 6. Mechanism of I_{Ks} current suppression in KCNQ1-asssociated long QT syndrome (LQTS-1). To obtain a clearly arranged presentation, KCNE1 subunits are not shown. A. Cardiomyocytes of healthy subjects; B. heterozygous KCNQ1-ΔF339 gene carriers. C. JLNS gene carriers. D. JLNS patients (see text for details).

KCNQ1-isoform 2 subunits. As illustrated by the results presented in Fig. 5, in KCNQ1-ΔF339-associated RWS patients I_{Ks} currents are reduced by 93.9% compared to healthy individuals, leading to severely impaired cardiac repolarization.

If the balanced expression of both KCNQ1-isoforms (isoforms 1 and 2) in hearts of healthy subjects is a basic requirement for I_{Ks} function, changes that influence and destabilize the balance of isoforms would strongly affect repolarization reserve and cardiac function. The presented RWS patients carrying the ΔF339 mutation express two mutated subunits, namely the mutated isoform 1 (KCNQ1-iso1-ΔF339) and the mutated isoform 2 (KCNQ1-iso2-ΔF339). Both mutant subunits strongly affect the balance of isoforms and, by dominant-negative suppression of I_{Ks} currents, may lead to severely impaired cardiac repolarization.

4.4. Mechanisms of KCNQ1-iso2-induced dominant-negative current suppression

In contrast to KCNQ1-iso1-ΔF339, dominant-negative current suppression by wild type and mutant KCNQ1-iso2 proteins did not require the presence of KCNE1. This suggests that in addition to the mechanisms proposed earlier for KCNQ1-iso1 (see Section 4.2), different modes of dominant-negative current suppression are responsible for the effects of KCNQ1-iso2. While it is reasonable to assume that KCNQ1-iso2-ΔF339 also impairs the interaction of KCNQ1 and KCNE1, the lack of the KCNQ1 N-terminus by itself leads to strong current suppression. However, disruption of KCNQ1 subunit tetramerization is unlikely, since the cytoplasmic N-terminus of KCNQ1 does not contain a tetramerization domain like *Sh* channels [11]. In contrast, a region required for multimerization has been mapped to amino acids 590–

620 [25] which is neither interrupted by deletion of the N-terminus (isoform 2) nor by deletion of F339.

Trafficking defects as explanation for KCNQ1 current suppression induced by isoform 2 seem to be unlikely as well, since a motif within the C-terminus (as opposed to the N-terminus) determines KCNQ1 cell surface expression [26]. But still, incorrect protein folding caused by deletion of the KCNQ1 N-terminus might prevent the formation of functional KCNQ1 channel multimers. Furthermore, impaired posttranslational protein modifications due to the absence of the cytoplasmic N-terminus might account for markedly reduced channel function in the presence of KCNQ1-iso2. In particular, the single protein kinase A (PKA) phosphorylation site and two protein kinase C (PKC) phosphorylation sites are located in the N-terminus. Thus, we may speculate that KCNQ1-iso2 causes dominant-negative current reduction via the lack of current enhancement by baseline phosphorylation through PKA and/or PKC. The mechanism(s) by which JLNS-associated KCNQ1-iso2 mutations offset the dominant-negative isoform 2 effects remain to be revealed.

Based upon these considerations, it is reasonable to assume that at least two independent mechanisms lead to dominant-negative I_{Ks} suppression in RWS patients carrying the KCNQ1-ΔF339 mutation. Firstly, deletion of F339 in the presence of KCNE1 may disrupt the KCNQ1/KCNE1 interaction (see Section 4.2). Secondly, the lack of the N-terminus in KCNQ1-iso2 reduces KCNQ1 currents through mechanisms independent of KCNE1 expression.

4.5. Sinus node dysfunction associated with long QT syndrome 1

Sinus bradycardia was observed in the index patient with severe long QT syndrome, while all other family members analyzed in this study displayed regular heart rates (Fig. 1C). The association of sinus bradycardia with KCNQ1-associated long QT syndrome is in line with previous studies on LQTS-1 patients [27,28] and transgenic mice with reduced I_{Ks} current amplitudes [29], suggesting that I_{Ks} contributes to automaticity in human sinus node, with suppression of I_{Ks} leading to decreased heart rates. The cellular electrophysiological mechanism by which pacemaker activity is modified by I_{Ks} remains to be elucidated.

4.6. Limitations of the study

Electrophysiological studies in heterologous expression systems such as *Xenopus* oocytes provide valuable information on ion channel electrophysiology and biophysics. However, it has to be taken into account that the human cardiac phenotype might still display differences, and expansions to in vivo genotype–phenotype correlations have to be done with care. Because of the inability to measure LQTS patients-derived cardiomyocytes, heterologous expression of the respective subunits is mandatory and reflects the state-of-the-art method to assess cellular electrophysiological effects of ion channel mutations linked to hereditary long QT syndrome.

Xenopus oocytes may display small I_{Ks} currents when injected with KCNE1 cRNA (Fig. 3D) due to endogenous *Xenopus* KCNQ1 channels [8]. In some experiments, KCNQ1-isoform 2 disrupted not only KCNQ1 channel function of heterologously expressed channels, but also of endogenous KCNQ1 (see Section 3.5, Fig. 4B). However, we suggest that the weak expression of endogenous KCNQ1 did not affect the experimental results obtained from the present study. Finally, moderate loss of voltage control during the last voltage step of the protocol and possible changes in intracellular potassium homeostasis during the recordings might have contributed to the results shown in Figs. 2 and 3. But still, they are not sufficient as sole explanation for the marked effect of KCNQ1-iso1-ΔF339 on current amplitudes and half-maximal activation voltage.

4.7. Conclusion

This study highlights the significance of dominant-negative KCNQ1-isoform 2 proteins in the pathophysiology of hereditary long QT syndrome linked to KCNQ1. By analyzing the cellular electrophysiological effects of the KCNQ1-ΔF339 mutation linked to RWS, we provide evidence that in addition to dominant-negative effects of mutant KCNQ1-isoform 1, wild type and mutant subunits of the truncated KCNQ1 splice variant isoform 2 led to pronounced current reduction of KCNQ1 and I_{Ks} channels. We confirm that the amplitude of KCNQ1 and I_{Ks} currents in heterozygous carriers depends strongly on the properties of KCNQ1-isoform 2 proteins.

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