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Cardiac Potassium Channel Dysfunction in Sudden Infant Death Syndrome

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Abstract

Life-threatening arrhythmias have been suspected as one cause of the sudden infant death syndrome (SIDS), and this hypothesis is supported by the observation that mutations in arrhythmia susceptibility genes occur in 5–10% of cases. However, the functional consequences of cardiac potassium channel gene mutations associated with SIDS and how these alleles might mechanistically predispose to sudden death are unknown. To address these questions, we studied four missense *KCNH2* (encoding HERG) variants, one compound *KCNH2* genotype, and a missense *KCNQ1* mutation all previously identified in Norwegian SIDS cases. Three of the six variants exhibited functional impairments while three were biophysically similar to wild-type channels (*KCNH2* variants V279M, R885C, S1040G). When coexpressed with WT-HERG, R273Q and K897T/R954C generated currents resembling the rapid component of the cardiac delayed rectifier current (I_{Kr}) but with significantly diminished amplitude. Action potential modeling demonstrated that this level of functional impairment was sufficient to evoke increased action potential duration and pause-dependent early afterdepolarizations. By contrast, *KCNQ1*-I274V causes a gain-of-function in I_{Ks} characterized by increased current density, faster activation, and slower deactivation leading to accumulation of instantaneous current upon repeated stimulation. Action potential simulations using a Markov model of heterozygous I274V- I_{Ks} incorporated into the Luo-Rudy (LRd) ventricular cell model demonstrated marked rate-dependent shortening of action potential duration predicting a short QT phenotype. Our results indicate that certain potassium channel mutations associated with SIDS confer overt functional defects consistent with either LQTS or SQTS, and further emphasize the role of congenital arrhythmia susceptibility in this syndrome.

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Keywords

sudden death; genetics; ion channels; long QT syndrome; short QT syndrome

1. Introduction

Life-threatening arrhythmias including the congenital long QT syndrome (LQTS) have been proposed to contribute to some cases of the sudden infant death syndrome (SIDS), the leading cause of death among infants 1 month to 1 year of age [1–3]. Supporting this theory are recent observations that mutations in genes responsible for LQTS are found in approximately 5–10% of SIDS cases [4–6]. Congenital LQTS is an inherited disorder associated with mutations in genes predominantly encoding subunits of cardiac ion channels [7]. The disorder is caused by impaired ventricular repolarization leading to prolongation of the QT interval and an increased risk for life-threatening ventricular arrhythmias. Other mutations in some of the same genes (*KCNH2*, *KCNQ1*) can enhance repolarization and cause shortening of the QT interval [8–10]. In the short QT syndrome (SQTS) there is also increased risk for cardiac arrhythmias and sudden death [11,12].

The data linking cardiac potassium channel mutations with SIDS include anecdotal reports and population surveys. In 2001, Schwartz and colleagues reported discovery of a *de novo* *KCNQ1* mutation (P117L) in a SIDS victim [13]. The same mutation was also observed to segregate with autosomal dominant LQTS at reduced penetrance in an unrelated Italian family [13]. Another missense *KCNQ1* variant (H105L) without functional consequences was discovered among 41 German SIDS cases [14]. Two novel *KCNH2* mutations (K101E, P1157L) have also been reported in single SIDS probands [5,15], and in one case this finding led to discovery of undiagnosed LQTS in family members [15]. But, there have been no studies demonstrating that any of these mutant channels are dysfunctional.

Ackerman *et al.* reported a systematic survey of autopsied SIDS cases for mutations in the LQTS genes, an effort that originally identified two *SCN5A* mutations in 93 subjects [4]. The study later reported novel mutations in cardiac potassium channel genes (*KCNH2*-G294V, *KCNQ1*-T600M) occurring in two SIDS victims [5]. Three other potassium channel variants were identified, but these alleles were also observed in ethnically-matched population controls, albeit rarely. Most recently, the largest series of cases screened for mutations in arrhythmia-susceptibility genes revealed novel missense *KCNH2* and *KCNQ1* mutations in Norwegian SIDS victims [6]. Importantly, the electrophysiological consequences of these novel SIDS-associated potassium channel mutations were previously unknown.

We report here the functional characterization of novel *KCNH2* and *KCNQ1* mutations identified in the Norwegian SIDS study. Our findings demonstrated a diversity of functional phenotypes that revealed plausible explanations for sudden death in this clinical setting.

2. Materials and Methods

2.1. Mutagenesis and heterologous expression

Four single *KCNH2* variants (V279M, R273Q, R885C, S1040G), one compound genotype (K897T/R954C) and a *KCNQ1* mutation (I274V) were constructed in HERG or *KCNQ1* cDNA vectors, respectively, using recombinant PCR mutagenesis (primer sequences available upon request). The final constructs were assembled in bicistronic mammalian expression plasmids (pIRES2-EGFP or pIRES2-DsRed, BD Biosciences-Clontech, Palo Alto, CA) to enable monitoring of transfection success by co-expression of fluorescent proteins. All constructs were sequenced to verify the mutation and to exclude polymerase errors. Recombinant human

KCNE1 cDNA was subcloned into pIRES2-DsRed for use in I_{Ks} expression experiments. In co-expression experiments, the WT channel (either KCNQ1 or HERG) was coupled to DsRed while mutants were expressed from vectors encoding EGFP.

Chinese hamster ovary cells (CHO-K1, ATCC) cells were grown as previously described [16,17]. For HERG experiments, cells were transiently co-transfected with 3 μ g of both WT and mutant plasmid DNA using Fugene-6 (Roche Diagnostics, Indianapolis, IN). For KCNQ1 experiments, cells were transiently transfected with 1 μ g KCNQ1 plasmid DNA, whereas to study I_{Ks} , cells were co-transfected with 1 μ g KCNQ1 and 1 μ g KCNE1 plasmids. In some experiments, we performed transient transfection of WT or mutant KCNQ1 in a stable I_{Ks} cell line [17]. For these experiments, we initially determined by plasmid DNA titration that 1 ng of WT-KCNQ1 was sufficient to boost current amplitude in stable I_{Ks} cells by 2–3 fold and thus used this amount in transfections to achieve approximately equal levels of stably and transiently expressed KCNQ1 alleles. Following transfection (48–72 hours), fluorescent cells were selected by epifluorescence microscopy (green for single transfections, yellow for co-transfections) for use in whole-cell patch clamp recording experiments. Non-transfected cells grown under these conditions did not exhibit measurable endogenous potassium currents.

2.2. Electrophysiology

Whole-cell currents were measured in the whole-cell configuration of the patch clamp technique [18] using an Axopatch 200B amplifier (Molecular Devices, Corp., Sunnyvale, CA, USA) as previously described [16,17]. Patch pipettes were pulled from thick-wall borosilicate glass (World Precision Instruments, Inc., Sarasota, FL, USA) with a multistage P-97 Flaming-Brown micropipette puller (Sutter Instruments Co., San Rafael, CA, USA) and fire-polished to a pipette resistance of 2–4 M Ω . A 1–2% agar-bridge composed of bath solution was utilized as a reference electrode. For HERG recording, the bath solution consisted of (in mM): NaCl 145, KCl 4, MgCl₂ 1, CaCl₂ 1.8, glucose 10, HEPES 10, adjusted to pH 7.35 with NaOH, ~275 mosm/kg. The pipette solution consisted of (in mM): KCl 110, ATP (dipotassium salt) 5, MgCl₂ 2, EDTA (ethylenediaminetetraacetic acid) 10, HEPES 10, adjusted to pH 7.2 with KOH, ~265 mosmol/kg. For KCNQ1 and I_{Ks} recording, the bath solution consisted of (in mM): NaCl 132, KCl 4.8, MgCl₂ 1.2, CaCl₂ 2, glucose 5, HEPES 10, pH 7.4, ~275 mosm/kg. The pipette solution consisted of (in mM): K-aspartate 110, ATP (dipotassium salt) 5, MgCl₂ 1, EGTA 11, HEPES 10, MgCl₂ 1, pH 7.3, ~265 mosm/kg. Pipette solutions were diluted with distilled water (to ~250 mosm/kg) to prevent activation of swelling-activated currents. Chemicals were purchased from SIGMA (St. Louis, MO, USA).

For HERG experiments, the holding potential was –80 mV and whole-cell currents were measured from –80 to +70 mV (in 10 mV steps) 1990 ms (activation) and 2200 ms (tail currents) after the start of the voltage pulse. For KCNQ1 and I_{Ks} experiments, the holding potential was –80 mV and whole-cell currents were measured from –80 to +60 mV (in 10 mV steps) 1990 ms after the start of the voltage pulse. Whole-cell current traces were filtered at 5 kHz and acquired at 2 kHz. The access resistance and apparent membrane capacitance were estimated as described by Lindau and Neher [19]. Pulse generation, data collection and analyses were done with Clampex 8.1 (Molecular Devices, Corp.). Specific voltage pulse protocols are described in the figures and legends. The apparent voltage-dependence of activation for I_{Ks} was obtained by fitting data with the Boltzmann function: $I = 1/(1 + \exp((V - V_{1/2})/k))$. To quantify the time course of deactivation, tail currents were fit with a single exponential function, $f(t) = A_1 \cdot \exp(-t/\tau_1) + C$. Statistical comparisons were made using Student's t-test and significance was assumed for $P < 0.05$.

2.3. Computational Modeling

Markov models of human WT and mutant I_{Ks} and I_{Kr} were applied to the LRd simulation of ventricular action potentials. In studies of I_{Ks} , only I_{Ks} was modified in the simulation; similarly, in studies of I_{Kr} , only I_{Kr} was modified. Simulations were performed at 310°K (37° C), with a Q_{10} of 3 applied to each I_{Ks} and I_{Kr} Markov transition rate to account for the experimental data being recorded at 296°K (23°C).

Markov models of wild-type and mutant I_{Kr} were generated using the 5-state formulation described by Clancy and Rudy (illustrated as an inset in Fig. 2A) [20]. The WT- I_{Kr} model was generated by optimizing simultaneously all 6 Markov transition rates to minimize the least-squared difference between modeled and experimental current density curves (see supplemental Fig. S1). To generate R273Q- I_{Kr} , each Markov transition rate was optimized independently. The single transition rate optimization that gave the best match with experimental data (assessed by χ^2) was chosen. An amplitude normalization factor was chosen for WT- I_{Kr} such that, when applied to LRd model, an APD of 180 ms was achieved at a paced cycle length of 1000 ms. The same amplitude normalization factor was then applied to the R273Q- I_{Kr} model. The model parameters and transition rate equations are provided in supplemental Table S1.

Markov models of wild-type I_{Ks} (WT- I_{Ks}) and I274V- I_{Ks} were generated using the 17-state formulation described by Silva and Rudy [21] (see supplemental Fig. S2). The model parameters and transition rate equations are provided in supplemental Table S2. To generate the WT- I_{Ks} model, all 17 Markov transition rates were optimized to minimize the least-squared difference between modeled and experimental current density curves. To model heterozygous I274V- I_{Ks} , we started with the WT- I_{Ks} model and then each Markov transition rate was optimized independently. The single transition rate optimization that gave the best match with experimental data (assessed by χ^2) was chosen. Because a main feature of repetitive I274V- I_{Ks} channel stimulation was current accumulation, multiple traces within a single voltage protocol were compared within each optimization iteration. Capacitive transients were not allowed to contribute to the χ^2 comparison. The set of adjustments to the transition rates and conductance (see supplemental Table S2) that minimized the χ^2 difference between experimental and simulated data (see supplemental Fig. S3) was used to form the model for all subsequent WT- I_{Ks} simulations. An amplitude normalization factor was chosen for WT- I_{Ks} such that, when applied to the Luo-Rudy (LRd) model, an action potential duration (APD) of 200 ms was achieved at a paced cycle length of 1000 ms. The same amplitude normalization factor was then applied to the I274V- I_{Ks} model.

Steady-state action potential simulations were recorded with pacing cycle lengths ranging from 300 to 10000 ms. The effects of a preceding pause on action potential duration was examined for WT and mutant I_{Kr} . For these simulations, a 1000 ms pacing cycle length protocol was conducted to steady state. The final pacing stimulus was preceded by a pause of specified duration, and the difference between the duration of that action potential and that of the preceding action potential (without an antecedent pause) was recorded. All simulations were implemented using Matlab v7.3 (The Mathworks, Inc., Natick, MA) with C++ code generously provided by Jonathan Silva and Yoram Rudy and run on a Macintosh G5-quad.

3. Results

We examined the biophysical properties of four single missense *KCNH2* variants, one compound *KCNH2* genotype, and a missense *KCNQ1* mutation using whole-cell patch clamp recording of recombinant potassium channels heterologously expressed in cultured mammalian cells. All mutations were originally identified in a large, retrospective series of 201 Norwegian SIDS cases that were screened for genetic variants in the major LQTS genes

[6]. Three *KCNH2* alleles (V279M, R885C, S1040G) exhibited biophysical properties indistinguishable from WT-HERG and we infer that these are probably benign variants. However, a range of functional defects were observed among the other mutations.

3.1. Partial loss-of-function *KCNH2* variants

Two *KCNH2* mutations, R273Q and R954C, alter highly conserved amino acid residues in the N-terminus or C-terminus of the HERG potassium channel, respectively. The proband in whom R954C was discovered also carried the common polymorphism K897T on the mutant allele giving rise to the compound genotype, K897T/R954C. Since each proband also carried a WT allele, we functionally characterized each mutant by co-expression with WT-HERG. To demonstrate expression of both constructs in the same cells, bicistronic vectors coupling the variant HERG allele to EGFP and WT-HERG to DsRed enabled the identification of co-expressing cells by yellow fluorescence (combined EGFP and DsRed).

Figure 1A illustrates representative whole-cell currents in cells co-expressing WT-HERG with either HERG-R273Q or HERG-K897T/R954C. When co-expressed with WT-HERG, both mutants generated currents resembling the rapid component of the cardiac delayed rectifier current (I_{Kr}). However, the amplitude of the activating (Fig. 1B) and peak tail currents (Fig. 1C) were significantly smaller at positive potentials in cells expressing the mutants as compared with WT-HERG alone. Tail current density was reduced ~35% and ~50% for cells expressing HERG-R273Q and HERG-K897T/R954C, respectively. Our results indicated that these two SIDS-associated *KCNH2* variants confer partial loss-of-function.

To determine if this degree of HERG functional impairment was sufficient to cause abnormal myocardial repolarization, we employed computational modeling of cardiac action potentials using the LRD model. Specifically, we modeled the level of HERG dysfunction observed for R273Q which was less severe. We first optimized Markov models for both WT and the R273Q using data derived from Fig. 1 (see supplemental Fig. S1), and then performed action potential simulations using different cycle lengths. Figure 2A illustrates that the mutant exhibits increased APD across a wide range of simulated cycle lengths. Because impaired HERG function is often associated with pause-dependent ventricular arrhythmias [22], we simulated the impact of an inter-pulse pause of varying duration. In Fig. 2B, we illustrate that progressive lengthening of the pause evokes lengthening of the APD and the spontaneous occurrence of an early afterdepolarization (EAD). We never observed an EAD in action potential simulations using the WT I_{Kr} model. These findings support the notion that reduced repolarization reserve conferring increased risk of arrhythmogenic electrical activity can occur with certain *KCNH2* mutations associated with SIDS.

3.2. *KCNQ1*-I274V is a gain-of-function mutation

The mutation *KCNQ1*-I274V, which alters a highly conserved amino acid residue in the S5 segment of the *KCNQ1* channel, confers a unique biophysical phenotype. Figure 3A illustrates representative whole-cell currents evoked by a series of depolarizing test potentials in cells expressing either WT-*KCNQ1* or *KCNQ1*-I274V. In the absence of KCNE1, *KCNQ1*-I274V generated a voltage dependent, rapidly activating, and slowly deactivating outward potassium current with current density (Fig. 3B) and biophysical properties similar to WT-*KCNQ1*. However, when expressed with KCNE1 (Fig. 3C), *KCNQ1*-I274V generated typical slowly activating outward currents resembling I_{Ks} , but with significantly greater peak current density than WT- I_{Ks} at test potentials positive to -30 mV (Fig. 3D). The increase in peak current density observed for I274V- I_{Ks} could not be explained by a simple shift in the voltage dependence of activation (apparent $V_{1/2}$ values from Boltzmann fits of isochronal activation curves: WT- I_{Ks} , 40.4 ± 2.4 mV, $N = 9$; I274V- I_{Ks} , 36.8 ± 2.0 mV, $N = 10$; no significant difference).

3.3. Altered activation and deactivation kinetics of I274V-I_{Ks}

In whole cell recordings, I274V-I_{Ks} appeared to have more rapid activation compared to WT-I_{Ks} (Fig. 3C). These differences in activation are further illustrated in Fig. 4A with representative WT-I_{Ks} and I274V-I_{Ks} traces that were normalized to their respective peak current levels. We quantified activation kinetics by measuring the time to half-maximal current at each voltage and observed significantly faster activation for I274V-I_{Ks} at positive test potentials between 20 and 50 mV (Fig. 4B).

The time course of deactivation was also slower for I274V-I_{Ks} compared to WT-I_{Ks} (Fig. 3D). To further investigate the kinetics and voltage dependence of deactivation for I274V-I_{Ks}, cells were depolarized to 40 mV followed by repolarization to a range of potentials from −40 to −100 mV. Figure 5A illustrates tail current deactivation for WT-I_{Ks} and I274V-I_{Ks} in representative traces normalized to the peak current measured at each voltage. Differences in the deactivation time course between WT-I_{Ks} and I274V-I_{Ks} are further illustrated in Fig. 5B. Tail currents were fit by a single exponential equation to obtain deactivation time constants (τ_{deact}). The time course of deactivation was significantly slower for I274V-I_{Ks}, as evidenced by the larger time constants measured at −80 to −100 mV (Fig. 5C). Deactivation time constants could not be measured at −70 mV since this voltage is close to the equilibrium potential for potassium under our experimental conditions, and no measurable deactivation was observed for I274V-I_{Ks} at −60 mV.

3.4. Gain-of-function effect of KCNQ1-I274V is dominant

Because KCNQ1-I274V was heterozygous in the SIDS proband [6], we tested whether this mutation conferred a dominant gain-of-function effect by co-expressing either WT-KCNQ1 or KCNQ1-I274V channels together with KCNE1 in the same cells. To avoid cell-to-cell variability associated with the transient transfection of three independent plasmids, we transiently expressed KCNQ1-I274V (engineered in an IRES2-EGFP vector) in a cell line stably expressing WT-KCNQ1 and KCNE1 (I_{Ks} cells). This strategy permitted reliable examination of cells expressing all three cDNAs. We initially optimized transfection conditions to determine the amount of WT-KCNQ1 plasmid DNA necessary to boost I_{Ks} current density by just 2–3 fold to avoid a large allelic imbalance. Figure 6 illustrates a comparison of whole-cell currents from I_{Ks} cells transiently transfected with either WT or I274V. Expression of the mutant channel causes a significantly greater current density consistent with a dominant gain-of-function phenotype.

The abnormal activation and deactivation kinetics observed for I274V-I_{Ks} (Fig. 4 and Fig. 5) suggested that the mutation may modulate rate-dependent responses of the current. To test this hypothesis, we examined the behavior of WT and mutant channels in response to a series of 50 depolarizing pulses (2 s, 60 mV) using the I_{Ks} cell expression strategy described above (Fig. 7A). With each successive pulse, I_{Ks} cells transfected with WT-KCNQ1 generated typical slowly activating outward currents with only a slight potentiation of current amplitude. By contrast in I_{Ks} cells transfected with I274V, the typical slowly activating outward current was observed only with the first pulse, and with subsequent pulses, a rapidly activating current was observed that progressively increased in amplitude. Deactivation during the post-pulse recovery step to −30 mV was also noticeably slower in cells expressing I274V.

To further quantify the abnormal rate dependent behavior exhibited by cells expressing I274V, currents were measured at 150 ms (“instantaneous”) and 1990 ms (“late”) after the start of the depolarizing pulse (see arrows on voltage protocol, Fig. 7A). The accumulation of instantaneous and late current was much larger for cells expressing I274V (Figs. 7B), consistent with the slower deactivation kinetics observed for the mutant. These studies support the idea

that impaired deactivation of I_{274V} can contribute to accumulation of the instantaneous current with repeated stimulation.

3.5. Action potential modeling of I_{274V}

The electrophysiological data indicated that in the presence of KCNE1, KCNQ1-I_{274V} causes a gain-of-function in I_{Ks} characterized by increased current density, faster activation, slower deactivation, and accumulation of instantaneous current during repeated stimulation. Therefore, we hypothesized that I_{274V} may promote a short QT syndrome phenotype. To test this hypothesis, computational modeling of ventricular action potentials was performed using a modified LRd model [21] in which a Markov modeling approach was applied to simulate WT-I_{Ks} and I_{274V}-I_{Ks} from data collected from the I_{Ks} cell experiments (Fig. 6 and Fig. 7). Implementation of WT-I_{Ks} alone into the LRd model evoked simulated action potentials similar to those published previously [21]. To model I_{274V}-I_{Ks}, we systematically modified each individual rate constant and evaluated the resulting simulated current profiles to determine the best fit to experimental data. Changing a single Markov transition rate was sufficient to best approximate steady-state I_{274V}-I_{Ks} behavior (see supplemental Table S2). Comparison of LRd simulations between WT-I_{Ks} and heterozygous I_{274V}-I_{Ks} revealed action potential shortening at all cycle lengths with little change in action potential morphology (Fig. 8). These simulations support our prediction that *KCNQ1-I274V* will cause a short QT phenotype, a plausible explanation for sudden death in an infant carrying this mutation.

4. Discussion

Sudden unexplained death of an infant is a tragic event. In the absence of an obvious cause of death including intentional injury as demonstrated by a negative autopsy and a thorough death scene investigation, a diagnosis of sudden infant death syndrome (SIDS) can be considered [23]. Although, the diagnosis of SIDS is one of exclusion, ongoing efforts to define preventable causes have great importance for understanding the pathogenesis of this syndrome and ultimately for reducing infant mortality. But despite intense research efforts to uncover its etiologies, SIDS remains a leading cause of death during the first year of life in industrialized nations [24–27]. In this study, our findings demonstrate that mutations in the cardiac potassium channel genes *KCNQ1* and *KCNH2* discovered in SIDS cases can exhibit functional defects consistent with either LQTS or SQTS. These observations strengthen the notion that congenital arrhythmia susceptibility may contribute to the pathogenesis of sudden infant death and that these disorders may present clinically as SIDS.

4.1. Cardiac potassium channel dysfunction in SIDS

The consequences of *KCNQ1* and *KCNH2* mutations associated with SIDS were not previously known [5,13–15], but we hypothesized that significant functional impairments were likely in some cases. However, it was difficult to predict *a priori* which variants would cause potassium channel dysfunction and promote arrhythmia susceptibility. In our report on the prevalence of LQTS gene mutations in Norwegian SIDS subjects [6], the three *KCNH2* variants (V279M, R885C, S1040G) that did not exhibit overt functional effects were not considered in calculating the percentage of SIDS cases associated with a pathological mutation but this had been based on preliminary work. Here we present the complete functional characterization of several mutants including those that exhibit abnormal functional properties that plausibly contributed to an increased risk of sudden infant death.

4.2. Loss-of-function *KCNH2* variants in SIDS

Our studies demonstrated that two *KCNH2* variant genotypes conferred partial loss-of-function consistent with LQTS and potentially contributed to unexplained death in the SIDS victims who carried these alleles. In comparison to other mutations associated with LQTS, the

functional impairments observed for *KCNH2*-R273Q and the compound variant *KCNH2*-K897T/R954C are considerably less severe. We speculate that the degree of functional loss exhibited by these SIDS-associated genotypes may require additional genetic or environmental factors to promote arrhythmogenesis. This notion was first demonstrated in the context of *SCN5A* variants associated with SIDS by Plant and colleagues for African-American carriers of *SCN5A*-S1103Y [28] and later emphasized by our study of several *SCN5A* mutations identified in the Norwegian SIDS cohort [29]. In those reports, exposure of seemingly benign sodium channel variants to intracellular acidosis unmasked a latent functional defect compatible with increased arrhythmia susceptibility. Other genetic factors may also contribute to sudden death in SIDS victims carrying *KCNH2* variants that exhibit mild functional impairments [16].

4.3. Novel gain-of-function *KCNQ1* mutation in SIDS

The most well studied congenital arrhythmia susceptibility syndrome is LQTS and a subset of SIDS cases may be caused by similar pathophysiological mechanisms. However, other inherited arrhythmia syndromes may also contribute to sudden death during infancy. Here, we demonstrated that *KCNQ1*-I274V is a novel gain-of-function mutation that is predicted to cause shortening of the ventricular action potential and the SQTs, another recognized predisposition for re-entrant arrhythmias and associated with an increased risk of sudden cardiac death. Indeed, aborted cardiac arrest in an 8-month old infant was reported in a carrier of *KCNH2*-N588K in one of the first described SQTs families [8,12]. However, sudden infant death has not been previously associated with gain-of-function *KCNQ1* mutations in either SQTs (V141M, V307L) [9,30] or atrial fibrillation (S140G) [31], but the proband first identified with *KCNQ1*-V141M presented *in utero* with an irregular bradyarrhythmia, which was recognized as atrial fibrillation, and SQTs manifested in the postnatal period [30]. Unfortunately, we cannot confirm SQTs in the Norwegian SIDS victim in whom *KCNQ1*-I274V was discovered or in first degree family members because all Norwegian SIDS victims were anonymous. But a fatal ventricular arrhythmia promoted by an abbreviated action potential seems a likely explanation for sudden death in this infant.

The structural basis for a gain-of-function associated with *KCNQ1*-I274V was not specifically explored by our study. The mutation affects a conserved residue in the S5 segment which contributes to the pore domain directly, but is also connected to the voltage-sensor domain by the S4-S5 linker. It is possible that gain-of-function is the result of increased single channel conductance, increased numbers of channels in the plasma membrane, or an increase in channel open probability. Based on the abnormal deactivation kinetics observed for I274V, we speculate that an increase in open probability is quite likely. We also observed that the gain-of-function was dependent upon the presence of the accessory subunit KCNE1. A similar KCNE1-dependence was reported for the SQTs mutant *KCNQ1*-V141M [30]. A molecular explanation for how the I274V mutation in the S5 segment alters *KCNQ1* gating modulation by KCNE1 is beyond the scope of our current study, but future studies aimed at understanding this phenomenon may provide information about fundamental aspects of this subunit interaction.

4.4. Functional impact of altered I_{Ks} deactivation

The kinetics of I_{Ks} deactivation may determine the rate of I_{Ks} accumulation during faster heart rates accounting for action potential duration adaptation in some species [32]. However, a recent computational study demonstrated that negligible open-state I_{Ks} accumulation occurs at rapid heart rates in human heart, but instead, the channels accumulate in pre-open closed states as an available reserve [21]. Our findings demonstrate that impaired deactivation of I274V- I_{Ks} leads to rate-dependent accumulation of open channels. At rapid heart rates, such as those typical of infancy, impaired deactivation may lead to I_{Ks} accumulation and pronounced

shortening of the action potential duration. This effect may exaggerate the transmural dispersion of repolarization that normally exists between epicardial and endocardial layers and thus promote arrhythmia susceptibility [33].

4.5. Study limitations

Our study utilizes heterologous expression of recombinant human ion channels. Although this approach is widely employed to assess functional consequences of ion channel mutations, we recognize that the *in vivo* effect of mutant potassium channels may be further influenced by factors in the native human cardiac myocyte environment that can't be replicated *in vitro*. Also, our computational studies used an established action potential model based upon guinea pig cardiac electrophysiology and direct relevance to human heart is not absolutely certain. However, we view these experiments collectively as supporting the hypothesis that certain cardiac potassium channel mutations identified in SIDS victims can confer increased arrhythmia susceptibility and this advances the concept that undiagnosed congenital arrhythmia syndromes can present as SIDS. Lastly, our work did not address the more complex issue of arrhythmia triggering mechanisms that may be important for understanding why some LQTS and SQTs mutation carriers do not survive infancy.

In conclusion, we demonstrated that there is a spectrum of cardiac potassium channel dysfunction associated with *KCNH2* and *KCNQ1* variants associated with SIDS that predict susceptibility to ventricular arrhythmias on the basis of either impaired or enhanced myocardial repolarization. These findings further establish that certain *KCNH2* and *KCNQ1* mutations are risk factors for SIDS, and emphasize SQTs as a potential cause of sudden infant death.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Functional characterization of *KCNH2* variants R273Q and K897T/R954C. (A) Representative whole-cell current traces recorded from CHO cells transiently transfected with plasmids encoding WT-HERG, R273Q, and K897T/R954C expressed in tandem with either EGFP or DsRed. In each experiment, the allele listed first in the inset legend is coupled to EGFP while the second is coupled to DsRed. Horizontal and vertical scale bars represent 1000 ms and 250 pA, respectively. The voltage protocol employed is shown in the inset (arrows indicate the time points at which currents were compared). (B) Current-voltage relationship for potassium current densities (normalized to membrane capacitance) measured in CHO cells expressing WT-HERG + WT-HERG (closed circles, $N = 19$), R273Q + WT-HERG (open circles, $N = 8$), or K897T/R954C + WT-HERG (closed triangles, $N = 7$). (C) Current-voltage relationship for amplitude of peak tail current densities following repolarization to -50 mV for WT-HERG + WT-HERG (closed circles, $N = 19$), R273Q + WT-HERG (open circles, $N = 8$), or K897T/R954C + WT-HERG (closed triangles, $N = 7$). Data shown are mean $\pm$ SEM. Significant differences between R273Q + WT-HERG and WT + WT-HERG at the $P < 0.05$ level are indicated by *. Significant differences between K897T/R954C + WT-HERG and WT + WT-HERG ($P < 0.05$) are indicated by #.

Fig. 2.

Action potential modeling of HERG-R273Q. (A) Predicted APD at various cycle lengths predicted from LRd modeling. The mutant was modeled using data from co-expression with WT-HERG. Inset figures illustrate the Markov scheme used to simulate I_{Kr} , and a representative overlay of action potentials generated at cycle length 1000 ms. (B) Pause-dependent lengthening of APD is illustrated by the relative change in APD at various pause durations. In these experiments, the pause occurred after 50 simulated action potentials when the model was at steady-state. The inset illustrates an EAD occurring following a pause of 7.5 s for the heterozygous mutant.

Fig. 3.

Functional characterization of KCNQ1-I274V. (A) Representative whole-cell current traces recorded from CHO cells transiently transfected with WT-KCNQ1 or KCNQ1-I274V. The voltage protocol employed is shown in the inset (arrow indicates the time point at which currents were compared). Horizontal and vertical scale bars represent 500 ms and 250 pA, respectively. (B) Current-voltage relationship for potassium current densities (normalized to membrane capacitance) measured in CHO cells expressing WT-KCNQ1 (closed circles, N = 9) and KCNQ1-I274V (open circles, N = 10). (C) Representative traces illustrating potassium currents observed in CHO cells transiently transfected with WT KCNQ1 + KCNE1 (WT-I_{Ks}) or KCNQ1-I274V + KCNE1 (I274V-I_{Ks}). Horizontal and vertical scale bars represent 500 ms and 2000 pA, respectively. (D) Current-voltage relationship for potassium current densities (normalized to membrane capacitance) measured in CHO cells expressing WT-I_{Ks} (closed circles, N = 9) and I274V-I_{Ks} (open circles, N = 10). Data shown are mean $\pm$ SEM. Significant differences between WT-I_{Ks} and I274V-I_{Ks} ($P < 0.05$) are indicated by *.

Fig. 4.

Activation kinetics of I274V-I_{Ks}. (A) Representative current traces normalized to peak current illustrating activation of WT-I_{Ks} and I274V-I_{Ks} during a 2 second pulse to 40 mV. Horizontal and vertical scale bars represent 500 ms and 0.25 arbitrary units (normalized current), respectively. (B) Voltage dependence of activation. The rate of activation was determined by measuring the time to half-maximal current at each voltage for WT-I_{Ks} (black bars, N = 9) and I274V-I_{Ks} (grey bars, N = 10) from experiments shown in Fig. 2C. Data shown are mean $\pm$ SEM. Significant differences between WT-I_{Ks} and I274V-I_{Ks} ($P < 0.05$) are indicated by *.

Fig. 5.

Deactivation kinetics of I274V-I_{Ks}. (A) Representative traces illustrating deactivation in CHO cells expressing WT-I_{Ks} or I274V-I_{Ks}. Currents were evoked by depolarization to 40 mV followed by repolarization to varying negative potentials for 1.5 s. Horizontal and vertical scale bars represent 500 ms and 0.25 arbitrary units (normalized current), respectively. (B) Averaged, normalized tail current traces illustrating deactivation of WT-I_{Ks} and I274V-I_{Ks}. Deactivation was examined after a 2 s pulse to 40 mV followed by repolarization to -40 mV. Tail currents were normalized to the peak tail current and are the average of 7 cells. Horizontal and vertical scale bars represent 250 ms and 0.2 arbitrary units (normalized current), respectively. (C) Voltage dependence of deactivation. Time constants of deactivation were obtained after fitting the tail current with a single exponential equation for WT-I_{Ks} (black bars, N = 8) or I274V-I_{Ks} (grey bars, N = 6). Data shown are mean $\pm$ SEM. Significant differences between WT-I_{Ks} and I274V-I_{Ks} ($P < 0.05$) are indicated by *.

Fig. 6.

Properties of KCNQ1-I274V in the heterozygous state. (A) Representative whole-cell currents recorded from I_{Ks} cells transiently transfected with either WT-KCNQ1 or I274V. The voltage protocol used was the same as illustrated in Fig. 3A. (B) Current-voltage relationship for potassium current densities (normalized to membrane capacitance) measured in I_{Ks} cells expressing either WT-KCNQ1 (closed circles, $N = 15$) or KCNQ1-I274V (open circles, $N = 13$). Current density values are different at the $P < 0.01$ level for all voltages between -20 and 60 mV.

Fig. 7.

Use dependent behavior of I274V in I_{Ks} cells. (A) Averaged whole-cell current traces recorded from I_{Ks} cells transfected with either WT ($N = 8$) or I274V ($N = 6$) during a series of 50 depolarizing pulses (2 s, 60 mV) delivered at 13.3 pulses/min. Traces shown correspond to pulse numbers 1–10, and every 5th pulse thereafter. The voltage protocol employed is shown in the inset (arrow indicates the time point at which 'instantaneous' and 'late' currents were compared). (B) Instantaneous current density measured 150 ms after the start of each depolarizing pulse. Data shown are mean $\pm$ SEM. (C) Late current density measured 1990 ms after the start of each depolarizing pulse. Data shown are mean $\pm$ SEM.

Fig. 8.

Action potential simulations with WT- I_{Ks} and I274V- I_{Ks} (A) Simulated action potentials using WT- I_{Ks} (black line) and heterozygous I274V- I_{Ks} (red line) at cycle lengths of 300 ms and 1000 ms. Data for these simulations were derived from the expression experiments in I_{Ks} cells (Fig. 6 and Fig. 7) and the modeled currents are illustrated in supplemental Fig. S3. (B) Relationship of APD with cycle length for WT- I_{Ks} and heterozygous I274V- I_{Ks} .