A Common Single Nucleotide Polymorphism Can Exacerbate Long-QT Type 2 Syndrome Leading to Sudden Infant Death

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Background—Identification of infants at risk for sudden arrhythmic death remains one of the leading challenges of modern medicine. We present a family in which a common polymorphism (single nucleotide polymorphism) inherited from the father, combined with a stop codon mutation inherited from the mother (both asymptomatic), led to 2 cases of sudden infant death.

Methods and Results—KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2, CACNA1c, CACNB2b, and KCNJ2 genes were amplified and analyzed by direct sequencing. Functional electrophysiological studies were performed with the single nucleotide polymorphism and mutation expressed singly and in combination in Chinese ovary (CHO-K1) and COS-1 cells. An asymptomatic woman presenting after the death of her 2-day-old infant and spontaneous abortion of a second baby in the first trimester was referred for genetic analysis. The newborn infant had nearly incessant ventricular tachycardia while in utero and a prolonged QTc (560 ms). The mother was asymptomatic but displayed a prolonged QTc. Genetic screening of the mother revealed a heterozygous nonsense mutation (P926afsX14) in KCNH2, predicting a stop codon. The father was asymptomatic with a normal QTc but had a heterozygous polymorphism (K897T) in KCNH2. The baby who died at 2 days of age and the aborted fetus inherited both K897T and P926afsX14. Heterologous coexpression of K897T and P926afsX14 led to loss of function of HERG current much greater than expression of K897T or P926afsX14 alone.

Conclusions—Our data suggest that a common polymorphism (K897T) can markedly accentuate the loss of function of mildly defective HERG channels, leading to long-QT syndrome–mediated arrhythmias and sudden infant death.

Key Words: genetics ■ arrhythmias ■ sudden cardiac death ■ electrophysiology ■ HERG

Long-QT syndrome (LQTS) is a congenital disorder that predisposes affected individuals to sudden cardiac death (SCD).1,2 To date, mutations in 12 genes have been identified,1–5 although some affected LQT patients have symptoms ranging from syncope to severe arrhythmias such as torsade de pointes (TdP), in most cases patients are asymptomatic.3–5 In some, the QT interval is within normal range.6 LQTS has also been linked to sudden infant death syndrome (SIDS). In a study done by Arnestad et al.,6 9.5% of the cases diagnosed as SIDS had functional genetic variants in 1 of the LQT genes. Other studies have also found cardiac ion channel mutation in SIDS cases.7–13 Although evaluation of family members clearly demonstrated that the parents were in some cases not affected, in most studies genetic or ECG screening of the parents was not available and therefore there was no definitive evidence as to whether these mutations were inherited or de novo. Interestingly, in a study by Maron et al.,14 ECG data of family members of SIDS victims showed QT prolongation in >25% of the first-degree relatives. Thus, there are different phenotypes and varying degrees of QT prolongation in LQTS patients.

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Variations of phenotype expression are thought to be attributable to the severity of the disease-causing mutation as well as the possible coexistence of other genetic variations,15,16 including single nucleotide polymorphisms (SNP), which are not disease-causing by definition but which can alter arrhythmia susceptibility. This has been demonstrated with several SNPs such as D85N (in KCNE1),16 K897T (in KCNH2),17 and H558R (in SCN5A).18–20 In all cases, the patients also had mutations in the same gene causing the disease phenotype. SNPs have been shown to modify clinical expression either by aggravating the clinical phenotype16,18,21 or by attenuating the clinical phenotype.19,20 We present a family in which an inherited common polymorphism in KCNH2 when combined with a loss of function mutation on separate alleles of the same gene led to infant death. Family
members with the only polymorphism or mutation alone did not have any events of syncope or SCD.

Methods

ECG Analysis

QT interval was measured and adjusted to heart rate (QTc), according to the Bazett formula.22 The end of the T wave was defined as the intersection with the isoelectric line of a tangent drawn to the descending portion of the T wave.

Genetic Evaluation

After informed consent was obtained, blood was collected from family members. Tissue obtained from the stillborn fetus was provided with written consent of the parents. Genomic DNA was extracted from peripheral blood leukocytes and from fresh and frozen tissue with a commercial kit (Puregene, Gentra Systems, Inc, Minneapolis, Minn). The genomic DNA was amplified by polymerase chain reaction (PCR) on GeneAmp PCR System 9700 (Applied Biosystems, Foster City, Calif). All exons and intron borders of the KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2, CACNA1c, CACNB2b, and KCN2 genes were amplified and analyzed by direct sequencing. PCR products were purified with a commercial reagent (ExoSAP-IT, USB Corporation, Cleveland, Ohio) and directly sequenced from both directions using ABI PRISM 3100 Automatic DNA sequencer (Applied Biosystems).

Electrophysiology

Voltage clamp recordings were made as previously described23 using patch pipettes fabricated from borosilicate glass capillaries (1.5 mm OD, Fisher Scientific, Pittsburgh, Pa). The pipettes were pulled using a gravity puller (Narishige Co Ltd, Tokyo, Japan) and filled with pipette solution of the following composition (mmol/L): 10 KCl, 125 K-aspartate, 1.0 MgCl2, 10 HEPES, 10 NaCl, 5 MgATP, and 10 EGTA, pH 7.2 (KOH). The pipette resistance ranged from 1 to 4 MΩ when filled with the internal solution. The perfusion solution contained (mmol/L): 130 NaCl, 5 KCl, 1.8 CaCl2, 1.0 MgCl2, 2.8 Na acetate, and 10 HEPES, pH 7.3 with NaOH. Current signals were recorded using MultiClamp 700A and Axopatch 200B amplifiers (Axon Instruments Inc, Foster City, Calif), and series resistance errors were reduced by ~60% to 70% with electronic compensation. All signals were acquired at 10 to 50 kHz (Digidata 1322, Axon Instruments) and analyzed with a microcomputer running pClamp 9 software (Axon Instruments). All recordings were made at room temperature.

Immunofluorescence and Confocal Analysis

Twenty-four to 48 hours after transfection, cells were washed with phosphate buffered saline (PBS) and then fixed with 4% formaldehyde in PBS for 10 minutes. Cells were then permeabilized with 0.1% Triton-X for 5 minutes. Quenching was performed by 30-minute incubation with 0.1% bovine serum albumin (BSA) in PBS. The cells were then incubated for 1 to 2 hours with primary antibodies diluted in 0.1% BSA solution in PBS. Cells were then washed with PBS-BSA followed by 1-hour incubation with the fluorophore-conjugated secondary antibody at room temperature. After the final wash, the coverslips were mounted with Prolong Antifade (Molecular Probes, Eugene, Ore). XYZ images of labeled cells were collected as previously described16,21 with a Fluoview confocal microscope. An argon or krypton-argon laser (dependent on fluorophore) provided the excitation light. Fluorescence signals were collected with a ×40 oil-immersion objective lens. XY frame was set to 512×512 pixels and laser intensity was set to 6% to 10% power. The Z-axis was changed in approximately 0.5-μm increments by computer control through the entire volume of the cell. Analysis of labeled cells was performed using both Fluoview and Image J software.

The primary antibodies used in this study were rabbit polyclonal antihuman Ether-a-go-go Related Gene (HERG) recognizing an extracellular epitope (1:100, Alomone Labs, Jerusalem, Israel). For fluorescence detection, a secondary donkey anti-rabbit antibody, conjugated with Alexa Fluor 594 (1:1000; Invitrogen, Carlsbad, Calif) was added.

Statistical Analysis

Electrophysiological data are presented as mean±SEM, and statistical comparisons were made with ANOVA followed by a Student-Newman-Keuls test or with a Student t test, as appropriate. Significance was defined as P<0.05.

Results

Clinical Findings

A 24-year-old woman was evaluated for the loss of a female baby shortly after birth and a first-trimester loss of a male fetus in her second pregnancy (Figure 1). Her first pregnancy resulted in a female baby born at 33 weeks who had incessant ventricular tachycardia throughout her last trimester as evidenced by fetal echocardiography showing atrioventricular (AV) dissociation with a V-rate of 190 to 270/min (mainly ~200/min) and an atrial rate of 110 to 120/min. The arrhythmia was treated with sotalol; propranolol; propranolol plus mexiletine; and propranolol plus flecainide, all in attempt to treat the fetal tachycardia but without significant benefit to the fetus. The fetus’ arrhythmia responded only to magnesium therapy administered in the last 2 weeks of pregnancy. After delivery, the baby had no arrhythmia but had severe hydrops and ultimately died. Her 12-lead ECG demonstrated a QTc of 560 ms (QT of 200 ms corrected for a heart rate of
130 bpm). Postmortem autopsy revealed no evidence of any congenital or any structural heart disease.

The mother had no structural heart disease and had no apparent clinical history of syncope, near syncope, or palpitations. She had been on telemetry in a high-risk unit during her pregnancy for weeks without any documented arrhythmia of her own (or during and after her c-section). Twelve-lead ECG of the mother showed QTc prolongation at baseline and displayed repolarization abnormalities with a markedly prolonged QTc after administration of sotalol to treat the fetal arrhythmias. She did not develop TdP (Figure 2). There is no known family history of long QT or syncope in the family. Of note, 1 cousin had SIDS.

The mother’s second pregnancy ended on the 9th week. The fetal tissue was sent for genetic screening. The mother remained asymptomatic throughout without any symptoms of palpitations, dizziness, or syncope. She was subsequently maintained on a low dose of propranolol, and a decision was made by the treating physician to implant an automatic implantable cardioverter-defibrillator. The mother’s third and fourth pregnancies resulted in delivery of a full-term healthy baby with no documented arrhythmias; however, the fourth child had a prolonged QTc of 505 (Figure 2). The father was asymptomatic with a normal QTc interval of 380 ms.

**Genetic Studies**

Genetic analysis of the mother revealed a heterozygous nucleotide insertion of G at position 2775 of KCNH2, predicting a substitution of proline for alanine acid at position 926 of HERG and leading to a frame shift resulting in a stop codon (TAG) 14 amino acids later (P926AfsX14) (Figure 3 and Figure 4). Genetic analysis of the father (number I-1 in pedigree; Figure 1) revealed a heterozygous nucleotide change from A to C at position 2690 of KCNH2, predicting a substitution of lysine with threonine at position 897 of HERG (K897T) (Figure 3). K897T is a common polymorphism found in approximately 32% of the general White population. Genetic analysis of the first child and

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**Figure 1.** Pedigree of the family.

**Figure 2.** Twelve-lead ECG of mother (I-2 in Figure 1) and daughter (II-4 in Figure 1) showing repolarization abnormalities with a prolonged QTc. The mother’s T-wave morphology is suggestive of LQT2.
the second child (fetal tissue) (II-1 and II-2 in pedigree) demonstrated that both children inherited both P926AfsX14 and K897T. The third child (II-3) inherited neither the mutation nor the K897T polymorphism. She had a normal ECG with a QTc of 406 ms. The fourth child (II-4), who was found to have a QTc of 505 ms, inherited only the P926AfsX14 mutation and not the K897T polymorphism.

Expression Studies
To determine how the mutation P926AfsX14 and K897T altered the biophysical properties of HERG current and contributed to the clinical phenotype, we expressed HERG channels (WT and mutants) in CHO-K1 cells and performed patch-clamp experiments. Currents activated rapidly during step depolarizations to positive potentials and displayed the characteristic tail current generated by channels recovering on repolarization (Figure 5). Homozygous K897T and P926AfsX14 both demonstrated a reduction in tail current density to 59.0±8.5% (n=9) and 60.2±14.4% (n=11), respectively, compared with WT (Figure 5B). The P926AfsX14 mutant yielded current even though a large portion of the C-terminal was truncated. To mimic the heterozygote state of family members I-1 and I-2 and II-4, K897T and P926AfsX14 were coexpressed with WT. Coexpression of either K897T or P926AfsX14 with WT resulted in no significant difference in current density compared with WT alone. In contrast, coexpression of K897T with P926AfsX14 as found in family member II-2, the deceased infant, severely reduced tail current density to 38.7±4.7% of WT (n=10; P<0.05).

Channel availability was assessed using a standard triple-pulse protocol (Figure 6). Compared with WT, K897T currents showed a small positive shift in the voltage dependence of midinactivation values (−60.7±1.15 mV, n=13, and −53.1±1.13 mV, n=9, respectively, P<0.05). Coexpression with P926AfsX14 resulted in a tendency toward a leftward shift of midinactivation voltage (−63.3±1.13 mV, n=12, P=NS), which was most pronounced when P926AfsX14 was coexpressed with K897T, resulting in a midinactivation potential of (−69.2±1.91 mV, n=8, P<0.05).

Hence, K897T, together with P926AfsX14, reduced HERG channel function to a greater degree than homozygous expression of P926AfsX14, both with regard to tail current density and the steady-state availability of rapidly activating delayed rectifier potassium current (Ikr) channels.

Figure 3. Electropherograms. A, Insertion of G at position 2775 of KCNH2 causing a frame shift that leads to a stop codon 14 amino acids downstream (P926AfsX14). B, Heterozygous nucleotide change from A to C at position 2690 of KCNH2 predicts a substitution of lysine with threonine at position 897 of HERG (K897T).
Because the truncated section of the mutant channel contains the C-termini endoplasmic reticulum retention signal (Figure 4), we sought to determine whether the loss of function is due in part to a trafficking defect. We transfected COS cells with all possible combinations: WT, K897T, P926AfsX14, WT/K897T, WT/P926AfsX14, and K897T/P926AfsX14. The cells were probed with rabbit antibodies against HERG. XYZ scans of the cells using confocal microscopy showed normal distribution of HERG channels with no evidence of abnormal trafficking of the K897T and P926AfsX14 mutated channels (alone or combined) to the cell membrane. Figure 7 shows normal distribution of HERG resulting from transfecting K897T/P926AfsX14. Similar images were obtained when other combinations of WT, K897T, and P926AfsX14, were used for transfection (not shown).

Discussion

The results of our study suggest that a common polymorphism such as K897T-KCNH2 can potently accentuate the effects of a mutation in HERG, thus resulting in a lethal form of long QT syndrome. SNPs have previously been implicated in acquired forms of LQTS, leading to the development of drug-induced TdP.19 Westenskow et al20 and Crotti et al17 were the first to provide evidence that a common SNP (KCNE1-D85N and HERG-K897T, respectively) may modify the disease phenotype of congenital LQTS. The role of K897T in the LQTS, however, is not entirely straightforward. In large-scale population studies, the 897T allele has been associated with a shorter QTc interval compared with the 897K allele.24–28 In one study,29 Finnish women with K897T had a longer QTc than those with K897K. In contrast to the population studies, in functional studies performed using cells expressing K897T HERG channels, most investigators found that K897T alters the channel biophysical properties leading to a reduction in HERG current.17,30 Our results suggest that the loss of function of K897T is relatively small, consistent with the results of large population studies,24–29 and that its manifestation is generally subclinical, as in the case of the father of the family described in this report. CHO-K1 cells expressing K897T HERG channels demonstrated only a mild reduction in tail current compared with WT, and the heterozygous expression of K897T and P926AfsX14 channels shows a reduction to 38.7±4.7% (n=10) versus WT. Intermediate current values can be observed for the homozygous expression of K897T and P926AfsX14 (59.0±8.5%, n=9; and 60.2±14.4%, n=8, respectively).
from the mother. The first child had incessant ventricular tachycardia and a prolonged QTc on her ECG and eventually died on her second day of life. The second child died in utero in the first trimester. The third child (subject II-3 in family pedigree; Figure 1), who inherited neither the polymorphism nor the mutation, is healthy and has a normal QTc. Consistent with the phenotype, coexpression of K897T and P926AfsX14 produced a major reduction of HERG current (to 38.7 ± 4.7% of WT), whereas expression of homozygous P926AfsX14 produced only a modest reduction in HERG current. Our results suggest that expression of the K897T polymorphism and the stop codon mutation in KCNH2 on separate alleles leads to a lethal form of LQT2 that can result in in utero ventricular tachycardia and sudden infant death.

Our findings are congruent to those of Crotti et al,17 in which K897T combined with a KCNH2 mutation modified the clinical expression of a LQT2 mutation, leading to SCD. Numerous mechanisms have been proposed to underlie SIDS. Filiano and Kinney31 suggested that SIDS results from the intersection of 3 overlapping factors. Our study, like that of Crotti et al, suggests the convergence of at least 2 genetic factors. It is possible that still others such as ischemia, acidosis, or other environmental factors contribute as well.

SNPs have been associated with SIDS in several studies.6–13 Recently, NOS1AP, a genetic modifier found to increase the risk of SCD in LQTS patients,32 was also found to be involved in SIDS.33 However, the precise mechanisms by which these SNPs are linked to SIDS are not clear. SNPs have been shown to contribute to drug-induced TdP.19 Genetic variations can create a subclinical form of LQTS, which may manifest only after exposure to drugs with QT-prolonging actions. Similarly, SNPs may alter the repolarization reserve of the infants, rendering them more vulnerable to LQTS mutations. Triggering events or factors such as sleep (specifically in SIDS cases with SCN5A SNPs),34 sympathetic surge, other “de novo” polymorphisms/mutations,6 or drugs used by the mother, may trigger a life-threatening arrhythmia. Wang et al34 demonstrated that certain genetic variants in SCN5A found to be associated with SIDS are not sufficient by themselves to cause pathological manifestations but may become pathological in vitro when exposed to acidosis.

To our knowledge, this report is the first to demonstrate how a common SNP may contribute to SIDS or lethal infant arrhythmias. Both parents often showed a normal QTc, although careful scrutiny revealed that the QTc of the mother was at times prolonged. Loss of function of HERG channel current was mild when the genetic variants of the individual parents were expressed alone but resulted in a severe loss of function that was associated with a severe clinical phenotype when the 2 variants were combined. It is noteworthy that K897T was reported to be associated with a HERG mutation in a Norwegian SIDS study leading to a 50% reduction in the HERG tail current when coexpressed.35 However, no evidence was presented as to whether the phenotype was the result of the mutation alone or from the combination of K897T and the mutation. Moreover, no evidence was presented showing that K897T accentuates the effect of the mutation to reduce HERG current. Also of note is the fact that the allele frequency of K897T is similar in SIDS cases and control subjects.6

Identification of infants at risk for SIDS or for fetal life-threatening arrhythmias remains one of the leading challenges of modern medicine. One difficulty is that SIDS is generally not a familial disease. Some of these cases are due to de novo mutations.7–13,36 Other possibilities may be a parental mosaicism for LQT-associated mutations37 or homozygous missense mutations.38 Our results suggest that
even a common SNP may place an infant born to asymptomatic parents at risk, emphasizing the importance of identifying SNPs when screening families for SCD and SIDS.

Another interesting finding is that CHO-K1 cells coexpressing K897T and P926AfSx14 had a significant reduction in HERG activity compared with cells expressing homoygous P926AfSx14. Previous studies\(^ {18,38,39}\) describing mutations resulting in truncation of the HERG C-terminus also did not find major reductions in HERG function when expressed in heterologous systems. Similar to our results, the truncated HERG C-terminus proteins reached the cell surface with no apparent trafficking defect.\(^ {18,39}\) It is noteworthy that the truncation removes the endoplasmic retention signal as well as a motif involved in masking the retention\(^ {18}\) (Figure 4). The truncated HERG proteins in all of these reports as in our report contain all 6 transmembrane segments and the cyclic nucleotide-binding domain and therefore it is not surprising that the function of these channels is relatively preserved in expression studies. The reason why some of the patients carrying these mutations have a severe phenotype has long been puzzling. Bhuiany et al\(^ {38}\) suggested that in their case, the mutant HERG was destroyed by the nonsense-mediated decay pathway regulating mRNA. Another possibility suggested by Choe et al\(^ {39}\) is that the truncation of the protein leads to the loss of the protein kinase A phosphorylation site (Figure 4) and therefore cannot respond to \(\beta\)-adrenergic stimulation properly. Our report suggests that in some cases the severity of the clinical phenotype may be due to the influence of a common polymorphism such as K897T. When combined with the HERG mutation, K897T alters the functional properties of the channel to reduce HERG current, because trafficking of K897T/P926AfSx14 was unaffected.

**Study Limitations**

Although it is tempting to conjecture that the spontaneous abortion of the second sibling was due to a genetic profile similar to that responsible for the death of the neonate (subject II-1), it is possible that causes other than an arrhythmic event associated with LQT2 were responsible.

Our expression studies were performed without cotransfection of \(\text{KCN2}\) along with \(\text{KCNH2}\). The role of this subunit in modifying \(\text{KCNH2}\) function continues to be debated. Relevant to this issue is the observation that the results of the functional studies correlated well with the clinical phenotypes.

Our results present further compelling evidence implicating a common polymorphism (K897T) in the accentuation of a loss of function of mildly defective HERG channels leading to LQTS-mediated arrhythmias and sudden infant death and suggest that further investigations into similar relationships are warranted.

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**Disclosures**

None.

**References**


An estimated 10% to 15% of sudden infant death cases are thought to be due to ion channelopathies giving rise to life-threatening cardiac arrhythmias. Identification of infants at risk for sudden arrhythmic death remains one of the leading challenges of modern medicine. We present a family in which a common polymorphism (K897T) in KCNH2 inherited from the father combined with a stop codon mutation in the same gene inherited from the mother, both asymptomatic, led to early onset of sudden infant death syndrome. The data suggest that a common polymorphism (K897T), found in 30% of the population, can markedly accentuate the loss of function of a mildly defective potassium channel leading to lethal long-QT syndrome–mediated arrhythmias and sudden infant death.
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