Sudden Death Associated With Short-QT Syndrome Linked to Mutations in HERG

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**Background**—Sudden cardiac death takes the lives of more than 300 000 Americans annually. Malignant ventricular arrhythmias occurring in individuals with structurally normal hearts account for a subgroup of these sudden deaths. The present study describes the genetic basis for a new clinical entity characterized by sudden death and short-QT intervals in the ECG.

**Methods and Results**—Three families with hereditary short-QT syndrome and a high incidence of ventricular arrhythmias and sudden cardiac death were studied. In 2 of them, we identified 2 different missense mutations resulting in the same amino acid change (N588K) in the S5-P loop region of the cardiac $I_{Kr}$ channel HERG (KCNH2). The mutations dramatically increase $I_{Kr}$ leading to heterogeneous abbreviation of action potential duration and refractoriness, and reduce the affinity of the channels to $I_{Kr}$ blockers.

**Conclusions**—We demonstrate a novel genetic and biophysical mechanism responsible for sudden death in infants, children, and young adults caused by mutations in KCNH2. The occurrence of sudden cardiac death in the first 12 months of life in 2 patients suggests the possibility of a link between KCNH2 gain of function mutations and sudden infant death syndrome. KCNH2 is the binding target for a wide spectrum of cardiac and noncardiac pharmacological compounds. Our findings may provide better understanding of drug interaction with KCNH2 and have implications for diagnosis and therapy of this and other arrhythmogenic diseases. (Circulation. 2004;109:r151-r156.)

**Key Words:** genetics ■ death, sudden ■ arrhythmia ■ ion channels

Sudden cardiac death in individuals with structurally normal heart accounts for approximately 20% of sudden cardiac death cases. Idiopathic sudden cardiac death syndromes are gradually coming into focus as forms of inherited ion channelopathies. Ion channel proteins are responsible for the currents that generate the cardiac action potential, and alterations of their function are known to be associated with a wide spectrum of clinical phenotypes. Gain of function in SCN5A, the gene that encodes for the $\alpha$ subunit of the cardiac sodium channel, is associated with the LQT3 form of the long-QT syndrome (LQTS), whereas a decrease in function of the same channel is associated with Brugada syndrome and familial conduction disease. Loss of function in $I_{Kr}$ and $I_{Ks}$ is also linked to other forms of LQTS, whereas an increase in $I_{Kr}$ current, caused by a mutation in the $\alpha$ subunit KCNQ1, is linked to familial atrial fibrillation. The final outcome is similar, involving alteration of ion channel activity, leading to the development of an arrhythmogenic substrate. Although arrhythmogenic diseases have been linked to gain of function in SCN5A (late $I_{Kr}$) and KCNQ1 ($I_{Ks}$), to our knowledge no disease had been associated with a gain of function in KCNH2 encoding for $I_{Kr}$.

Short-QT syndrome (SQTS) is a new clinical entity originally described as an inherited syndrome by Gussak et al in 2000. The familial nature of the disease was recently confirmed in 2 additional families. We have identified and genetically screened a total of 3 families with SQTS associ-
ated with sudden death. The 3 families are white, of European
descent, and not related. In this report, we identify
the mutations responsible for the disease in families 30-335 and
30-371, demonstrate genetic heterogeneity of the syndrome,
delineate the biophysical mechanisms involved in the gener-
ation of the phenotype, and investigate a potential pharma-
cological approach to therapy at both the basic and clinical
levels.

Methods
This study was approved by the regional institutional review board,
and the patients gave informed consent for participation.

Clinical Analysis
A detailed description of the clinical characteristics of families
30-335 and 30-371 was recently reported.8 Briefly, both families
displayed a very short QT interval in the ECG (Bazzett-corrected QT
interval [QTc] of 300 ms) and episodes of paroxysmal atrial
fibrillation, ventricular arrhythmias, and sudden death in patients
with structurally normal hearts; ventricular tachyarrhythmias were
inducible during electrophysiological study. In family 30-335, sud-
den death and malignant arrhythmias were observed before the first
year of life. One family member suffered averted sudden death at age
8 months and had severe neurological damage, and his brother died
suddenly at 3 months of age and was diagnosed as having SIDS
(Figure 1A).

The proband in family 30-339 is a 51-year-old male who suffered
cardiac arrest in a domestic airport and was resuscitated with the use
of an automatic external defibrillator (Figure 1A). His ECG showed
a QTc of 288 ms (Figure 1B, left). He underwent electrophysiolog-
cal study and was found to be easily inducible with a single
extrastimulus and was implanted with an automatic cardioverter
defibrillator. His son, 20 years of age, was asymptomatic and
presented with an ECG displaying a QTc of 293 ms (Figure 1B,
right). The daughter and wife had normal QT intervals.

Genetic Analysis
Genomic DNA was isolated from peripheral blood leukocytes using
a commercial kit (Genta System, Puregene). The exons of KCNH2
were amplified and analyzed by direct sequencing using previously
published primers.9 Polymerase chain reaction products were purified
with a commercial reagent (ExoSAP-IT, USB) and were directly
sequenced from both directions with the use of ABI PRISM
3100-Avant Automatic DNA Sequencer.

Site-Directed Mutagenesis
C1764A mutation was constructed with the use of GeneTailor site-directed
mutagenesis system (Invitrogen Corp) with the use of plasmid pcDNA3.1
containing KCNH2 cDNA. The primers for mutagenesis were the follow-
ing: 1764F (5'-GACTACGCGTCCGCTGGCTGCACAAACTGGGC-
GACCAG-3') and 1764R (5'-GTGCAGCCAGCCGATGCGTGAGTC-
CATGTGT-3'). The mutated plasmid was sequenced to ensure the pres-
ence of the C1764A mutation as well as the absence of other substitutions
introduced by the DNA polymerase.

In Vitro Transcription and Mammalian
Cell Transfection
KCNH2 and KCNE2 were a kind gift from Drs A.M. Brown
(Chantest, Cleveland, Ohio) and S.A. Goldstein (Yale University,
New Haven, Conn), respectively. Both gene constructs were re-
cloned from their original vector into pcDNA3.1 (Invitrogen). For
transfection, KCNH2 and KCNE2 cDNA were kept at a constant molar ratio of 1:20 to ensure proper expression of both subunits. Modified human embryonic kidney cells (TSA201) were cotransfected with the same amounts of pcDNA-KCNH2/KCNE2 and pcDNA-N588K.KCNE2 complex using the calcium phosphate precipitation method, as previously described. Cells were grown on polylysine-coated 35-mm culture dishes and placed in a temperature-controlled chamber for electrophysiological study (Medical Systems) 2 days after transfection.

**Electrophysiology**

Standard whole-cell patch-clamp technique was used to measure currents in transfected TSA201 cells. All recordings were made at room temperature using an Axopatch 1D amplifier equipped with a CV-4 1/100 headstage (Axon Instruments). Cells were superfused with HEPES-buffered solution containing (in mmol/L) NaCl 130, KCl 5, CaCl2 1.8, MgCl2 1, Na acetate 2.8, and HEPES 10, pH 7.3, with NaOH/HCl. Patch pipettes were pulled from borosilicate (7740) or flint glass (1161) (PP89 Narahige Japan) to have resistances between 2 and 4 MΩ when filled with a solution containing (in mmol/L) KCl 20, KF 120, MgCl2 1.0, HEPES 10, and EGTA 5, pH 7.2 (KOH/HCl). Currents were filtered with a 4-pole Bessel filter at 0.5 to 1 kHz, digitized at 1 kHz, and stored on the hard disk of an IBM-compatible computer. All data acquisition and analysis was performed using the suite of pCLAMP programs V7 or V6 (Axon Instruments).

**Results**

Because the 3 families were not large enough to undertake a genome scan, we used a candidate gene approach to perform the genetic analysis. We screened by direct sequencing the exons and intron-exon boundaries of candidate genes encoding ion channels contributing to repolarization of the ventricular action potential, including HERG (KCNH2), KCNE2, KCNQ1, KCNE1, SCN5A, and KCNJ2. In the case of family 30-339, we also included Kv4.3, Kv4.2, Kv1.5, KChIP2, KChAP, KChIP1, KCNJ3, KCNJ6, SUR1, KCNJ11, ANKB, and CHRM1, 4 and 5. We identified a missense mutation (C to G substitution at nucleotide 1764) in family 30-371 in KCNH2. Analysis of family 30-335 identified a different missense mutation in the same residue (C to A substitution at nucleotide 1764) in KCNH2. Both mutations, however, substituted the asparagine at codon 588 in KCNH2 for a positively charged lysine (Figure 2). This residue is located in the S5-P loop region of HERG at the outer mouth of the channel. The mutation was present in all affected members in families 30-371 and 30-335 but in none of the unaffected members. Given the pattern of transmission, the 2 individuals who died suddenly in family 30-371 were obligate carriers of the mutation. The mutations were confirmed by restriction analysis (data not shown). These mutations were not present in 400 control chromosomes of the same ethnic background (data not shown). No other mutations were detected in HERG or the other genes screened in the 2 individuals who died suddenly in family 30-371 were obligate carriers of the mutation. The mutations were confirmed by restriction analysis (data not shown). These mutations were not present in 400 control chromosomes of the same ethnic background (data not shown). No other mutations were detected in HERG or the other genes screened in affected individuals from these 2 families or in those from family 30-339.

To determine the mechanism by which mutation N588K modulates $I_{Kr}$, reduces the duration of the ventricular action potential, and shortens the QT interval, we coexpressed the mutated KCNH2 channels (N588K) with and without the ancillary β-subunit KCNE2 (MIRP1) in human embryonic kidney cells (TSA201) and performed patch-clamp experiments. Whole-cell recordings (Figure 3) showed that wild-type (WT) HERG/KCNE2 currents elicited by sequential depolarizing pulses reached a maximum steady-state current at 0 mV and started to decrease because of the rapid onset of inactivation (rectification) at more positive potentials. WT recordings also displayed the typical large tail currents generated by inactivated channels rapidly reopening (recovery) on repolarization (Figure 3A). In contrast, N588K/KCNE2 steady-state current continued to increase linearly well over +40 mV, and we did not observe significant tail currents after repolarization (Figure 3C). Because recent studies have questioned the contribution of KCNE2 to $I_{Kr}$ function, we repeated the expression studies with N588K and KCNH2 expressed without KCNE2. The mutation had similar effects on currents expressed by KCNH2 and N588K alone (Figures 3B and 3D), although the augmentation of the developing current and the diminution of the tail current (total charge) were not as pronounced as in the presence of KCNE2 (Figures 3B and 3D). Analysis of the current-voltage relationships (Figures 3E and 3F) showed that both N588K and N588K/KCNE2 currents failed to rectify significantly within a physiological range of potentials.

To determine how the mutation altered the kinetics of the current during an action potential, we elicited KCNH2 N588K currents with and without KCNE2 (Figures 3G and 3H, respectively) using a stimulus generated by a previously...
recorded AP. KCNH2 and KCNH2/KCNE2 currents displayed a typical hump-like waveform with slow activation kinetics and a rapid increase during the repolarization phase of the action potential as inactivated channels quickly recovered. In sharp contrast, N588K currents displayed a dome-like configuration resulting in a much larger relative current during the initial phases of the action potential.

Block of $I_{Kr}$ by methanesulfonanilides, phosphodiesterase inhibitors, 11 macrolide antibiotics, 12 antifungal agents, 13 and antihistamines 14 is the basis for the QT-prolonging effects and potential arrhythmogenicity of these compounds. Because QT abbreviation is likely attributable to an abbreviation of the ventricular AP secondary to an increase in $I_{Kr}$, we reasoned that blocking $I_{Kr}$ with class III antiarrhythmic drugs could be a potential therapeutic approach for the treatment of SQTS.

We administered Sotalol, a class III antiarrhythmic with potent $I_{Kr}$ blocking actions, to the proband as a preliminary test of this hypothesis. Sotalol was administered according to standard and recommended dosage of 1 to 1.5 mg/kg body weight. Because of hypotension, we decided to apply a dosage of 1 mg/kg (Figures 4A and 4B illustrate the response of patient IV-5 of family 30-371 to 1 mg/kg IV sotalol after 5 minutes). QTc at baseline (Figure 4A) was 291 ms and remained practically unchanged after administration of sotalol (Figure 4B), suggesting that this patient was not responsive to this dose of the $I_{Kr}$ blocker. We obtained similar results in 2 other short-QT patients (1 from family 30-371 and the other from family 30-335). We next evaluated the response of the heterologously expressed KCNH2/KCNE2 currents to sotalol in the WT and mutated channels. Figure 4C shows a representative experiment in which extracellular application of 100 µmol/L D-sotalol reduced WT current at −100 mV by 48%, as expected from previously published EC50 values. 15–19 N588K currents, on the other hand, were only reduced by 9.0 ± 0.3% and 27.0 ± 0.3% after application of 100 and 500 µmol/L D-sotalol, respectively (Figure 4D). Thus, N588K reduced the ability of D-sotalol to block the channel, a result consistent with the clinical findings. A similar decreased sensitivity to the drug was also observed when N588K was expressed without KCNE2 (data not shown).
**Discussion**

KCNH2 has a shaker-like tetrameric structure composed of homologous core units each containing 6 membrane-spanning segments. Coassembly with the β-subunit MiRP1 (KCN2E) is required to fully reproduce the biophysical and pharmacological properties of the native \( I_{Kr} \). KCNH2 has previously been linked to a decrease in outward repolarizing current responsible for the hereditary (LQT2) and acquired forms of LQTS. A common polymorphism in KCNH2 (K897T) has been reported to produce a modest abbreviation of QTc to 388.5 ± 2.9 ms by shifting the voltage of activation of \( I_{Kr} \) by -7 mV. KCNH2 is also the primary target of class III antiarrhythmic agents, many of which contribute to generation of an acquired form of the long-QT syndrome. Chimeric studies of KCNH2 showed that replacing the S5-S6 linker, which contains the pore region, with the corresponding area from the bovine ether-a-go-go removes the high-affinity block by dofetilide. Binding of dofetilide and sotalol occurs primarily in the open state, but the inactivation of the channel stabilizes the drug on its receptor site and increases its apparent affinity. Abolition of the current rectification by N588K additionally supports the hypothesis that residues in the outer mouth of the pore of the channel are important for C-type inactivation and block of KCNH2 by methanesulfonylanilides. Consistent with these observations, a recent study reported that N588C mutation of HERG also disrupts C-type inactivation.

Genetic analysis of KCNH2 failed to identify the mutation in family 30-339, indicating that SQTS probably is, as is the case for the other inherited arrhythmogenic disorders, a genetically heterogeneous disorder. We have screened the exons and intron-exon boundaries of several genes involved in cardiac repolarization and have not been successful at identifying the mutation. There are several possible explanations: (1) that we missed the mutation because of the reliability of the technique used; (2) that the mutation is not present in the coding region of the gene of interest; (3) that it is in an alternative spliced fragment that is not screened; and (4) that it is in a gene that is unknown at this time.

Our results identify the first KCNH2 mutation to produce a remarkable gain of function and provide for the first time a genetic basis for the SQTS, a disease characterized by marked QTc prolongation and a high incidence of atrial and ventricular arrhythmias and sudden death. Our data also demonstrate the first link of a cardiac disease to a gain of function in KCNH2, which encodes for rapidly activating delayed-rectifier current \( I_{Kr} \).

A N588K missense mutation is shown to abolish rectification of the current and reduce the affinity of the channel for drugs with class III antiarrhythmic action. The net effect of the mutation is to increase the repolarizing currents active during the early phases of the AP, leading to abbreviation of the action potential and thus to abbreviation of the QT interval. Because of the heterogeneous distribution of ion currents within the heart, we speculate that the AP shortening in SQTS is heterogeneous, leading to accentuation of dispersion of repolarization and the substrate for the development of both atrial and ventricular arrhythmias. Given the young age of occurrence of events in some patients (3 years of age, because the families will probably be rather small. It will be difficult to prove causality cannot be proven completely. It is possible that the mutations described are not the causative mutations but rare functional polymorphisms in linkage disequilibrium with the true mutation. Arguments against this are the fact that 2 different variations have been identified in 2 families, that the variations are not present in control chromosomes, and that their functional characterization is consistent with the phenotypical and clinical data. It will be difficult to prove causality by linkage in a disease with such a high mortality at a young age, because the families will probably be rather small.

Although we used a constant molar ratio of KCNE2 to HERG to ensure proper expression of both gene products, it is difficult to determine whether an individual cell actually expressed the 2 subunits in the same proportion based on the methodology used involving binding of CD8 beads. We take some comfort in the knowledge that cotransfection of the 2 genes consistently yielded tail currents with more rapid deactivation than transfection with HERG alone.
months), our data also suggest the possibility of a link between KCNH2 mutations and sudden infant death syndrome.

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