Table 3. Multivariate Cox models. Channel activation time ($\tau_{act}/\tau_{act\text{-}WT}$ > 1.20) is dichotomized at median values. (A) Results from the entire population. (B) Results on the 212 patients with QTc <500 ms. CI, confidence interval.

Parameter	Hazard ratio	95% CI	*P*
A. Cox model including all 387 patients*			
$\tau_{act}/\tau_{act\text{-}WT}$ >1.20	2.02	1.44–2.82	<0.001
QTc ≥500 ms	1.86	1.32–2.62	<0.001
B. Cox model including only the 212 patients with QTc <500 ms*			
$\tau_{act}/\tau_{act\text{-}WT}$ >1.20	2.22	1.35–3.63	0.002
QTc ≥470 ms	0.89	0.56–1.41	0.62

*The models are adjusted for male gender ages 0 to 12 years and time-dependent β-blocker therapy, which significantly contributed to all multivariate results.

>1.20) and fast-activating ($\tau_{act}/\tau_{act\text{-}WT} \leq 1.20$) channels, we calculated the median value of the distribution of change in τ_{act}. After exclusion of channels containing KCNQ1(V254M), the median increase in τ_{act} was 20% for the remaining mutant channels. Exclusion of any individual mutation from the analysis did not significantly alter the results, indicating that no individual mutation drove the increase in cardiac risk observed for slow-activating channels. With the separate analysis of KCNQ1(V254M), slow-activating channels remained a strong predictor of cardiac events for the remaining study population and for patients with QTc <500 ms (Table 4 and Fig. 3C).

I_{Ks} activation is faster after stimulation by protein kinase A (PKA) and at higher temperatures (*14*). To determine whether the differences in activation time course recorded at room temperature in unstimulated cells (Table 1) are also observed at physiological temperatures and after PKA stimulation, we measured changes in activation time course caused by increasing temperature and after PKA activation

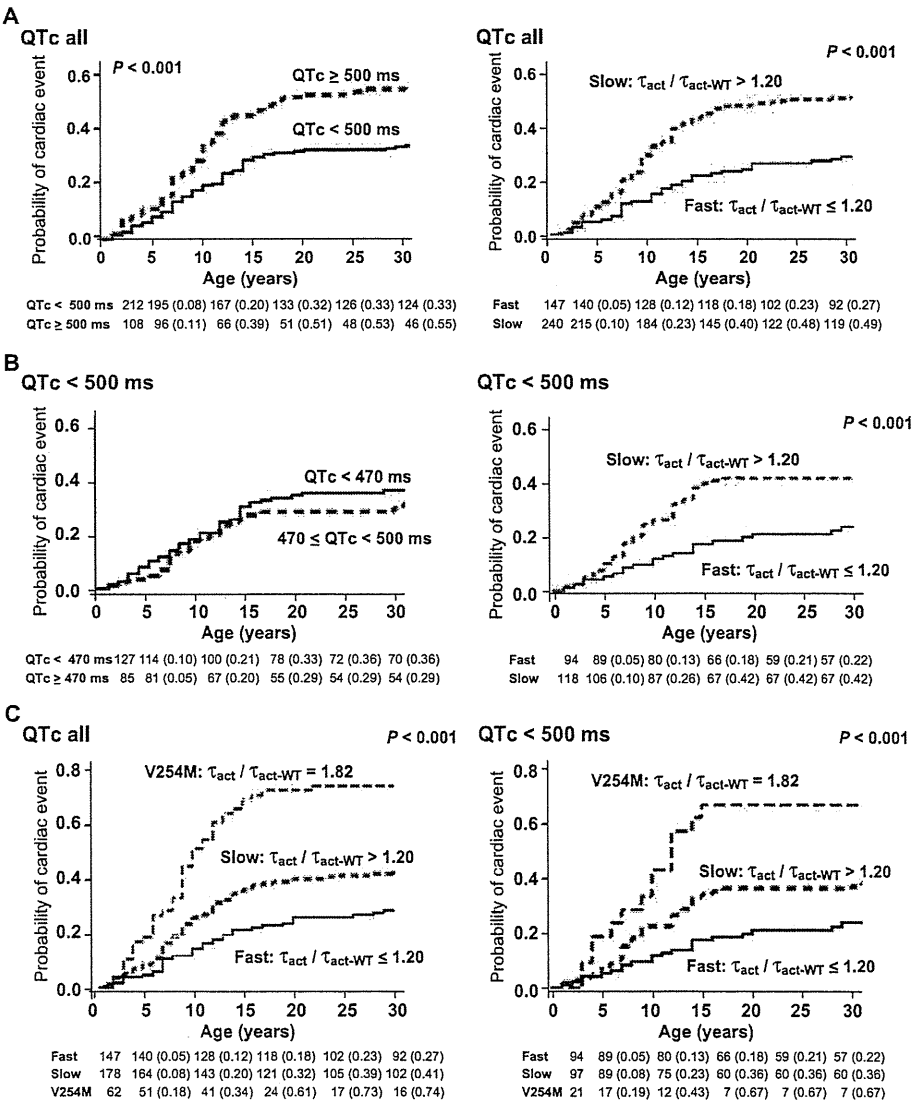

Fig. 3. Contribution of slow activation and QTc for the cumulative risk of first cardiac event. Kaplan-Meier graphs showing the probability of first cardiac event before age 30 in carriers of mutations. Number of patients at risk at 0, 5, 10, 15, 20, and 25 years of age and the respective cardiac event rate at that age are indicated below the graph [number of patients (event rate)]. (**A**) Left: Patients with QTc <500 ms and QTc ≥500 ms have significantly different cardiac risk. Right: Patients carrying mutations that produce fast-activating ($\tau_{act}/\tau_{act\text{-}WT} \leq 1.20$) and slow-activating ($\tau_{act}/\tau_{act\text{-}WT} > 1.20$) channels have significantly different cardiac risk (n = 387). (**B**) For patients with moderate prolongation of QTc. Left: Patients with QTc <470 ms and 470 ≤ QTc < 500 ms do not show differences in cardiac risk. QTc = 470 is the median QTc value for this population. Right: Patients carrying mutations that produce fast-activating ($\tau_{act}/\tau_{act\text{-}WT} \leq 1.20$) and slow-activating ($\tau_{act}/\tau_{act\text{-}WT} > 1.20$) channels have significantly different cardiac risk (n = 212). (**C**) Kaplan-Meier graphs showing the probability of first cardiac event before age 30 in carriers of mutations with $\tau_{act}/\tau_{act\text{-}WT} \leq 1.20$ and $\tau_{act}/\tau_{act\text{-}WT} > 1.20$, with adjustment for carriers of V254 ($\tau_{act}/\tau_{act\text{-}WT}$ = 1.82). Left: All patients. Right: Patients with QTc <500 ms.

Table 4. Multivariate Cox models with carriers of V254M modeled individually. Channel activation time ($\tau_{act}/\tau_{act\text{-}WT}$ > 1.20) is dichotomized at median values. (A) Results from the entire population. (B) Results on the 212 patients with QTc <500 ms.

Parameter	Hazard ratio	95% CI	P
A. Cox model including all 387 patients*			
$\tau_{act}/\tau_{act\text{-}WT}$ >1.20	1.55	1.08–2.23	0.016
QTc ≥500 ms	1.64	1.16–2.31	0.004
V254M mutation carrier	2.91	1.75–4.86	<0.001
B. Cox model including only the 212 patients with QTc <500 ms*			
$\tau_{act}/\tau_{act\text{-}WT}$ >1.20	1.80	1.06–3.03	0.029
QTc ≥470 ms	0.82	0.51–1.32	0.42
V254M mutation carrier	3.03	1.84–4.98	<0.001

*The models are adjusted for male gender ages 0 to 12 years and time-dependent β-blocker therapy, which significantly contributed to all multivariate results.

for channels formed by wild type and wild type coexpressed with mutant subunits (G168R, S225L, R243C, and V254M) in HEK293T cells. We measured the activation time course when the temperature was raised from 22°C to 37°C (fig. S3) and before and after application of the PKA activator forskolin (fig. S4). For all channels, activation time course at 37°C and after PKA activation was faster, consistent with the previously reported data for the wild-type channel. Both temperature- and PKA-mediated changes were not significantly different between wild-type and mutant channels, indicating that channels with slow kinetics of activation will remain slower than wild-type channels under both conditions.

Modeling of early after-depolarization in response to premature beats

To investigate the potential role of altered channel kinetics to generate pro-arrhythmic events, we used a mathematical reconstruction of a humanized model of the cardiac action potential based on the canine endocardium model of Flaim-Giles-McCulloch (FGM) (*15*) (for details, see the Supplementary Material). We chose this model over others, including some based on human data, because the model formulation contains a more advanced representation of Ca^{2+} release mechanisms and reproduces large differences in epicardial, mid-myocardial, and epicardial action potential shapes, including rate-dependent changes in action potential duration.

First, we generated a train of endocardium action potentials paced at 1 Hz and showed that slow channel activation in the range observed in the mutants studied (Table 2) can cause prolongation of action potential (Fig. 4A). Second, to investigate the mechanism of arrhythmia generation in slow-activating channels, we compared action potentials in cells with slow-activating I_{Ks} channels and channels with decreased conductance, which caused an equivalent prolongation of the action potential. We imposed high β-adrenergic stimulation because arrhythmias are generally triggered during exercise for LQT1 patients (*16*). Although the exact mechanism of arrhythmias is not known, in experimental studies in canine drug-induced LQT1, the ventricular arrhythmia Torsade de pointes (TdP) can be reproducibly triggered by β-adrenergic stimulation (*17*). Moreover, this study showed that TdP is preceded by systolic after-contractions, presumably from early after-depolarizations that occurred predominantly in the endocardium but not the epicardium.

In our simulation studies, endocardial cells with β-adrenergic drive showed early after-depolarizations under the fast-pacing protocol with one premature beat. Both decreases in channel conductance (G_{max} = 0.65) and activation rate (τ_{act} = 1.75) prolonged action potential duration (Fig. 4A). For channels where conductance alone was decreased, premature beats caused an early after-depolarization. For slowly activating channels, the premature beat caused early after-depolarizations associated with a more prolonged depolarization and incomplete depolarization following the early after-depolarization (Fig. 4B). Our results suggest that the slow repolarization of the cardiomyocytes caused by slow I_{Ks} activation affects the ability of the cell to recover from early after-depolarizations, potentially contributing to arrhythmogenesis. These results are consistent with our clinical data showing that slow activation of I_{Ks} increases cardiac risk for patients independent of QTc prolongation.

DISCUSSION

Here, we investigated the association between conventional measures of ion channel function from expression studies and the clinical phenotype in LQT1 subjects with a verified KCNQ1 channel mutation. Prolonged QTc in carriers of the mutation was highly correlated with decreased magnitude of channel current. Despite this, channel current amplitude correlated poorly with the risk of cardiac events in the carriers (syncope, ACA, or sudden death). In contrast, mutations causing a slow activation of the channel were strongly associated with increased risk for cardiac events. In patients with modest QTc prolongation (<500 ms), slow channel activation remained a strong independent predictor for cardiac events, whereas the QTc interval carried no predictive value. Thus, knowledge of the effect of the mutation on the channel rate of activation may help to identify individuals at an increased risk of cardiac events independent of clinical risk factors, and this may be especially important in the large group of LQT1 patients presenting with modest QTc prolongation. The appropriate clinical care for LQT1 patients with modest QTc prolongation is not well established, with many patients remaining untreated. Identification of high-risk mutations in this population could lead to more aggressive treatment in the population at risk and better patient care.

Mutations with a dominant-negative effect on channel function are associated with an increase in cardiac risk (*6*). Nonetheless, several previous studies have reported a poor correlation between current magnitude and the observed QTc interval in patients harboring the mutation (*7–9*). In particular, Wang and colleagues compared median QTc in patients to decrease in current for five LQT1 mutations, three of which were studied in the present study (*9*). Despite a decrease in current similar to that reported here, QTc_m prolongation for those patients did not significantly correlate to current decrease. This difference may have been a result of the low number of LQT1 mutations and/or subjects. We found a high inverse correlation between the measured magnitude of the mutant current and the median value for the observed QTc in carriers. However, for almost all mutations, carriers exhibited QTc durations over a broad range. This is a common finding (*18*) that indicates substantial influence of other modifiers on the QT interval and underscores the need for large populations in this type of study.

The V254M mutation prolonged τ_{act} excessively compared to 16 other mutations in this study. This is in agreement with previous results (*19*) and reports that the S4-S5 linker is important for K^+ channel gating (*9*, *19*–*21*). However, as illustrated in Fig. 1, mutations causing changes in τ_{act} >20% and arrhythmias were found at several other locations in the subunit such as the S4, S5, and S6 transmembrane domains, as well as the pore region. For instance, A341V, previously associated with high cardiac risk (*22*, *23*), also results in channels with slower activation rate. Removal of each individual mutation from the analysis did not significantly alter the results, indicating that slow activation is a robust predictor of cardiac risk in the study population.

Although decrease in current and G_{max}, slower activation, faster deactivation, and shifts in voltage dependence of activation are expected to contribute to loss of channel function, here we showed that only slower activation predicts cardiac risk in this patient population independently from patient QTc. To study the mechanism underlying the pro-arrhythmic state, we introduced slow-activating I_{Ks} channels into a cardiomyocyte action potential model with simulated β-adrenergic stimulation. In a rapid pacing protocol with a premature beat, cells with channels having slow activation rate showed prolonged action potentials, early after-depolarizations, and incomplete depolarization on the subsequent beat. Our simulation shows that both slow activation and decrease in conductance can similarly prolong action potential duration. Nonetheless, impaired recovery from early after-depolarizations is observed for slow-activating channels, suggesting that channel activation rate affects the ability to effectively repolarize the membrane. Both early after-depolarizations and heterogeneity of action potential durations are thought to foster TdP (*24*). However, the precise mechanism of generation of whole-heart arrhythmias is not yet clear.

We found that several mutations, with both severe and mild clinical phenotype, significantly altered the rate of deactivation. In previous studies, a faster rate of channel deactivation was found to be associated with channel dysfunction, leading to a severe form of LQT1 (*25*) and atrial fibrillation (*26*). During β-adrenergic stimulation, I_{Ks} channel deactivation is slowed (in addition to channel activation being accelerated). This regulation is thought to be important for the regulation of cardiac repolarization in response to increased heart rate (*27*). Our study found that changes in the basal rate of deactivation caused by LQT1 mutations did not correlate either with the median resting QTc interval or with risk of cardiac arrhythmias, suggesting that impairment of current accumulation at high heart rates does not underlie the arrhythmias in LQT1.

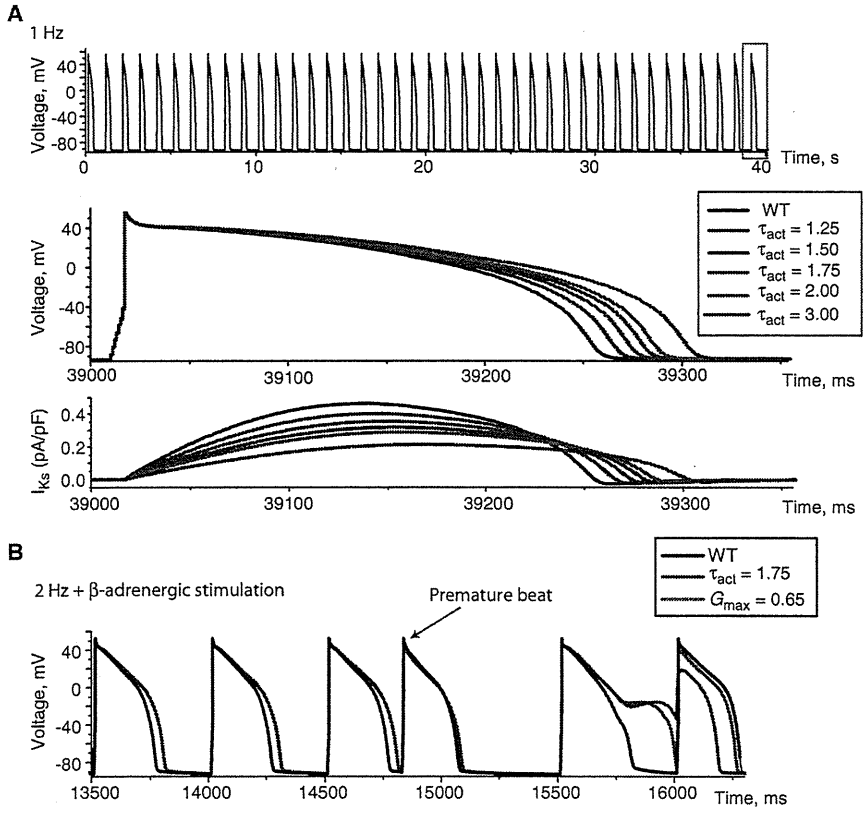

Fig. 4. Effect of slow channel activation on modeled cardiac action potential. (**A**) Cardiac action potential model with WT I_{Ks} channels (top graph). Fortieth action potential in the simulation incorporating I_{Ks} channels with slower activation as indicated (middle graph). Corresponding I_{Ks} current during the action potential for each of the conditions (lower graph). (**B**) Effect of a premature beat on modeled action potential. Cardiac action potential model with WT I_{Ks} channels, I_{Ks} channels with decreased conductance (G_{max} = 0.65), and/or I_{Ks} channels with slow activation (τ_{act} = 1.75) as indicated. Note that before the premature beat, the action potentials for G_{max} = 0.65 and τ_{act} = 1.75 virtually overlay. Cells with channels with slow activation show an increased prolongation of the action potential during early after-depolarizations and incomplete depolarization after the premature beat. Stimulation rate of 2 Hz was used to mimic the fast heart rates seen during β-adrenergic stimulation.

Some mutations were only found in 10 subjects, which make assessment of the survival rate in these subjects less accurate. Also, some of the mutations were only found in members of one family, making other genetic traits possible confounders. However, the models in this study are adjusted by a jackknife approach, the covariance sandwich estimator, which removes members of each family from the model one by one, making the reported *P* values conservative (Tables 3 and 4). On top of this, we treated the mutation with the most radical results independently, so we believe that the reported results are robust.

The *Xenopus laevis* oocyte system is a simple, cost-effective, and higher-throughput technique than other heterologous expression systems. Here, we show that it captures the changes in both current activation and deactivation rates and current decrease, reflecting changes observed in the mammalian system. We showed that the changes measured in the oocyte system can be used to predict ion channel cardiac risk. Our data suggest that measurement of ion channel function in this system can be extended to assess the cardiac risk of the hundreds of known mutations associated with LQT1, for which limited clinical data are available.

The occurrence of syncope and cardiac arrest is variable in LQT1, and proper risk stratification is needed to optimize

patient treatment (*6*, *28*–*30*). β-Adrenergic blockers are the treatment of choice for preventing cardiac events for patients with LQT1. Implantable cardioverter defibrillator (ICD) therapy may be offered to patients who remain symptomatic despite β-adrenergic blocker therapy (*28*–*30*). However, this is an invasive procedure that may be associated with technical difficulties (especially in young children) and complications. Risk stratification approaches to identify LQTS patients who have a high risk for cardiac arrhythmias are currently based mainly on the phenotypic expression of the disease (assessed the magnitude of prolongation of QTc duration and clinical symptoms) and may therefore fail to identify high-risk patients with a moderate-range QTc, who make up ~70% of the LQTS population (*13*). Our results will allow improved risk stratification for LQT1 patients. Specifically, β-blocker treatment may be indicated for patients with slow-activating channels, despite the lack of clinical symptoms and the presence of a moderately prolonged QTc. Furthermore, our findings suggest that patients who carry mutations that are associated with slow-activating channels should be carefully followed, regardless of additional risk factors, with more invasive interventions (including ICD implantation or left cardiac sympathetic denervation) if symptoms occur during medical therapy. In addition, our results may prove useful in assessing drug-induced cardiac risk for drugs that affect channel activation time course.

In conclusion, prolonged QTc values were correlated with decreases in ion channel current caused by the mutation. In addition, a slower rate of channel activation was significantly associated with an increased risk of cardiac events, independent of clinical measures such as gender, QTc, and β-adrenergic blocker treatment. These associations were significant in the large group of patients who have a QTc less than 500 ms, a group that includes most LQT1 patients, for which the QTc interval provides little prognostic information. Thus, mutation-induced changes in the rate of I_{Ks} activation may provide an important tool for the risk assessment and management of LQTS patients, and are the first step toward a mutation-specific risk stratification for patients with LQTS. Our findings also suggest that development of drugs that affect the kinetics of channel activation may be beneficial for LQT1 patients.

MATERIALS AND METHODS

An expanded Materials and Methods section is available in the Supplementary Material.

Population

The population involved patients with genetically confirmed LQT1 mutations or if they had died suddenly at a young age of suspected LQTS and were from a family with a genetically confirmed mutation. Patients were drawn from four LQTS registries: the U.S. part of the International LQTS Registry (n = 319), the Netherlands LQTS Registry (n = 44), the Japanese LQTS Registry (n = 29), and the Danish LQTS Registry (n = 1). To evaluate the clinical course of carriers with each mutation, we included mutations with at least 10 subjects. We identified patients with 17 different KCNQ1 missense mutations. For correlation with values for measures on the WT-KCNQ1/KCNE1 channel, we included 480 family members from the U.S. part of the International LQTS registry who tested negative for LQT1 and not positive for any other LQTS genotype. All subjects or their guardians provided informed consent for the genetic and clinical studies.

The patients were enrolled during childhood, adolescence, or, for a few individuals, during adulthood. The clinical course from birth up to enrollment was then reconstructed, and the individual was followed prospectively from that point. This method allows modeling the clinical course from date of birth.

Genotype characterization

The KCNQ1 mutations were identified with genetic tests performed in academic molecular-genetic laboratories including the Functional Genomics Center, University of Rochester Medical Center, Rochester, NY; Baylor College of Medicine, Houston, TX; Mayo Clinic College of Medicine, Rochester, MN; Boston Children's Hospital, Boston, MA; Laboratory of Molecular Genetics, National Cardiovascular Center, Suita, Japan; Department of Clinical Genetics, Academic Medical Center, Amsterdam, Netherlands; and Statens Serum Institut, Copenhagen, Denmark. For the proband in each family, the five most common LQTS loci (*KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, and *KCNE2*) were fully sequenced to identify the mutation by comparing the sequence with sequence of healthy individuals without LQTS. Once mutation was identified, other family member gene was sequenced to confirm the presence or absence of the mutation.

Phenotype characterization

The ECG parameters were obtained from the baseline ECG recorded at the time of enrollment in each of the registries. The QT and R-R intervals were measured in milliseconds, with QT corrected for heart rate by Bazett's formula (QTc). A small minority of individuals died suddenly before an ECG was recorded. Follow-up was censored at age 41 years to avoid the influence of coronary heart disease. In all four registries, clinical data were collected on prospectively designed forms for demographic characteristics, personal and family medical history, ECG findings, therapy, and endpoints during long-term follow-up. Data common to all four LQTS registries involving genetically identified patients with LQT1 were electronically merged into a common database for the present study.

Endpoints

LQTS-related cardiac events included syncope (transient loss of consciousness that is abrupt in onset and offset), ACA requiring defibrillation, and SCD, whichever occurred first. Information on endpoint events was determined from the clinical history ascertained by routine follow-up contact with the patient, family members, attending physician, or the medical records. Syncope has been shown to be a major predictor and an excellent surrogate for cardiac death (*31*). The number of events is as follows (176 total): syncope = 149, ACA = 9, SCD = 18. No ICD events were recorded in this population.

Plasmids and reagents

Human KCNQ1 and KCNE1 in pcDNA3.1(+) plasmid were used as described (*32*). All reagents were purchased from Sigma-Aldrich Corp. unless otherwise indicated.

Molecular biology

Human KCNQ1 and KCNE1 were subcloned in the pGEMsh vector (modified from PGEMHE vector) for oocyte expression (*32*, *33*). Polymerase chain reaction (PCR)–based site-directed mutagenesis was performed with PfuUltra DNA polymerase. Construct sequences were confirmed by DNA sequencing. Complementary RNAs (cRNAs)

were transcribed using the "message machine" kit (Ambion). RNA concentration was estimated with RNA markers (Invitrogen).

Electrophysiology

***Xenopus* oocyte experiments.** *Xenopus* oocytes were harvested, dissociated, and defolliculated by collagenase type I treatment. Mutant channel subunits were expressed in combination with WT subunits to mimic heterozygous mutations. KCNQ1/KCNE1 cRNA was injected at a molar ratio of either 1:1 (2 ng/0.4 ng) or 0.5:1 (1 ng/0.4 ng) to mimic the haploinsufficient phenotype. Mutant KCNQ1 cRNA was injected at a molar ratio of 1:1 (WT/mutant) or 1 ng of WT-KCNQ1/1 ng of mutant KCNQ1/0.4 ng of KCNE1. Mutations with currents significantly different from that of the haploinsufficient control were considered dominant-negative mutations. Whole-oocyte currents were measured with a GeneClamp 500 amplifier (Axon Instruments). Agarose-cushioned microelectrodes were used with resistances between 0.1 and 1.0 megohm. Oocytes were constantly superfused with the following: 91 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, and 5 mM NaOH/Hepes (pH 7.5). Currents of the mutant channels (I_{mut}) were evaluated 1 min after oocyte impailment. At least 10 oocytes of the same batch and 2 to 3 oocyte batches were used. One-way analysis of variance (ANOVA) followed by Dunnett's post hoc test was applied for the assessment of statistical significance when comparing the channel expressing the mutant subunits to WT channel function. KCNE1 expressed by itself at the concentrations used for these experiments yielded currents at least 10 times smaller than any of the mutant currents measured. This current did not significantly affect our analysis of the expressed channels. Because I_{Ks} does not reach a steady level even after long depolarizations at room temperature, we constructed isochronal ($t = 8$ s) activation curves to determine the voltage dependence of I_{Ks}. Experiments with longer depolarizing pulses (18 s compared to 2.7 s) showed that the length of the depolarizing pulse affects the voltage dependence of I_{Ks}, but relative shifts in the voltage dependence persist independent of the length of the pulse (*7*). All experiments were performed at room temperature.

To assess the effect of the expression of the mutant channel subunit on the current magnitude, we measured the current at +40 mV after 4-s depolarization from −80 mV (I_{mut}). To determine the voltage dependence of channel activation, we measured the I_{Ks} tail current at −40 mV after depolarization to a series of voltage steps from −50 to +80 mV every 10 mV. A Boltzmann fit ($G = G_{max}/(1 + \exp[-(V - V_{1/2})]/k)$ of the data was used to determine (i) the steepness or slope factor (k), (ii) the voltage that elicits half of the maximal activation ($V_{1/2}$) of activation, and (iii) the maximal conductance (G_{max}). The $V_{1/2}$ and k values indicate channel sensitivity to activation by voltage. Time constant for activation (τ activation: τ_{act}) and for deactivation (τ deactivation: τ_{deact}) were determined by fitting the activation current and tail current with a single exponential. Human I_{Ks} channels activated to +40 mV depolarizing voltage are well fit by a single exponential ($R > 0.98$ for all data points), although double exponentials improve the fit ($R > 0.99$). Nonetheless, for the purposes of this study, a single-exponential fit was sufficient to capture the increase in cardiac risk, so we used the simplest model. This is illustrated in detail in fig. S1.

HEK293T cell experiments. HEK293T cells were maintained in high-glucose (4.5 g/liter) Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 1% L-glutamax in a humidified incubator with 5% CO_2. Each mutant KCNQ1 was expressed in combination with WT-KCNQ1 to mimic heterozygous mutation. Mutant KCNQ1 plasmid was transfected with WT-KCNQ1 and KCNE1 plasmids at a ratio of 0.5 ng of mutant KCNQ1/0.5 ng of WT-KCNQ1/1 ng of KCNE1. Each mutant current was compared to the current observed from haplosufficient control channel [0.5 μg of WT-KCNQ1/1 μg of KCNE1 cotransfected with 0.5 μg of pcDNA3.1(+) vector] or WT control channel (1 μg of WT-KCNQ1/1 μg of KCNE1) from the same passage cells. Cells were also cotransfected with 0.2 μg of pEGFP-N1 for positive identification of transfected cells by fluorescence. All transfections were done by using Fugene HD transfection kit (Roche). Cells were replated on glass cover slides coated with 0.02% gelatin 24 hours after transfection using Accutase (Innovative Cell Technologies) and used for experiments 48 hours after transfection.

The composition of the extracellular solution for the I_{Ks} measurements was as follows: 145 mM NaCl, 5.4 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 10 mM Hepes, and 10 glucose (pH 7.40 adjusted by NaOH). The composition of the pipette solution was as follows: 130 mM K-aspartate, 11 mM EGTA, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM Hepes, and 5 mM K-ATP (adenosine triphosphate) (pH 7.20 adjusted by KOH). All experiments were performed at room temperature (≅22°C) unless otherwise indicated. For investigating temperature-dependent changes in channel functions, extracellular solution was heated up to 37°C and applied by local ejection using In-line Solution Heater (Model SH-27R, Warner Instruments) controlled by Automatic Temperature Controller (Model TC-344B, Warner Instruments).

Action potential model

The cell model is adapted from the FGM reconstruction of the canine cardiac cell (*15*). The I_{Ks} current is described by a Hodgkin-Huxley–type formulation with an infinity function (XKs_inf) and a time constant function (XKs_tau). This is a typical type of formalism in cardiac models and provides a compact and computationally efficient representation of the complex behavior of channel gating kinetics [for a review, see (*34*)]. Two critical changes were made in our model: (i) I_{Ks} current proportional to XKs_inf to the first power instead of second power in the original formulation. The change is made to match the electrophysiological data that were collected in this study and fit with XKs_inf to the first power. Hence, there is an essentially one-to-one correspondence between the experimental channel data and the modifications to the model parameters. (ii) Ito1 and Ito2 are reduced to 50 and 0%, respectively, of their original values to match published human data (*35*, *36*). These changes produce an action potential shape that more resembles human than dog. (iii) Parameters for voltage dependence of I_{Ks} conductance, activation time course, and deactivation time course were changed to matched human I_{Ks} channel data (*14*, *37*). I_{Ks} in human ventricles varies significantly in different studies and has been suggested to be strongly dependent on cell isolation techniques (*37*). We used I_{Ks} densities measured in the canine myocardium, which are consistent with the published reports in humans (*37*).

Formulation of I_{Ks} for computer model

$$I_{Ks} = G_{Ks} \times x_{Ks} \times (V - E_{Ks})$$

$$\text{xKs\_inf} = 1/(1 + \exp(-(V - 9.4)/11.8)$$

$$a_1 = 0.0000719 \times V/(1 + \exp(-0.74V))$$

$$a_2 = 0.000786 \times (V + 40)/(\exp(0.103(V + 40) - 1)$$

$$\text{xKs\_tau} = 1/(a_1 + a_2)$$

Because arrhythmias are generally triggered during exercise for LQT1 patients (*16*), we modeled a high state of β-adrenergic stimulation so

that I_{Ks} current densities were effectively twice as large as the unstimulated case suggested by experimental studies (*14*, *38*). To simulate the rise in the action potential plateau and the increased Ca^{2+} load, we made two changes to the Ca-handling mechanisms (*39*). First, the uptake of the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pumps is increased by increasing the forward rate (vmaxf) by 60% (*40*). Second, the influx via L-type channels is increased by decreasing the closure rate (gL) by 60% (*41*). The rationale is to simulate the effects of β-adrenergic stimulation that produces a gate mode with open times that are roughly twice that of the unstimulated case (*39*, *42*, *43*).

Statistics

The values of I_{mut}, τ_{act}, τ_{deact}, $V_{1/2}$, and G_{max} measured for channels expressing the mutant subunits (0.5 KCNQ1/0.5 mutant KCNQ1/1.0 KCNE1) were compared to the control mimicking the haploinsufficient phenotype (0.5 KCNQ1/1.0 KCNE1), and changes in these parameters are reported as mean ± SE. The distributions of QTc intervals measured in carriers of different mutations were not normally distributed and are expressed as median (range). Unpaired Student's *t* test was performed to compare two data sets. For multiple comparisons, one-way ANOVA followed by post hoc Tukey test or Dunnett's test was performed. Statistical significance was set as a *P* value of <0.05.

Several approaches were used to compare the characteristics of the channels formed with the mutant subunits with the clinical observations in the study population. To obtain measures of correlation that were independent of the varying population size of the carriers with each mutation, we compared the average biophysical parameters with median values of QTc and the 30-year Kaplan-Meier survival rates to first cardiac event for the carriers of each mutation using linear regression. The 30-year Kaplan-Meier limit was chosen due to the low event rate after this time point. Next, linear regression was used to test how well each channel characteristic influenced the variability of the absolute QTc measured at baseline in all carriers. Confidence intervals and *P* values were adjusted for the sample size. Only parameters that were significant in the linear regression models were included in the further analysis. Cox proportional hazards survivorship model was used to evaluate the independent contribution of the biophysical parameters compared to clinical and genetic factors to the first occurrence of time-dependent cardiac events from birth through end of follow-up at age 40 years. Because the V254M mutation was found to have radically altered parameters compared to the 16 other mutations, we treated carriers of V254M as an independent risk group. Because the slope of voltage dependence of activation curve (*k*) was largely unchanged by the mutations studied, this parameter was not included in the analyses. The remaining ion channel parameters were dichotomized at the median (50%) values according to the frequency distributions in the carriers excluding V254M carriers. To account for the influence of family membership between individuals, we fit all Cox models using a jackknife approach, the covariance sandwich estimator (*44*) that removes members of each family from the model one by one. The genotype-negative family members were not included in the Cox models. Patients who died suddenly at a young age from suspected LQTS and who did not have an ECG for QTc measurement were identified in the Cox models as "QTc missing."

Modeling

The cell model is adapted from the FGM reconstruction of the canine cardiac cell (*15*). The I_{Ks} current is described by a Hodgkin-Huxley–type formulation (see the Supplementary Material for details of current formulation). This is a typical type of formalism in cardiac models and provides as compact and computationally efficient representation of the complex behavior of channel gating kinetics (*45*). We modified the WT I_{Ks} current parameters in the model to better correspond to the electrophysiological data collected in this study. Specifically, the formulation is changed so that the current is proportional to x_{Ks}, the infinity gating variable, to the first power. Other changes were made to the time constants to make the channel properties more similar to experimental characterizations (see the Supplementary Material for details). These modifications allow the relative changes in I_{Ks} as assessed in the electrophysiology section to be mapped into model studies in a straightforward fashion (for example, a 50% increase in τ_{act} for a given mutant in the experimental study was simulated by a 50% increase in τ_{act} in the model). β-Adrenergic stimulation simulates increases in I_{Ks} current densities and the increased Ca^{2+} load observed experimentally during β-adrenergic stimulation (for details, see the Supplementary Material).

The pacing protocol consists of 40 beats at a 1000-ms interval and 30 beats at 500-ms interval to bring the model to steady state, followed by one premature beat at after 350 ms and then return to the initial rate. The premature beat produces a decreased Ca^{2+} transient that is followed by a larger Ca^{2+} transient on the subsequent beat.

SUPPLEMENTARY MATERIAL

www.sciencetranslationalmedicine.org/cgi/content/full/3/76/76ra28/DC1

Fig. S1. Determination of ion channel characteristics.

Fig. S2. Changes in ion channel parameters observed in LQT1-mutant channels expressed in mammalian system.

Fig. S3. Effect of PKA activation on channel activation kinetics in LQT1-mutant channels expressed in mammalian system.

Fig. S4. Effect of temperature on channel activation kinetics in LQT1-mutant channels expressed in mammalian cells.

Table S1. Changes in voltage dependence parameters for channels expressing LQT1-mutant subunits.

Table S2. Results from simple linear regression.

Code1: Code for Fig. 4A.

Code2: Code for Fig. 4B.

REFERENCES AND NOTES

1. Q. Wang, M. E. Curran, I. Splawski, T. C. Burn, J. M. Millholland, T. J. VanRaay, J. Shen, K. W. Timothy, G. M. Vincent, T. de Jager, P. J. Schwartz, J. A. Toubin, A. J. Moss, D. L. Atkinson, G. M. Landes, T. D. Connors, M. T. Keating, Positional cloning of a novel potassium channel gene: *KVLQT1* mutations cause cardiac arrhythmias. *Nat. Genet.* **12**, 17–23 (1996).
2. S. G. Priori, P. J. Schwartz, C. Napolitano, R. Bloise, E. Ronchetti, M. Grillo, A. Vicentini, C. Spazzolini, J. Nastoli, G. Bottelli, R. Folli, D. Cappelletti, Risk stratification in the long-QT syndrome. *N. Engl. J. Med.* **348**, 1866–1874 (2003).
3. A. J. Sauer, A. J. Moss, S. McNitt, D. R. Peterson, W. Zareba, J. L. Robinson, M. Qi, I. Goldenberg, J. B. Hobbs, M. J. Ackerman, J. Benhorin, W. J. Hall, E. S. Kaufman, E. H. Locati, C. Napolitano, S. G. Priori, P. J. Schwartz, J. A. Towbin, G. M. Vincent, L. Zhang, Long QT syndrome in adults. *J. Am. Coll. Cardiol.* **49**, 329–337 (2007).
4. J. B. Hobbs, D. R. Peterson, A. J. Moss, S. McNitt, W. Zareba, I. Goldenberg, M. Qi, J. L. Robinson, A. J. Sauer, M. J. Ackerman, J. Benhorin, E. S. Kaufman, E. H. Locati, C. Napolitano, S. G. Priori, J. A. Towbin, G. M. Vincent, L. Zhang, Risk of aborted cardiac arrest or sudden cardiac death during adolescence in the long-QT syndrome. *JAMA* **296**, 1249–1254 (2006).
5. I. Goldenberg, A. J. Moss, D. R. Peterson, S. McNitt, W. Zareba, M. L. Andrews, J. L. Robinson, E. H. Locati, M. J. Ackerman, J. Benhorin, E. S. Kaufman, C. Napolitano, S. G. Priori, M. Qi, P. J. Schwartz, J. A. Towbin, G. M. Vincent, L. Zhang, Risk factors for aborted cardiac arrest and sudden cardiac death in children with the congenital long-QT syndrome. *Circulation* **117**, 2184–2191 (2008).

6. A. J. Moss, W. Shimizu, A. A. Wilde, J. A. Towbin, W. Zareba, J. L. Robinson, M. Qi, G. M. Vincent, M. J. Ackerman, E. S. Kaufman, N. Hofman, R. Seth, S. Kamakura, Y. Miyamoto, I. Goldenberg, M. L. Andrews, S. McNitt, Clinical aspects of type-1 long-QT syndrome by location, coding type, and biophysical function of mutations involving the KCNQ1 gene. *Circulation* **115**, 2481–2489 (2007).
7. L. Bianchi, S. G. Priori, C. Napolitano, K. A. Surewicz, A. T. Dennis, M. Memmi, P. J. Schwartz, A. M. Brown, Mechanisms of I_{Ks} suppression in LQT1 mutants. *Am. J. Physiol. Heart Circ. Physiol.* **279**, H3003–H3011 (2000).
8. A. Murray, F. Potet, C. Bellocq, I. Baró, W. Reardon, H. E. Hughes, S. Jeffery, Mutation in *KCNQ1* that has both recessive and dominant characteristics. *J. Med. Genet.* **39**, 681–685 (2002).
9. Z. Wang, M. Tristani-Firouzi, Q. Xu, M. Lin, M. T. Keating, M. C. Sanguinetti, Functional effects of mutations in KvLQT1 that cause long QT syndrome. *J. Cardiovasc. Electrophysiol.* **10**, 817–826 (1999).
10. C. Jons, A. J. Moss, C. M. Lopes, S. McNitt, W. Zareba, I. Goldenberg, M. Qi, A. A. Wilde, W. Shimizu, J. K. Kanters, J. A. Towbin, M. J. Ackerman, J. L. Robinson, Mutations in conserved amino acids in the KCNQ1 channel and risk of cardiac events in type-1 long-QT syndrome. *J. Cardiovasc. Electrophysiol.* **20**, 859–865 (2009).
11. J. A. Capra, M. Singh, Predicting functionally important residues from sequence conservation. *Bioinformatics* **23**, 1875–1882 (2007).
12. W. S. Valdar, J. M. Thornton, Conservation helps to identify biologically relevant crystal contacts. *J. Mol. Biol.* **313**, 399–416 (2001).
13. W. Zareba, A. J. Moss, P. J. Schwartz, G. M. Vincent, J. L. Robinson, S. G. Priori, J. Benhorin, E. H. Locati, J. A. Towbin, M. T. Keating, M. H. Lehmann, W. J. Hall, Influence of genotype on the clinical course of the long-QT syndrome. International Long-QT Syndrome Registry Research Group. *N. Engl. J. Med.* **339**, 960–965 (1998).
14. J. P. Imredy, J. R. Penniman, S. J. Dech, W. D. Irving, J. J. Salata, Modeling of the adrenergic response of the human I_{Ks} current (hKCNQ1/hKCNE1) stably expressed in HEK-293 cells. *Am. J. Physiol. Heart Circ. Physiol.* **295**, H1867–H1881 (2008).
15. S. N. Flaim, W. R. Giles, A. D. McCulloch, Arrhythmogenic consequences of Na^+ channel mutations in the transmurally heterogeneous mammalian left ventricle: Analysis of the I1768V *SCN5A* mutation. *Heart Rhythm* **4**, 768–778 (2007).
16. P. J. Schwartz, S. G. Priori, C. Spazzolini, A. J. Moss, G. M. Vincent, C. Napolitano, I. Denjoy, P. Guicheney, G. Breithardt, M. T. Keating, J. A. Towbin, A. H. Beggs, P. Brink, A. A. Wilde, L. Toivonen, W. Zareba, J. L. Robinson, K. W. Timothy, V. Corfield, D. Wattanasirichaigoon, C. Corbett, W. Haverkamp, E. Schulze-Bahr, M. H. Lehmann, K. Schwartz, P. Coumel, R. Bloise, Genotype-phenotype correlation in the long-QT syndrome: Gene-specific triggers for life-threatening arrhythmias. *Circulation* **103**, 89–95 (2001).
17. D. J. Gallacher, A. Van de Water, H. van der Linde, A. N. Hermans, H. R. Lu, R. Towart, P. G. Volders, In vivo mechanisms precipitating torsades de pointes in a canine model of drug-induced long-QT1 syndrome. *Cardiovasc. Res.* **76**, 247–256 (2007).
18. P. A. Brink, L. Crotti, V. Corfield, A. Goosen, G. Durrheim, P. Hedley, M. Heradien, G. Geldenhuys, E. Vanoli, S. Bacchini, C. Spazzolini, A. L. Lundquist, D. M. Roden, A. L. George Jr., P. J. Schwartz, Phenotypic variability and unusual clinical severity of congenital long-QT syndrome in a founder population. *Circulation* **112**, 2602–2610 (2005).
19. L. Franqueza, M. Lin, J. Shen, I. Splawski, M. T. Keating, M. C. Sanguinetti, Long QT syndrome-associated mutations in the S4-S5 linker of KvLQT1 potassium channels modify gating and interaction with minK subunits. *J. Biol. Chem.* **274**, 21063–21070 (1999).
20. M. C. Sanguinetti, Q. P. Xu, Mutations of the S4-S5 linker alter activation properties of HERG potassium channels expressed in *Xenopus* oocytes. *J. Physiol.* **514**, 667–675 (1999).
21. M. Tristani-Firouzi, J. Chen, M. C. Sanguinetti, Interactions between S4-S5 linker and S6 transmembrane domain modulate gating of HERG K^+ channels. *J. Biol. Chem.* **277**, 18994–19000 (2002).
22. M. J. Heradien, A. Goosen, L. Crotti, G. Durrheim, V. Corfield, P. A. Brink, P. J. Schwartz, Does pregnancy increase cardiac risk for LQT1 patients with the KCNQ1-A341V mutation? *J. Am. Coll. Cardiol.* **48**, 1410–1415 (2006).
23. L. Crotti, C. Spazzolini, P. J. Schwartz, W. Shimizu, I. Denjoy, E. Schulze-Bahr, E. V. Zaklyazminskaya, H. Swan, M. J. Ackerman, A. J. Moss, A. A. Wilde, M. Horie, P. A. Brink, R. Insolia, G. M. De Ferrari, G. Crimi, The common long-QT syndrome mutation KCNQ1/A341V causes unusually severe clinical manifestations in patients with different ethnic backgrounds: Toward a mutation-specific risk stratification. *Circulation* **116**, 2366–2375 (2007).
24. Y. Aizawa, K. Ueda, F. Scornik, J. M. Cordeiro, Y. Wu, M. Desai, A. Guerchicoff, Y. Nagata, Y. Iesaka, A. Kimura, M. Hiraoka, C. Antzelevitch, A novel mutation in *KCNQ1* associated with a potent dominant negative effect as the basis for the LQT1 form of the long QT syndrome. *J. Cardiovasc. Electrophysiol.* **18**, 972–977 (2007).
25. L. Arbour, S. Rezazadeh, J. Eldstrom, G. Weget-Simms, R. Rupps, Z. Dyer, G. Tibbits, E. Accili, B. Casey, A. Kmetic, S. Sanatani, D. Fedida, A KCNQ1 V205M missense mutation causes a high rate of long QT syndrome in a First Nations community of northern British Columbia: A community-based approach to understanding the impact. *Genet. Med.* **10**, 545–550 (2008).
26. L. Restier, L. Cheng, M. C. Sanguinetti, Mechanisms by which atrial fibrillation-associated mutations in the S1 domain of KCNQ1 slow deactivation of I_{Ks} channels. *J. Physiol.* **586**, 4179–4191 (2008).
27. C. Terrenoire, C. E. Clancy, J. W. Cormier, K. J. Sampson, R. S. Kass, Autonomic control of cardiac action potentials: Role of potassium channel kinetics in response to sympathetic stimulation. *Circ. Res.* **96**, e25–e34 (2005).
28. S. G. Priori, C. Napolitano, P. J. Schwartz, M. Grillo, R. Bloise, E. Ronchetti, C. Moncalvo, C. Tulipani, A. Veia, G. Bottelli, J. Nastoli, Association of long QT syndrome loci and cardiac events among patients treated with β-blockers. *JAMA* **292**, 1341–1344 (2004).
29. A. J. Moss, Long QT syndrome. *JAMA* **289**, 2041–2044 (2003).
30. W. Zareba, A. J. Moss, G. Sheu, E. S. Kaufman, S. Priori, G. M. Vincent, J. A. Towbin, J. Benhorin, P. J. Schwartz, C. Napolitano, W. J. Hall, M. T. Keating, M. Qi, J. L. Robinson, M. L. Andrews; International LQTS Registry, University of Rochester, Rochester, New York, Location of mutation in the KCNQ1 and phenotypic presentation of long QT syndrome. *J. Cardiovasc. Electrophysiol.* **14**, 1149–1153 (2003).
31. R. E. Lane, M. R. Cowie, A. W. Chow, Prediction and prevention of sudden cardiac death in heart failure. *Heart* **91**, 674–680 (2005).
32. A. Matavel, C. M. Lopes, PKC activation and PIP_2 depletion underlie biphasic regulation of IKs by Gq-coupled receptors. *J. Mol. Cell. Cardiol.* **46**, 704–712 (2009).
33. A. Matavel, E. Medei, C. M. Lopes, PKA and PKC partially rescue long QT type 1 phenotype by restoring channel-PIP_2 interactions. *Channels* **4**, 3–11 (2010).
34. M. Reumann, V. Gurev, J. J. Rice, Computational modeling of cardiac disease: Potential for personalized medicine. *Personalized Med.* **6**, 45–66 (2009).
35. E. Wettwer, G. Amos, J. Gath, H. R. Zerkowski, J. C. Reidemeister, U. Ravens, Transient outward current in human and rat ventricular myocytes. *Cardiovasc. Res.* **27**, 1662–1669 (1993).
36. G. R. Li, J. Feng, Z. Wang, B. Fermini, S. Nattel, Comparative mechanisms of 4-aminopyridine-resistant Ito in human and rabbit atrial myocytes. *Am. J. Physiol.* **269**, H463–H472 (1995).
37. G. R. Li, J. Feng, L. Yue, M. Carrier, S. Nattel, Evidence for two components of delayed rectifier K^+ current in human ventricular myocytes. *Circ. Res.* **78**, 689–696 (1996).
38. K. B. Walsh, R. S. Kass, Regulation of a heart potassium channel by protein kinase A and C. *Science* **242**, 67–69 (1988).
39. I. Findlay, S. Suzuki, S. Murakami, Y. Kurachi, Physiological modulation of voltage-dependent inactivation in the cardiac muscle L-type calcium channel: A modelling study. *Prog. Biophys. Mol. Biol.* **96**, 482–498 (2008).
40. S. Morimoto, J. O-Uchi, M. Kawai, T. Hoshina, Y. Kusakari, K. Komukai, H. Sasaki, K. Hongo, S. Kurihara, Protein kinase A-dependent phosphorylation of ryanodine receptors increases Ca^{2+} leak in mouse heart. *Biochem. Biophys. Res. Commun.* **390**, 87–92 (2009).
41. D. T. Yue, S. Herzig, E. Marban, β-Adrenergic stimulation of calcium channels occurs by potentiation of high-activity gating modes. *Proc. Natl. Acad. Sci. U.S.A.* **87**, 753–757 (1990).
42. I. Findlay, β-Adrenergic stimulation modulates Ca^{2+}- and voltage-dependent inactivation of L-type Ca^{2+} channel currents in guinea-pig ventricular myocytes. *J. Physiol.* **541**, 741–751 (2002).
43. I. Findlay, Physiological modulation of inactivation in L-type Ca^{2+} channels: One switch. *J. Physiol.* **554**, 275–283 (2004).
44. D. Y. Lin, L. J. Wei, The robust inference for the Cox proportional hazards model. *J. Am. Stat. Assoc.* **84**, 1074–1078 (1989).
45. M. Reumann, B. G. Fitch, A. Rayshubskiy, D. U. Keller, G. Seemann, O. Dossel, M. C. Pitman, J. J. Rice, Orthogonal recursive bisection data decomposition for high performance computing in cardiac model simulations: Dependence on anatomical geometry. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* **2009**, 2799–2802 (2009).
46. P. Ridefelt, K. Yokote, L. Claesson-Welsh, A. Siegbahn, PDGF-BB triggered cytoplasmic calcium responses in cells with endogenous or stably transfected PDGF β-receptors. *Growth Factors* **12**, 191–201 (1995).
47. R. Jongbloed, C. Marcelis, C. Velter, P. Doevendans, J. Geraedts, H. Smeets, DHPLC analysis of potassium ion channel genes in congenital long QT syndrome. *Hum. Mutat.* **20**, 382–391 (2002).
48. R. J. Jongbloed, A. A. Wilde, J. L. Geelen, P. Doevendans, C. Schaap, I. Van Langen, J. P. van Tintelen, J. M. Cobben, G. C. Beaufort-Krol, J. P. Geraedts, H. J. Smeets, Novel KCNQ1 and HERG missense mutations in Dutch long-QT families. *Hum. Mutat.* **13**, 301–310 (1999).
49. O. S. Baker, H. P. Larsson, L. M. Mannuzzu, E. Y. Isacoff, Three transmembrane conformations and sequence-dependent displacement of the S4 domain in shaker K^+ channel gating. *Neuron* **20**, 1283–1294 (1998).
50. Y. H. Chen, S. J. Xu, S. Bendahhou, X. L. Wang, Y. Wang, W. Y. Xu, H. W. Jin, H. Sun, X. Y. Su, Q. N. Zhuang, Y. Q. Yang, Y. B. Li, Y. Liu, H. J. Xu, X. F. Li, N. Ma, C. P. Mou, Z. Chen, J. Barhanin, W. Huang, KCNQ1 gain-of-function mutation in familial atrial fibrillation. *Science* **299**, 251–254 (2003).
51. N. Neyroud, I. Denjoy, C. Donger, F. Gary, E. Villain, A. Leenhardt, K. Benali, K. Schwartz, P. Coumel, P. Guicheney, Heterozygous mutation in the pore of potassium channel gene KvLQT1 causes an apparently normal phenotype in long QT syndrome. *Eur. J. Hum. Genet.* **6**, 129–133 (1998).
52. R. Du, J. G. Yang, W. Li, L. Gui, G. H. Yuan, C. L. Kang, F. X. Ren, S. Y. Zhang, Mutation analysis of a Chinese family with inherited long QT syndrome. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* **22**, 68–70 (2005).

53. A. Kobori, N. Sarai, W. Shimizu, Y. Nakamura, Y. Murakami, T. Makiyama, S. Ohno, K. Takenaka, T. Ninomiya, Y. Fujiwara, S. Matsuoka, M. Takano, A. Noma, T. Kita, M. Horie, Additional gene variants reduce effectiveness of β-blockers in the LQT1 form of long QT syndrome. *J. Cardiovasc. Electrophysiol.* **15**, 190–199 (2004).
54. A. Anastasakis, C. M. Kotta, S. Kyriakogonas, B. Wollnik, A. Theopistou, C. Stefanadis, Phenotype reveals genotype in a Greek long QT syndrome family. *Europace* **8**, 241–244 (2006).
55. D. J. Tester, L. B. Cronk, J. L. Carr, V. Schulz, B. A. Salisbury, R. S. Judson, M. J. Ackerman, Allelic dropout in long QT syndrome genetic testing: A possible mechanism underlying false-negative results. *Heart Rhythm* **3**, 815–821 (2006).
56. M. Grunnet, E. R. Behr, K. Calloe, J. Hofman-Bang, J. Till, M. Christiansen, W. J. McKenna, S. P. Olesen, N. Schmitt, Functional assessment of compound mutations in the *KCNQ1* and *KCNH2* genes associated with long QT syndrome. *Heart Rhythm* **2**, 1238–1249 (2005).
57. M. Yamaguchi, M. Shimizu, H. Ino, H. Terai, K. Hayashi, T. Kaneda, H. Mabuchi, R. Sumita, T. Oshima, N. Hoshi, H. Higashida, Compound heterozygosity for mutations Asp611→Tyr in *KCNQ1* and Asp609→Gly in *KCNH2* associated with severe long QT syndrome. *Clin. Sci.* **108**, 143–150 (2005).
58. **Acknowledgments:** We thank J. Sorenson for her technical support. **Funding:** This work was supported by NIH R01(HL033843) and Mirowski-Moss Career Development Award at the University of Rochester Medical Center, Rochester (to C.J.); AHA Postdoctoral Fellowship (09POST2310079) (J.O.-U.); and Foreign Study Grant Award of Kanae Foundation (J.O.-U.). **Author contributions:** C.J. and J.O.-U. performed the experiments and data analysis and wrote the paper; C.M.B.L. guided the design of experiments and analysis; W.Z., A.A.M.W., W.S., J.K.K., and N.H. provided clinical data and contributed to analysis interpretation; A.J.M., I.G., S.M., and J.L.R. performed clinical data analysis and contributed to analysis interpretation; M.R. and J.J.R. performed computer simulation. **Competing interests:** A.A.M.W. is a member of the Advisory Board of PGxHealth. I.G. has been a paid consultant for Boston Scientific. The other authors declare that they have no competing interests.

Submitted 2 August 2010
Accepted 11 March 2011
Published 30 March 2011
10.1126/scitranslmed.3001551

Citation: C. Jons, J. O-Uchi, A. J. Moss, M. Reumann, J. J. Rice, I. Goldenberg, W. Zareba, A. A. M. Wilde, W. Shimizu, J. K. Kanters, S. McNitt, N. Hofman, J. L. Robinson, C. M. B. Lopes, Use of mutant-specific ion channel characteristics for risk stratification of long QT syndrome patients. *Sci. Transl. Med.* **3**, 76ra28 (2011).

Mutation and gender-specific risk in type 2 long QT syndrome: Implications for risk stratification for life-threatening cardiac events in patients with long QT syndrome

Dimitry Migdalovich, BS,* Arthur J. Moss, MD,* Coeli M. Lopes, PhD,† Jason Costa, MA,* Gregory Ouellet, MA,* Alon Barsheshet, MD,* Scott McNitt, MS,* Slava Polonsky, MS,* Jennifer L. Robinson, MS,* Wojciech Zareba, MD, PhD,* Michael J. Ackerman, MD, PhD,‡ Jesaia Benhorin, MD,§ Elizabeth S. Kaufman, MD,□ Pyotr G. Platonov, MD,¶ Wataru Shimizu, MD, PhD,# Jeffrey A. Towbin, MD,** G. Michael Vincent, MD,†† Arthur A.M. Wilde, MD, PhD,‡‡ Ilan Goldenberg, MD*

*From the *Cardiology Division, University of Rochester Medical Center, Rochester, New York; †Cardiovascular Research Institute, University of Rochester School of Medicine and Dentistry, Rochester, New York; ‡Department of Pediatrics, Division of Pediatric Cardiology, Mayo Clinic, Rochester, Minnesota; §Department of Cardiology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel; □Heart and Vascular Research Center, MetroHealth Campus of Case Western Reserve University, Cleveland, Ohio; ¶Department of Cardiology, Lund University, Lund, Sweden; #Division of Cardiology, Department of Internal Medicine National Cardiovascular Center, Suita, Japan; **Department of Pediatrics, University of Cincinnati Children's Hospital, Cincinnati, Ohio; ††LDS Hospital, Salt Lake City, Utah; and ‡‡Department of Cardiology Academic Medical Center, Amsterdam, The Netherlands.*

BACKGROUND Men and women with type 2 long QT syndrome (LQT2) exhibit time-dependent differences in the risk for cardiac events. We hypothesized that data regarding the location of the disease-causing mutation in the *KCNH2* channel may affect gender-specific risk in LQT2.

OBJECTIVE This study sought to risk-stratify LQT2 patients for life-threatening cardiac events based on clinical and genetic information.

METHODS The risk for life-threatening cardiac events from birth through age 40 years (comprising aborted cardiac arrest [ACA] or sudden cardiac death [SCD]) was assessed among 1,166 LQT2 male (n = 490) and female (n = 676) patients by the location of the LQTS-causing mutation in the *KCNH2* channel (prespecified in the primary analysis as pore-loop vs. non-pore-loop).

RESULTS During follow-up, the cumulative probability of life-threatening cardiac events years was significantly higher among LQT2 women (26%) as compared with men (14%; $P < .001$). Multivariate analysis showed that the risk for life-threatening cardiac events was not significantly different between women with and without pore-loop mutations (hazard ratio 1.20; $P = .33$). In contrast, men with pore-loop mutations displayed a significant >2-fold higher risk of a first ACA or SCD as compared with those with non-pore-loop mutations (hazard ratio 2.18; $P = .01$). Consistently, women experienced a high rate of life-threatening events regardless of mutation location (pore-loop: 35%, non-pore-loop: 23%), whereas in men the rate of ACA or SCD was high among those with pore-loop mutations (28%) and relatively low among those with non-pore-loop mutations (8%).

CONCLUSION Combined assessment of clinical and mutation-specific data can be used for improved risk stratification for life-threatening cardiac events in LQT2.

KEYWORDS Long QT syndrome; Pore-loop mutations; Sudden cardiac death; Gender

ABBREVIATIONS ACA = aborted cardiac arrest; **ECG** = electrocardiogram; **ICD** = implantable cardioverter-defibrillator; **LQTS** = long QT syndrome; **LQT2** = long QT syndrome type 2; **QTc** = corrected QT; **SCD** = sudden cardiac death

(Heart Rhythm 2011;8:1537–1543)

Conflict of interests/disclosure: Dr. Ackerman is a consultant for Biotronik, Boston Scientific, Medtronic, PGx Health, and St. Jude Medical; and has intellectual property in PGx Health. Dr. Kaufman receives grant support from CardioDx, Cambridge Heart Inc., and St. Jude Medical. All other authors have reported that they have no relationships to disclose. This work was supported by research grants HL-33843 and HL-51618 from the National Institutes of Health and by a research grant from GeneDx to the Heart Research Follow-Up Program in support of the LQTS Registry. **Address reprint requests and correspondence:** Dr. Ilan Goldenberg, Heart Research Follow-Up Program, Box 653, University of Rochester Medical Center, Rochester, NY 14642. E-mail address: Ilan.Goldenberg@heart.rochester.edu. (Received February 9, 2011; accepted March 20, 2011.)

1547-5271/$ -see front matter doi:10.1016/j.hrthm.2011.03.049

Introduction

Long QT syndrome (LQTS) is an inherited arrhythmogenic disorder caused by mutations in several cardiac ion channel genes.[1] Clinically, LQTS is identified by abnormal QT interval prolongation on the electrocardiogram (ECG) and is associated with arrhythmogenic syncope and sudden arrhythmic death (SCD).[1,2] Type 2 long QT (LQT2), the second most common variant of LQTS, is characterized by mutations in the α subunit of the *KCNH2* channel, which conducts the rapid delayed rectifier potassium current (I_{Kr}) in cardiac myocytes.[1,2–4] Recent data show that mutations in the *KCNH2* pore-loop region, which is responsible for forming the ion conduction pathway of the channel, are associated with a significantly higher risk of cardiac events as compared with mutations that are located in other regions of the channel.[5,6] Furthermore, the clinical course of LQT2 patients was shown to be associated with major time-dependent gender differences, wherein women display a significantly higher risk for cardiac events than men after the onset of adolescence.[7] Prior studies in LQT2 patients, however, evaluated mainly the combined end point of any cardiac event during follow-up (comprising mostly nonfatal syncopal episodes) and did not relate gender-specific risk to mutation location in this population. Accordingly, the present study was carried out in a population of 1,166 genetically confirmed LQT2 patients from Multinational LQTS Registries and was designed to: (1) evaluate time-dependent gender differences in the risk of life-threatening cardiac events (comprising aborted cardiac arrest [ACA] or SCD) in LQT2 patients; (2) relate gender-specific risk for life-threatening events in this population to the location of the LQT2-causing mutation in the *KCNH2* channel; and (3) develop a risk stratification scheme among LQT2 patients that combines clinical and mutation-specific data.

Methods

Study population

The study population was composed of 1,166 subjects derived from (n = 263) proband-identified families with genetically confirmed *KCNH2* mutations. Patients were drawn from the Rochester, New York, enrolling center (center 1) of the International LQTS Registry (n = 761), the Netherlands LQTS Registry (n = 214), and the Japanese LQTS Registry (n = 95), as well as from data submitted by other investigators specifically for this collaborative mutation analysis project: Denmark (n = 62), Israel (n = 24), and Sweden (n = 10). The proband in each family had otherwise unexplained diagnostic QTc prolongation or experienced LQTS-related symptoms. Patients were excluded from the study if they had >1 LQTS-causing mutation (n = 11).

Data collection and management

For each patient, personal history including cardiac events, ECGs, and therapies, as well as family history, were obtained at enrollment. Clinical data were then collected yearly on prospectively designed forms with information on demographic characteristics, personal and family medical history, ECG findings, medical therapies, left cardiac sympathetic denervation, implantation of a pacemaker or an implantable cardioverter-defibrillator (ICD), and the occurrence of LQTS-related cardic events. The QT interval was corrected for heart rate using the Bazett formula.[8] Data common to all LQTS registries involving genetically tested individuals were electronically merged into a common database for the present study.

Genotype characterization

KCNH2 mutations were identified with the use of standard genetic tests performed in academic molecular genetic research laboratories and/or in commercial laboratories. Genetic alterations of the amino acid sequence were characterized by location in the channel protein and by the type of mutation (missense, splice site, in-frame insertions/deletions, nonsense [stop codon], and frameshift).[9] The transmembrane region of the *KCNH2* encoded protein was defined as the coding sequence involving amino acid residues from 404 through 659 (pore-loop region: 548-659), with the N-terminus region defined before residue 404, and the C-terminus region after residue 659.

Pore-loop mutations disrupt normal channel gating[10] and were shown to be associated with a significantly higher risk of cardiac events as compared with mutations in each of the other regions of the *KCNH2* channel.[5,6] Accordingly, mutation location was categorized in the primary analysis of the present study as pore-loop vs. non–pore-loop. In a secondary analysis, non–pore-loop mutations were further subcategorized into those located in the transmembrane (non–pore-loop) region and in the C/N-terminus regions. Mutation type was categorized as missense vs. nonmissense. The specific mutations included in the present study, by location, type, and number of patients, are detailed in Supplementary Table 1. The distribution of study mutations in the *KCNH2* channel, by the relative number of patients, is shown in Figure 1.

End point

The primary end point of the study was the occurrence of a first life-threatening cardiac event, comprising ACA (requiring defibrillation as part of resuscitation), or LQTS-related SCD (abrupt in onset without evident cause, if witnessed, or death that was not explained by any other cause if it occurred in a nonwitnessed setting such as sleep). To further validate the consistency of the results among patients who received an ICD during follow-up, we also assessed a secondary end point comprising the first occurrence of ACA, SCD, or appropriate ICD shock during follow-up.

Statistical analysis

The baseline and follow-up clinical characteristics of the study population were evaluated using the χ^2 test for categorical variables, and the t test and the Mann-Whitney-Wilcoxon test for continuous variables. The cumulative probability of a first ACA or SCD by gender and by muta-

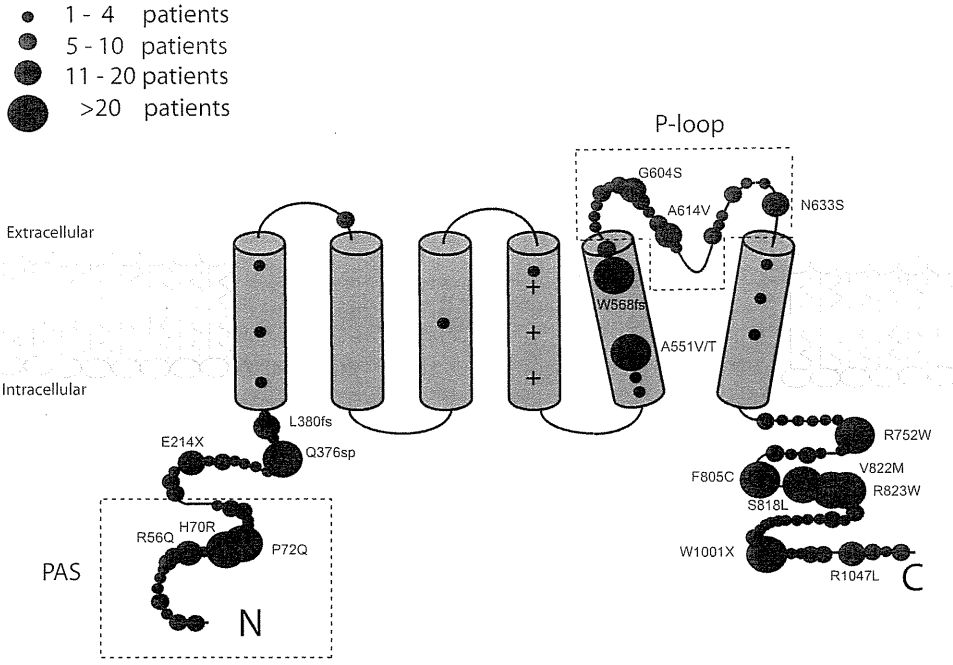

Figure 1 Distribution of mutations in the *KCNH2* potassium channel among study patients.

tion location was assessed by the Kaplan-Meier method, and significance was tested by the log-rank test. Follow-up data were censored at age 40 to avoid confounding by acquired cardiovascular disease. Multivariate Cox proportional hazards regression models were used to evaluate the independent contribution of clinical and genetic factors to the first occurrence of ACA or SCD. Prespecified covariates in the total population model included gender, QTc duration (categorized as ≥500 ms vs. <500 ms), mutation location and type (as defined above), the occurrence of syncope during follow-up, and medical therapy with blockers. Syncope and β-blocker therapy were assessed as time-dependent covariates in the multivariate models. The effect of each covariate in male and female subjects was assessed by interaction-term analysis (i.e., by including a gender-by-risk factor interaction term in the multivariate models), with interactions tested one at a time. To avoid violation of the proportional hazards assumption due to gender-risk crossover during adolescence, we used an age-gender interaction term in the multivariate models. Patients without available baseline QTc data (n = 150) were included as a separate (QTc-missing) covariate in the multivariate models.

Using the Cox model that included interactions among gender, mutation location, QTc duration, and time-dependent syncope, covariate patterns with similar estimated hazard ratios were united to form time-dependent risk groups.

Because almost all the subjects were first- and second-degree relatives of probands, the effect of lack of independence between subjects was evaluated in the Cox model with grouped jackknife estimates for family membership.[11] All grouped jackknife standard errors for the covariate risk factors fell within 3% of those obtained from the unadjusted Cox model, and therefore only the Cox model findings are reported. The statistical software used for the analyses was SAS version 9.20 (SAS Institute Inc, Cary, North Carolina). A 2-sided 0.05 significance level was used for hypothesis testing.

Results

The clinical characteristics of the study patients by gender are shown in Table 1. Baseline QTc was somewhat higher among women; however, the frequency of patients with prolonged QTc (≥500 ms) was similar in men and women. In addition, the frequency of patients with pore-loop mutations was the same in the 2 groups. During follow-up, there was no statistically significant difference between men and women in the frequency of medical therapy with β-blockers, whereas the frequency of device therapy (including pacemakers and ICDs) was significantly higher among women. The frequency of both nonfatal syncopal episodes and life-threatening cardiac events during follow-up was significantly higher among women as compared with men (Table 1).

Risk factors for ACA or SCD in the total LQT2 population

During follow-up, 179 (15%) study patients experienced the primary end point of a first ACA or SCD. Event rates were similar between men and women during childhood, whereas after onset of adolescence and during adulthood, LQT2 women experienced a significantly higher rate of ACA or SCD as compared with LQT2 men (Fig. 2). Accordingly, the cumulative probability of a first ACA or SCD from birth through age 40 years was significantly higher in women (26%) as compared with men (14%; $P < .001$ [Fig. 2]).

Table 1 Baseline and follow-up characteristics of the study population by gender

Characteristics	Male N = 490	Female N = 676	*P* value
QTc (ms)			
Continuous, means ± SD	478 ± 57	484 ± 52	.02
≥500, %	32	34	.44
RR (s), means ± SD	860 ± 250	856 ± 216	.91
Location of mutation			
Pore-loop, %	28	28	.93
Non-pore-loop:			
TM, %	4	4	.98
N-term/C-term, %	35	34	.98
Type of mutation			
Missense, %	65	68	.33
Nonmissense, %	35	32	
LQTS therapies			
β-blockers, %	52	55	.22
Pacemaker, %	3	6	.02
LSCD, %	0.6	2	.12
ICD, %	8	16	<.001
Cardiac events during follow-up			
Syncope, %	24	46	<.001
ACA, %	3	9	<.001
SCD, %	8	12	.02
Appropriate ICD shocks, %	1.5	1.9	.58
First SCD or ACA, %*	10	19	<.001

ACA = aborted cardiac arrest; ICD = implantable cardioverter-defibrillator; LCSD = left cervical sympathetic denervation; LQTS = long QT syndrome; SCD = sudden cardiac death; TM = transmembrane; QTc = corrected QT; RR = relative risk.
*Only the first event for each patient was considered.

Multivariate analysis in the total study population (Table 2) showed that during childhood (ages 0 to 13 years), the risk of ACA or SCD was similar between women and men (hazard ratio [HR] 1.53; $P = .33$), whereas after the onset of adolescence (age >13 years), women showed a significantly higher risk for ACA or SCD as compared with men (HR 2.23; $P < .001$). Mutations located in the pore-loop region of the *KCNH2* channel were shown to be associated with a significant 39% ($P = .04$) increase in the risk for ACA or SCD as compared with other ion channel mutations (Table 2). Results were similar when the secondary end point of a first ACA, SCD, or appropriate ICD shock was assessed.

Table 2 Multivariate analysis: risk factors for ACA/SCD among all LQT2 patients*

Risk factor	Relative risk		
	Hazard ratio	95% confidence interval	*P* value
Gender: female vs. male			
Age group: 0 to 13 years	1.53	0.72–3.26	.33
Age group: 14 to 40 years	2.23	1.55–3.21	<.001
Mutation location			
Pore-loop vs. non-pore-loop	1.39	1.02–1.91	.04
Pore-loop vs. C/N-term	1.44	1.06–1.97	.02
TM (nonpore) vs. C/N-term	0.91	0.45–1.87	.80
Mutation type			
Missense vs. nonmissense	0.87	0.62–1.23	.43
QTc duration (ms)			
≥500 vs. <500	3.24	2.05–5.12	<.001
Time-dependent syncope			
Syncope vs. no syncope	3.15	2.26–4.38	<.001

Abbreviations as in Table 1.
*Models were further adjusted for missing QTc values, time-dependent β-blocker therapy, and the occurrence of syncope prior to the end point (assessed as a time-dependent covariate).

Gender-specific risk factors for life-threatening cardiac events in LQT2 patients

Kaplan-Meier survival analysis showed that the cumulative probablity of ACA or SCD by age 40 years was high in women with or without pore-loop mutations (35% and 23%, respectively; $P = .02$ [Fig. 3]). In contrast, in men the rate of ACA or SCD was high among those with pore-loop mutations (28%) and relatively low among non–pore-loop mutations carriers (8%; $P < .001$ [Fig. 4]). Consistent with these findings, gender-specific multivariate analysis (Table 3) showed that the risk for ACA or SCD was not significantly different among women with or without pore-loop mutations (HR 1.20; $P = .33$), whereas men with pore-loop mutations showed a significantly higher risk for ACA or SCD as compared with

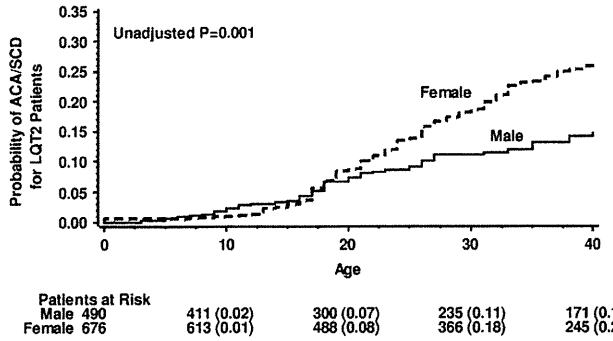

Figure 2 Kaplan-Meier estimates of the cumulative probability of aborted cardiac arrest or sudden cardiac death in LQT2 patients by gender. ACA = aborted cardiac arrest; LQT2 = long QT syndrome type 2; SCD = sudden cardiac death.

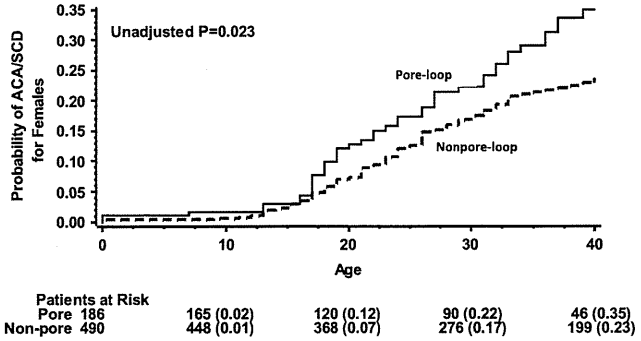

Figure 3 Kaplan-Meier estimates of the cumulative probability of aborted cardiac arrest or sudden cardiac death in LQT2 women by mutation location. ACA = aborted cardiac arrest; LQT2 = long QT syndrome type 2; SCD = sudden cardiac death.

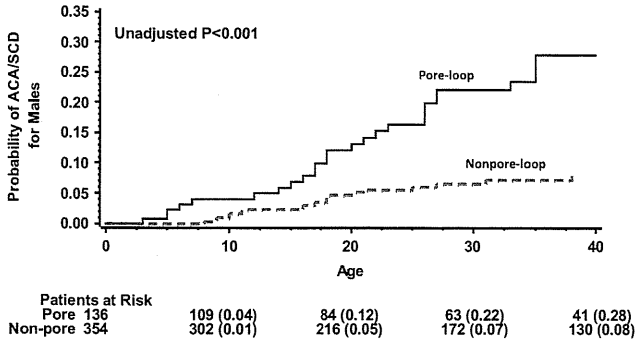

Figure 4 Kaplan-Meier estimates of the cumulative probability of a first aborted cardiac arrest or sudden cardiac death in LQT2 men by mutation location. ACA = aborted cardiac arrest; LQT2 = long QT syndrome type 2; SCD = sudden cardiac death.

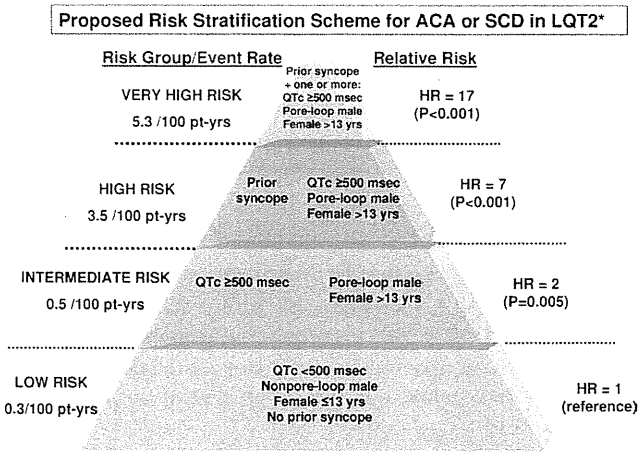

Figure 5 Proposed scheme for risk stratification for the end point of ACA or SCD in LQT2 patients by gender, mutation location, QTc, and a history of prior syncope. *Hazard ratios and score estimates were obtained from a multivariate Cox model that included interactions among the identified risk factors (categorized by QTc duration, time-dependent syncope, gender, and mutation location); decimal points in HRs are rounded to the nearest whole number; event rates per 100 person-years were calculated by dividing the number of life-threatening cardiac events (comprising ACA or SCD) in each risk category by the total follow-up time in the category (with follow-up censored after the occurrence of a ACA) and multiplying the result by 100. ACA = aborted cardiac arrest; HR = hazard ratio; LQT2 = long QT syndrome type 2; SCD = sudden cardiac death; QTc = corrected QT.

men without pore-loop mutations (HR 2.18; P = .01). Results for both men and women were consistent when the reference group of non–pore-loop mutations was further subcategorized into the transmembrane (non–pore-loop) and C/N-terminus regions (Table 3).

QTc ≥500 ms was associated with >2-fold and >4-fold risk increase in men and women, respectively, whereas the mutation-type was not associated with a statistically significant risk increase (Table 3). Similarly, the occurrence of syncope during follow-up was associated with nearly a 3-fold increase in the risk of subsequent ACA or SCD in men, with a >3-fold risk increase in women (Table 3).

Time-dependent medical therapy with β-blockers was associated with a significant 61% reduction in the risk of ACA or SCD in the total study population (HR 0.39 [95% confidence interval 0.20 to 0.74]). The benefit of treatment with β-blockers was not significantly different between men and women (P value for β-blocker–by–gender interaction = 0.23).

Risk stratification for ACA or SCD in LQT2 patients

Using interaction terms among risk factors related to gender, mutation location, QTc duration, and time-dependent syncope in the time-dependent Cox models, we identified 4 risk groups with significantly different risk for the end point of ACA or SCD (Fig. 5): (1) a low-risk group, comprising LQT2 patients with no risk factors (i.e., QTc <500 ms, no prior syncope, male subjects without pore-loop mutations or female subjects ≤13 years of age); (2) an intermediate-risk

Table 3 Multivariate analysis: risk factors for ACA/SCD among LQT2 patients by gender*†

	LQT2 male subjects		LQT2 female subjects	
	Hazard ratio (95% confidence interval)	P value	Hazard ratio (95% confidence interval)	P value
Mutation location				
Pore-loop vs. non-pore-loop	2.18 (1.28–3.72)	.01	1.20 (0.83–1.74)	.33
Pore-loop vs. C/N-term	2.04 (1.15–3.61)	.01	1.18 (0.81–1.70)	.39
TM (nonpore) vs. C/N-term	NA‡		1.25 (0.60–2.58)	.56
Mutation type				
Missense vs. nonmissense	0.56 (0.29–1.06)	.08	1.29 (0.82–1.74)	.25
QTc duration (ms)				
≥500 vs. <500	2.16 (1.08–5.06)	.03	4.05 (2.33–7.04)	<.001
Time-dependent syncope				
Syncope vs. no syncope	2.83 (1.36–5.58)	.01	3.32 (2.19–4.87)	<.001

Abbreviations as in Table 1.

*Findings were further adjusted for missing QTc values, time-dependent β-blocker therapy, and the occurrence of syncope prior to the end point (assessed as a time-dependent covariate).

†Models were carried out in the total population using interaction-term analysis, with interactions tested one at a time; all interaction P values were >.05.

‡Hazard ratio was not computed due to a low event rate in male patients with TM mutations.

group (HR vs. low-risk group = 2.14; P =.005), including (a) male subjects with pore-loop mutations or women >13 years of age (regardless of mutation location) and no additional risk factors; and (b) patients with QTc ≥500 ms and no additional risk factors; (3) a high-risk group (HR vs. low-risk group = 7.22; P <.001), including (a) patients with prior syncope and no additional risk factors, and (b) male subjects with pore-loop mutations or female subjects >13 years of age with QTc ≥500 ms, but without prior syncope; and (4) a very-high-risk group (HR vs. low-risk group = 17.01; P <.001), comprising patients who experienced prior syncope and also had 1 or more additional risk factor (i.e., QTc ≥500 ms, male with a pore-loop mutation or female >13 years old).

The nature of time-dependent covariates precludes assessment of cumulative event rates based only on the covariate pattern at the time origin. Therefore, to obtain an estimate of event rates, we adjusted the number of events for the follow-up time in each risk group. Thus, among very-high-risk patients the rate of ACA or SCD was 5.3 per 100 patient-years; high-risk patients experienced 3.5 life-threatening cardiac events per 100 patient-years; intermediate-risk patients had an event rate of 0.5 per 100 patient-years, whereas among low-risk patients the rate of ACA or SCD was only 0.3 per 100 patient years (Fig. 5).

Discussion

The present study is the first to assess gender differences in the risk of life-threatening cardiac events in LQT2, and to relate gender-specific risk in this population to the location of the disease-causing mutation. We have shown that among patients with LQT2: (1) both men and women have a relatively low rate of ACA or SCD during childhood, whereas after the onset of adolescence and throughout adulthood women show a significantly higher rate of life-threatening events as compared with men; (2) the risk of ACA or SCD in women is high regardless of the location of the disease-causing mutation in the *KCNH2* channel, whereas pore-loop mutations identify increased risk for ACA or SCD in men; and (3) combined assessment of clinical and mutation-specific risk factors can be used for improved risk stratification for life-threatening cardiac events in patients with LQT2.

In a prior study, Zareba et al.[7] assessed age-dependent gender differences in the risk of cardiac events (comprising mostly nonfatal syncopal episodes) among 533 genotyped patients from the International LQTS Registry. The study included 209 LQT2 patients, and showed that in this population no significant gender-related differences in the risk of cardiac events were present duirng childhood, whereas in the age range of 16 through 40 years, LQT2 women had >3-fold higher risk of cardiac events as compared with men.[7] Possibly due to sample size limitations, the study did not identify a significant gender-related risk difference when the more severe end point of a first life-threatening cardiac event was assessed. The present study comprises the largest LQT2 population reported to date of 1,166 patients. We have shown that after the onset of adolescence there is a pronounced increase in the risk of ACA or SCD among LQT2 women (resulting in a cumulative event rate of 26% by age 40 years), whereas the risk of ACA or SCD among LQT2 men remains significantly lower throughout follow-up (resulting in a cumulative event rate of 14% by age 40 years). These age-gender risk differences in the clinical course of LQT2 patients may be mediated by the opposing effects of male and female sex hormones on the potassium channel. Testosterone was found to shorten the action potential duration and the QT interval through enhancement of the I_{Kr} current,[12,13] and thus may be associated with QT shortening in male subjects after childhood. In contrast, estrogen was shown to exhibit both acute and genomic effects on I_{Kr}, including reduction in channel function and prolongation of ventricular repolarization.[14,15] Thus, LQT2 women who harbor mutations impairing potassium channel activity may be specifically sensitive to estrogen activity that may result in an increase in the risk for arrhythmic events after the onset of adolesence.

Recent data from the International LQTS Registry show that the location of the mutation in the ion channel is an important determinant of arrhythmic risk in LQTS patients. In a study of 201 LQT2 subjects with a total of 44 different *KCNH2* mutations, Moss et al.[5] showed that subjects harboring pore mutations exhibited a more severe clinical course and experienced a higher frequency of cardiac events, occurring at an earlier age, than did subjects with nonpore mutations. Consistent with these findings, in a more recent study, Shimizu et al.[6] showed that mutations in the pore region were associated with a greater risk of cardiac events as compared with mutations located in other regions in the *KCNH2* channel. The pore region forms the potassium conductance pathway, and most mutations present in this region have a dominant-negative effects on I_{Kr},[10] suggesting that the pore region is critical for channel function. The findings of the present study are consistent with the previous link of high cardiac risk to pore-domain mutations, and show that the presence of pore-loop mutations was independently associated with a significant 39% increase in the risk of ACA or SCD in the total LQT2 population. Our findings, however, extend prior data and show a differential effect of mutation-related risk between LQT2 men and women. Thus, among men the presence of pore-loop mutations was associated with >2-fold (P = .01) increase in the risk of ACA or SCD, whereas women with pore-loop mutations did not display a significant increase in risk as compared with those with non–pore-loop mutations. Accordingly, by age 40 years the rate of life-threatening cardiac events among men with pore-loop mutations was >3-fold higher as compared with those with other mutations (28% vs. 8%, respectively), whereas the corresponding event rates among women were high regardless of mutation location (35% and 23%, respectively). Possible mechanisms that may explain the observed gender-related differences include the fact that estrogen increases I_{Kr} independently of mutation location, thereby increasing ar-

rhythymic risk even among women who carry lower-risk (nonpore) mutations in the *KCNH2* channel. In contrast, the protective effects of testosterone on I_{Kr} and ventricular repolarization in postadolescent male subjects result in a reduction in the risk of arrhytmic events among carriers of low-risk mutations, with a possible remaining residual risk in men who harbor higher-risk mutations in the functionally more important pore-loop region.

In a prior study, Priori et al.[16] proposed a risk stratification scheme for LQTS patients that is based on the LQTS genotype, QTc, and gender. This study, however, assessed a composite end point of cardiac events of any type, comprising mostly nonfatal symcopal episodes,[16] whereas the large sample size of genotyped LQT2 patients in the present study facilitated for the first time the development of a risk stratification scheme for the end point of life-threatening cardiac events within the LQT2 population. We show that combined assessment of clinical and genetic data, related to mutation location, can be used to identify risk groups of LQT2 patients with a significantly different risk of ACA or SCD and with a pronounced difference in the rate of ACA or SCD during follow-up. These findings suggest that risk stratification in LQTS should combine clinical and mutation-related risk factors that are specific for each of the 3 main LQTS genotypes.

Prior data suggest that LQT2 patients expereince a relatively high rate of cardiac events during β-blocker therapy.[17] In the present study, medical therapy with β-blockers was associated with a pronounced 61% reduction in the risk of ACA or SCD in the total LQT2 population. However, the present findings also suggest that careful follow-up, with consideration of ICD therapy for primary prevention, is warranted in high- and very-high-risk LQT2 patients. These patient subsets were shown to experience 3.5 to 5.3 events per 100 patient years (which corresponds to a high rate of 1.5 to 2.1 life-threatening cardiac events per patient from birth through age 40 years) despite frequent usage of β-blocker therapy (>80%).

Study limitations

We did not carry out expression studies to assess the effects of estrogen and testosterone on ion channel mutations by their location. Therefore, further studies are necessary to evaluate the mechanism related to the observed gender-specific risk related to mutation location.

Because of sample size limitations, we did not carry out comprehensive analysis of the relation between all function regions of the *KCNH2* channel (including the PAS, CNBD, and other C-terminus and and N-terminus domains) and gender-specific risk. However, the results from the secondary analysis in which non–pore-loop mutations were further subcategorized into mutations in the transmembrane and C/N-terminus regions support the consistency of our findings.

Conclusions and clinical implications

The present study shows a distinct association between mutation characteristics and time-dependent differences in the clinical course of LQT2 patients. We have shown that after the onset of adolescence, women with and without high-risk mutations show increased risk for life-threatening cardiac events, whereas the risk of ACA or SCD in men is increased only among carriers of the higher-risk pore-loop mutations. Thus, a comprehensive approach that combines clinical and genetic data should be used for risk assessment and management of LQTS patients.

Appendix

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.hrthm.2011.03.049.

References

1. Moss AJ, Kass RS. Long QT syndrome: from channels to cardiac arrhythmias. J Clin Invest 2005;115:2018–2024.
2. Goldenberg I, Zareba W, Moss AJ. Long QT syndrome. Curr Probl Cardiol 2008;33:629–694.
3. Sanguinetti MC, Tristan-Firouzi M. hERG potassium channels and cardiac arrhythmia. Nature 2006;440:463–469.
4. Sanguinetti MC. HERG1 channelopathies. Eur J Physiol 2010;460:265–276.
5. Moss AJ, Zareba W, Kaufman ES, et al. Increased risk of arrhythmic events in long-QT syndrome with mutations in the pore region of the human ether-a-go-go-related gene potassium channel. Circulation 2002;105:794–799.
6. Shimizu W, Moss AJ, Wilde AAM, et al. Genotype-phenotype aspects of type 2 long QT syndrome. J Am Coll Cardiol 2009;54:2052–2062.
7. Zareba W, Moss AJ, Locati EH, et al. Modulating effects of age and gender on the clinical course of cong QT syndrome by genotype. J Am Coll Cardiol 2003;42:103–109.
8. Bazett HC. An analysis of the time relations of electrocardiograms. Heart 1920;7:353–367.
9. Splawski I, Shen J, Timothy KW, et al. Spectrum of mutations in long-QT syndrome genes: KVLQT1, HERG, SCN5A, KCNE1, and KCNE2. Circulation 2000;102:1178–1185.
10. January CT, Gong Q, Zhou Z. Long-QT syndrome: cellular basis and arrhythmia mechanism in LQT2. J Cardiovasc Electrophysiol 2000;11:1413–1418.
11. Therneau TM, Grambsch PM. Modeling survival data: extending the Cox model. New York, NY: Springer-Verlag, 2000.
12. Bidoggia H, Maciel JP, Capalozza N, et al. Sex differences on the electrocardiographic pattern of cardiac repolarization: possible role of testosterone. Am Heart J 2000;140:678–683.
13. Ridley JM, Shuba YM, James AF, Hancox JC. Modulation by testosterone of an endogenous hERG potassium channel current. J Physiol Pharmacol 2008;59: 395–407.
14. Hara M, Danilo P Jr, Rosen MR. Effects of gonadal steroids on ventricular repolarization and on the response to E4031. J Pharmacol Exp Ther 1998;285: 1068–1072.
15. Song M, Helguera G, Eghbali M, et al. Remodeling of Kv4.3 potassium channel gene expression under the control of sex hormones. J Biol Chem 2001;276: 31883–31890.
16. Priori SG, Schwartz PJ, Napolitano C, et al. Risk stratification in the long-QT syndrome. N Engl J Med 2003;348:1866–1874.
17. Priori SG, Napolitano C, Schwartz PJ, et al. Association of long QT syndrome loci and cardiac events among patients treated with β-blockers. JAMA 2004; 292:1341–1344.

Journal of the American College of Cardiology
© 2011 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 57, No. 22, 2011
ISSN 0735-1097/$36.00
doi:10.1016/j.jacc.2011.01.026

Flecainide Therapy Reduces Exercise-Induced Ventricular Arrhythmias in Patients With Catecholaminergic Polymorphic Ventricular Tachycardia

Christian van der Werf, MD,* Prince J. Kannankeril, MD, MSCI,‡ Frederic Sacher, MD,‖ Andrew D. Krahn, MD,¶ Sami Viskin, MD,# Antoine Leenhardt, MD,** Wataru Shimizu, MD, PHD,†† Naokata Sumitomo, MD,‡‡ Frank A. Fish, MD,‡ Zahurul A. Bhuiyan, MD, PHD,† Albert R. Willems, MD, PHD,* Maurits J. van der Veen, MD, PHD,§§ Hiroshi Watanabe, MD, PHD,‖‖ Julien Laborderie, MD,¶¶ Michel Haïssaguerre, MD,‖ Björn C. Knollmann, MD, PHD,§ Arthur A. M. Wilde, MD, PHD*

Amsterdam and Ede, the Netherlands; Nashville, Tennessee; Bordeaux, Paris, and Bayonne, France; London, Ontario, Canada; Tel Aviv, Israel; and Suita, Tokyo, and Niigata, Japan

Objectives This study evaluated the efficacy and safety of flecainide in addition to conventional drug therapy in patients with catecholaminergic polymorphic ventricular tachycardia (CPVT).

Background CPVT is an inherited arrhythmia syndrome caused by gene mutations that destabilize cardiac ryanodine receptor Ca^{2+} release channels. Sudden cardiac death is incompletely prevented by conventional drug therapy with β-blockers with or without Ca^{2+} channel blockers. The antiarrhythmic agent flecainide directly targets the molecular defect in CPVT by inhibiting premature Ca^{2+} release and triggered beats in vitro.

Methods We collected data from every consecutive genotype-positive CPVT patient started on flecainide at 8 international centers before December 2009. The primary outcome measure was the reduction of ventricular arrhythmias during exercise testing.

Results Thirty-three patients received flecainide because of exercise-induced ventricular arrhythmias despite conventional (for different reasons, not always optimal) therapy (median age 25 years; range 7 to 68 years; 73% female). Exercise tests comparing flecainide in addition to conventional therapy with conventional therapy alone were available for 29 patients. Twenty-two patients (76%) had either partial (n = 8) or complete (n = 14) suppression of exercise-induced ventricular arrhythmias with flecainide ($p < 0.001$). No patient experienced worsening of exercise-induced ventricular arrhythmias. The median daily flecainide dose in responders was 150 mg (range 100 to 300 mg). During a median follow-up of 20 months (range 12 to 40 months), 1 patient experienced implantable cardioverter-defibrillator shocks for polymorphic ventricular arrhythmias, which were associated with a low serum flecainide level. In 1 patient, flecainide successfully suppressed exercise-induced ventricular arrhythmias for 29 years.

Conclusions Flecainide reduced exercise-induced ventricular arrhythmias in patients with CPVT not controlled by conventional drug therapy. (J Am Coll Cardiol 2011;57:2244–54) © 2011 by the American College of Cardiology Foundation

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a malignant inherited arrhythmia syndrome characterized by physical or emotional stress-induced bidirectional or polymorphic ventricular tachycardia (VT) in structurally

From the *Department of Cardiology, Heart Failure Research Center, Academic Medical Center, Amsterdam, the Netherlands; †Department of Clinical Genetics, Academic Medical Center, Amsterdam, the Netherlands; ‡Department of Pediatrics, Vanderbilt University School of Medicine, and the Monroe Carell Jr. Children's Hospital at Vanderbilt, Nashville, Tennessee; §Departments of Medicine and Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee; ‖Service de Rythmologie, CHU de Bordeaux, Université Bordeaux 2, Bordeaux, France; ¶Arrhythmia Service, Division of Cardiology, University of Western Ontario, London, Ontario, Canada; #Department of Cardiology, Tel Aviv Sourasky Medical Center, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel; **Service de Cardiologie, Hôpital Lariboisière, Assistance Publique–Hôpitaux de Paris, Université Paris Diderot, INSERM U942, Paris, France; ††Division of Arrhythmia and Electrophysiology, Department of Cardiovascular Medicine, National Cerebral and Cardiovascular Center, Suita, Japan; ‡‡Department of Pediatrics and Child Health, Nihon University School of Medicine, Tokyo, Japan; §§Department of Cardiology, Gelderse Vallei Hospital, Ede, the Netherlands; ‖ ‖Division of Cardiology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan; and the ¶¶Service de Cardiologie, Hôpital de Bayonne, Bayonne, France. This work was

normal hearts, with a high fatal event rate in untreated patients (1–3). Approximately 60% of CPVT patients have mutations in genes encoding the cardiac ryanodine receptor Ca^{2+} release channel (RyR2) or cardiac calsequestrin (4–6), and these cause spontaneous RyR2 channel openings (7,8). The resulting increase in cytosolic Ca^{2+} triggers delayed afterdepolarizations, ventricular premature beats (VPBs), and ventricular tachycardia, especially under conditions of β-adrenergic stimulation (9,10).

Hence, β-blockers are considered first-line therapy, but unfortunately they are not completely effective in preventing life-threatening arrhythmias (1–3,11–16). An implantable cardioverter-defibrillator (ICD) is often used in patients who continue to have ventricular arrhythmias despite β-blocker therapy. However, ICDs are not fully protective and can be proarrhythmic in CPVT patients because both appropriate and inappropriate ICD shocks can trigger catecholamine release, subsequently resulting in multiple shocks (arrhythmic storm), and death (17,18). Thus, additional therapy is desired for CPVT. Small case series show that left cardiac sympathetic denervation is effective in patients who are insufficiently protected by β-blocker therapy and/or experiencing too many ICD shocks (19–22).

Recently, we discovered that the antiarrhythmic agent flecainide directly blocks RyR2 channels, prevents RyR2-mediated premature Ca^{2+} release, and suppresses triggered beats in myocytes isolated from mouse hearts lacking calsequestrin, an animal model of CPVT (23). This effect is not mediated by Na^{+}-channel block, the conventional mode of action thought to underlie flecainide activity, but rather can be attributed to open state block of RyR2 channels (that is, flecainide directly targets the molecular defect responsible for the arrhythmogenic Ca^{2+} waves that trigger CPVT in vivo) (24). In preliminary work, flecainide also appeared to be effective in 2 highly symptomatic CPVT patients (23).

Here we collate the data from every CPVT patient started on flecainide at 8 international centers and report on the efficacy and safety of flecainide treatment in CPVT.

Methods

Participants and study design. To better understand the efficacy and safety of flecainide in CPVT, we reviewed the chart of each consecutive CPVT patient in whom flecainide was started at 8 tertiary referral centers in the Netherlands, Canada, France, Israel, Japan, and the United States before December 2009. All patients had a clinical diagnosis of CPVT (based on exercise-induced bidirectional or polymorphic VT in the absence of structural cardiac disease) and a putative pathogenic mutation in the gene encoding RyR2 or cardiac calsequestrin. Determination of flecainide starting dose and dosing increases were made by the treating physician as part of specialized clinical care. Data collection and analysis were done retrospectively by chart review and were approved by the institutional review board at each participating institution.

Abbreviations and Acronyms

CPVT = catecholaminergic polymorphic ventricular tachycardia

ICD = implantable cardioverter-defibrillator

NSVT = nonsustained ventricular tachycardia

RyR2 = cardiac ryanodine receptor Ca^{2+} release channel

VPB = ventricular premature beat

VT = ventricular tachycardia

Primary and secondary outcome measures. Couplets or VT during exercise are significantly associated with future arrhythmic events in CPVT (2). Because all patients were monitored by repeat exercise testing as part of routine clinical care, we used the reduction of ventricular arrhythmias during exercise testing as the primary outcome measure. The effect of flecainide was quantified by comparing the ventricular arrhythmia score (see later text) of the last exercise test on conventional therapy with the ventricular arrhythmia score of the first exercise test after a minimum of 5 days on the stable flecainide dose. Only patients on an unchanged or lower β-blocker dose during flecainide treatment were included in the primary analysis. Depending on the site, exercise testing was performed using a treadmill (standard or modified Bruce protocols) or bicycle ergometer.

Secondary outcome measures were the incidence of arrhythmic events (defined as syncope, aborted cardiac arrest, appropriate ICD shocks, and sudden cardiac death), assessment of well-being and side effects of flecainide, and monitoring of proarrhythmic effects of flecainide, in particular QRS duration during exercise and increase in the ventricular arrhythmia burden (25,26).

Definitions of ventricular arrhythmia. Exercise testing was analyzed and scored using the following pre-defined parameters (modified from Rosso et al. [27]): 1) ventricular arrhythmia score, defined by the worst ventricular arrhythmia (1, no or isolated VPBs; 2, bigeminal VPBs and/or frequent VPBs [>10 per min]; 3, couplet; and 4, nonsustained ventricular tachycardia [NSVT], ≥3 successive VPBs); 2) the presence of either of the parameters of the ventricular arrhythmia score or the presence of bidirectional VT (>3 successive VPBs with a beat-to-beat alternating right and left QRS axis); 3) sinus rate at the onset of ventricular arrhythmias, most often an isolated VPB; 4) maximum number of VPBs during a 10-s period; and 5)

supported by ZorgOnderzoek Nederland Medische Wetenschappen (ZonMW, grant 120610013 to Drs. van der Werf and Wilde), by the U.S. National Institutes of Health (grants HL88635, HL71670 to Dr. Knollmann and HL076264 to Dr. Kannankeril), by a grant from the Heart and Stroke Foundation of Ontario (grant NA3397 to Dr. Krahn), by a grant from the French national government named Programme Hospitalier de Recherche Clinique (grant AOR04070, P040411 to Dr. Leenhardt), by a Research Grant for Cardiovascular Diseases (21C-8) from the Ministry of Health, Labor, and Welfare, Japan (to Dr. Shimizu), by the American Heart Association Established Investigator Award (grant 0840071N to Dr. Knollmann), and by the Fondation Leducq Trans-Atlantic Network of Excellence, Preventing Sudden Death (grant 05-CVD-01 to Dr. Wilde). Dr. Leenhardt is a consultant to Sanofi-Aventis and MedaPharma. Dr. Wilde is on the Advisory Board of PGX Health. All other authors have reported that they have no relationships to disclose.

Manuscript received November 27, 2010; revised manuscript received December 17, 2010, accepted January 3, 2011.

Table 1 Baseline Characteristics and Flecainide Therapy Parameters

Patient #	Sex	Mutation*	Age at First Symptom, yrs	Proband or Relative	Presenting Symptom	Age at Diagnosis, yrs	Aborted Cardiac Arrest	ICD	Age at Baseline, yrs	Drug Therapy at Baseline, mg (mg/kg body weight)	Indication for Starting Flecainide Treatment	Daily Starting/Stable Flecainide Dose, mg (mg/kg body weight)†	Follow-Up, months	Response to Flecainide Treatment	Side Effects of Flecainide
1‡	F	A4091T	5	Proband	Seizure	6	Yes	Yes	13	Nadolol 160 (2.4), verapamil 180 (2.7)§	NSVT (on Holter recordings)	300 (4.5)	25	Complete	None
2	F	R2401H	6	Proband	Syncope	6	No	No	7	Nadolol 15 (0.9)	NSVT (on Holter recordings)	96 (5.6)/120 (7.1)	22	None	None
3‡	M	CASQ2: 532+ 1G>A	NA	Relative	None	3	No	Yes	12	Metoprolol 125 (2.3), verapamil 120 (2.2)§	NSVT (on ICD recordings) + frequent ICD shocks	100 (1.9)/150 (2.8)	28	Complete	None
4‡	F	E4076K	28	Relative	Syncope	31	No	No	37	Metoprolol 100 (1.6)	Couplets + side effects	100 (1.6)/150 (2.4)	23	Partial	None
5	F	S4124G	NA	Relative	None	31	No	No	36	Bisoprolol 5 (0.08), verapamil 240 (3.7)§	NSVT + side effects	100 (1.5)/150 (2.3)	28	Partial	None
6	F	S4124G	45	Proband	Syncope	50	No	No	68	Bisoprolol 2.5 (0.04)	NSVT + side effects	75 (1.2)/150 (2.4)	13	Partial	Sinus arrest and dizziness
7	F	S4124G	26	Relative	Aborted cardiac arrest	26	Yes	No	41	None‖	NSVT	150 (2.2)	22	Partial	Dizziness
8‡	M	S4124G	8	Relative	Syncope	8	No	No	10	Metoprolol 50 (1.9)	Couplets	50 (1.9)/100 (3.7)	22	Partial	None
9‡	M	E4187Q	NA	Proband	None (detected by cardiological examination after SCD of his son)	47	No	No	53	Metoprolol 200 (2.4)	NSVT + side effects	150 (1.7)	20	Partial	None
10‡	M	E4187Q	NA	Relative	None	19	No	Yes	25	Metoprolol 200 (2.7)	NSVT	150 (2.0)	20	None	None
11‡	F	E4187Q	NA	Relative	None	14	No	Yes	20	Metoprolol 150 (2.6)	NSVT	100 (1.8)	20	Complete	None
12‡	M	E4187Q	NA	Relative	None	11	No	Yes	17	Metoprolol 100 (1.6)	NSVT	100 (1.6)/300 (4.8)	20	Partial	None
13	F	E1724K	13	Relative	Syncope	13	No	No	25	Metoprolol 25 (0.4)	Couplets	100 (1.3)¶#	NA#	NA#	Fatigue, dizziness, chest pain
14	F	E1724K	9	Proband	Syncope	15	No	No	50	Sotalol 160 (2.1)	Bigeminy/frequent VPBs + side effects	100 (1.3)	20	None	None
15‡	M	R420W	NA	Relative	None	38	No	No	49	Metoprolol 100 (1.3)	Couplets	150 (1.9)/300 (3.9)	19	Complete	None
16‡	M	R420W	NA	Relative	None	12	No	No	16	Metoprolol 100 (1.7)	NSVT	100 (1.7)	19	Complete	None
17	F	Y4962C	NA	Relative	None	41	No	No	45	Atenolol 25 (0.4)	NSVT	150 (2.5)	12	Complete	None
18‡	F	M2605V, A4510T, 14757-6_7CT>TA	NA	Proband	None (detected by exercise testing at pre-participation screening)	40	No	No	40	Metoprolol 100 (1.4)	Couplets	200 (2.9)	18	Partial	None

Continued on next page

JACC Vol. 57, No. 22, 2011
May 31, 2011:2244–54

Table 1 Continued

Patient #	Sex	Mutation*	Age at First Symptom, yrs	Proband or Relative	Presenting Symptom	Age at Diagnosis, yrs	Aborted Cardiac Arrest	ICD	Age at Baseline, yrs	Drug Therapy at Baseline, mg (mg/kg body weight)	Indication for Starting Flecainide Treatment	Daily Starting/Stable Flecainide Dose, mg (mg/kg body weight)†	Follow-Up, months	Response to Flecainide Treatment	Side Effects of Flecainide
19	F	R420W	33	Proband	Syncope	33	No	Yes	36	Bisoprolol 5 (0.08)	Bigeminy/frequent VPBs	100 (1.5)	17	Complete	None
20	M	R420W	NA	Relative	None	11	No	No	12	Atenolol 25 (0.7)	Couplets	100 (2.6)	23	Complete	None
21‡	F	G3946S	14	Proband	Syncope	15	No	No	34	Nadolol 160 (2.7)	Couplets	200 (3.3)	18	Complete	None
22	F	R420Q	14	Proband	Syncope	15	No	Yes	20	Bisoprolol 1.25 (0.03)	Couplets	200 (4.0)	17	None	None
23‡	F	R2474G	1	Proband	Convulsion without fever	11	No	Yes	18	Atenolol 100 (2.1), verapamil 120 (2.6)	NSVT	150 (3.2)	20	Complete	None
24	F	R420W	NA	Relative	None	20	Yes	No	24	Metoprolol 25 (0.4)#	Bigeminy/frequent VPBs + side effects	100 (1.8)	17	Complete	None
25	F	E1724K	10	Proband	Syncope	31	No	No	39	Carvedilol 2.5 (0.05)	NSVT	100 (2.2)	14	Partial	None
26‡	F	F2215L	5	Proband	Cardiac arrest	10	Yes	No	24	Propranolol 140 (2.8)	NSVT (on Holter recordings) + syncope + palpitations	100 (2.0)	13	None	None
27	F	R4157H	56	Relative	Palpitations	57	No	Yes	57	Bisoprolol 5 (0.08)**	NSVT	150 (2.3)	31	NA**	None
28	F	M3978I	14	Relative	Syncope	15	No	Yes	25	Nadolol 40 (0.7)	Frequent VPBs + syncope	150 (2.5)	31	Complete	Nausea and dizziness
29	F	M3978I	14	Proband	Syncope	14	No	Yes	26	Bisoprolol 5 (0.06)††	Bigeminy/frequent VPBs	150 (3.1)	32	None	None
30	F	M3978I	13	Relative	Syncope	32	No	No	45	None‡‡	Bigeminy/frequent VPBs	150 (2.3)	NA§§	Partial	Nausea and dizziness
31	F	M3978I	13	Relative	Syncope	38	No	No	50	Bisoprolol 5 (0.09)	VPBs + palpitations	100 (1.8)	NA‖‖	None	Nausea and dizziness
32	M	V4771I	4	Proband	Syncope with seizure	18	No	No	18	Sotalol 240 (3.2)	NSVT	200 (2.7)	29 yrs¶¶	Complete	None
33‡	F	R2401H	9	Proband	Syncope	9	No	Yes	17	Nadolol 160 (2.5)	Syncope with VF and arrhythmic storm (recorded on ICD log)	150 (2.3)	40	Complete	None
Total	F: 24 (73%)	*RyR2*: 32 (97%)	Median: 13 (range 1–56)	Probands: 15 (45%)	Symptoms: 21 (64%)	Median: 18 (range 3–57)	Yes: 4 (12%)	Yes: 12 (36%)	Median: 25 (range 7–68)	β-blocker: 31 (94%); Ca^{2+} channel blocker: 4 (12%)	Severe ventricular arrhythmia: 26 (79%); symptoms: 5 (15%)	Median: 100 (range 50–300)/150 (range 100–300)	Median: 20 (range 12–40)	Complete: 14/31 (45%); partial: 10/31 (32%)	Yes: 6 (18%)

*RYR2 mutations unless otherwise indicated. †Stable dose was identical to starting dose when only 1 dose is displayed. ‡Patients who were treated with a first-line β-blocker at an optimal dose (n = 15). §Verapamil was discontinued when flecainide was started. ‖This patient discontinued β-blocker therapy during 3 consecutive pregnancies, and thereafter agreed with her treating cardiologist to permanently discontinue β-blocker therapy and avoid exercise. ¶Flecainide was discontinued within a few days and before exercise testing on flecainide could be performed. #Metoprolol was discontinued and flecainide was started in this patient because of intolerable side effects. **This patient was not included in the primary analysis because the bisoprolol dose was also increased. ††This patient discontinued β-blocker therapy on her own initiative after flecainide treatment was started and before an exercise test on combined therapy could be performed. The ventricular arrhythmia score on flecainide monotherapy did not change compared with that on the baseline exercise test while taking a β-blocker. ‡‡This patient discontinued β-blocker therapy because of side effects. §§This patient discontinued flecainide and restarted β-blocker therapy on her own initiative. ‖‖This patient discontinued flecainide because of side effects after exercise testing while taking a β-blocker and flecainide was performed. ¶¶This patient was excluded from the follow-up calculation.

ICD = implantable cardioverter defibrillator; NA = not applicable; NSVT = nonsustained ventricular tachycardia; SCD = sudden cardiac death; VF = ventricular fibrillation; VPB = ventricular premature beat.

JACC Vol. 57, No. 22, 2011
May 31, 2011:2244–54

ratio of VPBs to sinus beats during the 10-s period with the maximum number of VPBs.

Reaching a ventricular arrhythmia score of 1 was considered complete suppression of ventricular arrhythmias. Other ventricular arrhythmia score improvements were considered partial suppression.

Statistical analysis. Continuous data are presented as mean ± SD or median (range), and categorical variables as number (percentage). Related samples were compared using the paired Wilcoxon signed-rank test for continuous and ordinal variables and the McNemar test for dichotomous variables. Independent continuous variables were compared by means of the Mann-Whitney *U* test. A 2-tailed p value <0.05 was considered statistically significant. Statistical analysis was performed with SPSS software package, version 15.0 (SPSS, Inc., Chicago, Illinois).

Results

Patient characteristics. A total of 33 genotype-positive CPVT patients from 21 families were started on flecainide at 8 tertiary care centers (Table 1). All patients had persistent physical or emotional stress-induced ventricular arrhythmias documented by exercise testing, Holter recordings, or ICD interrogation and/or persistent symptoms of palpitations, syncope, aborted cardiac arrest, or appropriate ICD shocks, while taking β-blockers with or without Ca^{2+}-channel blockers. Twenty-four of the patients (73%) were female. The median age at the start of flecainide therapy was 25 years (range 7 to 68 years). Thirty-one patients (94%) were treated with β-blockers, and 4 (12%) of them also received Ca^{2+}-channel blockers (Table 1).

In 1 patient (Patient #13), flecainide was stopped because of side effects before exercise testing could be repeated; in another patient (Patient #27) the β-blocker dose was increased during flecainide treatment; and 2 patients (Patients #7 and #30) did not receive β-blocker therapy when flecainide was started (Table 1). In the remaining 29 patients, exercise tests on combination therapy of flecainide with conventional drugs at unchanged or lower doses were available for analysis. In 17 patients (59%), baseline exercise testing was performed <48 h before flecainide initiation.

Flecainide therapy reduces exercise-induced ventricular arrhythmias. Flecainide treatment improved the ventricular arrhythmia score in 22 patients (76%) ($p < 0.001$) (Fig. 1A). Fourteen patients (48%) had complete suppression of ventricular arrhythmias (including 7 patients without any VPBs), and 8 (28%) had partial suppression. None of the patients experienced significant (i.e., couplet or VT) worsening of the exercise-induced ventricular arrhythmia score.

Flecainide treatment also significantly improved all other predefined parameters of exercise-induced ventricular arrhythmia (Table 2). For example, patients receiving flecainide therapy achieved significantly higher heart rates before ventricular arrhythmias occurred. Independently, flecainide caused a significant reduction in maximum sinus rate during exercise, even though a higher mean workload was achieved. As expected (28), flecainide prolonged the PR interval (149 ± 21 ms vs. 160 ± 24 ms; $p = 0.003$), and the QRS duration (83 ± 9 ms vs. 89 ± 11 ms; $p = 0.005$), but did not change the QTc interval (399 ± 26 ms vs. 405 ± 19 ms; $p = 0.171$) at rest. These parameters remained within the normal range at rest and during peak exercise in all patients, except for a slightly prolonged resting PR interval (220 ms) in 1 patient (Patient #20).

We next assessed the reproducibility of exercise testing as a measure of the ventricular arrhythmia burden in CPVT. Although not available for all patients, a subset of patients underwent repeated exercise testing either at the same dose of conventional therapy (n = 14) or at the same flecainide dose (n = 16). In both cases, the ventricular arrhythmia score of the second exercise test was not statistically different from that on the first exercise test (Fig. 2). Similarly, all other predefined parameters of exercise-induced ventricular arrhythmia also did not change significantly (e.g., the maximum number of VPBs during a 10-s period was 5 ± 5 on the first exercise test at the stable flecainide dose and 6 ± 6 on the second exercise test at the same flecainide dose [$p = 0.556$]), suggesting that ventricular arrhythmia scores obtained from exercise testing are reproducible measures of drug efficacy in CPVT and that tachyphylaxis was not present.

We found that 14 of the 29 patients included in the primary analysis received drug therapy that could be considered suboptimal (i.e., an unusual β-blocker for CPVT [bisoprolol, carvedilol, or sotalol]) or a relatively low β-blocker dose (atenolol, metoprolol, or nadolol <1 mg/kg body weight daily) (2). These patients had either side effects on other β-blockers and/or a higher β-blocker dose, or nadolol was not available in their country. To assess whether flecainide was also effective in CPVT patients on optimal conventional therapy, we next analyzed the 15 patients who were treated with a first-line β-blocker at an optimal dose (Table 1). Flecainide significantly improved the ventricular arrhythmia score ($p = 0.003$) (Fig. 1B), and all other pre-defined arrhythmia parameters in this subgroup to a similar extent as in the primary analysis.

The ventricular arrhythmia score in the 2 patients (Patients #7 and #30) who did not receive β-blocker therapy when flecainide was started improved from NSVT to couplet and from NSVT to bigeminal VPBs and frequent VPBs, respectively.

Flecainide dose in CPVT. To estimate the optimal dosing of flecainide in CPVT, we analyzed the relationship between starting dose and VT suppression during the first exercise test on flecainide. Patients without suppression of exercise-induced ventricular arrhythmias on the starting flecainide dose received a significantly lower dose (113 ± 39 mg, n = 13; $p = 0.038$) compared with patients with either partial (142 ± 38 mg, n = 6) or complete ventricular arrhythmia suppression (150 ± 60 mg, n = 12). Eight