Mutations in Cytoplasmic Loops of the KCNQ1 Channel and the Risk of Life-Threatening Events

Implications for Mutation-Specific Response to β-Blocker Therapy in Type 1 Long-QT Syndrome

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Background—β-Adrenergic stimulation is the main trigger for cardiac events in type 1 long-QT syndrome (LQT1). We evaluated a possible association between ion channel response to β-adrenergic stimulation and clinical response to β-blocker therapy according to mutation location.

Methods and Results—The study sample comprised 860 patients with genetically confirmed mutations in the KCNQ1 channel. Patients were categorized into carriers of missense mutations located in the cytoplasmic loops (C loops), membrane-spanning domain, C/N terminus, and nonmissense mutations. There were 27 aborted cardiac arrest and 78 sudden cardiac death events from birth through 40 years of age. After multivariable adjustment for clinical factors, the presence of C-loop mutations was associated with the highest risk for aborted cardiac arrest or sudden cardiac death (hazard ratio versus nonmissense mutations = 2.75; 95% confidence interval, 1.29–5.86; P = 0.009). β-Blocker therapy was associated with a significantly greater reduction in the risk of aborted cardiac arrest or sudden cardiac death among patients with C-loop mutations than among all other patients (hazard ratio = 0.12; 95% confidence interval, 0.02–0.73; P = 0.02; and hazard ratio = 0.82; 95% confidence interval, 0.31–2.13; P = 0.68, respectively; P for interaction = 0.04). Cellular expression studies showed that membrane spanning and C-loop mutations produced a similar decrease in current, but only C-loop mutations showed a pronounced reduction in channel activation in response to β-adrenergic stimulation.

Conclusions—Patients with C-loop missense mutations in the KCNQ1 channel exhibit a high risk for life-threatening events and derive a pronounced benefit from treatment with β-blockers. Reduced channel activation after sympathetic activation can explain the increased clinical risk and response to therapy in patients with C-loop mutations. (Circulation. 2012;125:1988-1996.)

Key Words: adrenergic beta-antagonists • ion channels • long QT syndrome • mutation

Long-QT syndrome type 1 (LQT1) is the most common type of inherited long-QT syndrome (LQTS), accounting for ≈35% of all patients and >50% of genotyped patients.1 LQT1 arises from a decrease in repolarizing potassium current resulting from mutations in the KCNQ1 gene. Four KCNQ1-derived α-subunits assemble to form the \( I_{Ks} \) channel along with obligatory auxiliary subunits derived from KCNE1. Exercise is the main trigger for cardiac arrhythmic

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1988
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Extra cellular

intracellular

C-loops

1-4
5-9
10-20
>20

N

C

Figure 1. Frequency and location of mutations in the KCNQ1 potassium channel. Diagrammatic location of 99 different mutations in the KCNQ1 potassium channel involving 860 subjects. The α-subunit involves the N-terminus (N), 6 membrane-spanning segments, 2 cytoplasmic loops (S2–S3 and S4–S5), and the C-terminus portion (C). The size of the circles reflects the number of subjects with mutations at the respective locations.

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The KCNQ1 protein consists of 676 amino acid residues with an intracellular N-terminus region, 6 membrane-spanning segments with 2 connecting cytoplasmic loops (C loops), and an intracellular C-terminus region.7 Prior genotype–phenotype studies have provided important information on the effect of location and coding type of the channel mutations on the phenotypic manifestations and clinical course of LQT1 patients. These studies have shown that missense mutations and mutations located at the transmembrane region (including the C loops) were associated with greater risk for cardiac events.8 However, the mechanism related to the increased risk associated with transmembrane mutations has not been studied. C loops, part of the transmembrane region, were suggested to affect adrenergic channel regulation by PKA.8 We therefore hypothesized that the previously reported finding about the risk associated with transmembrane mutations8 is related to the effect of C-loop mutations within this region. Accordingly, the present study was carried out in a large cohort of subjects having a spectrum of KCNQ1 mutations from the International LQTS Registry and was designed to investigate the clinical outcomes among KCNQ1 mutation carriers by further dividing the transmembrane region into membrane-spanning and C-loop domains, to determine a possible differential response to β-blocker therapy depending on mutation location and function related to PKA regulation, and to relate the clinical data to functional studies of changes in Iks function and β-adrenergic receptor regulation in mammalian cells.

Methods

Study Sample

The study comprised 860 patients with genetically confirmed KCNQ1 mutations derived from 170 proband-identified families. The proband in each family had QTc prolongation not resulting from a known secondary cause. The subjects were drawn from the Rochester (n=637), the Netherlands (n=94), the Japanese (n=82), the Danish (n=43), and the Swedish (n=4) portions of the Multicenter Mutation Registry. All subjects or their guardians provided informed consent for the genetic and clinical studies. Patients with congenital deafness and patients with multiple LQTS-associated mutations were excluded from the study.

Phenotype Characterization

On enrollment, routine clinical and ECG information was obtained from birth to the participants’ enrolled age, and ongoing clinical information was obtained at yearly intervals thereafter. For each patient, data on personal and family histories, cardiac events, and therapy were systematically recorded at enrollment and at each visit or medical contact. Clinical data, recorded on prospectively designed forms, included patient and family histories and demographic, ECG, therapeutic, and cardiac event information. Data on β-blocker therapy included the starting date and discontinuation date if appropriate. Information on the end point of ACA or sudden cardiac death (SCD) was also verified through requested medical records. Every effort was made to confirm an underlying life-threatening arrhythmia when observed or documented by medical staff.

Genotype Characterization

The KCNQ1 mutations were identified with the use of standard genetic tests performed in academic molecular genetics laboratories. Genetic alterations of the amino acid sequence were characterized by location and by the specific type of mutation (missense, splice site, in-frame insertions/deletions, nonsense, stop codon, and frameshift).

We evaluated the risk associated with 4 main prespecified subgroups: C- or N-terminus missense, membrane-spanning missense, C-loop missense, and nonmissense (ie, splice sites, in-frame insertions, in-frame deletions, stop codons, and frameshift). The membrane-spanning region of the KCNQ1-encoded channel was defined as the coding sequence involving amino acid residues between 124 and 170 (S1–S2), 196 and 241 (S3–S4), and 263 and 355 (S5–S6), with the C-loop region between residues 171 to 195 (S2–S3) and 242 to 262 (S4–S5; Figure 1). The N-terminus region was defined before residue 124 and the C-terminus region after residue 355.

To minimize survival bias, we included patients who died before they were genotyped (n=64). They were assumed to have the mutation that their first-degree relatives had. All other patients were confirmed through genotyping.
Cellular Expression Studies
To study the mechanism underlying the risk for cardiac events in patients with missense C-loop mutations, we measured channel function and regulation for channels formed with wild-type (WT) subunits coexpressed with 4 mutant subunits present in C loops (G189R, R190Q, R243C, and V254M) and 4 mutant subunits present in the non–C-loop domains: 3 in the membrane-spanning domain (T312I, G166R, and S225L) and 1 in the C terminus (R555C). The mutations chosen included the most common mutations in the LQT1 registry. WT and mutant KCNQ1 subunit cDNA and KCNE1 subunit cDNA were transfected into HEK293T cells.10–12 Mutant KCNQ1 cDNA was transfected in combination with WT-KCNQ1 to mimic the heterozygous nature of the disease (WT-KCNQ1:mutant KCNQ1:KCNE1=0.5:0.5:1). Fluorescence-conjugated and -tagged constructs were used to evaluate the efficiency of the cotransfection of WT and mutant subunits13 (see the online-only Data Supplement). Of the HEK293T cells cotransfected with both WT and mutant subunits, 85% to 90% showed fluorescence of at least 1 subunit transfected, and 85% to 95% of transfected cells expressed all the subunits transfected (Figure I in the online-only Data Supplement). All electrophysiology determinations were performed with the untagged construct. Expression of WT and mutant subunits was confirmed by Western blot (Figure II in the online-only Data Supplement). Expression levels were not significantly decreased for the mutant subunits compared with WT. We measured ion channel currents after channel depolarization to 20 mV for 4 seconds from −80-mV holding potential before and after application of forskolin, a PKA activator (10 μmol/mL), with standard electrophysiological techniques and physiological solutions. Current was normalized for all voltages to cell capacitance, and further normalization was accomplished by use of WT cell currents transfected, and 85% to 95% of transfected cells expressed all the subunits transfected (Figure I in the online-only Data Supplement).

Results
Study Sample
The spectrum of mutations as categorized by location and type and their respective number of carriers are presented in Table I-A in the online-only Data Supplement. The location and frequency of missense mutations are presented diagrammatically in Figure 1. Of the 99 total different KCNQ1 mutations identified, 77 were missense mutations and 22 were nonmissense mutations. Missense mutations were further categorized according to their location: 28 different mutations in C-terminus or N-terminus regions (26 in C terminus), 34 mutations in membrane-spanning regions, and 15 mutations in the C-loop regions (8 in S2-S3 loop and 7 in S4-S5 loop). The clinical characteristics of patients in the 4 mutation location/type subgroups are presented in Table 1. Of the 860 study subjects, 20% had C/N terminal missense mutations, 44% had membrane-spanning missense mutations, 15% had C-loop missense mutations, and 22% had nonmissense mutations. Patients with C-loop missense mutations exhibited the longest QTc interval at enrollment, were treated with β-blockers more frequently during follow-up, and had a higher frequency of cardiac events of any type, including
Clinical Outcome of Patients According to Mutation Location and Type

There were 105 first life-threatening cardiac events (27 first ACA events and 78 first LQTS-related SCD events) among the 860 study patients. Patients were enrolled in the registry between 1978 and 2007 with follow-up through 2008; the last reported life-threatening cardiac event occurred in 2005. Figure 2 presents the cumulative probabilities of first life-threatening cardiac events in the 4 subgroups. There was a significantly higher event rate in the C-loop missense subgroup compared with the other 3 subgroups (log-rank \( P < 0.001 \)). Thus, at 40 years of age, the rate of life-threatening cardiac events was 33% in patients with C-loop missense mutations compared with \( \leq 16\% \) in patients with other mutations.

The findings from the multivariable analysis for the end point of a first life-threatening cardiac event are shown in Table 2. Notably, the adjusted hazard ratio for C-loop missense versus nonmissense mutation was 2.75 (\( P < 0.009 \)), and there was no statistically significant difference in the risk among the other mutation location/type subgroups.

Secondary confirmatory analyses (Table II in the online-only Data Supplement) showed that patients with C-loop missense mutations had an adjusted hazard ratio of 2.74 (95% confidence interval, 1.68–4.46; \( P < 0.001 \)) for life-threatening events compared with patients with other mutations. The results were consistent when the biophysical function of the mutations was added as a covariate to the multivariable model. To show that our results do not depend on the C-loop V254M mutation, which is the most common mutation in the C-loop subgroup (Table I in the online-only Data Supplement), accounting for 50% of C-loop patients, we...
have carried out an additional separate analysis excluding patients who carried this mutation. Results were consistent, with patients with C-loop mutations having a greater risk for life-threatening events, demonstrating that our findings were independent of this mutation. The results were also consistent after inclusion of appropriate implantable cardioverter-defibrillator shocks in the composite end point (adjusted hazard ratio for C-loop missense mutations versus nonmissense mutations: 2.64; 95% confidence interval, 1.64–4.23; P=0.001) and after stratifying patients by enrolling center.

To assess whether fuller adjustment for family membership was important, regression models that included frailty terms (ie, random effects) for family were fit in the multivariable models. Models with gamma and gaussian frailty terms (ie, random effects) for family were fit in the multivariable models while remaining statistically significant. Furthermore, in both of these models, the frailty terms were nonsignificant. The consistency of the results provides further support for the higher risk associated with C-loop mutations.

**β-Blocker Therapy**

In the present study, the effect of β-blocker therapy on the risk of life-threatening events among the different mutation subgroups was assessed as a time-dependent covariate (ie, β-blockers were given to patients at different time points during follow-up, and this information was taken into account in the multivariable models). Multivariable analysis showed a significant differential effect of β-blocker therapy on the outcome of patients with C-loop missense mutations compared with those who had other mutations (Table 3). β-Blocker therapy was associated with a significant 88% reduction (P=0.02) in the risk of life-threatening events among patients with C-loop missense mutations, whereas the benefit of β-blocker therapy was significantly attenuated among patients with other mutations in the KCNQ1 channel.

![Figure 3](http://circ.ahajournals.org/)

**Figure 3.** Risk for life-threatening cardiac events by mutation location and β-blocker treatment. Sixty-three of the 125 subjects (50%) with C-loop missense mutations were treated with β-blockers during a mean follow-up of 26.2 years; 305 of the 735 subjects (42%) with non-C-loop missense mutations were treated by β-blockers during a mean follow-up of 27.5 years. Event rates per 100 person-years were calculated by dividing the number of events during the period of β-blocker therapy or the absence of β-blocker therapy by person-years and multiplying the results by 100. ACA indicates aborted cardiac death; LQTS, long-QT syndrome.
Cellular Expression Studies

To understand the mechanism underlying the increase in risk associated with C-loop mutations, we measured channel basal function and regulation in 8 mutant channels associated with LQT1: 3 in the membrane-spanning domains (T312I, G168R, and S225L), 4 located in C loops (G189R, R190Q, R243C, and V254M), and 1 in the C terminus (R555C; Figure 4A). WT and mutant subunits were coexpressed for all experiments. Basal channel current was decreased for all mutations studied compared with WT subunits (Figure 4B). In addition, because activation by PKA is thought to be particularly important for \( I_{Ks} \) function and to underlie arrhythmogenesis in LQT1, we measured the effect of the PKA activator forskolin. All C-loop mutations tested showed a dramatic activation by forskolin, as did the WT KCNQ1 channel (Figure 4C and 4D).

Discussion

The present analysis of 860 LQT1 patients with a wide range of mutations in the KCNQ1 channel provides several important implications regarding risk assessment and management in this study sample. First, patients with missense mutations located in the C loop exhibit the highest risk for life-threatening cardiac events independently of clinical and ECG variables. Second, \( \beta \)-blocker therapy is associated with a pronounced reduction in the risk of ACA or SCD among carriers of missense mutations in the C loop, whereas the benefit of this mode of medical therapy is significantly attenuated in LQT1 patients with other mutations. Third, expression studies of C-loop mutations suggest that an impaired regulation by PKA is the mechanism underlying the increased risk for cardiac events independently of patient QTc and may explain the pronounced response to medical therapy with \( \beta \)-blockers among patients with C-loop mutation carriers.

We have recently shown that patients with mutations located in the transmembrane region have a significantly higher rate of cardiac events than those with mutations located in the C terminus. In addition, mutations in the transmembrane domain were suggested to be associated with...
important functional role in modifying the function of other mutations in the KCNQ1 channel. The S2-S3 and S4-S5 C loops have previously been suggested to have an important functional role in modifying the function of voltage-gated potassium channels. In particular for $I_{Ks}$, the S4-S5 loop has been suggested to mediate a functional interaction with the auxiliary KCNE1 subunits. Most recently, LQT1 mutations in C loops, when expressed in the absence of WT subunits, were suggested to affect adrenergic channel regulation. Our results showed that even when expressed in the presence of WT subunits, C-loop mutations can dramatically affect channel regulation. Consistent with our results, induced pluripotent stem cells differentiated into cardiomyocytes from a patient carrying R190Q were recently shown to lack adrenergic regulation of their $I_{Ks}$ current. Also consistent with our results, for haploinsufficient mutations, not tested here, a simple lack of mutant subunit expression is expected to maintain normal adrenergic regulation, contributing to the milder phenotype of these mutations. It is conceivable that a decrease in channel regulation, as observed for the C-loop mutations, will lead to an increase in the burden of the mutation during adrenergic stimulus. The increase in cardiac risk associated with C-loop mutations is independent of traditional clinical variables; this can be explained by a blunted PKA-mediated activation because QTc is generally measured at rest. Thus, our results suggest that exercise may exacerbate the QTc prolongation for C-loop mutants. It has recently been suggested that the mutation KCNQ1(A341V) also caused an impairment in $\beta$-adrenergic activation. This mutation is located at the end of the S6 domain, a region suggested to interact with the S4-S5 loop. It is possible that other mutations causing functional impairment similar to that of the C-loop mutations may also carry the increased cardiac risk and $\beta$-blocker efficacy.

Current guidelines recommend empirical therapy with $\beta$-blockers in all LQTS patients. The present study shows, for the first time, a mutation-specific response to $\beta$-blocker therapy in LQT1, demonstrating that $\beta$-blockers were associated with a significantly greater reduction in the risk of life-threatening cardiac events among patients with mutations located in the C loops compared with all other mutations. It is conceivable that during $\beta$-adrenergic stimulation, patients with mutations located in the C loops have an unopposed increase in inward $\text{Ca}^{2+}$ currents and prolongation of repolarization caused by blunted PKA-mediated activation of $I_{Ks}$. $\beta$-Blockers may decrease these unopposed inward $\text{Ca}^{2+}$ currents, shorten repolarization, and reduce the risk of ventricular arrhythmias, whereas patients with other mutations do not exhibit such an effect.

**Study Limitations**

Clinical history was obtained on enrollment in the registry, so follow-up data in the current study comprised historical data from birth to enrollment and prospective information collected at yearly intervals after enrollment.

The International LQTS Registry records therapies that are prescribed at the discretion of the treating physicians to enrolled subjects; therefore, $\beta$-blocker administration was not randomized. However, because the patient’s physician would have been blinded to whether the patient had a C-loop mutation, the interaction of this with $\beta$-blocker therapy is still compelling. Prior studies from the International LQTS Registry have shown that $\beta$-blocker therapy is associated with a significant reduction in the risk of cardiac events in LQTS patients. However, the present study is the first to assess the benefit of $\beta$-blocker therapy for the reduction in the risk of ACA or SCD among LQT1 patients. We have shown that $\beta$-blocker therapy is associated with a significant 88% ($P=0.02$) reduction in the risk of life-threatening cardiac events among LQT1 carriers of the higher-risk C-loop mutations. Risk reduction associated with $\beta$-blocker therapy in the total study sample and among carriers of the low-risk non-C-loop mutations did not reach statistical significance. The lack of a significant $\beta$-blocker effect may be due to sample size limitation and a more limited number of events among carriers of lower risk mutations. Thus, lower-risk patients should still be treated with $\beta$-blocker therapy according to guidelines because the cumulative probability of ACA or SCD from birth through 40 years of age among patients with non-C-loop missense mutations was still considerable (between 11% and 16%). These limitations also suggest that further studies in independent populations are needed before the results can be extrapolated to clinical practice.

The present results, derived from LQTS families enrolled in the registry, may be confounded by familial factors such as ethnicity. To minimize bias, we adjusted for family membership in the multivariable models and carried out a secondary analysis in which additional adjustment was made for proband status. These analyses yielded similar results, further supporting the consistency of our findings. Of the 127 LQT1 mutation carriers without available ECG data, 58 (46%) died suddenly at a young age without a documented ECG. To minimize this bias related to exclusion of higher-risk patients, all multivariable analyses included adjustment for a QTc-missing category in addition to the category of QTc >500 milliseconds.

Channel current and response to forskolin were analyzed for 8 mutations of 99 mutation types observed in this study, but the robust findings in these expression studies strongly support our suggested mechanism. Experimental data were performed at room temperature; results may be different at physiological temperature.

**Conclusions**

We used a combination of clinical analysis and cellular electrophysiology experiments to investigate the molecular determinants and mechanisms underlying the clinical outcomes of a large cohort of subjects having a spectrum of KCNQ1 mutations categorized by their code type and location. Patients with KCNQ1 missense mutations located in the cytoplasmic loops had a significantly greater risk for life-threatening cardiac events and gained greater benefit when...
treated with β-blockers compared with patients having other KCNQ1 missense or nonmissense mutations independently of clinical risk factors. We suggest that a combination of a decrease in basal function and altered adrenergic regulation of the $I_{Ks}$ channel underlies the increased cardiac risk in this subgroup of patients. Our results highlight the importance of understanding the molecular determinants and mechanisms underlying arrhythmogenesis to identify cardiac risk factors for LQT1 patients.

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Disclosures

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References


**CLINICAL PERSPECTIVE**

Long-QT syndrome type 1 (LQT1) arises from a decrease in repolarizing potassium current resulting from mutations in the KCNQ1 gene. The main trigger for cardiac arrhythmic events in patients with LQT1 is activation of β1-adrenergic receptors during exercise. Despite the observed reduction in the risk of cardiac events with β-blocker therapy among LQT1 patients, there is still a considerable cardiac residual event rate, suggesting that subgroups of LQT1 patients have differential response to β-blockers. The present study of 860 patients from the International LQTS Registry shows that the presence of missense mutations in distinct functional domains of the KCNQ1 protein, the S2-S3 and S4-S5 cytoplasmic loops (C loops), is associated with a significantly increased risk for life-threatening cardiac events compared with other mutations. Furthermore, patients with missense C-loop mutations gained greater benefit when treated with β-blockers compared with patients having other KCNQ1 mutations independently of clinical risk factors, demonstrating that LQT1 patients have differential response to β-blocker therapy depending on mutation location. Both a decrease in basal function and altered adrenergic regulation of the IKs channel underlie the increased cardiac risk and response to β-blockers in this subgroup of patients. Patients with missense C-loop mutations should be considered a high-risk group of patients but with a pronounced response to β-blockers.

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SUPPLEMENTAL MATERIAL

Supplemental Methods

Plasmid, antibodies and reagents

The following plasmids were used for whole experiments: Human KCNQ1 and KCNE1 in pcDNA3.1(+) vector (gifts from Dr. Robert S. Kass, Columbia University), GFP-tagged human KCNQ1 in pEGFP-N2 (kindly provided from Dr. Gildas Loussouarn, IRT UN, France)\(^1\) human KCNQ1 in modified pIRES2 vector in containing the fluorescent protein cDNA of DsRed-MST instead of GFP (generously provided by Dr. Alfred George, Vanderbilt University),\(^2\) pcDNA3.1(+) vector (Invitrogen) and pEGFP-N1 vector (Clontech). Mutations were introduced into KCNQ1 and GFP-tagged KCNQ1 using the PCR based site direct mutagenesis was performed using PFU ultra DNA polymerase (Agilent Technologies).\(^3\) Construct sequences were confirmed by DNA sequencing at Functional Genomics Center, University of Rochester Medical Center.

The following antibodies were used for immunoblot: anti-KCNQ1 antibody (goat polyclonal IgG, Santa Cruz Biotechnology) raised against the peptide mapping at the C-terminus of KCNQ1 of human origin, anti-β-actin antibody (mouse monoclonal IgG, GenScript USA), IRDye800-conjugated anti-goat antibody (Rockland Immunochemicals), and Alexa Fluor® 680 goat anti-mouse antibody (Invitrogen).

All reagents were purchased from Sigma-Aldrich Corporation unless otherwise indicated. Adenylyl cyclase activator, forskolin was dissolved in Dimethyl sulfoxide (DMSO) to make 25mM stock and stored at -20°C.
**Cell culture and transfection**

HEK293T cells (generously provided from Dr. Keigi Fujiwara, University of Rochester) were maintained in high glucose (4.5 g/L) Dulbecco's modified Eagle's medium (Mediatech) supplemented with 10% fetal bovine serum (Equitech-Bio) and 1% L-glutamax (Invitrogen) in a humidified incubator with 5% CO₂. Cells were transfected with FUGENE-HD transfection regents (Roche), re-plated 24 hours after transfection by using Acutase (Innovative Cell Technologies) and used for experiments 48 hours after transfection.

**Western blot analysis**

Expression of wild-type (WT)- and mutant-\textit{KCNQ1} subunits were confirmed by Western blot. Whole cell lysates were prepared from HEK293T cells, separated by 10% SDS-PAGE, transferred to nitrocellulose membrane (Bio-Rad) and incubated with primary antibodies, followed by incubation with fluorescence-conjugated secondary antibodies. Immunoreactive bands were visualized by Odyssey Infrared Imaging System (LI-COR Biotechnology, Lincoln NE). Densitometric analyses of immunoblots were performed with NIH Image J software. The expression of β-actin was used to control for protein loading in each condition. Mutant band intensity were normalized to wild-type intensity in each blot.

**Analysis of co-transfection efficiency**

GFP-tagged mutant constructs and wild-type subunits in a vector that can expressed DsRed fluorescent protein in addition to the WT subunit were used (Supplementary figure 1S top panel). Co-expression of WT- and mutant-\textit{KCNQ1} subunits were observed using laser scanning confocal microscope (Fluoview FV1000, Olympus) with images obtained using
Fluoview software (FV10-ASW ver2.1c, Olympus) in live cells at room temperature in Tyrode’s solution. The composition of Tyrode’s solution was as follows (mM): NaCl, 136.9; KCl, 5.4; CaCl₂, 2; MgCl₂, 0.5; NaH₂PO₄, 0.33; HEPES, 5; glucose, 5, pH 7.40 adjusted with NaOH. HEK293T cells were transfected with GFP-tagged mutant KCNQ₁, WT-KCNQ₁ in pIRES2- DsRed-MST vector and KCNE₁ at the ratio of 0.5: 0.5: 1.

In order to study the heterozygous mutant channel, expression of both wild-type and mutant channel subunits in the same cells is necessary. To address whether co-transfection of wild-type and mutant subunits in HEK293 cells yielded expression of both subunits, we used fluorescence tagged constructs (Top panel A). A typical group of cells showing mutant and wild type expression is depicted at the bottom of panel A. In panel B, green (mutant expressing) and red (wild-type expressing) cells were counted and separated into three groups: 1) only WT subunit-expressed cells despite co-transfection of the mutant subunit (only red fluorescence from DsRed), 2) only mutant subunit-expressed cells despite co-transfection of wild-type subunit (only green fluorescence from EGFP) and 3) the desired combination of WT- and mutant subunit-coexpressed cells (both green and red fluorescence). For 85-95% of cells, WT and mutant-KCNQ₁ were co-expressed, suggesting that this transfection protocol leads to expression of both subunits in the same cells and the heterozygous expression of the channel subunits (Supplementary Figure 1S bottom panel). Using electrophysiology measurements (see next section for details), we determined that for all heterozygous mutant channels transfected, in about 10% of total cells patched, fast current activation characteristic of KCNQ₁ expressed alone without KCNE₁ subunits was
observed. These cells were not used for determination of channel regulation. This number is consistent with a high co-transfection efficiency of the KCNE1 subunit.

**Electrophysiology**

Wild type \( KCNQ1:KCNE1 \) DNA were expressed in HEK293T cells either at a ratio of 1:1 or 0.5:1, mimicking the haploinsufficient phenotype. Empty plasmid was added to maintain total DNA concentration constant. Each mutant \( KCNQ1 \) plasmid was co-transfected with WT \( KCNQ1 \) and \( KCNE1 \) at the ratio of 0.5 \( \mu \)g mutant \( KCNQ1:0.5 \) \( \mu \)g WT-\( KCNQ1:1\)\( \mu \)g \( KCNE1 \) to yield heterozygous mutant channel expressed. Cells were also co-transfected with low amounts of pEGFP-N1 (0.2 \( \mu \)g) to allow identification of transfected cells using fluorescence. Selection of bright green cells, expressing high concentration of pEGFP, are expected to have higher concentration of the other transfected plasmids, decreasing the inherent variability of the subunit expression levels. In a small percentage of cells (about 10% of total cells patched for all mutants), fast current activation characteristic of \( KCNQ1 \) expressed alone without \( KCNE1 \) subunits was observed. These cells were not used for determination of channel regulation.

\( K^+ \) currents were measured using an Axon 200B amplifier (Axon Instruments) and conventional whole cell patch clamp techniques. The tip resistances of glass pipettes were of 2–6 M\( \Omega \). Voltage-clamp protocols and data acquisition and analyses were performed using Clampex software (Axon Instruments). The voltage drop across the access resistance was compensated \( >70\% \). Whole cell currents were recorded using a low-pass filter with an 1 kHz cutoff and sampled at 2–5 kHz.
The composition of the extracellular solution for the $I_{Ks}$ measurements was (in mmol/L): 145 NaCl, 5.4 KCl, 1. MgCl$_2$, 1.8 CaCl$_2$, 10 HEPES, 10 glucose (pH 7.40 adjusted with NaOH). The composition of the pipette solution was (in mmol/L): 130 K-aspartate, 11 EGTA, 1. MgCl$_2$, 1 CaCl$_2$, 10 HEPES, 5 K-ATP (pH 7.20 adjusted with KOH). Cell-plated glass cover slides were placed on recording chamber (Warner Instruments) and continuously perfused with extracellular solution. All experiments were performed at room temperature ($\approx 22^\circ$C).

Currents time course was measured using a 4-sec depolarization pulse to +20 mV from a holding potential of -80 mV, followed by a 2-sec pulse to -20mV repeated every 10 sec. Current-voltage (I-V) relationships were obtained using a series of test pulses between -40 mV and +120 mV in 10-mV increments before and after forskolin treatment. Baseline mutant current was compared to the current measured from haploinsufficient control channel (0.5 ng WT-$KCNQ1$: 1ng $KCNE1$). The time course of current regulation by forskolin for channels formed by mutant subunits co-expressed with WT subunits was normalized to the changes measured in the absence of forskolin application over the same time course. The time course of current regulation by forskolin in WT channels was measured from cells transfected with 1 $\mu$g WT-$KCNQ1$ and 1 $\mu$g $KCNE1$.

Statistics

One-way ANOVA followed by Tukey Post Hoc test was applied for the assessment of statistical significance for multiple group comparison by using SPSS Statistics ver 17 (IBM). Unpaired Student T-test was used for two group comparison. The significance was set at $p<0.05$. 
### Supplemental Tables

#### Table 1S. Distribution of Mutation Location and Type in LQT1 patients

<table>
<thead>
<tr>
<th>C/N terminal</th>
<th>Membrane spanning</th>
<th>Cytoplasmic loops</th>
<th>Nonmissense</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Missense</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Codon</strong></td>
<td><strong>n (%)</strong></td>
<td><strong>Dominant</strong></td>
<td><strong>Type</strong></td>
</tr>
<tr>
<td><strong>n (%)</strong></td>
<td><strong>Negative</strong></td>
<td></td>
<td><strong>n (%)</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Negative</strong></td>
<td><strong>Dominant</strong></td>
</tr>
<tr>
<td>All</td>
<td>172 (20)</td>
<td>-</td>
<td>All</td>
</tr>
<tr>
<td>R591H</td>
<td>19 (11)</td>
<td>No*</td>
<td>A344A/sp</td>
</tr>
<tr>
<td>H363N</td>
<td>17 (10)</td>
<td>Unknown</td>
<td>Q530X</td>
</tr>
<tr>
<td>R366W</td>
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<td>R518X</td>
</tr>
<tr>
<td>R594Q</td>
<td>14 (8)</td>
<td>No*</td>
<td>K598K/sp</td>
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<tr>
<td>D611Y</td>
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<td>Unknown</td>
<td>448 insG</td>
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<tr>
<td>K395N</td>
<td>10 (6)</td>
<td>Unknown</td>
<td>delF340</td>
</tr>
<tr>
<td>S373P</td>
<td>8 (5)</td>
<td>Unknown</td>
<td>IVS +5 G&gt;A</td>
</tr>
<tr>
<td>G568R</td>
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<td>Unknown</td>
<td>IVS +5 G&gt;A</td>
</tr>
<tr>
<td>I567S</td>
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<td>Unknown</td>
<td>L191fs/90</td>
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<tr>
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<tr>
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<td>Y171X</td>
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<tr>
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<td>S349X</td>
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<tr>
<td>R591C</td>
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<td>intron2,-2 G/A</td>
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<tr>
<td>S546L</td>
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<td>Unknown</td>
<td>S571fs/20</td>
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<tr>
<td>R562S</td>
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<td>639+5G&gt;A</td>
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<tr>
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<td>A150fs/133</td>
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<tr>
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<td>IVS2 +1G&gt;A</td>
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<td>Unknown</td>
<td>IVS4 +5 G&gt;A</td>
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<td>W305C</td>
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<tr>
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<td>Unknown</td>
<td>E284K</td>
</tr>
<tr>
<td>W120C</td>
<td>2 (1)</td>
<td>Unknown</td>
<td>F296S</td>
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<tr>
<td>A525T</td>
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<td>Unknown</td>
<td>G306R</td>
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<tr>
<td>G57V</td>
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<td>V310I</td>
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<tr>
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<td>I274V</td>
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<tr>
<td></td>
<td>P320H</td>
<td>1 (0)</td>
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<tr>
<td></td>
<td>P343S</td>
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<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>Y315S</td>
<td>1 (0)</td>
<td>Unknown</td>
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</tbody>
</table>

*The biophysical function of the mutations was categorized as dominant-negative (>50% reduction in ion channel repolarizing current), haploinsufficiency (<50% reduction in ion channel repolarizing current), and unknown.
†Based on the present study.
‡Assumption based on the nature of the mutation.
Table 2S. Secondary confirmatory multivariate analyses: risk factors for aborted cardiac arrest or sudden cardiac death

A. Including the variable Cytoplasmic loops- missense vs. other mutations.

<table>
<thead>
<tr>
<th></th>
<th>Hazard ratio</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender/age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males age &lt; 13 yrs</td>
<td>1.94</td>
<td>1.09-3.47</td>
<td>0.026</td>
</tr>
<tr>
<td>Females age 13 to 40 yrs</td>
<td>0.88</td>
<td>0.51-1.52</td>
<td>0.652</td>
</tr>
<tr>
<td>QTc ≥ 500 msec (vs. QTc&lt;500)</td>
<td>3.46</td>
<td>1.80-6.63</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cytoplasmic loops- missense vs. other mutations</td>
<td>2.74</td>
<td>1.68-4.46</td>
<td>&lt;0.001</td>
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</tbody>
</table>
B. With adjustment for biophysical function

<table>
<thead>
<tr>
<th></th>
<th>Hazard ratio</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender/age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males age &lt; 13 yrs</td>
<td>1.96</td>
<td>1.09-3.52</td>
<td>0.024</td>
</tr>
<tr>
<td>Females age 13 to 40 yrs</td>
<td>0.87</td>
<td>0.51-1.49</td>
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<tr>
<td>QTc ≥ 500 msec (vs. QTc&lt;500)</td>
<td>3.46</td>
<td>1.79-6.70</td>
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<tr>
<td>Cytoplasmic loops- missense vs. other mutations</td>
<td>2.87</td>
<td>1.74-4.75</td>
<td>&lt;0.001</td>
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<tr>
<td>Dominant negative vs. Haploinsufficiency.*</td>
<td>0.97</td>
<td>0.52-1.81</td>
<td>0.929</td>
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</tbody>
</table>

Adjusted also for unknown biophysical function.

* In a separate model adjusting only for biophysical function (including unknown function), Dominant negative vs. Haploinsufficiency HR= 2.15, 95% CI 1.15-4.02, p=0.017.
### C. Excluding patients with V254M mutation.

<table>
<thead>
<tr>
<th></th>
<th>Hazard ratio</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender/age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males age &lt; 13 yrs</td>
<td>2.14</td>
<td>1.09-4.24</td>
<td>0.028</td>
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<tr>
<td>Females age 13 to 40 yrs</td>
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<td>0.40-1.22</td>
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<tr>
<td>QTc ≥ 500 msec (vs. QTc&lt;500)</td>
<td>4.45</td>
<td>2.26-8.76</td>
<td>&lt;0.001</td>
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<td>Cytoplasmic loops- missense (excluding V254M) vs. other mutations</td>
<td>1.89</td>
<td>1.09-3.26</td>
<td>0.024</td>
</tr>
</tbody>
</table>

### D. Including appropriate ICD shocks in the composite end point.

<table>
<thead>
<tr>
<th></th>
<th>Hazard ratio</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender/age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males age &lt; 13 yrs</td>
<td>1.70</td>
<td>0.95-3.05</td>
<td>0.074</td>
</tr>
<tr>
<td>Females age 13 to 40 yrs</td>
<td>0.95</td>
<td>0.57-1.60</td>
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<tr>
<td>QTc ≥ 500 msec (vs. QTc&lt;500)</td>
<td>3.26</td>
<td>1.75-6.09</td>
<td>&lt;0.001</td>
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<tr>
<td>Cytoplasmic loops- missense vs. other mutations</td>
<td>2.64</td>
<td>1.65-4.23</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

All tables adjusted also for time-dependent β-blocker treatment and for QTc missing.
Supplementary Figure 1S.
Supplementary Figure 2S.
Figure Legends

Supplementary Figure 1S. Co-expression of WT- and mutant-KCNQ1 subunits in HEK293T cells.

A. Schematic pictures of the DNA constructs used for confirming co-expression of WT- and mutant-KCNQ1 subunits in HEK293T cells. GFP was tagged to mutant KCNQ1 at C-term (upper panel, left). WT-KCNQ1 was introduced in pIRES2-DsRed plasmid containing unlinked KCNQ1 and DsRed fluorescence cDNA (see also Supplementary Material and Methods) (upper panel, right). Each GFP-tagged mutant KCNQ1 plasmid was transfected with WT KCNQ1 in pIRES2-DsRed plasmid and KCNE1 at the ratio of 0.5 μg mutant KCNQ1: 0.5 μg WT-KCNQ1: 1 μg KCNE1 to mimic heterozygous mutation. Representative pictures obtained from confocal microscope shown in lower panels showed that most of the cells were expressing both WT-KCNQ1 and KCNQ1(G168R) subunits. Scale bar in each panel, 10 μm. B. Summary data obtained from all mutants tested. WT channels expression (GFP-tagged WT-KCNQ1: WT-KCNQ1 in pIRES2-DsRed plasmid: KCNE1= 0.5 μg :0.5 μg: 1μg) is shown as control experiments (showing as WT+WT). The number of the cells used for analysis is shown in parentheses. Untransfected cells show very low background green and red fluorescence compared to transfected cells (not shown).

Supplementary Figure 2S. Expression of WT- and mutant-KCNQ1 subunit proteins in HEK293T cells.

Representative Western blotting picture for WT- and mutant-KCNQ1 subunit proteins expressed in HEK293T cells. HEK293T cells were co-transfected with either WT- or
mutant KCNQ1 (1 μg), in addition to KCNE1 (1 μg) and pEGFP-N1 (0.2 μg). Lysates were subjected to 10% SDS-PAGE followed by immunoblotting with anti-KCNQ1 antibody and anti-β-actin antibody. B. Summary data obtained from 4-9 experiments. The band intensity of each mutant subunit was not significantly changed compared to that of WT (G168R, p=1.00; G189R, p=0.850; R190Q, p=1.00; S225L, p=0.492; R243C, p=0.309; V254M, p=0.222; T312I, p=0.994; R555C, 0.750). Normalized intensity was determined by measuring intensity of KCNQ1 protein and β-actin protein in each lane to control for protein loading. KCNQ1/β-actin ratio for each mutant was normalized to WT/β-actin ratio in each blot.
Supplemental References