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Compound Mutations
A Common Cause of Severe Long-QT Syndrome

Peter Westenskow, BS; Igor Splawski, PhD; Katherine W. Timothy, BS; Mark T. Keating, MD; Michael C. Sanguinetti, PhD

Background—Long QT syndrome (LQTS) predisposes affected individuals to sudden death from cardiac arrhythmias. Although most LQTS individuals do not have cardiac events, significant phenotypic variability exists within families. Probands can be very symptomatic. The mechanism of this phenotypic variability is not understood.

Methods and Results—Genetic analyses of KVLQT1, HERG, KCNE1, KCNE2, and SCN5A detected compound mutations in 20 of 252 LQTS probands (7.9%). Carriers of 2 mutations had longer QTc intervals (527±54 versus 489±44 ms; P<0.001); all had experienced cardiac events (20 of 20 [100%] versus 128 of 178 [72%]; P<0.01) and were 3.5-fold more likely to have cardiac arrest (9 of 16 [56%] versus 45 of 167 [27%]; P<0.01; OR, 3.5; 95% CI, 1.2 to 9.9) compared with probands with 1 or no identified mutation. Two-microelectrode voltage clamp of *Xenopus* oocytes was used to characterize the properties of variant slow delayed rectifier potassium (I_Ks) channels identified in 7 of the probands. When wild-type and variant subunits were coexpressed in appropriate ratios to mimic the genotype of the proband, the reduction in I_Ks density was equivalent to the additive effects of the single mutations.

Conclusions—LQTS-associated compound mutations cause a severe phenotype and are more common than expected. Individuals with compound mutations need to be identified, and their management should be tailored to their increased risk for arrhythmias. (Circulation. 2004;109:1834-1841.)

Key Words: arrhythmia ■ electrophysiology ■ long-QT syndrome ■ potassium

The long-QT syndrome (LQTS) is an inherited or acquired disorder of ventricular repolarization that predisposes affected individuals to sudden death. Although LQTS can cause severe arrhythmias such as torsade de pointes, most individuals do not have symptoms, and few die of cardiac events. However, within the same LQTS family, a gene carrier can have a markedly prolonged QT interval (QTc, 590 ms) and die of sudden death, whereas another can have a normal QTc of 410 ms and never experience symptoms. The causes of this phenotypic variability are not understood.

LQTS is caused by mutations in the cardiac ion channel genes KVLQT1, HERG, KCNE1, KCNE2, and SCN5A. Loss-of-function mutations of KVLQT1, HERG, KCNE1, and KCNE and gain-of-function mutations of SCN5A are responsible for LQTS. Recent clinical studies demonstrate that individuals with mutations in KVLQT1 have lower incidence of cardiac events, including cardiac arrest and sudden death, compared with carriers of HERG or SCN5A mutations. However, these subtle variations do not explain the dramatic phenotypic variability observed within families and cannot be used to predict who is at greatest risk for sudden death. Schwartz et al recently suggested that compound mutations are associated with increased arrhythmic risk.

Here, we show that compound mutations are a common cause of a severe phenotype in LQTS. Genetic analyses of HERG, KVLQT1, KCNE1, KCNE2, and SCN5A detected higher incidence of compound mutations than expected. Of 252 LQTS probands, 20 (7.9%) carried 2 mutations. When wild-type (WT) and variant subunits (KVLQT1 plus KCNE1) of I_Ks channels were coexpressed in Xenopus oocytes in the appropriate ratios to mimic the genotype of the proband, the reduction in I_Ks density was equivalent to the additive effects of the single mutations.

Methods

Informed consent was obtained from all individuals or their guardians according to standards established by local institutional review boards. Phenotypic criteria were identical to those used in our previous studies. Symptoms for this study were defined as syncope and cardiac arrest. Genotypic and DNA sequence analyses of KVLQT1, HERG, KCNE1, KCNE2, and SCN5A were performed as described.

Site-directed mutagenesis of KVLQT1 and KCNE1 cDNA subcloned into the pSP64 vector was performed by polymerase chain reaction as described. Constructs were linearized with EcoR1, and cRNA was transcribed in vitro with Capribe (Roche Applied Science). The amount of cRNA injected into individual oocytes is indicated in the figure legends.

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Oocytes were isolated from *Xenopus laevis* as previously described. For voltage clamp experiments, the oocytes were bathed in a nominal Cl⁻/H₂PO₄ saline solution as described. A GeneClamp 500B amplifier (Axon Instruments) was used to record currents at 24°C in oocytes 2 (*KVLQT1* or *KVLQT1/KCNE1*) or 3 (HERG) days after injection with cRNA with standard 2-electrode voltage clamp techniques. Data acquisition was performed with a personal computer, a Digidata 1322 A/D interface, and pClamp 8 software (Axon Instruments).

*KVLQT1* channel currents were elicited from a holding potential of −80 mV every 20 seconds with 3-second pulses to potentials ranging from −70 to 60 mV in 10-mV increments. Heteromeric

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**Figure 1.** Pedigree structures and phenotypic and genotypic information for families with mutation in *KVLQT1* and another LQT gene. A, Five families with 2 *KVLQT1* mutations. B, Four families with single mutations in *KVLQT1* and *KCNE1* and 1 family with single mutations in *KVLQT1* and HERG. Affected individuals are indicated by filled circles (females) and squares (males). Unaffected individuals are indicated by open symbols; individuals with uncertain phenotype, by stippled symbols; and deceased family members, by symbols with slashes. QTc intervals, presence of symptoms (syncope, seizure, or cardiac arrest), and genotypes are shown beneath each symbol. Probands are indicated with stars. SD indicates individuals who died suddenly; CA, cardiac arrest; and Syn, syncope.
KVLQT1/KCNE1 channel currents ($I_{KS}$) were elicited every 20 seconds with 7-second depolarizing pulses to the same potentials. Currents were always recorded from the same batch of oocytes for the comparisons shown in each panel of the figures. Currents were analyzed with pCLAMP 8 and Origin (OriginLab Corp) software, and data are reported as mean±SEM (n=number of experiments). The rate of $I_{KS}$ deactivation was determined with a Chebyshev least-squares fitting routine. The voltage dependence of $I_{KS}$ activation was determined by dividing peak current by $V_t-E_{rev}$, where $V_t$ is the test potential and $E_{rev}$ is the reversal potential for $I_{KS}$. The normalized current-voltage relationship was fit to a Boltzmann function to estimate $V_{1/2}$ (half-point) and $k$ (slope factor). A 1-way ANOVA or Student’s $t$ test was used to compare paired data. A 2-way repeated-measures ANOVA, followed by a Bonfer-

Figure 2. Pedigree structures and phenotypic and genotypic information for families with mutation in HERG or SCN5A and another LQT gene. A, Seven families with single mutations in HERG and KCNE1. B, Two families with a mutation in SCN5A and KCNE1 and a proband with 2 mutations in HERG. Symbols and abbreviations as described in Figure 1.
roni test, was applied for multiple comparisons. A value of $P<0.05$ was considered statistically significant.

**Results**

Of 252 LQTS probands, 152 had 1 mutation, 20 (7.9%) were found to have 2 mutations (Figures 1 and 2), and mutations were not found in the remaining 80 probands. When probands with 2 mutations were compared with probands with 1 or no identified mutations, they had longer QTc ($527 \pm 44$ ms; $P<0.001$) and all had experienced symptoms (20 of 20 [100%] compared with 128 of 178 [72%]; $P<0.01$). Carriers of 2 mutations were 3.5-fold more likely to experience cardiac arrest (9 of 16 [56%] versus 45 of 167 [27%]; $P<0.01$; OR, 3.5; 95% CI, 1.2 to 9.9). Carriers of 2 mutations had longer QTc ($530 \pm 53$ versus $449 \pm 31$ ms; $P<0.0001$) and were more symptomatic (12 of 12 versus 2 of 30 [7%]; $P<0.0001$; Figures 1 and 2) than family members with 1 known mutation. Half of these individuals had cardiac arrest compared with only 1 case among the carriers of 1 mutation (6 of 12 [50%] versus 1 of 26 [4%]; $P<0.005$). The presence of cardiac arrest in the remaining 4 individuals with 1 mutation was unknown. Finally, compared with probands with a single identified mutation, probands with compound mutations had a longer QTc ($527 \pm 54$ versus $491 \pm 42$ ms; $P<0.005$) and a greater incidence of syncope and cardiac arrest (20 of 20 [100%] versus 77 of 113 [68%]; $P<0.001$) and all had experienced symptoms ranging from −50 to 30 mV. B, KVLQT1/KCNE1 $I_{ks}$ recorded in oocytes injected with 6 ng WT, T391I, or Q530X KVLQT1 cRNA. Currents were elicited with 3-second pulses applied in 20-mV increments to potentials ranging from −50 to 30 mV. B, KVLQT1/KCNE1 $I_{ks}$ recorded in oocytes injected with 6 ng WT KVLQT1 plus 0.6 ng WT KCNE1 (E1) cRNA (left) or 3 ng T391I KVLQT1 plus 3 ng Q530X KVLQT1 plus 0.6 ng WT KCNE1 cRNA. $I_{ks}$ was elicited with 7-second pulses applied in 20-mV increments to potentials ranging from −40 to 40 mV.

We next determined the functional consequences of single or double mutations in KVLQT1 on the properties of $I_{ks}$. For these experiments, we coexpressed KVLQT1 $\alpha$-subunits with KCNE1 $\beta$-subunits in 3 different combinations. First, WT $I_{ks}$ was compared with currents induced by coexpression of single mutant KVLQT1 subunits plus WT KCNE1 subunits to determine whether the mutant protein was altered by coassembly with KCNE1 subunits (Figure 4E, 4F, and 4G). Second, WT $I_{ks}$ was compared with currents induced by coinjection of oocytes with equal amounts (3 ng) of mutant and WT KVLQT1 cRNA plus 0.6 ng of WT KCNE1 cRNA to mimic a heterozygous mutation (Figure 4H, 4I, and 4J; open symbols). Finally, we measured currents induced by coinjection of the 2 mutant forms of KVLQT1 (3 ng each) plus WT KCNE1 subunit cRNA (Figure 4H, 4I, and 4J, filled circles) to mimic the genotype of the proband.

To analyze the functional consequences of KVLQT1 mutations identified in K1000, we expressed WT KCNE1 and Q530X KVLQT1 subunits. $I_{ks}$ was indistinguishable from currents recorded from oocytes expressing KCNE1 alone (Figure 4E). Coexpression of WT KCNE1 plus T391I KVLQT1 induced currents $\sim15\%$ smaller than WT $I_{ks}$ (Figure 4E), consistent with partial loss of function observed for expression of the mutant subunit alone. Currents induced by WT KCNE1 plus equal amounts of T391I and WT KVLQT1 subunits were not significantly different than WT $I_{ks}$. However, when both mutant forms of KVLQT1 subunits were coexpressed with WT KCNE1, then $I_{ks}$ was reduced by 64% (Figures 3B and 4H).

$I_{ks}$ was then analyzed for the mutations identified in K1100 (Figure 4B, 4F, and 4I). Coexpression of either V310I or R594Q KVLQT1 with KCNE1 caused almost complete loss of function (Figure 4F). This was an unexpected result for V310I KVLQT1 because expression of this subunit alone reduced current by $\sim60\%$ relative to WT KVLQT1 (Figure 4B). However, it also appears that the V310I KVLQT1/WT...
Figure 4. Functional consequences of single or double KVLQT1 mutations. A, B and C, Pair of KVLQT1 (Q1) mutations identified in K1000 (A), K1100 (B), or K1200 (C) were evaluated singly and compared with WT KVLQT1 currents recorded from same batch of oocytes. Each oocyte was injected with 6 ng of single cRNA type, and resulting currents were measured at end of 3-second pulses to indicated test potential (V_t; n=8). †P<0.001, *P<0.005 vs WT KVLQT1. E, F, and G, Current-voltage relationships for WT KVLQT1/KCNE1, mutant KVLQT1/WT KCNE1, and WT KCNE1 channels for K1000 (E), K1100 (F), and K1200 (G). Current amplitudes were mea-
KCNE1 channels may activate at much more positive potentials because the current at 60 mV is much larger than expected for currents induced by KCNE1 alone (Figure 4F, triangles). Coexpression of either mutant KVLQT1 with WT KVLQT1 plus KCNE1 subunits reduced current by ≈30% relative to control $I_{ks}$ (Figure 4I), suggesting that neither of these mutations caused a dominant negative effect. Coexpression of WT KCNE1 with both KVLQT1 mutants together to mimic the compound heterozygous condition of the proband caused a large reduction in amplitude and an apparent rightward shift in the voltage dependence of $I_{ks}$ activation (Figure 4I, filled circles).

The experimental analysis for the variants found in the proband of K1200 is summarized in Figure 4C, 4G, and 4J. Coexpression of KVLQT1 and KCNE1 subunits to mimic the compound heterozygous condition of the proband indicated that $I_{ks}$ channel dysfunction was entirely attributable to the G314S pore mutation (Figure 4J, filled circles).

When the homozygous mutation G179S (K1400) was coexpressed with KCNE1, activation was shifted to more negative potentials, deactivation was slowed (the Table), and current magnitude was reduced at potentials positive to 20 mV (Figure 4K). However, the mutant was without effect when coexpressed with an equal quantity of WT KVLQT1 cRNA plus 0.6 ng KCNE1 cRNA (Figure 4K), consistent with a lack of symptoms in heterozygote carriers in this family (Figure 1A). The effects of mutations on channel deactivation and activation are summarized in the Table.

### Single Mutations in KVLQT1 and KCNE1 Combine to Decrease $I_{ks}$

We characterized 3 of the 4 probands found to have a mutation in KVLQT1 combined with the D85N KCNE1 variant (Figure 1B). A341V KVLQT1 and P127T KCNE1 were found in the proband of K1500 who presented with syncope and a longer QTc than his parents, who declined genotypic analysis. In K1600, both carriers of G168R KVLQT1 and D85N KCNE1 had symptoms and a longer QTc than his parents, who declined genotypic analysis. In K1700, carriers of G168R KVLQT1 and D85N KCNE1 had symptoms and a longer QTc compared with the individual who carried only G168R KVLQT1 (Figure 1B). The proband in K1700 carried 1 mutation in KVLQT1 (T312I) and KCNE1 who carried only G168R KVLQT1 (Figure 1B). No family members were available for phenotypic and genotypic analyses.
We first characterized the functional consequences of the mutations in KVLQT1 alone. All 3 mutations caused complete loss of channel function (Figure 5A, 5C, and 5E). When these KVLQT1 mutants were coexpressed with WT KCNE1, the resulting currents were indistinguishable from currents induced by KCNE1 alone (Figure 5B, 5D, and 5F, triangles). We next characterized the effect of the KCNE1 mutations when expressed with WT KVLQT1. The P127T mutation identified in K1500 had no apparent affect on $I_{Ks}$ (Figure 5B, upside-down triangles), whereas D85N, identified in the other 2 probands, reduced $I_{Ks}$ by about half (Figure 5D and 5F, upside-down triangles).

Finally, the currents induced by coexpression of equal amounts of WT and mutant forms of the KVLQT1 and KCNE1 subunits were studied to mimic the genotype of the probands. Mutant $I_{Ks}$ was reduced 60% to 78% compared with WT current (Figure 5B, 5D, and 5F, upside-down triangles).

**Mutations in KCNE1 and HERG or SCN5A Combine to Increase Symptoms and QTc**

Seven probands were found to have single mutations in HERG and KCNE1 (Figure 2A). In 6 of these probands, 1 of the variants was D85N KCNE1 with 2 homozygous carriers in K2200. The effect of this variant on $I_{Ks}$ was described above (Figure 4). In K2000, S74L KCNE1 was previously characterized and found to cause a 20-mV rightward shift in the voltage dependence of $I_{Ks}$ activation and an accelerated rate of deactivation. R922W HERG current was 25% smaller than WT HERG current at all test potentials ($n=10$; not shown). On the basis of the severe phenotypes of the probands, the combined mutations are predicted to decrease both $I_{Kr}$ and $I_{Ks}$ in all 7 probands.

Two probands were found to have single mutations in SCN5A and KCNE1 (Figure 2B). In K2800, the proband had severe arrhythmia and carried a de novo mutation in SCN5A (R1623Q, previously characterized by Makita et al10) and the D85N variant. In K2700, a child died of sudden death. His father and his brother (the proband) had mutations in SCN5A (R1644H) and KCNE1 (R32H). R1644H SCN5A channels were previously characterized11 and shown to have a sustained inward current typical of other LQTS-associated mutations in the cardiac Na channel. We expressed R32H KCNE1 together with
WT KCNE1 and KVLQT1 and found that \( I_{Ks} \) had an amplitude equivalent to 78% of WT \( I_{Ks} \) \((P<0.05; \text{data not shown})\) without any changes in gating (the Table). Finally, we also identified 1 proband with 2 mutations in HERG and a QTc of 510 ms (K2900; Figure 2B). The P347S mutation is located in the C terminus, as is H70R, which we previously reported alters the kinetics and voltage dependence of HERG gating.\(^\text{12}\)

Discussion

Compound mutations are relatively common in LQTS. In the present study, we reported that 20 of 252 LQTS probands (7.9%) had 2 variants in the ion channel genes HERG, KVLQT1, KCNE1, or SCN5A. QTc intervals were longer, the incidence of cardiac arrhythmia was higher, and symptoms were more severe in probands with 2 mutations. Carriers of 2 mutations were 3.5-fold more likely to have cardiac arrest. Moreover, probands with 2 mutations had longer QTc and were more symptomatic compared with family members with 1 mutation. We further demonstrated that the cause of this severe phenotype is the additive loss of channel function. Taken together, these findings indicate that 2 mutations in LQT genes are a common cause of severe LQTS. Recently, we proposed a multi-hit hypothesis for cardiac arrhythmias.\(^\text{3}\)

This hypothesis was based on the observation that individuals with Jervell and Lange Nielsen syndrome, which results from homozygous mutations of KVLQT1 or KCNE1, have a very severe phenotype and die in childhood unless treated. In contrast, their parents who carry 1 loss-of-function mutation of KVLQT1 or KCNE1 generally have few or no symptoms. These studies indicated that complete loss of 1 arrhythmia gene such as KVLQT1 has much more severe consequences than mutations in 1 allele. We also recently identified SCN5A Y1102.\(^\text{13}\)

This common variant in the black population causes a subtle effect on SCN5A function and has subtle phenotypic consequences. This might be considered a half-hit because most carriers of this variant will never have cardiac arrhythmia.

Here, we have defined another class of LQTS patients, individuals with compound mutations. These individuals are much more common than we expected. The phenotypic consequences of compound mutations are more severe than in individuals with 1 mutation alone. However, the phenotypes are not as severe as in individuals with complete loss of function of 1 gene such as KVLQT1 or HERG. These findings support the idea that arrhythmia risk is a multi-hit process and that genotype can be used to predict risk. While this article was in review, Schwartz et al\(^\text{4}\) reported a very similar observation: 4.6% of 130 patients with LQTS were noted to have compound mutations and more severe clinical symptoms than patients with a single mutation. As in any molecular genetic investigation of disease, a limitation of our study is the possibility that polymorphisms or mutations in promoter regions or intronic sequences of the genes chosen for study were not detected yet may contribute to clinical phenotype. Another limitation is the incomplete clinical data for many of the families.

We examined the molecular mechanisms of increased risk through compound mutations using heterologous expressions in Xenopus oocytes. In most cases, when WT and variant subunits were coexpressed in appropriate ratios to mimic the genotype of the probands with \( I_{Ks} \) mutations, the reduction in current density was equivalent to the additive effects of the single mutations. Coexpression of 2 mutant subunits caused a significant but incomplete reduction in \( I_{Ks} \) (Figure 4). Compound carriers of KVLQT1 mutations have a severe cardiac phenotype, but they are not deaf because \( I_{Ks} \) retains some function. Neural deafness in Jervell and Lange Nielsen syndrome is caused by complete loss of \( I_{Ks} \) activity. This additive effect of the mutations was expected because all the genes in which mutations were identified are critical for the cardiac action potential.

In summary, we have described a large sample of individuals with LQTS caused by compound mutations in ion channel genes. An unexpected finding was that carriers of 2 mutations in LQTS were relatively common. These individuals had longer QTc intervals and were severely symptomatic compared with carriers of single mutations. The cumulative effect of both mutations on \( I_{Ks} \) provided a mechanistic basis for this observation. Individuals with 2 hits are at increased risk of arrhythmia.

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