Circulation
JOURNAL OF THE AMERICAN HEART ASSOCIATION

American Heart Association.

Compound Mutations: A Common Cause of Severe Long-QT Syndrome

Peter Westenskow, Igor Splawski, Katherine W. Timothy, Mark T. Keating and Michael C. Sanguinetti

Circulation. 2004;109:1834-1841; originally published online March 29, 2004;
doi: 10.1161/01.CIR.0000125524.34234.13
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/109/15/1834

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Circulation* is online at:
http://circ.ahajournals.org//subscriptions/

Downloaded from http://circ.ahajournals.org/ by guest on August 14, 2013

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.[1] Although LQTS can cause severe arrhythmias such as torsade de pointes, most individuals do not have symptoms, and few die of cardiac events.[2] 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.[3] 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.[2] 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[4] 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, the reduction in current 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.[5] 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.[6]

Site-directed mutagenesis of KVLQT1 and *KCNE1* cDNA subcloned into the pSP64 vector was performed by polymerase chain reaction as described.[7] Constructs were linearized with *Eco*R1, and cRNA was transcribed in vitro with Capscribe (Roche Applied Science). The amount of cRNA injected into individual oocytes is indicated in the figure legends.

Received January 3, 2003; de novo received October 1, 2003; revision received December 12, 2003; accepted January 15, 2004.

From the Departments of Physiology (P.W., M.C.S.) and Human Genetics (K.T.W.), University of Utah, Salt Lake City, and Department of Cardiology, Children's Hospital, and Departments of Pediatrics and Cell Biology, HMS, HHMI, Boston, Mass (I.S., M.T.K.).

Correspondence to Michael C. Sanguinetti, Nora Eccles Harrison Cardiovascular Research and Training Institute, University of Utah, 95 South 2000 East, Salt Lake City, UT 84112-5000. E-mail sanguinetti@cvrti.utah.edu

© 2004 American Heart Association, Inc.

Circulation **is available at http://www.circulationaha.org** **DOI: 10.1161/01.CIR.0000125524.34234.13**

Downloaded from http://circ.ahajournals.org/ by guest on August 14, 2013

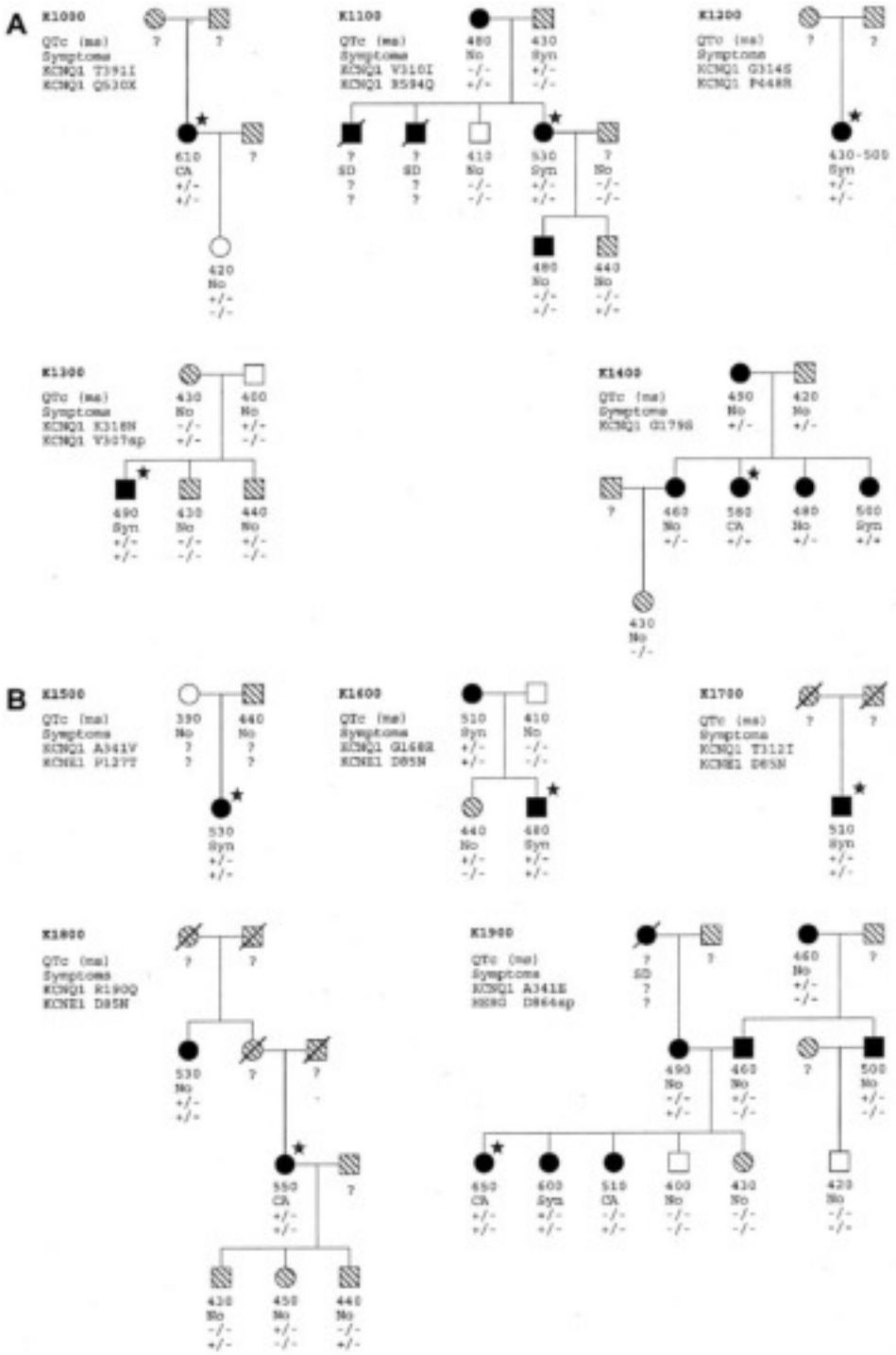

Figure 1. Pedigree structures and phenotypic and genotypic information for families with mutation in *KVLQT1* and another LQT gene. A, Five families with 2 *KVLQT1* mutations. B, Four families with single mutations in *KVLQT1* and *KCNE1* and 1 family with single mutations in *KVLQT1* and *HERG*. Affected 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.

Oocytes were isolated from *Xenopus laevis* as previously described.[7] For voltage clamp experiments, the oocytes were bathed in a nominal Cl^- saline solution as described.[8] 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

Downloaded from http://circ.ahajournals.org/ by guest on August 14, 2013

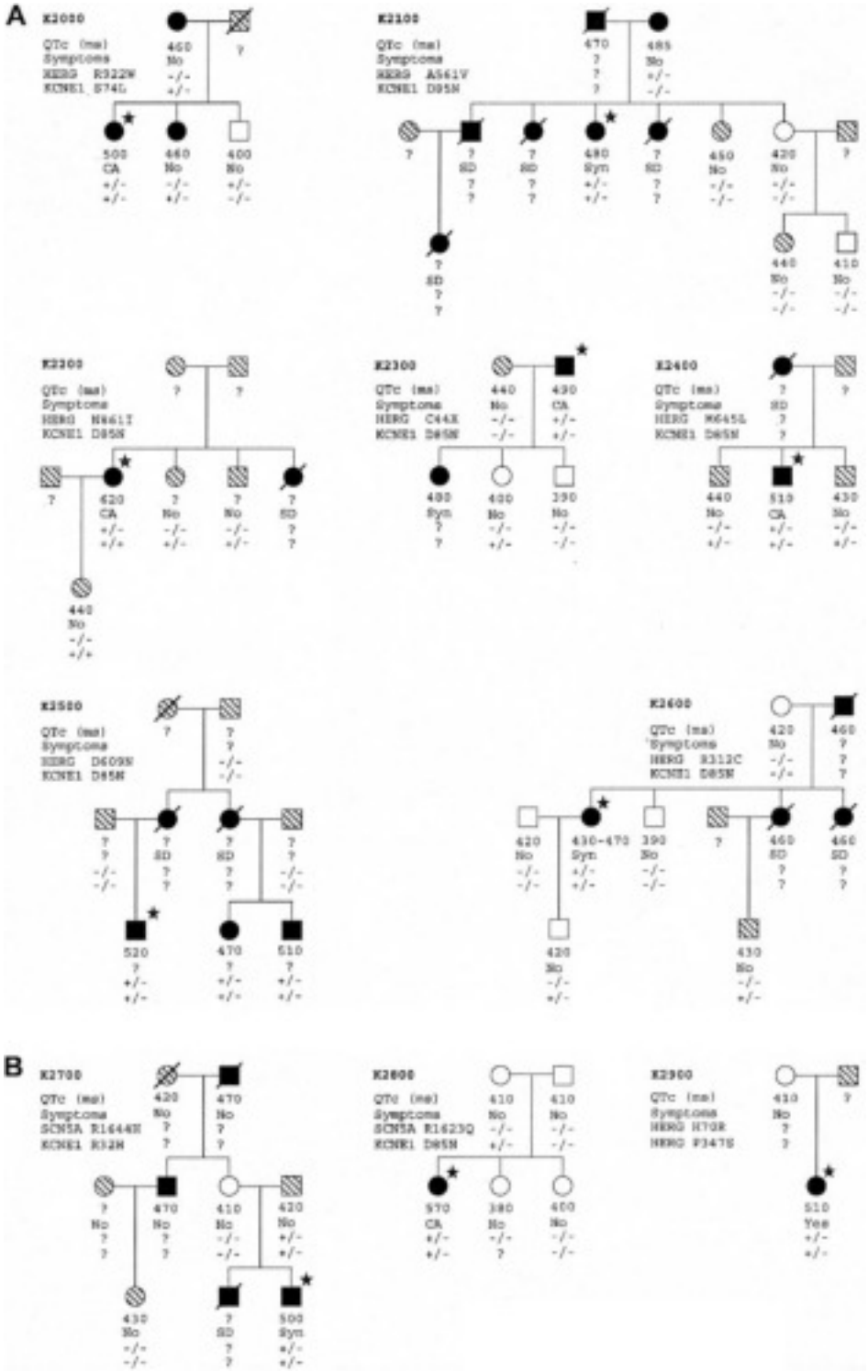

Figure 2. Pedigree structures and phenotypic and genotypic information for families with mutation in *HERG* or *SCN5A* and another LQT gene. A, Seven families with single mutations in *HERG* and *KCNE1*. B, Two families with a mutation in *SCN5A* and *KCNE1* and a proband with 2 mutations in *HERG*. Symbols and abbreviations as described in Figure 1.

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-

Downloaded from http://circ.ahajournals.org/ by guest on August 14, 2013

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 80 remaining probands. When probands with 2 mutations were compared with probands with 1 or no identified mutations, they had longer QTc (527±54 versus 489±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±53 versus 449±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±54 versus 491±42 ms; $P<0.005$) and a greater incidence of syncope and cardiac arrest (20 of 20 [100%] versus 77 of 113 [68%]; $P<0.005$).

In 10 probands, 1 of the 2 variants found was KCNE1 D85N (Figures 1 and 2). The variant was present in 22 of 462 control individuals and 15 of 252 affected individuals. In the other 5 probands, a second mutation was not found in our screen. However, 4 of these individuals exhibited syncope, 2 had cardiac arrest, and all had prolonged QTc (470 to 510 ms). It is likely that these 5 families harbor another mutation that has yet to be identified. Thus, D85N is present in ≈4.8% of the general population but is not overrepresented in LQTS individuals ($P=0.49$). All the mutations studied here were previously associated with LQTS.[6]

Two Mutations in *KVLQT1* Cause Incomplete Loss of I_{Ks} Function

Five probands harbored 2 mutations in *KVLQT1* (Figure 1A), but none were deaf. In K1000, K1100, K1300, and K1400, individuals carrying 2 mutant alleles had a more severe phenotype than individuals carrying 1. The proband in K1200 was adopted and phenotypic, and genotypic information on the parents of the proband was not available.

Voltage clamp experiments were performed to determine the functional effects of *KVLQT1* mutations. In K1000, T391I caused partial loss of function, whereas Q530X caused complete loss of KVLQT1 channel function (Figures 3A and 4A). In K1100, V310I caused partial loss, whereas R594Q caused complete loss of channel function (Figure 4B). In K1200, P448R is a polymorphism present in ≈20% of Asian individuals.[9] P448R caused a 2-fold increase in current (Figure 4C), whereas the mutation G314S caused complete loss of function. The homozygous KVLQT1 mutation (G179S) identified in K1400 caused partial loss of function when expressed alone (Figure 4D).

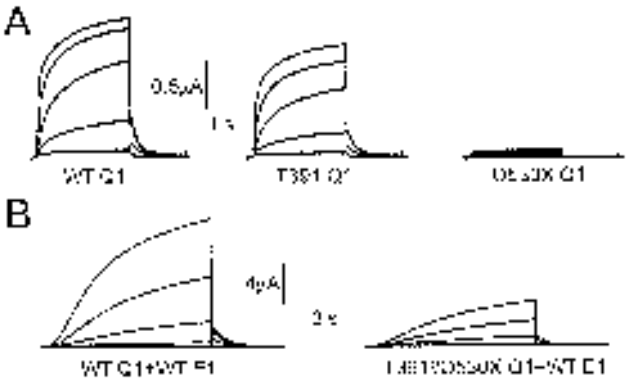

Figure 3. KVLQT1 and KVLQT1/KCNE1 channel currents recorded from voltage-clamped *Xenopus* oocytes. A, KVLQT1 (Q1) channel currents recorded in oocytes injected with 6 ng WT, T391I, or Q530X KVLQT1 cRNA. Currents were elicited with 3-second pulses applied in 20-mV increments to potentials ranging from −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 α-subunits with KCNE1 β-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 ≈15% 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 ≈60% relative to WT KVLQT1 (Figure 4B). However, it also appears that the V310I KVLQT1/WT

Downloaded from http://circ.ahajournals.org/ by guest on August 14, 2013

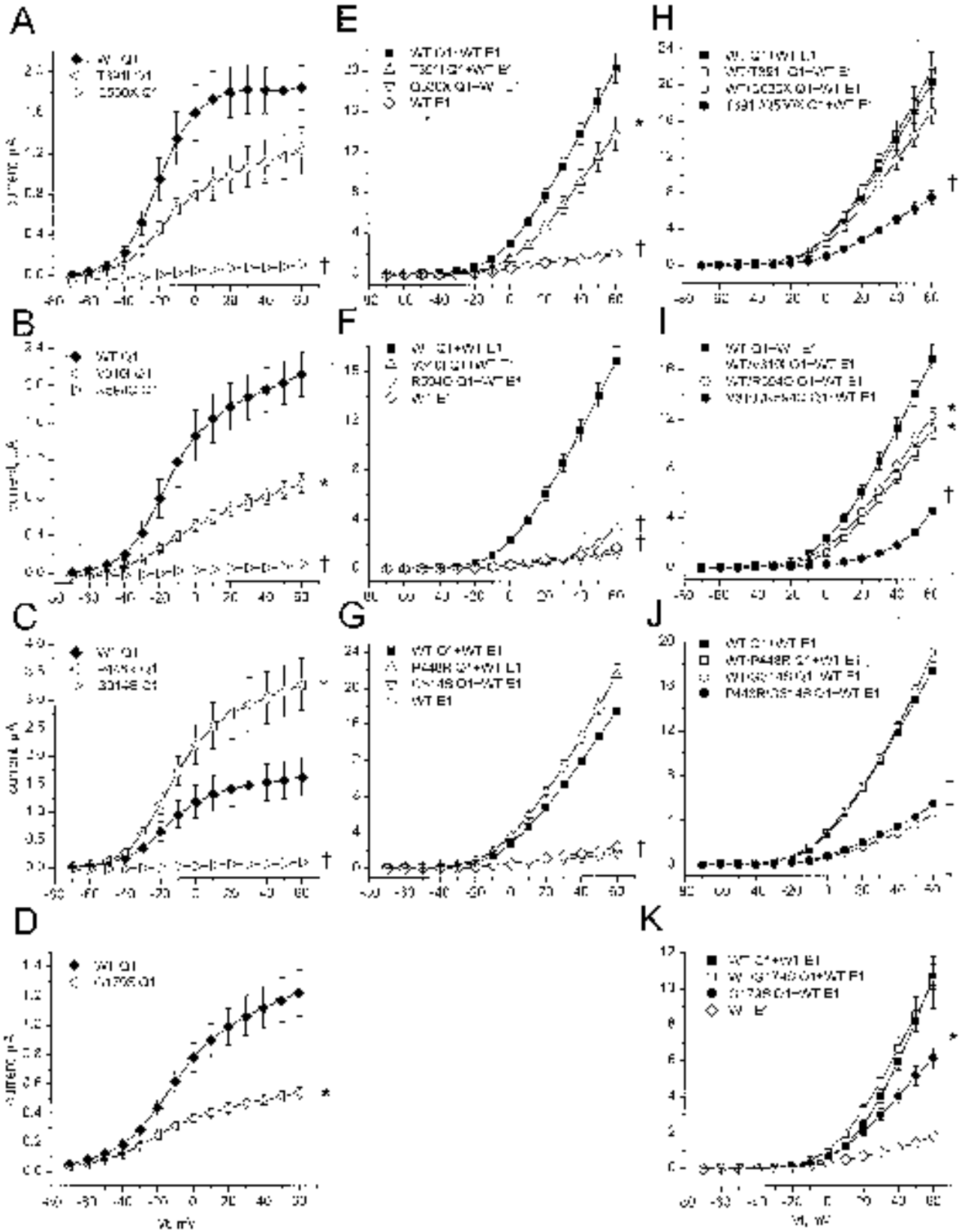

Figure 4. Functional consequences of single or double KVLQT1 mutations. A, B and C, Pair of KVLQT1 (Q1) mutations identified in K1000 (A), K1100 (B), or K1200 (C) were evaluated singly and compared with WT *KVLQT1* currents recorded from same batch of oocytes. Each oocyte was injected with 6 ng of single 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-

Downloaded from http://circ.ahajournals.org/ by guest on August 14, 2013

Biophysical Properties of WT and Mutant I_{Ks} Channels

(Kindred) Variants	Subunit Composition	τ Deactivation at −60 mV, ms	Activation, mV	
			$V_{1/2}$	k
(K1000)	WT Q1+WT E1	436±32*	23±1.7	16±0.3
T391I/Q530X KVLQT1	T391I/Q530X Q1+WT E1	511±10	24±0.9	15±0.6
(K1100)	WT Q1+WT E1	551±28	22±1.5	16±0.4
V310I/R594Q KVLQT1	V310I/R594Q Q1+WT E1	Not determined	>50†	Not determined
(K1200)	WT Q1+WT E1	514±19	23±0.8	17±0.5
G314S/P448R KVLQT1	G314S/P448R Q1+WT E1	503±10	30±3.1	19±0.8
(K1400)	WT Q1+WT E1	414±17	41±2.0	15±0.5
G179S KVLQT1	G179S+WT E1	551±26†	29±2.0†	16±0.4
(K1500)	WT Q1+WT E1	414±45	25±1.3	16±0.3
A314V KVLQT1 P127T KCNE1	WT/A314V Q1+WT/P127T E1	439±25	38±3.1‡	17±0.3‡
(K1600)	WT Q1+WT E1	574±34	33±2.8	18±0.8
G168R KVLQT1 D85N KCNE1	WT/G168R Q1+WT/D85N E1	575±23	46±3.7	22±1.2‡
(K1700)	WT Q1+WT E1	529±24	20±1.2	16±0.6
T312I KVLQT1 D85N KCNE1	WT/T312I Q1+WT/D85N E1	457±13‡	30±1.8†	17±0.6
(K2700)	WT Q1+WT E1	556±34	28±2.7	17±0.4
R32H KCNE1	WT Q1+WT/R32H E1	525±87	28±1.4	17±0.2

Q1 indicates KVLQT1; E1, KCNE1.
*Mean±SEM (n=8).
†$P<0.01$; ‡$P<0.05$.

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 compared with the individual who carried only G168R KVLQT1 (Figure 1B). The proband in K1700 carried 1 mutation in KVLQT1 (T312I) and KCNE1 D85N. No family members were available for phenotypic and genotypic analyses.

sured at end of 7-second pulses to indicated test potential (n=8 for each group). Oocytes were injected with 6 ng mutant *KVLQT1* cRNA plus 0.6 ng *KCNE1* cRNA or 0.6 ng *KCNE1* cRNA alone. H, I, and J, Current-voltage relationships for channels induced by coexpression of WT *KCNE1* subunits plus wilt-type and mutant or both mutant forms of *KVLQT1* channel subunits for K1000 (H), K1100 (I), and K1200 (J). WT I_{Ks} was replotted (filled squares) from right panels for ease of comparison (n=8 for each group). Oocytes were coinjected with 6 ng WT *KVLQT1* (or 3 ng of WT plus 3 ng mutant *KVLQT1*) cRNA plus 0.6 ng of *KCNE1* cRNA. K, Current-voltage relationship for G179S *KVLQT1* expressed with or without WT *KVLQT1* or *KCNE1*. †$P<0.001$, *$P<0.05$ vs WT I_{Ks}.

Downloaded from http://circ.ahajournals.org/ by guest on August 14, 2013

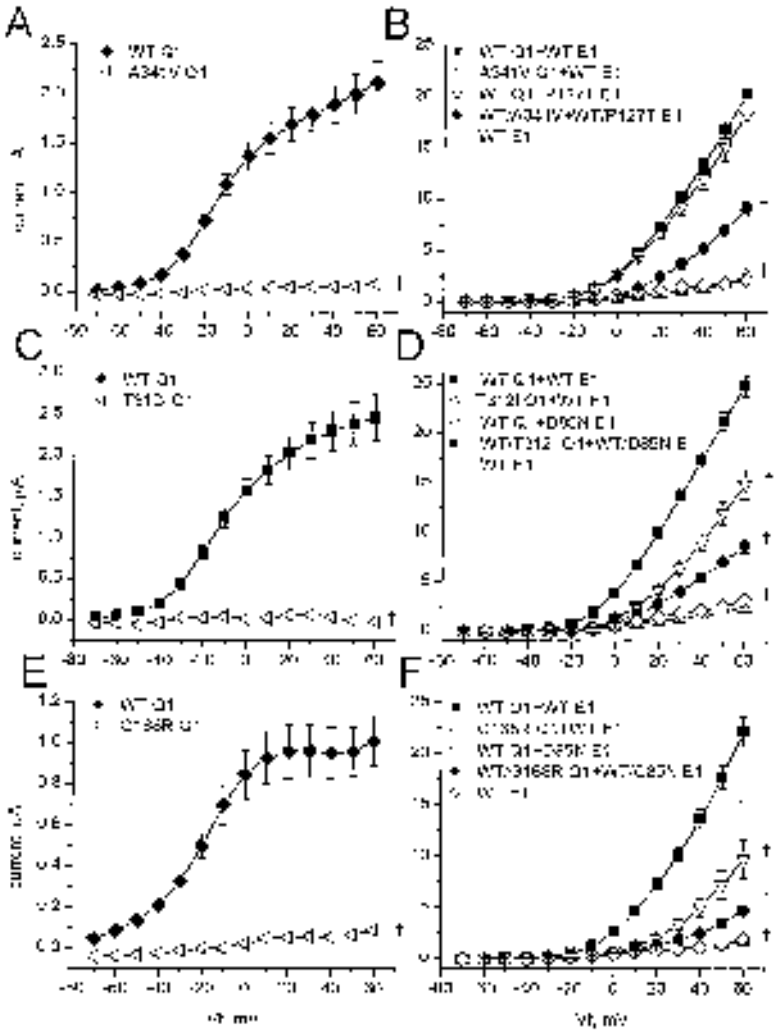

Figure 5. Functional consequences of single KVLQT1 plus single KCNE1 mutations. A, C, and E, Current-voltage relationships for WT or mutant KVLQT1 channels. Oocytes were injected with 6 ng of *KVLQT1* cRNA. B, D, and F, Current-voltage relationships in oocytes injected with WT or mutant KVLQT1 plus WT or mutant KCNE1 channel subunits. WT I_{Ks} is plotted as filled squares. Mutations were identified in K1500 (A, B), K1700 (C, D), and K1600 (E, F) (n=8 for each group). Oocytes were injected with a total of 6 ng *KVLQT1* cRNA and/or 0.6 ng *KCNE1* cRNA. †$P<0.001$ vs WT I_{Ks}.

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, filled circles), whereas only relatively minor effects were noted on the kinetics of current deactivation at −60 mV and the voltage dependence of activation (the Table). In the K1500 proband, P127T KCNE1 mutation had no functional effect, and the decrease in I_{Ks} was attributable solely to A341V KVLQT1. In contrast, the KVLQT1 mutants and the D85N KCNE1 variant both contribute to reduce I_{Ks} in the probands of K1600 and K1700.

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

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[5] 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 al[10]) 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 characterized[11] 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

Downloaded from http://circ.ahajournals.org/ by guest on August 14, 2013

WT KCNE1 and KVLQT1 and found that I_{Ks} had an amplitude equivalent to 78% of WT I_{Ks} ($P<0.05$; data not shown) without any changes in gating (the Table). Finally, we also identified 1 proband with 2 mutations in HERG and a QT_c 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.[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.[1] 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.[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[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.

Acknowledgments

This study was supported by grant HL-46401 and HL-52338 from the National Heart, Lung, and Blood Institute/National Institutes of Health. We thank Meng San Pun and Leah Sharpe for technical support.

References

1. Keating MT, Sanguinetti MC. Molecular and cellular mechanisms of cardiac arrhythmias. *Cell*. 2001;104:569–580.
2. Priori SG, Schwartz PJ, Napolitano C, et al. Risk stratification in the long-QT syndrome. *N Engl J Med*. 2003;348:1866–1874.
3. Vincent GM, Timothy KW, Leppert M, et al. The spectrum of symptoms and QT intervals in carriers of the gene for the long-QT syndrome. *N Engl J Med*. 1992;327:846–852.
4. Schwartz PJ, Priori SG, Napolitano C. Creative musings: how really rare are rare diseases? The intriguing case of independent compound mutations in the long QT syndrome. *J Cardiovasc Electrophysiol*. 2003;14:1120–1121.
5. Splawski I, Tristani-Firouzi M, Lehmann MH, et al. Mutations in the *hminK* gene cause long QT syndrome and suppress I_{Ks} function. *Nat Genet*. 1997;17:338–340.
6. 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.
7. Sanguinetti MC, Jiang C, Curran ME, et al. A mechanistic link between an inherited and an acquired cardiac arrhythmia: *HERG* encodes the I_{Kr} potassium channel. *Cell*. 1995;81:299–307.
8. Mitcheson JS, Chen J, Sanguinetti MC. Trapping of a methanesulfonanilide by closure of the HERG potassium channel activation gate. *J Gen Physiol*. 2000;115:229–240.
9. Yang P, Kanki H, Drolet B, et al. Allelic variants in long-QT disease genes in patients with drug-associated torsades de pointes. *Circulation*. 2002;105:1943–1948.
10. Makita N, Shirai N, Nagashima M, et al. A de novo missense mutation of human cardiac Na^+ channel exhibiting novel molecular mechanisms of long QT syndrome. *FEBS Lett*. 1998;423:5–9.
11. Wang DW, Yazawa K, George AL, et al. Characterization of human cardiac Na^+ channel mutations in the congenital long QT syndrome. *Proc Natl Acad Sci U S A*. 1996;93:13200–13205.
12. Chen J, Zou A, Splawski I, et al. Long QT syndrome-associated mutations in the Per-Arnt-Sim (PAS) domain of HERG potassium channels accelerate channel deactivation. *J Biol Chem*. 1999;274:10113–10118.
13. Splawski I, Timothy KW, Tateyama M, et al. Variant of SCN5A sodium channel implicated in risk of cardiac arrhythmia. *Science*. 2002;297:1333–1336.

Downloaded from http://circ.ahajournals.org/ by guest on August 14, 2013