

Novel LQT-3 Mutation Affects Na⁺ Channel Activity Through Interactions Between α - and β_1 -Subunits

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Abstract—The congenital long-QT syndrome (LQT), an inherited cardiac arrhythmia characterized in part by prolonged ventricular repolarization, has been linked to 5 loci, 4 of which have been shown to harbor genes that encode ion channels. Previously studied LQT-3 mutations of SCN5A (or hH1), the gene that encodes the human Na⁺ channel α -subunit, have been shown to encode voltage-gated Na⁺ channels that reopen during prolonged depolarization and hence directly contribute to the disease phenotype: delayed repolarization. Here, we report the functional consequences of a novel SCN5A mutation discovered in an extended LQT family. The mutation, a single A→G base substitution at nucleotide 5519 of the SCN5A cDNA, is expected to cause a nonconservative change from an aspartate to a glycine at position 1790 (D1790G) of the SCN5A gene product. We investigated ion channel activity in human embryonic kidney (HEK 293) cells transiently transfected with wild-type (hH1) or mutant (D1790G) cDNA alone or in combination with cDNA encoding the human Na⁺ channel β_1 -subunit (h β_1) using whole-cell patch-clamp procedures. Heteromeric channels formed by coexpression of α - and β_1 -subunits are affected: steady-state inactivation is shifted by -16 mV, but there is no D1790G-induced sustained inward current. This effect is independent of the β_1 -subunit isoform. We find no significant effect of D1790G on the biophysical properties of monomeric α - (hH1) channels. We conclude that the effects of the novel LQT-3 mutation on inactivation of heteromeric channels are due to D1790G-induced changes in α - and β_1 -interactions. (*Circ Res.* 1998;83:141-146.)

Key Words: long-QT syndrome ■ genetics ■ Na⁺ channel

The congenital long-QT syndrome (LQT) is an inherited cardiac arrhythmia that is defined in part by prolonged ventricular repolarization, an association with recurrent syncope, and a propensity to polymorphous ventricular tachycardia (torsade de pointes) and sudden death.^{1,2} LQT has been linked to 5 loci, 4 of which have been shown to harbor genes that encode ion channels.³⁻⁹ These discoveries have created the unique opportunity to develop molecular therapeutic approaches to disease management based on specific functional changes in the channel proteins encoded by mutant genes.¹⁰⁻¹⁵ This has been possible in part because all previously reported LQT ion channel mutations have been shown to cause functional changes in expressed channel activity that contribute directly to delayed ventricular repolarization.

SCN5A (or hH1) is the gene that encodes the human voltage-gated Na⁺ channel α -subunit.¹⁶ Recently, Benhorin et al¹⁷ reported a novel SCN5A mutation linked to LQT-3 in which there is a single A→G base substitution at nucleotide 5519 of the SCN5A cDNA.¹⁶ The mutation is expected to cause a nonconservative change from an aspartate to a glycine residue at position 1790 (D1790G) of the SCN5A gene product. The D1790G mutation was found in all patients (n=24) of an extended LQT family but not in >200 chromosomes carried by healthy individuals.¹⁷

Previously studied LQT-3 mutations of SCN5A have been shown to encode voltage-gated Na⁺ channels that fail to inactivate completely during prolonged depolarization and hence contribute directly to the disease phenotype: delayed repolarization.^{11,13,18,19} Because the position of glycine 1790 (D1790) is thought to be intracellular, near the C-terminus of the α -subunit, and not associated with major functional channel properties,²⁰ extrapolation to changes in the properties of encoded Na⁺ channels is not obvious. Here we report the functional consequences of the D1790G LQT-3 mutation as revealed by measuring ion channel activity in transiently transfected human embryonic kidney (HEK 293) cells. We find that the D1790G mutation has little effect on the biophysical properties of monomeric α - (hH1) channels, but it significantly affects the properties of heteromeric channels formed by coexpression of α - and β_1 -subunits. This result is important not only because it shows the functional consequences of a novel gene mutation linked to an inherited cardiac arrhythmia but also because it provides important insight into the physiological importance and key interaction residues for the human cardiac Na⁺ channel β_1 -subunit. Furthermore, the data suggest that therapeutic strategies designed to treat carriers of previously described LQT-3

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Summary of Effects of the D1790G Mutation and h β_1 Coexpression on Expressed Channel Activity

	hH1	hH1+h β_1	D1790G	D1790G+h β_1
Peak current density	-51.3±6.2 (n=14)	-63.8±6.7† (n=16)	-55.4±4.5* (n=16)	-52.8±5.0‡ (n=16)
Inactivation V _{1/2}	-70.2±1.3 (n=17)	-58.7±1.2† (n=16)	-73.1±0.9* (n=9)	-75.0±1.6§ (n=20)
Inactivation slope factor	6.3±0.3 (n=16)	7.8±0.3† (n=16)	5.8±0.2* (n=10)	4.9±0.2§ (n=20)

*P>0.05 vs hH1.

†P<0.01 vs hH1.

‡P>0.05 vs hH1+h β_1 .§P<0.01 vs hH1+h β_1 .

||P>0.05 vs D1790G.

mutations of SCN5A are not likely to be effective in treating carriers of the D1790G mutation of SCN5A.

Materials and Methods

HEK 293 cells (Cold Spring Harbor Laboratories) were grown under culture conditions and transfected by a lipofection procedure previously described by us.¹¹ hH1 cDNA was subcloned into the vector pcDNA3 (Invitrogen, Inc). The D1790G mutation was engineered with the QuickChange Site-Directed Mutagenesis Kit (Stratagene, Inc) and confirmed by sequence analysis. Plasmid cDNA for transfection was isolated by use of Qiagen (Plasmid Midi Kit) anion exchange column kits (Qiagen, Inc). Base-pair and deduced amino acid numbers are determined according to the Genebank sequence of SCN5A, accession number M77235. Wild-type cDNAs were gifts of Drs M. Keating (University of Utah School of Medicine), L. Isom (University of Michigan School of Medicine), P. Bennett (Vanderbilt University School of Medicine), and A. George (Vanderbilt University School of Medicine). Membrane currents were measured by the whole-cell application of the patch-clamp procedure²¹ and pulse protocols, and tests for control of Na⁺ channel currents were carried out as previously described.¹¹ Internal pipette solutions consisted of (mmol/L) cesium aspartate 60, Na-ATP 5, EGTA 11, HEPES 10, CaCl₂ 1, and MgCl₂ 1, with pH 7.2 adjusted with CsOH. External solutions (full Na) consisted of (mmol/L) CaCl₂ 2, MgCl₂ 1, NaCl 135, HEPES 10, and glucose 5, with pH 7.4 adjusted with HCl. HEK 293 cells are small (total membrane capacitance, ≤30 pF). However, to ensure adequate voltage control, in all experiments except those designed to measure sustained inward current during prolonged depolarization, extracellular Na⁺ was reduced to 30 mmol/L by substitution with *N*-methylglucamine. To quantify the voltage dependence of steady-state inactivation and activation, data from individual experiments were fitted with Boltzmann relationships, $y(V_m) = (A_1 - A_2) / (1 + \exp[(V_m - V_{1/2})/V_k])$, in which V_{1/2} is the voltage at which half the available channels are inactivated (or activated), V_k is the slope factor, y is the relative current, V_m is the membrane potential, and A₁ and A₂ are arbitrary constants. Averaged data for these parameters are reported in the Table. Steady-state inactivation curves shown in all figures were generated with the mean parameters from these fits (Table). Holding potentials were -90 mV unless otherwise noted.

Results

Because Na⁺ channels have been suggested to consist of α - (260-kDa) and β_1 - (36-kDa) subunits,²²⁻²⁴ we first tested for unique functional effects of the D1790G mutation in cells expressing heteromeric channels consisting of test α - (hH1, wild-type, D1790G) and human heart β_1 -subunits (h β_1).²⁵ Figure 1 shows functional consequences of the D1790G mutation that distinguish it from other previously described LQT-3 mutations, here illustrated by the SCN5A KPQ deletion mutant.⁷ Unlike previously described LQT-3 mutations of SCN5A,^{11,13,18,19} coexpression of h β_1 with D1790G encodes channels that do not conduct sustained inward

current with prolonged depolarization (Figure 1A). Figure 1A compares currents recorded during maintained depolarization from cells cotransfected with h β_1 plus D1790G cDNA (left) and h β_1 plus KPQ cDNA (right). The current traces in Figure 1A are shown at high gain to emphasize differences in small, sustained currents. Peak currents, too large to be resolved at this gain, were 1155 pA (D1849G) and 824 pA (KPQ). Thus, the sustained inward current through KPQ-h β_1 channels is on the same order of magnitude (2% to 4% of peak currents) as previously reported for monomeric KPQ channels.^{11,18} Despite similar expression levels of peak currents, maintained current could not be resolved for h β_1 -D1790G channels in this or in any of 15 other similar experiments.

In contrast, when compared with hH1-h β_1 channel activity, D1790G-h β_1 but not KPQ-h β_1 channels alter steady-state inactivation (Figure 1B). Figure 1B, which illustrates effects for steady-state inactivation measured with 100-ms prepulses (similar results were obtained with 500 ms but are not shown), compares inactivation curves measured for hH1-h β_1

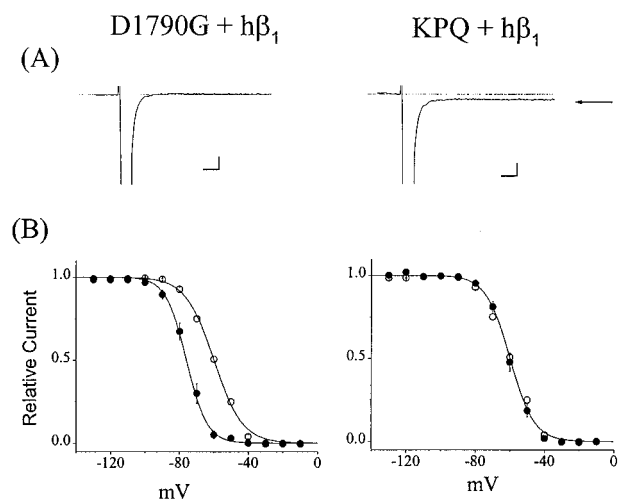


Figure 1. Heteromeric channels: unique functional consequences of the D1790G mutation. Currents recorded in cells cotransfected with h β_1 and either D1790G (left) or KPQ (right) cDNA. A, High-gain records (recorded at -10 mV) show sustained inward current for KPQ (arrow) but not D1849G α -subunits. Calibration: 2 ms, 50 pA. Peak currents in these experiments (off scale at this gain) were 1155 pA (D1790G) and 824 pA (KPQ). [Na⁺]_o = 135 mmol/L. B, Steady-state inactivation is negatively shifted for D1790G (left) but not KPQ (right) mutation. Plots: average (mean±SEM) normalized test-pulse current versus conditioning voltage. Left, ○, hH1+h β_1 , n=17; ●, D1790G+h β_1 , n=9; right, ○, hH1+h β_1 , n=17; ●, KPQ+h β_1 , n=7.

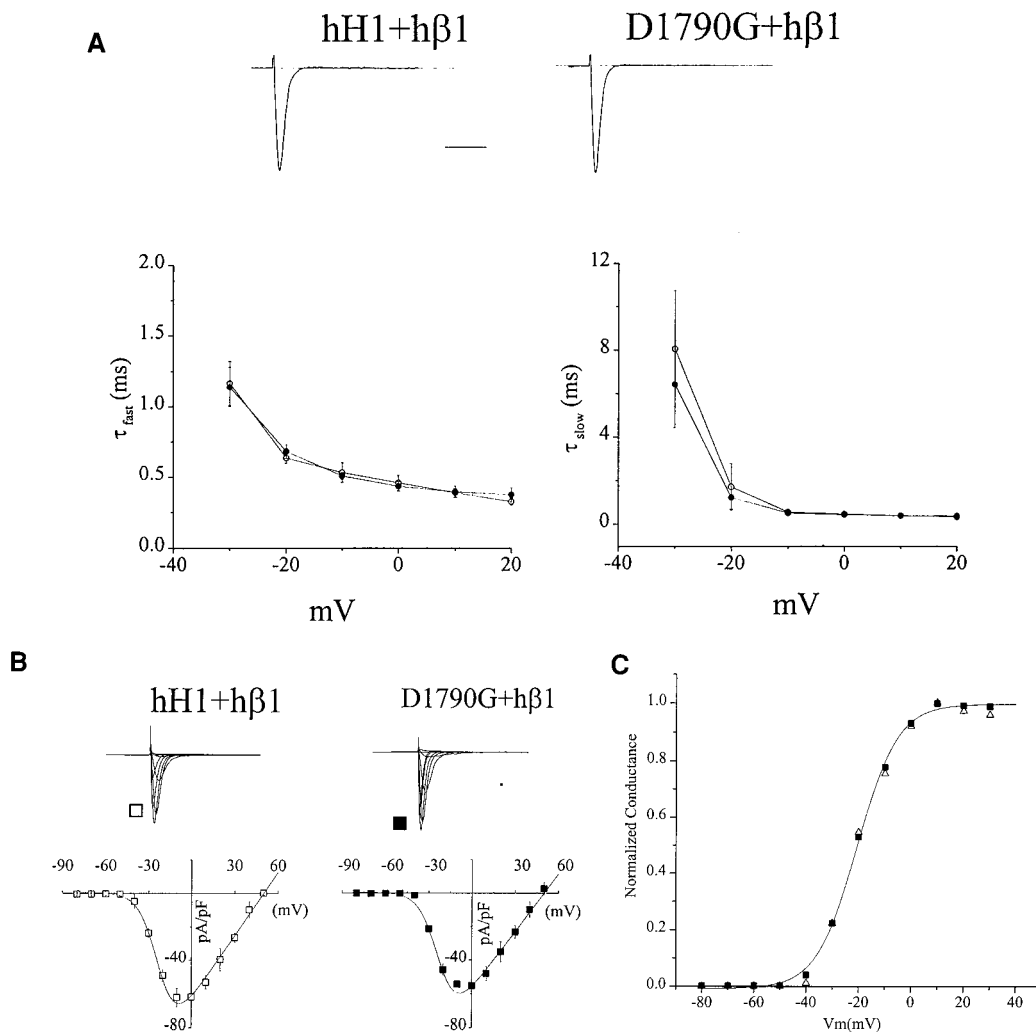


Figure 2. The D1790G mutation does not affect kinetics of onset of inactivation or voltage dependence of activation. **A**, Fast (left) and slow (right) time constants (mean \pm SEM) of onset of inactivation vs test-pulse voltage obtained by best fits of data to functions with 2 exponential components. Voltage pulses were chosen positive to threshold for Na channel activation only. Shown are results for hH1 + β ₁ (open symbols, n=6) and D1790G + β ₁ (solid symbols, n=6) channels. Traces: normalized currents recorded at -10 mV. Calibration: 5 ms. **B**, Peak current- (normalized to total cell capacitance) voltage relationship of hH1+h β ₁ (left, mean \pm SEM, n=16) and D1790G+h β ₁ (right, n=16) channels. Smooth curves are described below. **C**, Normalized mean conductance vs voltage relationships for D1890G+h β ₁ (Δ) and hH1+h β ₁ (\blacksquare). Data were obtained from mean data of Figure 2A with V_{rev} 50 mV. Smooth curve is best-fit Boltzmann relationship ($V_{1/2} = -22$ mV, slope factor = 6.6 mV) to data. Smooth curves in **A** were obtained by scaling this relationship by appropriate maximal conductances and driving forces.

channels (open circles in both panels), D1790G-h β ₁ channels (solid circles, left panel), and KPQ-h β ₁ channels (solid circles, right panel). The D1790G mutation alters both the half-maximal voltage and slope conductance of this relationship (Table).

We next compared the time course of inactivation of currents measured during depolarizing pulses for cells transfected with hH1 cDNA plus h β ₁ cDNA and D1790G cDNA plus h β ₁ cDNA. The results of these experiments are summarized in Figure 2A. Here, we analyzed the time course of currents in response to voltage pulses positive to -40 mV by fitting the measured currents at each test voltage with functions containing 2 exponential components. As shown in the figure, we found that the D1790G mutation does not affect inactivation kinetics over the voltage range studied. Similarly, the D1790G mutation does not affect the voltage

dependence of the peak of the current-voltage relationship or channel selectivity (judged by reversal potential) of heteromeric Na⁺ channels (Figure 2B). The D1790G mutation does not cause a significant difference between peak current densities of wild-type and D1790G heteromeric channels ($P > 0.05$, Table). Finally, we tested for an effect of the D1790G mutation on the voltage dependence of activation by normalizing the data presented in Figure 2A to driving force and peak conductance and then plotting the resulting normalized conductances versus test potential for wild-type and D1790G mutant heteromeric channels (Figure 2C). As shown in the figure, there is no effect of the D1790G mutation on the voltage dependence of activation. The smooth curves shown in Figure 2A were determined by scaling the normalized curves in Figure 2C by appropriate maximal conductances and driving forces.

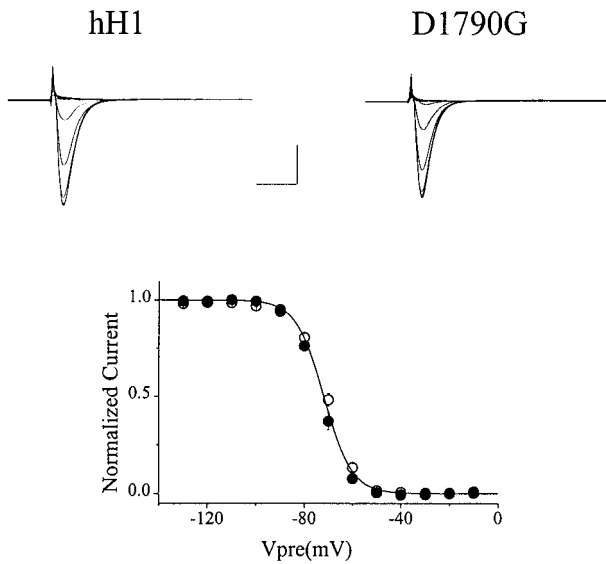


Figure 3. Insensitivity of steady-state inactivation of monomeric (α -subunit) Na⁺ channels to D1790G mutation. Bottom, Steady-state inactivation (mean \pm SEM; \circ , hH1, $n=17$; \bullet , D1790G, $n=9$). Curve: Boltzmann function ($V_{1/2} = -72$ mV; $V_h = 5.8$ mV). Top, Currents at -10 mV after conditioning pulses (100 ms, -130 to -10 mV, 10-mV increments). Calibration: 2 ms, 100 pA.

Because the location of the D1790G mutation is thought to be intracellular, near the C-terminus, and Makita et al²⁶ have shown that the carboxy-terminal half of the α -subunit may be important for coassembly of cardiac Na⁺ channel α - and β -subunits, we investigated the possibility that functional changes induced by the D1790G mutation in heteromeric channels might depend on subunit interactions. We thus compared the effects of the D1790G mutation on heteromeric (α and β) and monomeric (α) channels. As was the case for heteromeric channels, the D1790G mutation does not affect the kinetics of the onset of inactivation, the voltage dependence of activation, or reversal potential (data not shown). However, surprisingly, neither does the D1790G mutation affect the voltage dependence of steady-state inactivation (Figure 3) of monomeric channels. Thus, the D1790G-induced shift in inactivation (Figure 1B) of heteromeric channels depends on the presence of the β_1 -subunit.

Does the D1790G mutation affect α - and β_1 -interactions? To test this, we focused on steady-state inactivation and compared the effects of coexpression of h β_1 with both hH1 and D1790G α -subunits. Coexpression of h β_1 and hH1 causes a positive shift in $V_{1/2}$ and increases the slope factor of the

inactivation curve compared with expression of only hH1 subunits, and these effects are statistically significant (Table, Figure 4A). In contrast, although not statistically significant, coexpression of the β_1 -subunit with D1790G α -subunit, if anything, causes small opposite effects on steady-state inactivation: a slight positive shift in $V_{1/2}$ and a decrease in the slope (Figure 4B and Table).

Discussion

These results thus support the view that the D1790G mutation, discovered by linkage analysis and subsequent sequence analysis of LQT-3 in an extended LQT family,¹⁷ markedly affects the voltage dependence of inactivation by altering the interaction between the Na⁺ channel α - and β_1 -subunits. The fact that the mutation has little or no effect on other channel properties suggests that it causes specific changes in the interaction between the 2 Na⁺ channel subunits and not major conformational changes in the channel protein. Future experiments probing α - and β_1 -interaction domains should focus on this region of the cardiac Na⁺ channel α -subunit.

Experiments designed to identify the functional roles of the cardiac β_1 -subunit in other expression systems have produced inconsistent results. Some groups report no effects.^{25,27} Others report that coexpression of α - and β_1 -subunits enhances expression of Na⁺ channel currents with slight changes in gating kinetics and gating voltage dependencies^{28,29} and modifies expressed channel sensitivity to lidocaine block.³⁰ Our experiments show consistent and significant effects of coexpression of hH1 and h β_1 on steady-state inactivation (Table) that are not seen when h β_1 is coexpressed with D1790G in HEK 293 cells. We found that these effects were the same if we substituted the rat ($r\beta_1$)³¹ for the human (h β_1) isoform or if we measured inactivation in full (135 mmol/L) [Na⁺]_o (data not shown). It should be pointed out that the composition of native cardiac Na⁺ channels has not been firmly established, because message²⁸ for β_1 has been reported in heart but immunoprecipitation of β_1 protein has not been demonstrated.³²

Our results indicate that the D1790G mutation does not affect the voltage dependence of activation of heteromeric channels. Gating of cardiac Na⁺ channels differs from gating of neuronal Na⁺ channels in that evidence has been presented for voltage-dependent transitions from open to inactivated states for cardiac but not neuronal channels.³³⁻³⁶ In addition, for both cardiac and neuronal channels, multiple closed states precede transitions into the open state, and transitions from

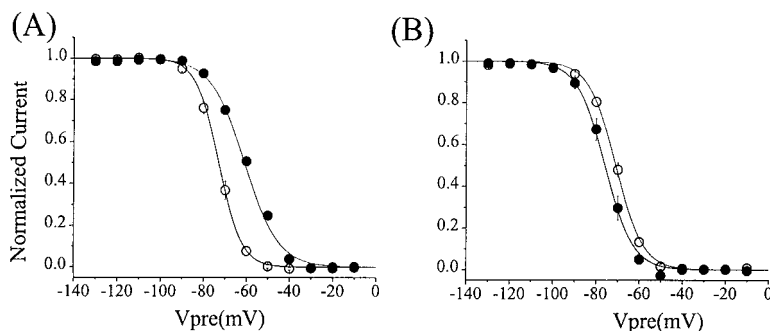


Figure 4. Effects of D1790G mutation on h β_1 -induced changes in steady-state inactivation. Steady-state inactivation determined as in Figures 1 and 3. Shown in plots are normalized inactivation curves (mean \pm SEM). A, hH1 (\circ , $n=17$); hH1+h β_1 (\bullet , $n=16$). B, D1790G (\circ , $n=10$); D1790G+h β_1 (\bullet , $n=20$). Parameters for smooth curves are summarized in the Table.

each of these closed states can occur directly into the inactivated state.³⁷ D1790G-induced alteration in the rate constants linking these early closed- to inactivated-state transitions or voltage-dependent transitions between open and inactivated states would be consistent with our experimental data. Furthermore, because our data strongly suggest that the D1790G mutation alters α - and β_1 -subunit interactions, our results imply that the β_1 -subunit specifically affects transitions between these states of the channel.

Unlike previously reported SCN5A mutations,^{7,8} we find that the D1790G mutation does not alter channel properties in a manner that would be expected to promote sustained inward Na⁺ channel current during the ventricular action potential. Hence, a causal link between the D1790G mutation and prolonged ventricular repolarization remains unclear. The facts that our data indicate that this mutation alters the interactions between Na⁺ channel α - and β_1 -subunits and that the subunit composition of native Na⁺ channels has not been firmly established add to the paradox of our results. Furthermore, it is possible that heterologous expression of the D1790G mutation with and without h β_1 in HEK 293 cells is not sufficient to express the phenotype of the mutation that is present in myocardial cells.

This new LQT-3 mutation (D1790G) was identified in a single large (n=131) family that originates in the isle of Jerba near Tunis.¹⁷ In this family, there have been 3 cases of sudden cardiac death (1 documented) and currently only a single symptomatic case (recurrent syncope) out of 26 mutation carriers. In addition, episodes of sinus arrest have been documented by Holter recording in 3 mutation carriers. Clinical and ECG data have been collected for 92 members of this family for >10 years, and blood samples for genetic analyses were available for 75 members. Strict ECG criteria for QT-interval prolongation, derived from a normal ECG database,³⁸ were used for phenotypic classification.³⁹ To accommodate uncertainty in phenotypic classification at intermediate QTc values, all family members were characterized according to 3 phenotypic subsets: affected, unaffected, and equivocal. To account for age and sex differences,² separate phenotypic definitions were used for 3 predefined demographic subsets: children (<16 years old), adult (>16 years old) men, and adult women. To account for QTc variability over time, each family member was classified according to his or her mean QTc value that was typically calculated from 4 to 6 QTc values measured in several ECGs recorded over several years. According to this phenotypic classification scheme, 40 family members were classified as unaffected, 23 as affected, and 12 as equivocal. Further genetic analysis¹⁷ identified the new D1790G mutation in all affected family members and in 3 members with an equivocal phenotypic status. The mutation was excluded in all unaffected members and in 300 unrelated control subjects. Hence, the mutation is clearly linked to the disease.

The D1790G inactivation shift in Figure 1B predicts that in ventricular cells in which resting potentials are expected to be near -90 mV (holding potentials in our experiments), the D1790G mutation will have little effect on channel availability. This prediction is supported by clinical data showing little change in impulse conduction (QRS duration) in carriers of

the D1790G mutation (J.B. et al, unpublished observations). Consequently, our results suggest that the D1790G mutation prolongs ventricular repolarization through an indirect effect on cardiac electrical activity. One possibility is that voltage-gated Na⁺ channels may participate in electrical activity in or near the sinoatrial node (SAN) and hence contribute to pacing, as has been suggested in several animal studies.⁴⁰⁻⁴² Chronic depolarization of the nodal area (SAN) compared with the ventricular cells would make cells of the SAN more susceptible to marked changes in D1790G-induced reduction in Na⁺ channel availability and hence pacing. Another possibility is that Na⁺ channels in sympathetic neurons innervating the sinus node may also express channels carrying the D1790G mutation, which in neuronal cells exhibiting repetitive activity would be expected to inhibit excitability and consequently sympathetic stimulation of the node. In either case, the subsequent reduction in heart rate would indirectly be expected to prolong ventricular repolarization. Interestingly, carriers of the D1790G mutation do in fact have low heart rates, and in some, sinus arrest has been documented (J.B. et al, unpublished observations). Confirmation of this possibility must await future experiments in which the effects of the D1790G mutation can be tested more directly in cells of the SAN region, perhaps in genetically altered murine models.

Finally, our results have important implications for strategies to treat LQT with a gene-specific approach. It is now clear that local anesthetic (lidocaine or mexiletine) treatment of LQT-3 in carriers of previously reported SCN5A mutations may prove to be a unique and specific therapeutic strategy to treat these gene defects.¹¹ The results of our investigation of the D1790G SCN5A mutation predict that such treatment will be ineffective in controlling this LQT-3 arrhythmia, because this mutation does not promote sustained inward current but instead causes a negative shift in steady-state inactivation. In fact, such treatment might even exacerbate rhythm disturbances in carriers of this gene defect by further shifting the Na⁺ channel inactivation curve.⁴³ Preliminary clinical data indicate that lidocaine does not correct QT prolongation in carriers of the D1790G mutation (J.B., unpublished observations). Thus, our results clearly show the importance of carrying out cellular functional studies before generalizing a molecular therapeutic approach to management of specific gene defects. Here we present examples of mutations in the same gene (SCN5A) linked to the same disorder (LQT-3) but for which distinct therapeutic strategies need to be developed.

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