

Drug-Induced Long-QT Syndrome Associated With a Subclinical SCN5A Mutation

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Background—Subclinical mutations in genes associated with the congenital long-QT syndromes (LQTS) have been suggested as a risk factor for drug-induced LQTS and accompanying life-threatening arrhythmias. Recent studies have identified genetic variants of the cardiac K⁺ channel genes predisposing affected individuals to acquired LQTS. We have identified a novel Na⁺ channel mutation in an individual who exhibited drug-induced LQTS.

Methods and Results—An elderly Japanese woman with documented QT prolongation and torsade de pointes during treatment with the prokinetic drug cisapride underwent mutational analysis of LQTS-related genes. A novel missense mutation (L1825P) was identified within the C-terminus region of the cardiac Na⁺ channel (*SCN5A*). The L1825P channel heterologously expressed in tsA-201 cells showed Na⁺ current with slow decay and a prominent tetrodotoxin-sensitive noninactivating component, similar to the gain-of-function phenotype most commonly observed for *SCN5A*-associated congenital LQTS (LQT3). In addition, L1825P exhibited loss of function Na⁺ channel features characteristic of Brugada syndrome. Peak Na⁺ current density observed in cells expressing L1825P was significantly diminished, and the voltage dependence of activation and inactivation was shifted toward more positive and negative potentials, respectively.

Conclusions—This study demonstrates that subclinical mutations in the LQTS-related gene *SCN5A* may predispose certain individuals to drug-induced cardiac arrhythmias. (*Circulation*. 2002;106:1269-1274.)

Key Words: long-QT syndrome ■ drugs ■ genetics ■ torsade de pointes ■ ion channels

Congenital long-QT syndrome (LQTS) is a rare inherited disorder of cardiac repolarization that predisposes affected individuals to life-threatening arrhythmias. The molecular basis of LQTS is the prolongation of action potential duration attributable to defects in several ion channel genes encoding delayed rectifier K⁺ currents (*I_K*) or Na⁺ current (*I_{Na}*).¹ The cardiac Na⁺ channel α subunit gene *SCN5A* is responsible for a subgroup of LQTS (LQT3),^{2,3} idiopathic ventricular fibrillation (IVF), or Brugada syndrome (BrS).^{4,5} The cellular mechanism for QT prolongation in most LQT3 is believed to be persistent Na⁺ current during the action potential plateau attributable to the defects in Na⁺ channel fast inactivation (gain of function) that delay repolarization.⁶ Pathophysiological mechanisms underlying BrS include diminished myocardial Na⁺ current (loss of function) and a resultant increase in transmural voltage gradient and ST-elevation on ECG.⁷

Acquired LQTS, on the other hand, is a more prevalent disorder and is often caused by several drugs that preferen-

tially block the rapid component of delayed rectifier K⁺ current (*I_{Kr}*).^{8,9} However, development of drug-induced LQTS is unpredictable and most likely occurs in a subset of susceptible individuals. It is therefore suggested that these individuals have a genetic substrate possibly involving cardiac ion channels that determine the susceptibility to life-threatening arrhythmias yet whose phenotype remains subclinical until drug exposure.

Recent studies indicate that drug-induced torsade de pointes (TdP) can be associated with silent mutations and common polymorphisms in K⁺ channel genes responsible for congenital LQTS.^{10–12} In this study we describe the first reported case of drug-induced LQTS associated with a novel *SCN5A* mutation (L1825P) and characterize its functional consequences. Despite the patient's normal QT interval before exposure to cisapride and the anticipated normal sodium channel function, the heterologously expressed Na⁺ channel mutant L1825P exhibited severe abnormalities characteristic of both LQT3 and IVF/BrS. Our study suggests that

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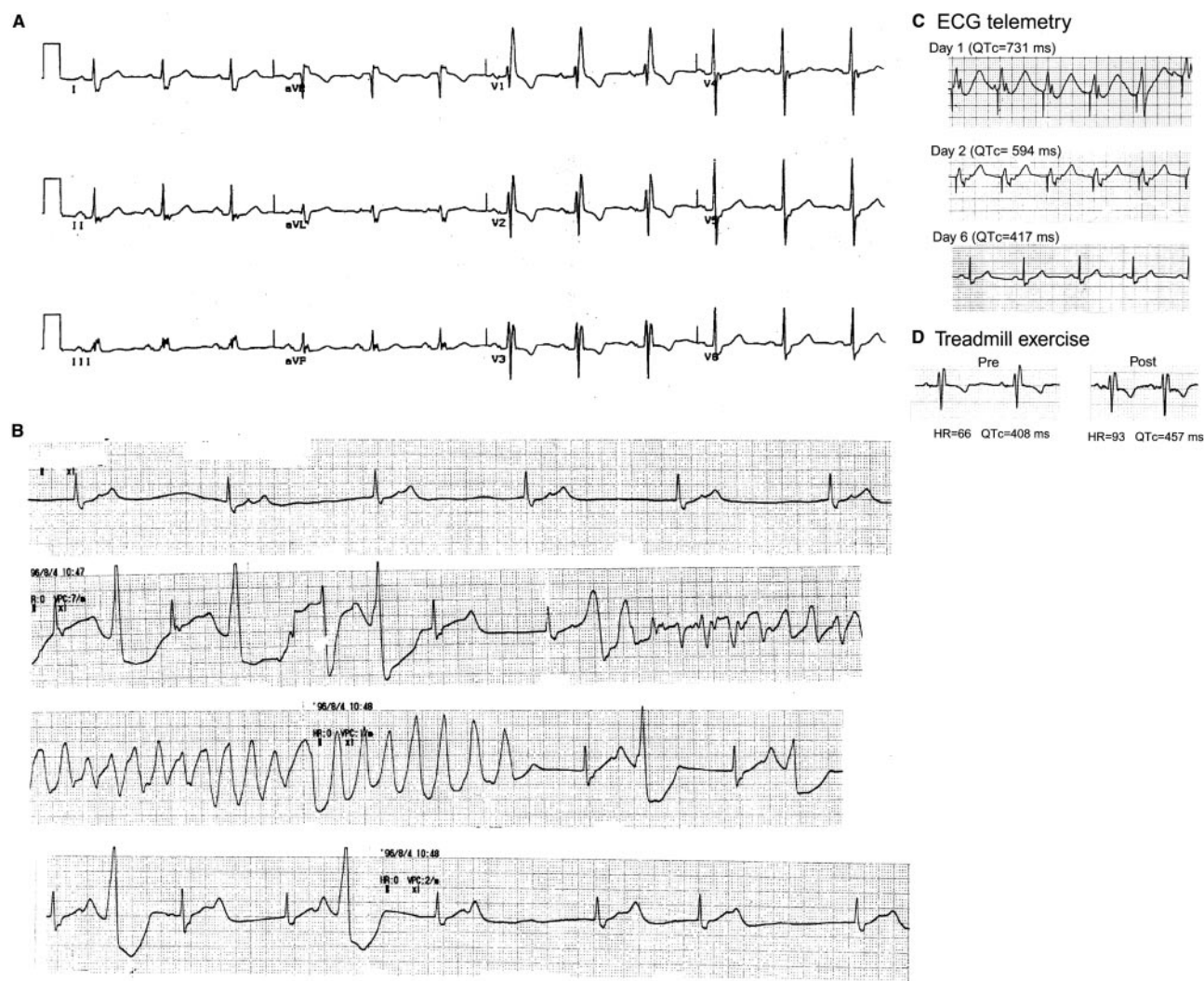


Figure 1. Electrocardiographic findings of the patient. A, ECG recording obtained from the proband during treatment with acebutolol and pirfenidone before prescription of cisapride. Normal sinus rhythm (heart rate, 73 bpm) with complete right bundle-branch block was evident, and the QT interval was within normal limits (QTc, 435 ms). B, ECG telemetry on admission showed severe bradycardia after prescription of cisapride (junctional rhythm, heart rate \approx 30 bpm) with QT prolongation (QTc, 480 ms) and repetitive TdP. C, Time course of QT interval. Emergency pacing was performed (pacing rate, 80 bpm) at the first day. Marked QT prolongation was observed (QTc, 731 ms). It was shortened the next day after withdrawal of drugs (QTc, 594 ms). QT interval was normalized at the sixth day (QTc, 417 ms). D, ECG recordings on V2 lead during treadmill exercise test before exercise and at the maximum exercise are shown. Heart rate was increased from 66 to 93 bpm, and the QTc was prolonged from 408 to 457 ms.

subclinical mutations or polymorphisms in *SCN5A* may predispose to life-threatening drug-induced arrhythmias.

Methods

Patient

A 70-year-old woman was admitted to the hospital because of recurrent syncope. She had been treated with acebutolol (200 mg/d) and pirfenidone (200 mg/d) for hypertension and frequent premature ventricular contractions, respectively. Her ECG while taking these medications exhibited complete right bundle branch block (CRBBB) and a normal QT interval (QTc, 435 ms, Figure 1A). Soon after the addition of cisapride (5 mg/d) because of bowel transit dysfunction, she experienced general malaise and syncope. ECG telemetry on admission showed severe bradycardia (junctional rhythm HR, 30 bpm) with prolonged QT interval (QTc, 480 ms) and repetitive torsade de pointes (TdP) (Figure 1B). Laboratory data, including serum electrolytes, were within normal limits. All medicines were

withdrawn, and emergency cardiac pacing was performed. QT prolongation was exaggerated by pacing (pacing rate, 80 bpm; QTc, 731 ms; Figure 1C), but the QT interval was substantially shortened the next day (QTc, 594 ms). The ECG taken after 6 days of drug-free condition, equivalent to the baseline ECG, showed normal sinus rhythm with CRBBB and normal QT interval (QTc, 417 ms); therefore, the temporary pacing was terminated. Oral administration of mexiletine (300 mg/d) did not additionally change the QT interval (data not shown). The QT interval prolonged during exercise stress testing, contrary to the QT shortening most commonly observed for LQT3 patients (Figure 1D). Rate-dependent ST elevation was not observed. Cardiac catheterization showed normal left ventricular function and normal coronary arteries.

The proband's paternal grandfather had sudden death of unknown cause, but the rest of her family members are asymptomatic. She has no offspring. Her sister showed normal QT interval (QTc, 440 ms) and was genetically unaffected (Figure 2A). The rest of her family members did not agree to additional examinations, including DNA diagnostics.

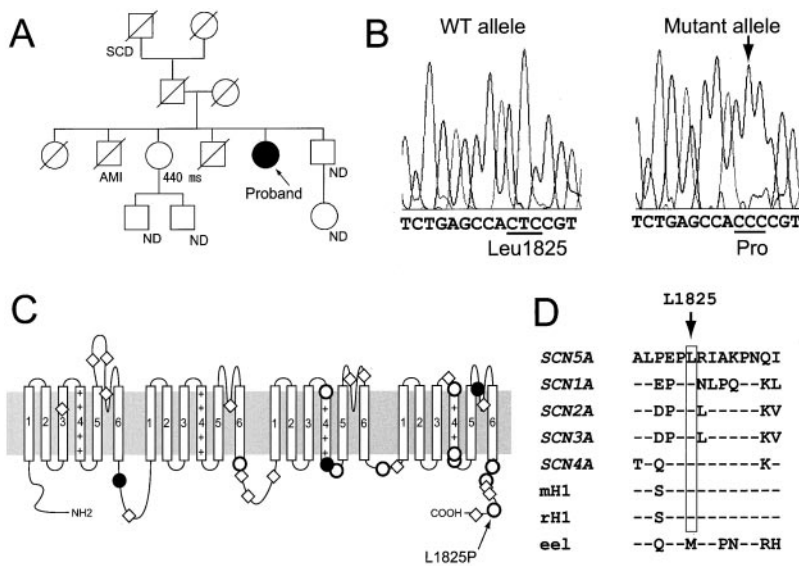


Figure 2. Pedigree, the results of DNA sequencing, and the location of the mutation. A, Pedigree of the cisapride-induced long-QT syndrome. ECG and DNA were only available from the proband and her sister. Her sister's QTc interval was 440 ms, and she was genetically unaffected. SCD and AMI denote sudden cardiac death and acute myocardial infarction, respectively. Genetic and electrocardiographic examinations were not carried out for the family members marked with ND. Arrow indicates the proband. B, DNA sequences of clones representing normal (left) and mutant alleles (right) are shown. The mutant clone had a T to C transition. C, Predicted topology of the cardiac Na⁺ channel is illustrated with location of known mutations associated with either LQTS (○) or IVF/BrS (◻) and cardiac conduction defect (●) indicated. D, Amino acid sequence alignments of *SCN5A* with related Na⁺ channel sequences are shown in the lower panel. *SCN5A* indicates human heart; *SCN1A*, human brain type I; *SCN2A*, human brain type II; *SCN3A*, human brain type III; *SCN4A*, human skeletal muscle; rH1, rat heart; mH1, mouse heart; and Eel, eel electroplax.

Molecular Genetics

Genomic DNA was extracted from peripheral blood leukocytes by a standard method. All exons of *SCN5A* were amplified by polymerase chain reaction (PCR) using primers designed by Wang et al¹³ and analyzed by single-strand conformational polymorphism (SSCP), as previously reported.⁵ Genetic screening of *KCNQ1*, *HERG*, *KCNE1*, and *KCNE2* was carried out as previously described.^{11,14} The PCR product showing an aberrant conformer was subcloned into pGEMTeasy (Promega), and multiple independent clones were sequenced using an ABI Prism 310 genetic analyzer (Applied Biosystems).

Site-Directed Mutagenesis and Electrophysiology

Wild-type (WT) human heart sodium channel α subunit (hH1) cDNA was subcloned into the pRcCMV plasmid (Invitrogen). Site-directed mutagenesis of hH1 was performed by an overlap-extension PCR strategy, as described.¹⁵ A 183-bp *HincII/SacII* fragment (No. 5290-5473 of hH1) of the mutant PCR fragment was subcloned back into the pRcCMV-WT plasmid to generate a mutant plasmid pRcCMV-L1825P. Correct assembly of the mutant was verified by restriction analysis, and the mutated regions generated by PCR were sequenced completely to identify clones without polymerase errors. The human cell line tsA-201 was transiently transfected with either pRcCMV-WT or pRcCMV-L1825P using standard calcium phosphate method in combination with a bicistronic plasmid (pCD8-IRES-h β_1) encoding CD8 and the human β_1 subunit (h β_1) to visually identify cells expressing heterologous h β_1 with Dynabeads M-450 CD8 (Dyna).

Na⁺ currents were recorded 24 to 72 hours after transfection using the whole-cell patch-clamp technique and analyzed, as we described elsewhere.¹⁶ The holding potential was -120 mV unless otherwise stated, and details of each pulse protocol are given schematically in the Figures and explained in the Results section. In some experiments, cells were superfused with bath solution containing either cisapride (0.01% in DMSO), pirlmenol, tetrodotoxin, or mexiletine to determine their pharmacological effects. Functional expression studies were performed on multiple independent recombinants. Results are presented as mean \pm SEM, and statistical comparisons were made using the unpaired Student's *t* test. Statistical significance was assumed for $P < 0.05$. Cisapride and pirlmenol were obtained from Welfide Co and Dainippon Pharmaceutical Inc, respectively.

Results

Molecular Genetics

Initial SSCP analysis was performed to screen for genetic variations in the LQTS-related K⁺ channel genes *KCNQ1*,

HERG, *KCNE1*, and *KCNE2*. Because aberrant conformers were not detected in these genes, we screened *SCN5A*. An aberrant SSCP conformer was identified in exon 28 of the proband. DNA sequencing confirmed a T to C transition leading to amino acid substitution of proline for leucine1825 (L1825P; Figure 2B) located within the C-terminal region of hH1 (Figure 2C). The amino acid residue corresponding to L1825 of *SCN5A* is highly conserved among different Na⁺ channel isoforms (Figure 2D). This sequence variation was not observed in 200 normal chromosomes, consistent with a disease-related mutation. Heterozygous state of the proband and the WT sequence of the proband's sister were confirmed by direct sequencing of the genomic DNA (data not shown).

Functional Analysis of the L1825P Mutation

Because the basal QT-interval of the patient was normal, it was expected that functional defects exhibited by the L1825P mutant channel, if any, might be minor when heterologously expressed in mammalian cells. Contrary to this assumption, the recombinant L1825P channel showed profound biophysical abnormalities. Figure 3A illustrates representative whole-cell current traces from cells expressing WT or L1825P Na⁺ channels in the presence of coexpressed h β_1 . Current density of L1825P channel measured at test pulse potential of -20 mV was approximately one third of WT (WT, 459 ± 99 pA/pF, $n = 13$; L1825P, 136 ± 21 pA/pF, $n = 12$; $P < 0.001$). L1825P showed a robust persistent late current and slower current decay. The amplitude of the persistent late current after 200 ms of depolarization at -20 mV was ≈ 8 -fold larger in L1825P (WT, $0.32 \pm 0.05\%$ of the peak current, $n = 15$; $2.56 \pm 0.27\%$, $n = 22$; $P < 0.001$), and it was almost completely abolished by either $30 \mu\text{mol/L}$ tetrodotoxin (Figure 3B) or $10 \mu\text{mol/L}$ mexiletine but was not affected by higher temperature at 34°C (data not shown). Macroscopic current decay fit with a biexponential function revealed that the fraction of slowly inactivating component (A_s) was significantly larger and the time constants for both fast and slow components (τ_f , τ_s) were significantly larger in L1825P relative to WT at all

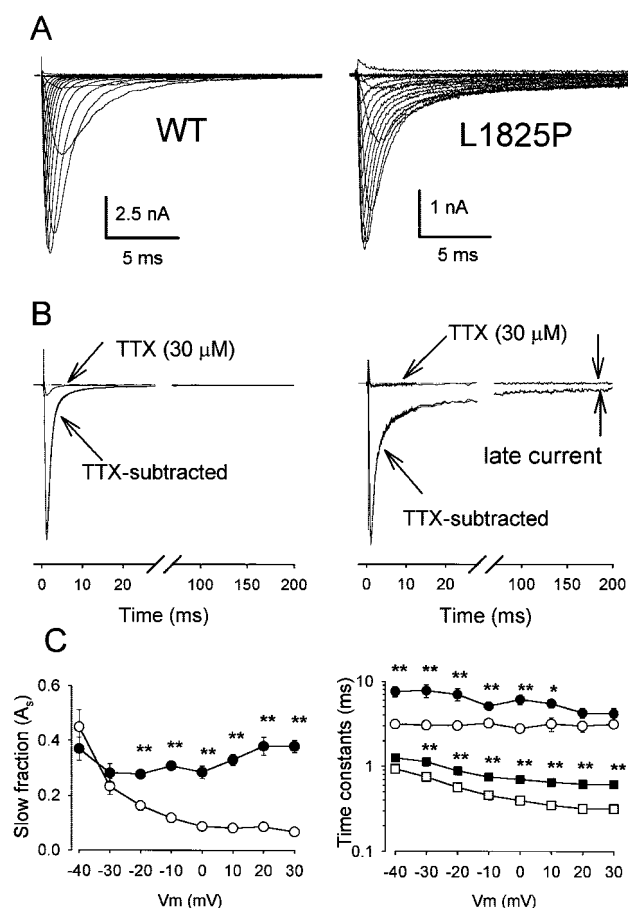


Figure 3. Macroscopic Na⁺ current of the mutant channel L1825P. **A**, Representative whole-cell current traces obtained from tsA-201 cells transfected with either WT or L1825P Na⁺ channels. Cells were cotransfected with human β₁ subunit. Currents were recorded from a holding potential of −120 mV and stepped from −90 mV to +90 mV during 20 ms in 10-mV increments. Currents were normalized and superimposed to illustrate differences in the time course of current decay. **B**, Na⁺ currents were recorded with test pulse potential −20 mV in the absence or presence of 30 μmol/L tetrodotoxin (TTX). TTX-sensitive current was calculated by digital subtraction. Note that prominent TTX-sensitive persistent current is observed in L1825P but not in WT. **C**, Time course of inactivation was fit with a two exponential function: $I/I_{\max} = A_0 + A_f \exp(-t/\tau_f) + A_s \exp(-t/\tau_s)$, where A_0 is a constant value, A_f and A_s are the fractions of fast and slow inactivating components, and τ_f and τ_s are the time constants of fast and slow inactivating components, respectively. Fraction of slow component (A_s) is shown in the top panel, and the fast time constants (τ_f , squares) and the slow time constant (τ_s , circles) are illustrated in the lower panel. Statistical significance in the difference between WT (open, n=13) and L1825P (closed, n=13) is shown (* $P < 0.05$; ** $P < 0.01$).

test pulse voltages between −40 and 30 mV (Figure 3C). These data suggest that the onset of inactivation was significantly slowed and was incomplete in L1825P.

The voltage dependence of steady-state inactivation of L1825P showed a significant hyperpolarizing shift ($V_{1/2}$: WT, -91.0 ± 1.3 mV, n=13; L1825P, -102.0 ± 1.1 mV, n=16; $P < 0.001$) (Figure 4A). Conversely, voltage dependence of activation of L1825P significantly shifted toward depolarizing direction ($V_{1/2}$: WT, -47.2 ± 1.1 mV; L1825P, -38.3 ± 0.74 mV; $P < 0.001$). Slope factors for both inactivation

and activation were significantly larger in L1825P (k_{inact} : WT, 6.93 ± 0.23 mV; L1825P, 8.78 ± 0.23 mV, $P < 0.001$; k_{act} : WT, -6.08 ± 0.26 mV; L1825P, -8.48 ± 0.16 mV; $P < 0.001$) (Figure 4A). Large negative shift of steady-state inactivation and positive shift of activation do not alter the point at which 2 curves cross; however, they do result in larger window current than WT, probably attributable to noninactivating Na⁺ channel even at higher prepulse potentials (Figure 4B). Recovery from inactivation was assessed by a standard double-pulse protocol using a recovery potential of −120 mV. Although the time constants of either fast or slow recovery components were comparable between WT (n=12) and L1825P (n=16) (τ_f : WT, 10.8 ± 2.0 ms; L1825P, 9.5 ± 0.4 ms; τ_s : WT, 154 ± 28 ms; L1825P, 181 ± 27 ms), the fraction of the slow component (A_s) was significantly larger in L1825P (WT, 0.16 ± 0.02 ; L1825P, 0.25 ± 0.02 ; $P < 0.01$), indicating that the recovery from inactivation was significantly slower in L1825P (Figure 4C). The severe abnormalities in activation and fast inactivation gating properties exhibited by L1825P will potentially result in greater reduction in Na⁺ channel availability during excitation.

In addition to the dysfunction of activation and fast inactivation, some mutant Na⁺ channels associated with IVF/BrS show defects in inactivation kinetics intermediate between fast and slow inactivation, referred to as I_M , which is now recognized as an important biophysical feature underlying certain Na⁺ channelopathies.¹⁷ We analyzed the onset of slow inactivation by prolonging the prepulses to from 1 ms to 10 s followed by a brief repolarization to allow channels to recover from fast inactivation before the test pulse. Distinct from the BrS mutation T1620 mol/L¹⁸ or the LQT3/BrS mutant channel 1795insD¹⁹ that have enhanced I_M , the time course of the onset of slow inactivation of L1825P was virtually identical to WT (Figure 4D).

Although L1825P channel shows slower open-state inactivation, a population of Na⁺ channels enters an inactivated state without channel opening by a mechanism called closed-state inactivation. Closed-state inactivation greatly affects the availability of the channels at voltages near the resting membrane potentials, thereby controlling the Na⁺ current amplitude of the action potential. The time course of the development of closed-state inactivation at −100 mV was significantly facilitated in L1825P (Figure 4E). These data suggest that a substantial fraction of L1825P channels are inactivated at voltages near the resting potential.

To test whether the I_{Kr} blocker cisapride has direct effects on Na⁺ channels, 1 μmol/L cisapride, a sufficient concentration to block I_{Kr} ,²⁰ was applied to the bath solution. However, cisapride did not change the peak Na⁺ current in either WT or L1825P channels (% control; WT, $99.8 \pm 3.6\%$, n=6; L1825P, $88.8 \pm 4.3\%$, n=8; NS). Cisapride failed to change the kinetics or the amplitude of the persistent Na⁺ current in either WT or L1825P, confirming that cisapride-induced QT prolongation in the proband was mediated through mechanisms other than direct effects on cardiac Na⁺ channels. We also examined the effect of the Na⁺ channel blocker pirlenol, which the proband had been actively taking by the time she developed TdP. However, 10 μmol/L pirlenol did not affect the persistent late current of L1825P, whereas it

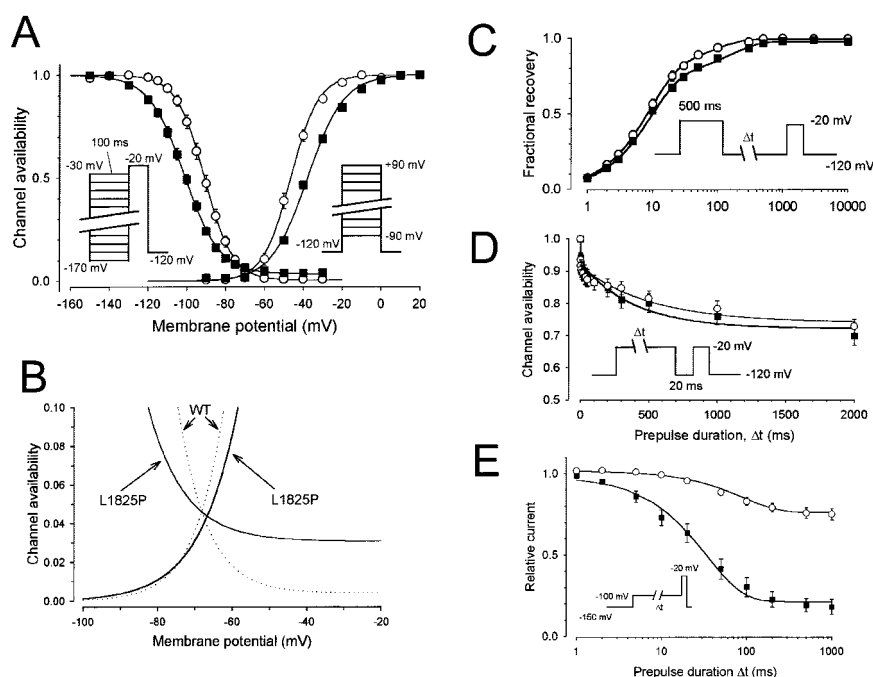


Figure 4. Gating properties of L1825P mutant channels. A, Steady-state availability for inactivation and conductance-voltage relationship were measured with standard pulse protocols, as shown in the inset. Curves were fit with the Boltzmann equation, $I/I_{\max} = [1 + \exp((V - V_{1/2})/k)]^{-1}$, to determine the membrane potential for half-maximal inactivation or activation ($V_{1/2}$) and the slope factor k . Data for WT and L1825P are shown with \circ and \blacksquare , respectively. B, Close-up of the window region. C, Recovery from inactivation was measured by a double-pulse protocol shown in the inset and analyzed by fitting data to two exponentials: $I/I_{\max} = A_{\text{fast}}[1 - \exp(-t/\tau_{\text{fast}})] + A_{\text{slow}}[1 - \exp(-t/\tau_{\text{slow}})]$. Data for WT and L1825P are shown with \circ and \blacksquare , respectively. D, Onset of slow inactivation was measured by a double-pulse protocol shown in the inset. A brief repolarization pulse was applied to allow most fraction of the channels recover from fast inactivation. Curve was well fit with a single exponential equation. Time courses of slow inactivation of WT (\circ) and L1825P (\blacksquare) were nearly identical. E, Closed-state inactivation was measured by a double-pulse

protocol in the inset. Cells were prepulsed to -100 mV for various durations from a holding potential of -20 mV to determine the availability of I_{Na} during the prepulse. Time course for development of closed-state inactivation were fit with the following monoexponential equation: $I/I_{\max} = y_0 + A \times \exp(-t/\tau)$. L1825P channels showed significantly larger extent of closed-state inactivation (WT, $A = 0.28 \pm 0.03$, $n = 20$; L1825P, $A = 0.80 \pm 0.03$, $n = 5$; $P < 0.001$) and smaller time constant (WT, $\tau = 96.4 \pm 9.9$ ms; L1825P, $\tau = 41.8 \pm 8.4$ ms; $P < 0.02$) than WT.

blocked the peak Na^+ current of L1825P to the extent comparable to WT (% control; WT, $95.7 \pm 4.0\%$, $n = 5$; L1825P, $86.4 \pm 3.7\%$, $n = 4$, NS). Therefore, it is unlikely that pirlmenol had been reducing persistent Na^+ current of L1825P, thereby protecting against QT prolongation before cisapride exposure.

Discussion

Drug-induced LQTS is a prevalent life-threatening disorder with uncertain etiologies but shares substantial clinical features with congenital LQTS. It has been inferred that a subset of individuals with normal or borderline QT-interval may carry subclinical mutations in LQTS disease genes and are susceptible to life-threatening arrhythmias on drug exposure.^{21,22} In fact, several lines of evidence have suggested genetic variations of LQTS-related K^+ channel genes.^{10–12} Our study suggests that Na^+ channel mutations can predispose apparently stable patients to life-threatening arrhythmias when treated with agents that inhibit K^+ channels.

Roden et al²³ hypothesized repolarization reserve as a potential mechanism underlying susceptibility to drug-induced LQTS. The cardiac action potential is orchestrated by a fine balance between inward and outward currents expressed in myocardial cells. The action potential duration is prolonged by either an increase of inward currents or a decrease of outward currents. Because the outward currents consist of multiple distinct K^+ currents, such as I_{to} , I_{Ks} , or I_{Kr} , K^+ channel dysfunction attributable to mutations or polymorphisms could be potentially compensated by other K^+ currents, and thereby the repolarization is tolerated (repolarization reserve). To extrapolate this hypothesis, it is assumed

that repolarization reserve allows the dysfunction of the L1825P mutation to be tolerated until the time of drug exposure. However, administration of cisapride blocked I_{Kr} and exhausted the repolarization reserve, leading to manifest the action potential prolongation and TdP. There are several lines of evidence supporting the existence of functional interplay between Na^+ and outward K^+ currents in the in vivo myocardium. Using a canine perfused ventricular wedge model, Shimizu et al²⁴ have shown that mexiletine reduced transmural dispersion of repolarization and prevented TdP in the LQT2 model as well as in LQT3. Moreover, K^+ channel opener nicorandil prevents TdP in congenital or acquired models of LQT1, LQT2, and LQT3.²⁵ The functional association between gain of function of I_{Na} and loss of function of I_{Kr} attributable to cisapride may be responsible for the manifestation of drug-induced QT prolongation in the present case.

Moreover, QT-prolonging drugs usually require multiple risk factors, such as hypokalemia, female sex, or slow heart rate to manifest life-threatening arrhythmias.¹ In the present case, bradycardia, presumably induced by acebutolol, was the additional risk factor to trigger QT prolongation and TdP. Another explanation is that the contribution to persistent Na^+ current of L1825P to the cardiac action potential duration may be relatively small, because the persistent current is offset by the concomitant loss of function phenotype (Figures 4A, 4D, and 4E). Alternatively, biophysical dysfunctions of the L1825P allele could be functionally compensated by the WT allele. Furthermore, manifestation of LQTS is variable and seems to be determined in genotype-specific, mutation-specific, or individual-specific manners, and up to 75% of the

gene carriers show normal QT interval.²⁶ It is speculated that the present case could be a congenital LQTS with an *SCN5A* mutation that exhibits reduced penetrance. Moreover, contribution of other genes, such as P450 3A4, a major metabolizing enzyme of cisapride, remains to be examined.

Mutations in *SCN5A* result in multiple arrhythmic syndromes, including LQT3,^{2,3} IVF/BrS,^{4,5} an inherited cardiac conduction defect,^{27,28} and sudden infant death syndrome,²⁹ constituting a spectrum of disease entities termed cardiac Na⁺ channelopathies.³⁰ Heterologously expressed L1825P channels exhibit biophysical properties strikingly similar to an *SCN5A* mutation 1795insD found in an unusual LQT3 family, in which affected individuals exhibited rate-dependent QT prolongation and ST elevation.³¹ The L1825P and 1795insD mutations, both located at the C-terminal of the Na⁺ channel, share the following biophysical properties: (1) prominent persistent late Na⁺ current; (2) negative shift of steady-state inactivation; and (3) decreased current density. These results suggest that LQT3 and IVF/BrS are closely related Na⁺ channelopathies with overlapping phenotypes and that L1825P is another example of a mutation that can explain this clinical overlap. Nonetheless, clinical manifestations of these 2 mutations are not identical. Exercise-induced ST elevation observed in 1795insD was not evident in L1825P despite the fact that both channels exhibited BrS-like properties. This phenotypic difference in ST elevation may be attributed to the difference in the kinetics of I_M, the physiological mechanism for the rate-dependent ST elevation in 1795insD,¹⁹ but it was not evident in L1825P.

In summary, we propose that subclinical mutations in the LQTS-related gene *SCN5A* may predispose the subset of individuals to life-threatening arrhythmias during drug therapy.

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References

- Keating MT, Sanguinetti MC. Molecular and cellular mechanisms of cardiac arrhythmias. *Cell*. 2001;104:569–580.
- Wang Q, Shen J, Splawski I, et al. *SCN5A* mutations associated with an inherited cardiac arrhythmia, long QT syndrome. *Cell*. 1995;80:805–811.
- 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.
- Chen Q, Kirsch GE, Zhang D, et al. Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. *Nature*. 1998;392:293–296.
- Akai J, Makita N, Sakurada H, et al. A novel *SCN5A* mutation associated with idiopathic ventricular fibrillation without typical ECG findings of Brugada syndrome. *FEBS Lett*. 2000;479:29–34.
- Bennett PB, Yazawa K, Makita N, et al. Molecular mechanism for an inherited cardiac arrhythmia. *Nature*. 1995;376:683–685.
- Naccarelli GV, Antzelevitch C. The Brugada syndrome: clinical, genetic, cellular, and molecular abnormalities. *Am J Med*. 2001;110:573–581.
- Roden DM, Lazzara R, Rosen M, et al. Multiple mechanisms in the long-QT syndrome: current knowledge, gaps, and future directions. The SADS Foundation Task Force on LQTS. *Circulation*. 1996;94:1996–2012.
- Mitcheson JS, Chen J, Lin M, et al. A structural basis for drug-induced long QT syndrome. *Proc Natl Acad Sci U S A*. 2000;97:12329–12333.
- Abbott GW, Sesti F, Splawski I, et al. MiRP1 forms I_{Kr} potassium channels with HERG and is associated with cardiac arrhythmia. *Cell*. 1999;97:175–187.
- Sesti F, Abbott GW, Wei J, et al. A common polymorphism associated with antibiotic-induced cardiac arrhythmia. *Proc Natl Acad Sci U S A*. 2000;97:10613–10618.
- Napolitano C, Schwartz PJ, Brown AM, et al. Evidence for a cardiac ion channel mutation underlying drug-induced QT prolongation and life-threatening arrhythmias. *J Cardiovasc Electrophysiol*. 2000;11:691–696.
- Wang Q, Li Z, Shen J, et al. Genomic organization of the human *SCN5A* gene encoding the cardiac sodium channel. *Genomics*. 1996;34:9–16.
- Splawski I, Shen J, Timothy KW, et al. Genomic structure of three long QT syndrome genes: KVLQT1, HERG, and KCNE1. *Genomics*. 1998;51:86–97.
- Makita N, Shirai N, Wang DW, et al. Cardiac Na⁺ channel dysfunction in the Brugada syndrome is aggravated by β₁ subunit. *Circulation*. 2000;101:54–60.
- Shirai N, Makita N, Sasaki K, et al. A mutant cardiac sodium channel with multiple biophysical defects associated with overlapping clinical features of Brugada syndrome and cardiac conduction disease. *Cardiovasc Res*. 2002;53:348–54.
- Balser JR. The cardiac sodium channel: gating function and molecular pharmacology. *J Mol Cell Cardiol*. 2001;33:599–613.
- Wang DW, Makita N, Kitabatake A, et al. Enhanced Na⁺ channel intermediate inactivation in Brugada syndrome. *Circ Res*. 2000;87:E37–E43.
- Veldkamp MW, Viswanathan PC, Bezzina C, et al. Two distinct congenital arrhythmias evoked by a multidysfunctional Na⁺ channel. *Circ Res*. 2000;86:E91–E97.
- Mohammad S, Zhou Z, Gong Q, et al. Blockage of the HERG human cardiac K⁺ channel by the gastrointestinal prokinetic agent cisapride. *Am J Physiol*. 1997;273:H2534–H2538.
- Moss AJ, Schwartz PJ. Delayed repolarization (QT or QTU prolongation) and malignant ventricular arrhythmias. *Mod Concepts Cardiovasc Dis*. 1982;51:85–90.
- Roden DM. Pharmacogenetics and drug-induced arrhythmias. *Cardiovasc Res*. 2001;50:224–231.
- Roden DM. Taking the “idio” out of “idiopathic”: predicting torsades de pointes. *Pacing Clin Electrophysiol*. 1998;21:1029–1034.
- Shimizu W, Antzelevitch C. Sodium channel block with mexiletine is effective in reducing dispersion of repolarization and preventing torsade des pointes in LQT2 and LQT3 models of the long-QT syndrome. *Circulation*. 1997;96:2038–2047.
- Shimizu W, Antzelevitch C. Effects of a K⁺ channel opener to reduce transmural dispersion of repolarization and prevent torsade de pointes in LQT1, LQT2, and LQT3 models of the long-QT syndrome. *Circulation*. 2000;102:706–712.
- Priori SG, Napolitano C, Schwartz PJ. Low penetrance in the long-QT syndrome: clinical impact. *Circulation*. 1999;99:529–533.
- Schott JJ, Alshinawi C, Kyndt F, et al. Cardiac conduction defects associate with mutations in *SCN5A*. *Nat Genet*. 1999;23:20–21.
- Tan HL, Bink-Boelkens MT, Bezzina CR, et al. A sodium-channel mutation causes isolated cardiac conduction disease. *Nature*. 2001;409:1043–1047.
- Ackerman MJ, Siu BL, Sturner WQ, et al. Postmortem molecular analysis of *SCN5A* defects in sudden infant death syndrome. *JAMA*. 2001;286:2264–2269.
- Grant AO. Molecular biology of sodium channels and their role in cardiac arrhythmias. *Am J Med*. 2001;110:296–305.
- Bezzina C, Veldkamp MW, van Den Berg MP, et al. A single Na⁺ channel mutation causing both long-QT and Brugada syndromes. *Circ Res*. 1999;85:1206–1213.