associated proteins, such as caveolin-3, dystrobrevin, and nitric oxide synthase 1,[24] are warranted.

Study Limitations

This study has several significant limitations : (1) the study population is small and linkage analysis could not be performed; (2) we could not perform functional studies on the KCNQ1 intronic variant, and therefore, we cannot exclude that this intronic variant or other unknown genes can be accountable for the patients' phenotypes although we examined and excluded all known LQTS genes; and (3) the data were obtained by in vitro experiments using cell lines expressing only the α- and β_1-subunit of the cardiac sodium channels or neonatal rat cardiomyocytes overexpressing human SNTA1, which is far different from the physiological conditions in the actual human heart. Although further studies are warranted to explore the links between our data and the phenotypes of the affected patients, our study does indicate that *SNTA1* now includes *ANKB, CAV3,* and *AKAP9* (yotiao) as "nonprimary channelopathic" mechanisms for LQTS.[2,3,5]

Conclusions

We identified a novel mutation of *SNTA1*, A257G, in 3 unrelated LQTS patients whose genetic screenings were negative for LQT1–9. Electrophysiological study suggests that biophysical modifications of the $Na_v1.5$ by A257G-SNTA1 can lead to "gain-of-function," through 3 mechanisms: (1) increase of the channel availability by leftward shift of activation kinetics; (2) delay of the current decay; and (3) increase in the current density. The molecular and electrophysiological evidences implicate A257G-SNTA1 in the pathogenesis of LQTS as a novel, albeit rare, LQTS-susceptibility gene. Although further studies are warranted to understand the detailed mechanisms leading to LQTS phenotypes in patients with the A257G-SNTA1, our study demonstrated that SNTA1, as an integral part of the DGC, plays an important role in regulating $hNa_v1.5$ function. Mutation in SNTA1 may not only account for the development of cardiac arrhythmias in affected patients but also potentially serve as novel target(s) of therapeutic interventions.

Acknowledgments

The authors are grateful to the patients and their family members who participated in this study. In addition, we are grateful to Jennifer L. Robinson, LQTS Study Coordinator for registry information assistance.

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Disclosures

None.

References

1. Shah M, Akar FG, Tomaselli GF. Molecular basis of arrhythmias. *Circulation*. 2005;112:2517–2529.
2. Saenen JB, Vrints CJ. Molecular aspects of the congenital and acquired long QT syndrome: clinical implications. *J Mol Cell Cardiol*. 2008;44:633–646.
3. Mohler PJ, Schott JJ, Gramolini AO, Dilly KW, Guatimosim S, duBell WH, Song LS, Haurogne K, Kyndt F, Ali ME, Rogers TB, Lederer WJ, Escande D, Le Marec H, Bennett V. Ankyrin-B mutation causes type 4 long-QT cardiac arrhythmia and sudden cardiac death. *Nature*. 2003;421:634–639.
4. Schwartz PJ. Management of long QT syndrome. *Nat Clin Pract Cardiovasc Med*. 2005;2:346–351.
5. Vatta M, Ackerman MJ, Ye B, Makielski JC, Ughanze EE, Taylor EW, Tester DJ, Balijepalli RC, Foell JD, Li Z, Kamp TJ, Towbin JA. Mutant caveolin-3 induces persistent late sodium current and is associated with long-QT syndrome. *Circulation*. 2006;114:2104–2112.
6. Cronk LB, Ye B, Kaku T, Tester DJ, Vatta M, Makielski JC, Ackerman MJ. Novel mechanism for sudden infant death syndrome: persistent late sodium current secondary to mutations in caveolin-3. *Heart Rhythm*. 2007;4:161–166.
7. Guglieri M, Magri F, Comi GP. Molecular etiopathogenesis of limb girdle muscular and congenital muscular dystrophies: boundaries and contiguities. *Clin Chim Acta*. 2005;361:54–79.
8. Chen L, Marquardt ML, Tester DJ, Sampson KJ, Ackermán MJ, Kass RS. Mutation of an A-kinase-anchoring protein causes long-QT syndrome. *Proc Natl Acad Sci U S A*. 2007;104:20990–20997.
9. Towbin JA. Cardiac arrhythmias: the genetic connection. *J Cardiac Electrophysiol*. 2000;11:601–602.
10. Adams ME, Butler MH, Dwyer TM, Peters MF, Murnane AA, Froehner SC. Two forms of mouse syntrophin, a 58 kd dystrophin-associated protein, differ in primary structure and tissue distribution. *Neuron*. 1993;11:531–540.
11. Adams ME, Dwyer TM, Dowler LL, White RA, Froehner SC. Mouse alpha 1- and beta 2-syntrophin gene structure, chromosome localization, and homology with a discs large domain. *J Biol Chem*. 1995;270:25859–25865.
12. Ahn AH, Yoshida M, Anderson MS, Feener CA, Selig S, Hagiwara Y, Ozawa E, Kunkel LM. Cloning of human basic A1, a distinct 59-kDa dystrophin-associated protein encoded on chromosome 8q23–24. *Proc Natl Acad Sci U S A*. 1994;91:4446–4450.
13. Yang B, Ibraghimov-Beskrovnaya O, Moomaw CR, Slaughter CA, Campbell KP. Heterogeneity of the 59-kDa dystrophin-associated protein revealed by cDNA cloning and expression. *J Biol Chem*. 1994;269:6040–6044.
14. Piluso G, Mirabella M, Ricci E, Belsito A, Abbondanza C, Servidei S, Puca AA, Tonali P, Puca GA, Nigro V. Gamma1- and gamma2-syntrophins, two novel dystrophin-binding proteins localized in neuronal cells. *J Biol Chem*. 2000;275:15851–15860.
15. Gee SH, Madhavan R, Levinson SR, Caldwell JH, Sealock R, Froehner SC. Interaction of muscle and brain sodium channels with multiple members of the syntrophin family of dystrophin-associated proteins. *J Neurosci*. 1998;18:128–137.
16. Ou Y, Strege P, Miller SM, Makielski J, Ackerman M, Gibbons SJ, Farrugia G. Syntrophin gamma 2 regulates SCN5A gating by a PDZ domain-mediated interaction. *J Biol Chem*. 2003;278:1915–1923.
17. Schwartz PJ, Moss AJ, Vincent GM, Crampton RS. Diagnostic criteria for the long QT syndrome: an update. *Circulation*. 1993;88:782–784.
18. Shuraih M, Ai T, Vatta M, Sohma Y, Merkle EM, Taylor EW, Li Z, Razavi M, Towbin JA, Cheng J. A common SCN5A variant alters its pharmacological responsiveness to class I antiarrhythmic agents. *J Cardiovasc Electrophysiol*. 2007;18:434–440.
19. Moss AJ, Zareba W, Benhorin J, Locati EH, Hall WJ, Robinson JL, Schwartz PJ, Towbin JA, Vincent GM, Lehmann MH. ECG T-wave patterns in genetically distinct forms of the hereditary long QT syndrome. *Circulation*. 1995;92:2929–2934.
20. Reese MG, Eeckman FH, Kulp D, Haussler D. Improved splice site detection in genie. *J Comp Biol*. 1997;4:311–323.
21. Gavillet B, Rougier JS, Domenighetti AA, Behar R, Boixel C, Ruchat P, Lehr HA, Pedrazzini T, Abriel H. Cardiac sodium channel Nav1.5 is

regulated by a multiprotein complex composed of syntrophins and dystrophin. *Circ Res*. 2006;99:407–414.
22. Wang DW, Yazawa K, George AL Jr, Bennett PB. Characterization of human cardiac Na$^+$ channel mutations in the congenital long QT syndrome. *Proc Natl Acad Sci U S A*. 1996;93:13200–13205.
23. Wehrens XH, Rossenbacker T, Jongbloed RJ, Gewillig M, Heidbuchel H, Doevendans PA, Vos MA, Wellens HJ, Kass RS. A novel mutation L619F in the cardiac Na$^+$ channel SCN5A associated with long-QT syndrome (LQT3): a role for the I-II linker in inactivation gating. *Hum Mutat*. 2003;21:552.
24. Aarnoudse AJ, Newton-Cheh C, de Bakker PI, Straus SM, Kors JA, Hofman A, Uitterlinden AG, Witteman JC, Stricker BH. Common NOS1AP variants are associated with a prolonged QTc interval in the Rotterdam Study. *Circulation*. 2007;116:10–16.

CLINICAL PERSPECTIVE

Long-QT syndrome (LQTS) is a primary arrhythmic disease often genetically determined by defects in major and ancillary subunits of cardiac ion channels. Ion channel mutations have been identified as the cause of LQTS since 1995, resulting in the definition of LQTS as a "cardiac channelopathy." To date, 10 LQTS-susceptibility genes have been discovered, accounting for approximately 75% of LQTS. Ion channels require membrane stability for proper activity, and cytoskeletal proteins acting as channel-interacting proteins represent a natural target for mutations hampering the ion channel function. In the present study, we provide evidence that the SNTA-encoded cytoskeletal protein, α-1-syntrophin (SNTA1), constitutes a novel LQTS-susceptibility gene. SNTA1, an abundantly expressed syntrophin subtype in human hearts, belongs to the dystrophin glycoprotein complex (DGC), which plays an important role in regulation of cardiac sodium channels (hNav1.5). Subsequently, we show that mutant SNTA alters hNav1.5 kinetics and confers a gain-of-function phenotype on the structurally intact sodium channel. Our findings still support LQTS as a "cardiac channelopathy," but shed new light on the critical roles of cytoskeletal proteins such as SNTA in modulating ion channel function. This observation not only suggests that other DGC factors may represent possible candidate to be considered for the remnant of LQTS (25%), but it also stresses the importance of the sarcolemma and subsarcolemmal proteins as an important functional intermediate for ion channel activity. In addition, these observations suggest that treatments aimed at scaffolding proteins rather than specific ion channels may represent an alternative antiarrhythmic strategy.

Malignant Perinatal Variant of Long-QT Syndrome Caused by a Profoundly Dysfunctional Cardiac Sodium Channel

Dao W. Wang, MD, PhD; Lia Crotti, MD, PhD; Wataru Shimizu, MD, PhD; Matteo Pedrazzini, BSc; Francesco Cantu, MD; Paolo De Filippo, MD; Kanako Kishiki, MD; Aya Miyazaki, MD; Tomoaki Ikeda, MD, PhD; Peter J. Schwartz, MD; Alfred L. George Jr, MD

Background—Inherited cardiac arrhythmia susceptibility contributes to sudden death during infancy and may contribute to perinatal and neonatal mortality, but the molecular basis of this risk and the relationship to genetic disorders presenting later in life is unclear. We studied the functional and pharmacological properties of a novel de novo cardiac sodium channel gene (*SCN5A*) mutation associated with an extremely severe perinatal presentation of long-QT syndrome in unrelated probands of different ethnicity.

Methods and Results—Two subjects exhibiting severe fetal and perinatal ventricular arrhythmias were screened for *SCN5A* mutations, and the functional properties of a novel missense mutation (G1631D) were determined by whole-cell patch clamp recording. In vitro electrophysiological studies revealed a profound defect in sodium channel function characterized by ≈10-fold slowing of inactivation, increased persistent current, slowing of recovery from inactivation, and depolarized voltage dependence of activation and inactivation. Single-channel recordings demonstrated increased frequency of late openings, prolonged mean open time, and increased latency to first opening for the mutant. Subjects carrying this mutation responded clinically to the combination of mexiletine with propranolol and survived. Pharmacologically, the mutant exhibited 2-fold greater tonic and use-dependent mexiletine block than wild-type channels. The mutant also exhibited enhanced tonic (2.4-fold) and use-dependent block (≈5-fold) by propranolol, and we observed additive effects of the 2 drugs on the mutant.

Conclusions—Our study demonstrates the molecular basis for a malignant perinatal presentation of long-QT syndrome, illustrates novel functional and pharmacological properties of *SCN5A*-G1631D, which caused the disorder, and reveals therapeutic benefits of propranolol block of mutant sodium channels in this setting. **(*Circ Arrhythmia Electrophysiol.* 2008;1:370-378.)**

Key Words: antiarrhythmia agents ■ arrhythmia ■ death, sudden ■ heart arrest ■ ion channels

Sudden unexplained death attributable to cardiac arrhythmia may occur at any age. When death occurs during infancy for no apparent reason, a diagnosis of the sudden infant death syndrome (SIDS) may be appropriate.[1,2] Recent evidence suggests that 9% to 10% of SIDS victims carry germ line mutations in arrhythmia susceptibility genes such as those associated with the congenital long-QT syndrome (LQTS).[3] Anecdotally, ventricular arrhythmias occurring during the perinatal or neonatal periods are associated with a poor prognosis and a low survival rate.[4–7] Whether cardiac arrhythmia susceptibility presenting in early life represents a biologically distinct disease is an unanswered question.

Clinical Perspective see p 378

Mutations in *SCN5A* encoding the cardiac voltage-gated sodium channel $Na_V1.5$ have been associated with a spectrum of increased sudden death risk extending from fetal life to adulthood. Recurrent third trimester fetal loss has been observed in the setting of occult *SCN5A* mutations.[8] In older children and adults with LQTS of known genotype, only ≈10% carry mutations in *SCN5A*,[9–11] but the proportion of *SCN5A* mutations among SIDS victims with an LQTS gene defect approaches 50%.[3] Further, among older children and adults with LQTS those individuals harboring *SCN5A* mutations exhibit a greater likelihood of severe symptoms including sudden death when compared with the majority of

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From the Department of Medicine (D.W.W., A.L.G.), Vanderbilt University, Nashville, Tenn; Section of Cardiology (L.C., P.J.S.), Department of Lung, Blood and Heart, University of Pavia; Department of Cardiology (L.C., P.J.S.) and Molecular Cardiology Laboratory (L.C., M.P., P.J.S.), IRCCS Fondazione Policlinico S. Matteo, Pavia, Italy; Division of Cardiology (W.S.), Department of Internal Medicine, Department of Pediatric Cardiology (K.K., A.M.), Department of Perinatology (T.I.), National Cardiovascular Center, Osaka, Japan; Department of Cardiology (F.C., P.D.F.), Ospedali Riuniti, Bergamo, Italy; Department of Pharmacology (A.L.G.), Vanderbilt University, Nashville, Tenn.

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Correspondence to Alfred L. George Jr, MD, Division of Genetic Medicine, 529 Light Hall, Vanderbilt University, Nashville TN 37232-0275. E-mail al.george@vanderbilt.edu

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individuals who carry mutations in 2 potassium channel genes (*KCNQ1*, *KCNH2*).[9,11] The higher proportion of *SCN5A* mutations among SIDS victims with known genotype when compared with older LQTS subjects might be explained by negative selection for more deleterious alleles. Support for this hypothesis requires evidence that mutations with greater functional consequences are responsible for severe and earlier onset arrhythmia syndromes.

Here, we present an extensive characterization of a novel *SCN5A* mutation that occurred de novo in unrelated and ethnically distinct newborns. In mutation carriers, life-threatening ventricular arrhythmias occurred within hours of birth. The mutation caused a profound degree of sodium channel dysfunction that was more severe than that observed for any previous *SCN5A* variant. Despite the extreme nature of the mutation and the associated dire clinical scenario, the subjects survived owing to prompt therapeutic interventions including treatment with the combination of mexiletine and propranolol, 2 drugs that exhibited enhanced and additive activity against the mutant allele. These observations illustrate the role of severe sodium channel mutations in a malignant perinatal variant of LQTS and successful use of combination pharmacotherapy to prevent perinatal mortality in this setting.

Methods

Molecular Genetics

Informed consent for performing genetic studies was obtained using methods approved by the Ethics Review Board of IRCCS Fondazione Policlinico San Matteo (Pavia, Italy) or by the Institutional Research Board and Ethics Committee and the Committee on Genetic Analysis and Gene Therapy of the National Cardiovascular Center (Suita, Japan). Genomic DNA was isolated from whole blood and coding exons of *SCN5A*, *KCNQ1*, *KCNH2*, *KCNE1*, and *KCNE2* were screened for genetic variants using previously described methods.[12,13]

Mutagenesis and Heterologous Expression of Na Channels

Mutations were engineered in a human heart sodium channel ($Na_V1.5$) cDNA (hH1) using recombinant polymerase chain reaction. Final constructs were assembled in the mammalian expression plasmid pRc/CMV-hH1 and then sequenced to verify creation of the mutation and to exclude polymerase errors. Cells (tsA201) were transiently transfected with pRc/CMV-hH1 or mutants using FuGene6 (Roche Diagnostics) combined with a bicistronic plasmid (pEGFP-IRES-hβ1) encoding enhanced green fluorescent protein and the human β1 subunit (hβ1) under the control of the cytomegalovirus immediate early promoter. Additional methods are provided in an online supplement.

Statement of Responsibility

The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Malignant Perinatal Arrhythmia Associated With a Novel *SCN5A* Mutation

We identified a novel *SCN5A* mutation in 2 unrelated newborns that experienced life-threatening perinatal ventricular arrhythmias. The first subject was an Italian male

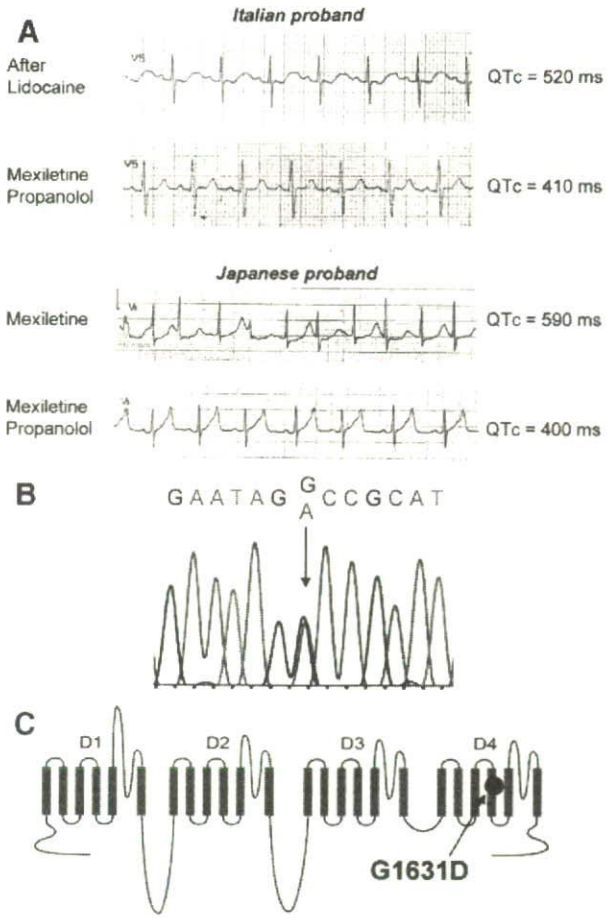

Figure 1. Electrocardiographic responses to pharmacotherapy and genotype of probands. A, Representative ECG traces (lead V5) showing responses to mexiletine and propranolol for the Italian and Japanese probands. Rate-corrected QT interval (QTc) measurements are indicated to the right of each tracing. B, Sequencing electropherogram (Italian proband) illustrating heterozygosity for a G to A mutation corresponding to G1631D. C, Location of G1631D in the predicted transmembrane topology of $Na_V1.5$.

delivered by emergency C-section at 32-weeks gestation for abnormal fetal heart rhythm. Initially, he appeared healthy (APGAR score 8) but then, within hours of his birth, developed polymorphic ventricular tachycardia with periods of bradycardia and frequent premature ventricular beats. Initial treatments with intravenous magnesium and isoproterenol were not effective, but administration of intravenous lidocaine suppressed ventricular arrhythmias and restored sinus rhythm revealing a prolonged QTc interval (520 ms). Empirical treatment with propranolol (1.3 mg/kg/d) and mexiletine (11 mg/kg/d) controlled arrhythmias and normalized the QTc (410 ms) (Figure 1A). One month after discharge, the infant survived an episode of ventricular fibrillation. Ventricular arrhythmia was further controlled by rapid pacing (120 bpm) with increased dosages of propranolol (3 mg/kg/d) and mexiletine (16 mg/kg/d). During the following 12 months, the child exhibited no further ventricular arrhythmias but required recurrent hospitalizations for paroxysmal atrial flutter that was eventually controlled by

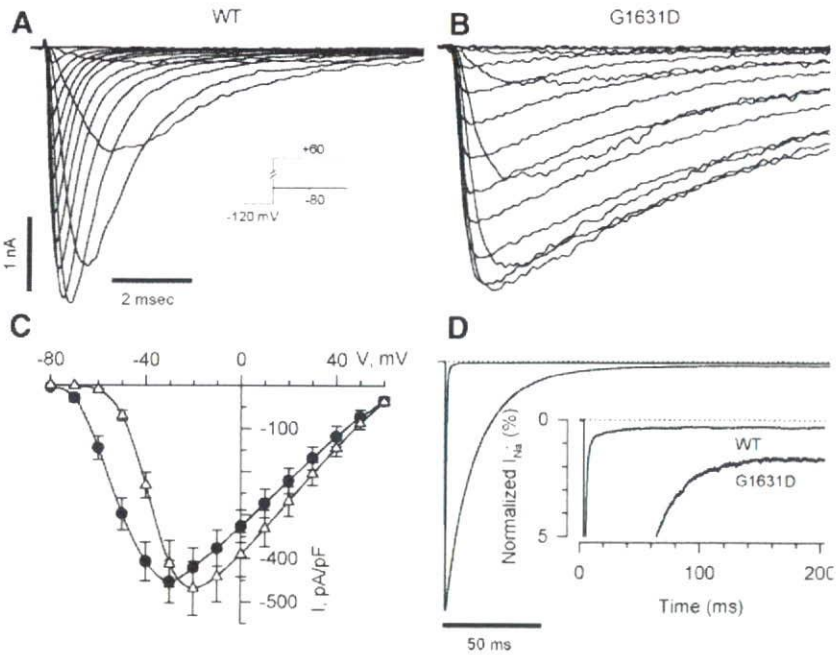

Figure 2. Whole-cell current recordings of WT and G1631D sodium channels. Representative sodium currents recorded from cells expressing WT (A) or G1631D (B) elicited by depolarizing steps from −80 mV to +60 mV in 10-mV increments from holding potential −120 mV. C, Comparison of current-voltage relationships for WT (n=15) and G1631D (n=16). Current is normalized to cell capacitance to give sodium current density. D, Increased tetrodotoxin (TTX)-sensitive persistent sodium currents for G1631D. Peak sodium currents were normalized. The zero-current level is indicated by a dotted line. The inset shows an expanded *y* axis scaled to emphasize the relative proportion of persistent current for WT (n=8) and G1631D (n=9).

ablation. The child has survived beyond the age of 26 months without further ventricular or atrial arrhythmias.

The second proband was a Japanese male delivered by emergency C-section at 34-weeks gestation because of ventricular arrhythmia (torsade de pointes, TdP) documented in utero by magnetocardiography. Initial APGAR scores were 8 and 9, but his QTc interval was 567 ms and he had multiple episodes of TdP. Intravenous injection (3 mg/kg) followed by continuous infusion of mexiletine abolished TdP. He was discharged on oral mexiletine (20 mg/kg/d) but was readmitted for treatment of recurrent TdP approximately 2 months later (QTc=590 ms). Continuous infusion of mexiletine combined with oral mexiletine (serum drug concentration: 1.3 to 1.4 μg/mL) considerably abbreviated the QTc (462 to 499 ms) but did not completely suppress episodes of TdP. The addition of continuous infusion propranolol (0.5 mg/kg/d, serum drug concentration: 18.4 to 25.1 ng/mL) further shortened QTc (395 to 424 ms) and fully suppressed ventricular arrhythmias. Finally, combination therapy with oral mexiletine and oral propranolol was effective in suppressing ventricular arrhythmias through age 8 months (Figure 1A).

A novel *SCN5A* missense mutation (G1631D) was discovered in both probands (Figure 1B). Family histories were negative for arrhythmia syndromes. The results of ECG testing were normal for both sets of parents, and they were mutation negative. Paternity testing demonstrated that the mutation was de novo in both cases. No other mutations were identified in *SCN5A*, *KCNQ1*, *KCNH2*, *KCNE1*, or *KCNE2* in either proband.

Profound Dysfunction of G1631D Channels

The mutation results in substitution of a highly conserved glycine residue with a negatively charged glutamic acid in the S4 segment of domain 4 (D4/S4; Figure 1C). This residue is 100% conserved in all known voltage-gated sodium channel sequences from several diverse phyla. This structural domain in sodium channels participates as a component of the voltage-sensor important for activation and inactivation.[14,15] Introduction of a negatively charged side group into this domain was predicted to have a significant functional effect. To test this hypothesis, we engineered G1631D in recombinant human $Na_V1.5$ for heterologous expression and then performed electrophysiological studies.

Figure 2 illustrates the general functional properties of wild-type (WT) and mutant $Na_V1.5$ channels expressed heterologously in human tsA201 cells. Representative whole-cell current tracings demonstrate that the mutant exhibits a profound level of dysfunction characterized by substantial delays in activation and inactivation. Overall current density was similar between cells expressing WT or mutant channels but there was a positive shift in the peak current-voltage (I-V) relationship for the mutant (Figure 2C). Mutant channels exhibited increased steady-state persistent current measured 200 ms after the peak transient current (Figure 2D; persistent current as % of peak current: WT, 0.31±0.04%, n=8; G1631D, 1.63±0.31%, n=9; $P<0.001$). Although increased persistent current is characteristic of *SCN5A* mutations associated with LQTS,[16,17] no previously characterized mutation had such a profound inactivation defect.

Figure 3 illustrates quantitative assessments of activation and inactivation. Mutant channels exhibited a global slowing of activation across the range of tested potentials as assessed by time to peak current (Figure 3A). Similarly, G1631D exhibited a profound slowing of inactivation as illustrated by the voltage dependence of inactivation time constants (Figure 3B). The degree of slowing of inactivation was approximately 10-fold compared with WT. The mutant also exhibited significant depolarizing shifts in the voltage dependence of activation (+12 mV) and steady-state inactivation (+14 mV; Figure 3C and 3D; supplemental Table I, available online). These asymmetrical depolarizing shifts in activation and steady-state inactivation predict an increased window current

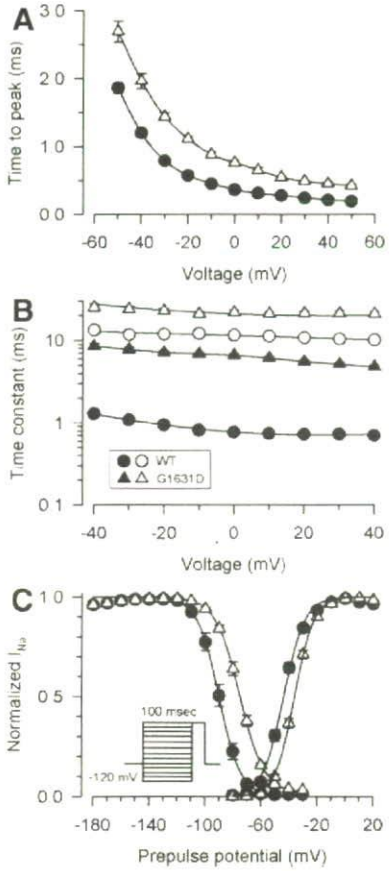

Figure 3. Activation and inactivation of WT and mutant channels. A, Time to peak activation in the voltage range of −50 to +50 mV. Differences between WT (n=15) and G1631D (n=16) were significant at the $P<0.001$ level for all tested voltages. B, Voltage dependence of inactivation time constants (same number of replicates as in A). Filled and open symbols indicate fast and slow component values, respectively. C, Voltage dependence of activation and steady-state inactivation elicited by a 100-ms conditioning pulse to various voltages (same number of replicates as in A).

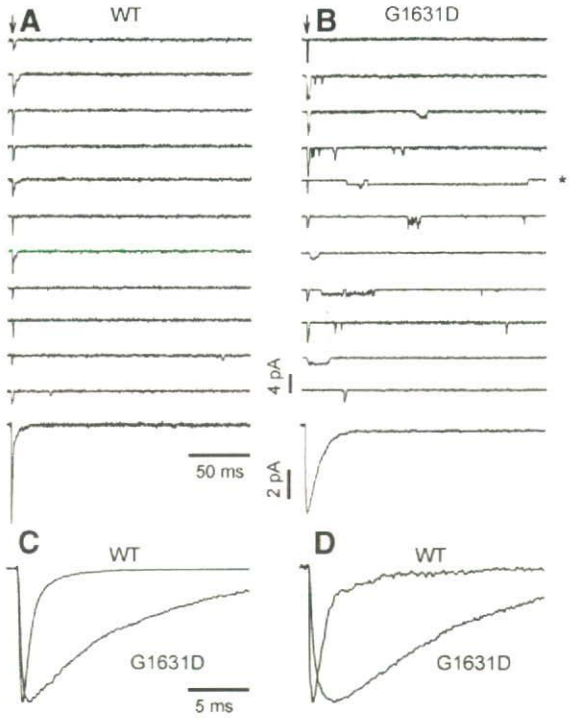

Figure 5. Single-channel properties of WT and G1631D channels. Sodium channel activities recorded at −20 mV from a multichannel outside-out patch excised from a cell expressing WT (A) or G1631D (B). Vertical arrows indicate the onset of patch depolarization from −120 mV to −20 mV. Lower traces show the ensemble averaged current obtained from 100 consecutive traces for WT and G1631D, respectively. C and D are comparisons of normalized and superimposed current traces of WT and G1631D at test potential of −20 mV from whole-cell recordings (C) and single-channel recordings (D).

defined as the overlap of these 2 curves (see Supplemental Figure I).

In Figure 4A, the time course of recovery from inactivation after a 100-ms conditioning pulse illustrates that the mutant has profound slowing of recovery. This difference was explained by a larger slow component of recovery from inactivation as determined by double exponential fitting (see Supplemental Table I). For WT channels, the majority of recovery occurs with a time constant of approximately 10 ms. By contrast for G1631D channels, the predominant fraction of channels recover from inactivation with a time constant of approximately 100 ms. The marked slowing of recovery from inactivation exhibited by G1631D correlated with a greater loss of channel availability during repetitive membrane depolarizations at frequencies exceeding 1 Hz (Figure 4B).

These profound gating abnormalities were correlated with aberrant single-channel events. Figure 5 illustrates representative single-channel recordings from cells expressing WT or mutant channels. Wild-type channels exhibited brief and transient openings clustered at the onset of the test depolarization. By contrast, the mutant exhibited a marked increase

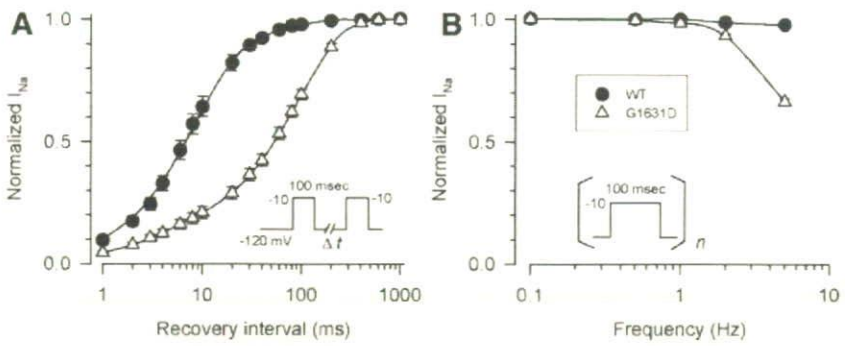

Figure 4. Recovery from inactivation. A, Time course of recovery from inactivation for WT (n=12) and G1631D (n=16) was elicited using the 2-pulse protocol shown in the inset. Time constants and fractional amplitudes are given in supplemental Table I. B, Activity dependent loss of channel availability following trains of 100 ms pulses to −10 mV from a holding potential of −120 mV applied at the frequency indicated (n=10 to 18 cells). Residual current following the 100th pulse was normalized to the first pulse current amplitude.

in probability of late reopenings and occasional prolonged openings (asterisk). Single-channel conductance levels were similar for WT (24pS) and G1631D (25pS), but mutant channels exhibited significantly longer latency to first opening (WT: 0.59±0.03 ms; G1631D: 1.15±0.03 ms; n=3; $P<0.001$), increased mean open time (WT: 0.34±0.09 ms; G1631D: 0.98±0.03 ms; n=3; $P=0.029$), and increased NP_o (WT: 0.14±0.02; G1631D: 0.22±0.03; n=3; $P=0.042$) when assessed at a test pulse of −20 mV. Ensemble averaged currents derived from single-channel records closely resemble those obtained from whole-cell recordings. These findings collectively indicate that G1631D causes a fundamental defect in channel activation and inactivation associated with dramatic clinical consequences.

Enhanced Mexiletine Sensitivity of G1631D Channels

Despite the profound nature of the sodium channel dysfunction caused by G1631D, both probands survived likely because of prompt intervention including pharmacological treatments. We compared the effect of mexiletine on WT and mutant channels. Figure 6A illustrates the responses of WT and G1631D to repetitive membrane depolarizations delivered at a frequency of 1 Hz in the presence of mexiletine (100 μmol/L). Both channels exhibited an initial drop in channel availability followed by further use-dependent loss of activity, but the effect is substantially greater for G1631D suggesting that the mutant has enhanced mexiletine sensitivity. Concentration-response relationships for tonic (Figure 6B) and use-dependent (Figure 6C) mexiletine block of WT and G1631D supported this hypothesis. Mexiletine block of WT channels exhibited EC_{50} values of 120.9 μmol/L and 50.9 μmol/L for tonic and use-dependent block, respectively. By contrast, G1631D was 1.8-fold and 2.8-fold more sensitive to tonic (EC_{50} 66.7 μmol/L) and use-dependent (EC_{50} 18.3 μmol/L) mexiletine block, respectively. Further, mexiletine induced a hyperpolarizing shift in steady-state inactivation of mutant channels such that this property became more similar to WT channels (G1631D $V_{½}$: no drug, −74.8±1.1 mV, n=16; 3 μmol/L mexiletine, −85.5±1.3 mV, n=9; $P<0.001$). By contrast, the same drug concentration has no significant effect on steady-state inactivation of WT channels (WT $V_{½}$: no drug, −89.3±1.1 mV, n=16; 3 μmol/L mexiletine, −86.6±3.2 mV, n=6; NS). Mexiletine also had moderate effects on the kinetics of G1631D inactivation (Figure 6B and 6C), illustrated by significant reductions in the time constants for inactivation, and significantly reduced the level of persistent current (no drug: 1.63±0.31%, n=9; 10 μmol/L mexiletine, 0.54±0.06%, n=8; $P=0.0098$).

Propranolol Block of WT and G1631D Channels

We also considered the role of propranolol in modulating mutant sodium channel behavior. Propranolol is a widely used β-adrenergic receptor antagonist, but early studies indicated that this drug also exhibits antiarrhythmic (membrane stabilizing) properties at high serum concentrations possibly from effects on voltage-gated sodium channels.[18,19] Figure 7A illustrates that both WT and G1631D channels are blocked by 3 μmol/L propranolol during repetitive stimula-

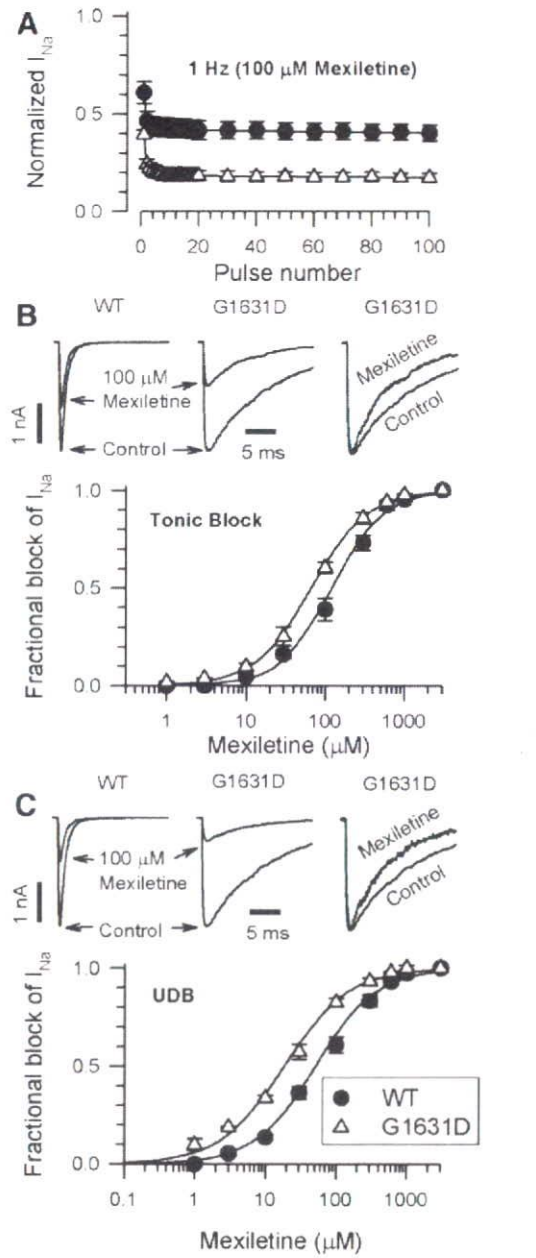

Figure 6. Effects of mexiletine on WT and G1631D. A, Mexiletine (100 μmol/L) block of WT (n=8) and G1631D (n=8) during a 1-Hz train of depolarizing pulses to −10 mV from a holding potential of −120 mV. B, Tonic mexiletine block of WT and G1631D. Upper traces (left, middle) illustrate the effects of 100 μmol/L mexiletine during a single depolarizing voltage step to −10 mV. Normalized traces (right) recorded in the absence (control) or presence of drug illustrate the effect of mexiletine on the inactivation time course. The plot illustrates the concentration-response relationships for tonic block by mexiletine (each data point represents the mean of 4 to 12 cells). C, Use-dependent mexiletine block of WT and G1631D. Upper traces (left, middle) illustrate the steady-state effects of 100 μmol/L mexiletine during a 1-Hz pulse train. Normalized traces (right) recorded in the absence (control) or presence of drug (100th pulse) illustrate the effect of mexiletine on the inactivation time course. Time constants in the absence of drug were: τ_1=7.7±0.7 ms, τ_2=19.7±0.6 ms, n=8; and in the presence of 100 μmol/L mexiletine: τ_1=4.5±0.7 ms, τ_2=10.9±1.0 ms, n=8 ($P=0.0095$ for τ_1; $P<0.0001$ for τ_2). The plot illustrates the concentration-response relationships for use-dependent block by mexiletine (each data point represents the mean of 4 to 12 cells). The lines in B and C were fit to the data according to the Hill equation.

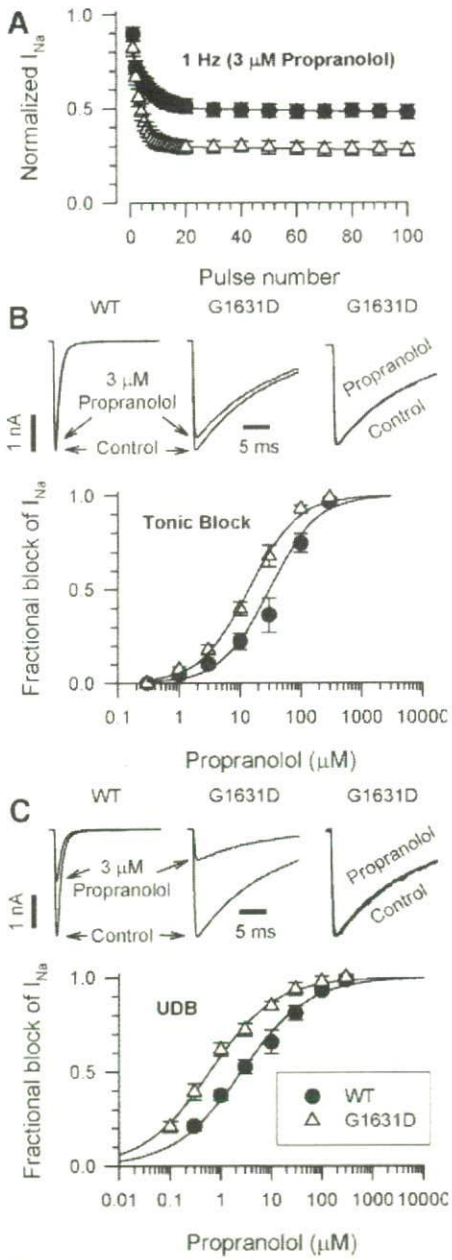

Figure 7. Effects of propranolol on WT and G1631D. A, Propranolol (3 μmol/L) block of WT (n=5) and G1631D (n=4) during a 1-Hz train of depolarizing pulses to −10 mV from a holding potential of −120 mV. B, Tonic propranolol block of WT and G1631D. Upper traces (left, middle) illustrate the effects of 3 μmol/L propranolol during a single depolarizing voltage step to −10 mV. Normalized traces (right) recorded in the absence (control) or presence of drug illustrate the effect of propranolol on the inactivation time course. The plot illustrates the concentration-response relationships for tonic block by propranolol (each data point represents the mean of 4 to 11 cells). C, Use-dependent propranolol block of WT and G1631D. Upper traces (left, middle) illustrate the steady-state effects of 3 μmol/L propranolol during a 1-Hz pulse train. Normalized traces (right) recorded in the absence (control) or presence of drug (100th pulse) illustrate the effect of propranolol on the inactivation time course. Time constants in the absence of drug were: τ_1=7.8±0.5 ms, τ_2=19.5±0.7 ms, n=4; and in the presence of 3 μmol/L propranolol: τ_1=6.9±1.1 ms, τ_2=18.1±1.0 ms n=4 (no significant differences in τ_1 or τ_2). The plot illustrates the concentration-response relationships for use-dependent block by propranolol (each data point represents the mean of 4 to 11 cells). The lines in B and C were fit to the data according to the Hill equation.

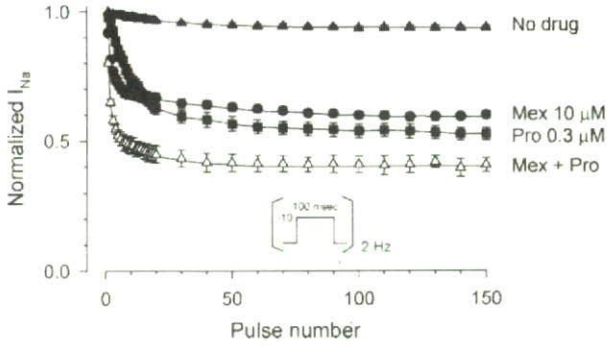

Figure 8. Effects of mexiletine with or without propranolol on G1631D channels. Sodium current was measured sequentially during a 2-Hz train of depolarizing pulses to −10 mV from a holding potential of −120 mV and values normalized to the current level after the initial pulse. Steady-state residual normalized sodium current after the 150th pulse was significantly lower for the combination of 10 μmol/L mexiletine with 0.3 μmol/L propranolol (fractional residual current, 0.4±0.03, n=3), when compared with either drug alone (mexiletine, 0.6±0.02; n=6; P=0.0039; propranolol, 0.5±0.03; n=6; P=0.041).

tion (1 Hz). Mutant channels exhibited a greater degree of steady-state block than WT channels under these conditions. Concentration-response curves demonstrated that propranolol exerts greater tonic (Figure 7B) and use-dependent (Figure 7C) block of G1631D than that of WT channels. Propranolol use-dependent block was enhanced 5-fold by the mutation (EC_{50}: WT, 3.0 μmol/L; G1631D, 0.6 μmol/L). The effect of propranolol, a racemic mixture, was not likely mediated through endogenous β-adrenergic receptors in the heterologous cell system because we observed similar blocking potency for R-(+)-propranolol, which has no receptor antagonist properties (see supplemental Figure II). Propranolol at a concentration similar to that observed in the Japanese proband (0.1 μmol/L) normalized steady-state inactivation of mutant channels (G1631D $V_{½}$: no drug, −74.8±1.1 mV, n=16; propranolol, −84.5±1.7 mV, n=10; P<0.001) but had no effect on steady-state inactivation of WT channels (WT $V_{½}$: no drug, −89.3±1.1 mV, n=16; propranolol, −88.8±0.9 mV, n=5; NS). Propranolol did not affect the kinetics of inactivation for WT or mutant channels (Figure 7B and 7C) or the level of persistent current observed for G1631D (no drug: 1.63±0.31, n=9; 1 μmol/L propranolol, 1.44±0.29, n=9; NS).

Because both probands responded clinically to the combination of mexiletine and propranolol, we tested the effects of both drugs together on G1631D channels. To closely simulate the clinical conditions, we tested use-dependent block at 2 Hz, which was the approximate resting heart rate of the Japanese proband and the frequency of cardiac pacing in the Italian child. The combination of mexiletine (10 μmol/L) and propranolol (0.3 μmol/L) caused a substantial loss of channel availability during a 2-Hz pulse train (Figure 8) when compared with the drug-free condition. The level of channel inhibition observed for the combination of mexiletine and propranolol was greater than either drug applied alone indicating additive effects.

Discussion

During late fetal development through shortly after birth, there is a vulnerable period when death occurs at a rate of 6 to 12 per 1000 live births per year,[20] and congenital arrhythmia susceptibility may be a significant contributor to this problem.[21,22] Life-threatening cardiac arrhythmias during infancy and the perinatal period may go unnoticed owing to the lack of routine use of electrocardiographic monitoring of the fetus and newborn. Studies of 2 large series of autopsied SIDS victims demonstrated that up to 10% of SIDS cases may represent genetic disorders of congenital arrhythmia susceptibility such as the LQTS,[3,23] short-QT syndrome,[24,25] and catecholaminergic polymorphic ventricular tachycardia.[26] Understanding the genetic risks for perinatal mortality should promote efforts to identify and treat at-risk newborns.

Malignant Perinatal Variant of LQTS

The profoundly dysfunctional *SCN5A* mutation, G1631D, produced a clinical entity distinct from typical LQTS (LQT3 subtype). Clinically, subjects with typical LQTS first develop symptoms (syncope, cardiac arrest, and sudden death) during late childhood, adolescence, or early adulthood.[9,27] Many mutation carriers may in fact be asymptomatic. The 2 probands we described seem to be affected by a very severe and life-threatening process.

At the molecular level, most *SCN5A* mutations associated with LQTS cause a subtle gain-of-function defect characterized by increased persistent current.[16,17] The markedly abnormal channel function we observed for G1631D including a 10-fold slowing of inactivation, substantial shifts in voltage dependence of activation and inactivation along with greatly impaired recovery from inactivation represent distinct molecular defects that distinguish this mutation from typical LQT3 alleles. Other *SCN5A* alleles may similarly predispose to early onset and severe perinatal arrhythmia syndromes,[4,5,28,29] but the functional aberrations associated with most of these reported alleles resemble mutations found in older individuals.

Negative Selection Against *SCN5A* Mutations

Mutations in *SCN5A* are represented disproportionately among SIDS victims who carry occult congenital arrhythmia susceptibility gene mutations when compared with older LQTS subjects. The lower proportion of *SCN5A* mutations among older children and young adults with LQTS when compared with the higher proportion in SIDS victims may be the result of negative selection against mutations in the sodium channel gene. Negative selection would cause an ascertainment bias for genotypes in living individuals in whom survival is favored when carrying mutations having less severe physiological consequences. In the case of SCN5A-G1631D, we assumed that without immediate treatment, this mutation would have been lethal. However, survival after successful treatment confounds the argument for negative selection.

Congenital arrhythmia susceptibility occurring in the perinatal and neonatal periods caused by *SCN5A* mutations appears biologically distinct from LQTS in older subjects. Carriers of certain *SCN5A* mutations may present with earlier onset and severe congenital arrhythmia syndromes. An illustration of this idea is recurrent third-trimester fetal loss attributable to inheritance of an *SCN5A* mutation (R1623Q) from a mother who was mosaic for this deleterious allele.[8] The R1623Q mutation, which affects a conserved residue in the D4/S4 segment nearby the location of G1631D, was originally identified in a Japanese child with a severe clinical presentation of LQTS,[30] and the molecular defect associated with this allele compromised inactivation to a greater extent than typical LQT3 mutations.[31] Our observations regarding the severity of biophysical defects associated with G1631D also support the idea that earlier onset cardiac symptoms may sometimes correlate with a severe molecular phenotype.

Genotype-Specific Pharmacological Treatment

The clinical consequences of G1631D were perinatal arrhythmias successfully managed in part by pharmacotherapy with the combination of mexiletine and propranolol. Mexiletine as well as other sodium channel blockers have been proposed as gene-specific therapeutic agents in LQT3.[32–34] In vitro studies have demonstrated the capability of these drugs to selectively suppress increased persistent current conducted by mutant channels[29,35] and to normalize ventricular repolarization in animal models.[36,37] One study suggested that certain biophysical properties of mutant $Na_v1.5$ channels may be predictive of mexiletine responsiveness. Specifically, Ruan et al[38] found that among 4 distinct *SCN5A* mutations, clinical benefit from mexiletine treatment was observed only in subjects carrying mutations that caused a hyperpolarizing shift in steady-state inactivation and this correlated with in vitro effects of the drug. However, this observation cannot be extrapolated to all *SCN5A* mutations as evidenced by the favorable response of G1631D to mexiletine both clinically and experimentally despite a depolarizing shift in steady-state inactivation (Figure 3). Similarly, another recently reported *SCN5A* mutation (F1473C) was also associated with a favorable clinical response to high-dose mexiletine despite having depolarized steady-state inactivation.[29] Additional factors besides those emphasized by Ruan et al[38] are likely to determine the clinical efficacy of mexiletine.

By contrast, use of β-blockers in the setting of *SCN5A* mutations has less certain benefits. Three studies have reported that β-blockers are generally less efficacious in LQT3 subjects, but the specific drug used varies considerably.[9,39,40] For example, in the report by Priori et al[40] the specific β-blocker was known in 69% of cases, and this was either propranolol or nadolol. As we have demonstrated in this study, propranolol may offer specific advantages in treating certain *SCN5A* mutations because of apparent local anesthetic-like properties of the drug.[18,19] By contrast, we recently determined that nadolol has no activity against sodium channels (Wang DW, unpublished observations, 2007). The role of propranolol in treating individuals with *SCN5A* mutations warrants further study.

Combination pharmacotherapy in the 2 probands with G1631D may have uniquely contributed to their survival. In the Japanese newborn, mexiletine alone was not adequate to control ventricular arrhythmia despite shortening of the QT interval. The addition of propranolol to the treatment regimen conferred better arrhythmia control and survival. In the Italian

proband, the coadministration of mexiletine with propranolol was efficacious, but this subject was also treated with ventricular pacing. Our study demonstrated additive effects of the 2 drugs at a pulsing frequency of 2 Hz (Figure 8). This observation suggested that a combination of mexiletine with propranolol in the setting of modest tachycardia were protective of ventricular arrhythmia caused by G1631D. We explain this effect by a combination of the intrinsic activity-dependent loss of channel availability observed for G1631D (Figure 4B) with the use-dependent drug effects.

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Disclosures

None.

References

1. Byard RW, Krous HF. Sudden infant death syndrome: overview and update. *Pediatr Dev Pathol*. 2003;6:112–127.
2. Rognum TO, Byard RW. Sudden infant death syndrome, etiology and epidemiology. In: Payne-James J, Byard RW, Corey TS, Henderson C, eds. *Encyclopedia of Forensic and Legal Medicine*. Boston: Elsevier; 2005:117–129.
3. Arnestad M, Crotti L, Rognum TO, Insolia R, Pedrazzini M, Ferrandi C, Vege A, Wang DW, Rhodes TE, George AL Jr, Schwartz PJ. Prevalence of long-QT syndrome gene variants in sudden infant death syndrome. *Circulation*. 2007;115:361–367.
4. Wedekind H, Smits JP, Schulze-Bahr E, Arnold R, Veldkamp MW, Bajanowski T, Borggrefe M, Brinkmann B, Warnecke I, Funke H, Bhuiyan ZA, Wilde AA, Breithardt G, Haverkamp W. De novo mutation in the SCN5A gene associated with early onset of sudden infant death. *Circulation*. 2001;104:1158–1164.
5. Chang CC, Acharfi S, Wu MH, Chiang FT, Wang JK, Sung TC, Chahine M. A novel SCN5A mutation manifests as a malignant form of long QT syndrome with perinatal onset of tachycardia/bradycardia. *Cardiovasc Res*. 2004;64:268–278.
6. Lupoglazoff JM, Denjoy I, Villain E, Fressart V, Simon F, Bozio A, Berthet M, Benammar N, Hainque B, Guicheney P. Long QT syndrome in neonates: conduction disorders associated with HERG mutations and sinus bradycardia with KCNQ1 mutations. *J Am Coll Cardiol*. 2004;43:826–830.
7. Schwartz PJ, Priori SG, Dumaine R, Napolitano C, Antzelevitch C, Stramba-Badiale M, Richard TA, Berti MR, Bloise R. A molecular link between the sudden infant death syndrome and the long-QT syndrome. *N Engl J Med*. 2000;343:262–267.
8. Miller TE, Estrella E, Myerburg RJ, Garcia d V, Moreno N, Rusconi P, Ahearn ME, Baumbach L, Kurlansky P, Wolff G, Bishopric NH. Recurrent third-trimester fetal loss and maternal mosaicism for long-QT syndrome. *Circulation*. 2004;109:3029–3034.
9. Schwartz PJ, Priori SG, Spazzolini C, Moss AJ, Vincent GM, Napolitano C, Denjoy I, Guicheney P, Breithardt G, Keating MT, Towbin JA, Beggs AH, Brink P, Wilde AA, Toivonen L, Zareba W, Robinson JL, Timothy KW, Corfield V, Wattanasirichaigoon D, Corbett C, Haverkamp W, Schulze-Bahr E, Lehmann MH, Schwartz K, Coumel P, Bloise R. Genotype-phenotype correlation in the long-QT syndrome: gene-specific triggers for life-threatening arrhythmias. *Circulation*. 2001;103:89–95.
10. Tester DJ, Will ML, Haglund CM, Ackerman MJ. Compendium of cardiac channel mutations in 541 consecutive unrelated patients referred for long QT syndrome genetic testing. *Heart Rhythm*. 2005;2:507–517.
11. Priori SG, Schwartz PJ, Napolitano C, Bloise R, Ronchetti E, Grillo M, Vicentini A, Spazzolini C, Nastoli J, Bottelli G, Folli R, Cappelletti D. Risk stratification in the long-QT syndrome. *N Engl J Med*. 2003;348:1866–1874.
12. Crotti L, Lundquist AL, Insolia R, Pedrazzini M, Ferrandi C, De Ferrari GM, Vicentini A, Yang P, Roden DM, George AL Jr, Schwartz PJ. KCNH2-K897T is a genetic modifier of latent congenital long-QT syndrome. *Circulation*. 2005;112:1251–1258.
13. Yokokawa M, Noda T, Okamura H, Satomi K, Suyama K, Kurita T, Aihara N, Kamakura S, Shimizu W. Comparison of long-term follow-up of electrocardiographic features in Brugada syndrome between the SCN5A-positive probands and the SCN5A-negative probands. *Am J Cardiol*. 2007;100:649–655.
14. Catterall WA. Cellular and molecular biology of voltage-gated sodium channels. *Physiologic Rev*. 1992;72:S15–S48.
15. Chahine M, George AL Jr, Zhou M, Ji S, Sun W, Barchi RL, Horn R. Sodium channel mutations in paramyotonia congenita uncouple inactivation from activation. *Neuron*. 1994;12:281–294.
16. Bennett PB, Yazawa K, Makita N, George AL Jr. Molecular mechanism for an inherited cardiac arrhythmia. *Nature*. 1995;376:683–685.
17. Dumaine R, Wang Q, Keating MT, Hartmann HA, Schwartz PJ, Brown AM, Kirsch GE. Multiple mechanisms of Na^+ channel-linked long-QT syndrome. *Circ Res*. 1996;78:916–924.
18. Dawson AK, Reele SB, Wood AJ, Duff HJ, Woosley RL, Smith RF. Electrophysiological effects of high-dose propranolol in dogs: evidence in vivo for effects not mediated by the β adrenoceptor. *J Pharmacol Exp Ther*. 1984;229:91–97.
19. Duff HJ, Roden DM, Brorson L, Wood AJ, Dawson AK, Primm RK, Oates JA, Smith RT, Woosley RL. Electrophysiologic actions of high plasma concentrations of propranolol in human subjects. *J Am Coll Cardiol*. 1983;2:1134–1140.
20. Strasburger JF, Cheulkar B, Wichman HJ. Perinatal arrhythmias: diagnosis and management. *Clin Perinatol*. 2007;34:627–652.
21. Berul CI. Neonatal long QT syndrome and sudden cardiac death. *Prog Pediatr Cardiol*. 2000;11:47–54.
22. Schwartz PJ. Stillbirths, sudden infant deaths, and long-QT syndrome: puzzle or mosaic, the pieces of the jigsaw are being fitted together. *Circulation*. 2004;109:2930–2932.
23. Ackerman MJ, Siu BL, Sturner WQ, Tester DJ, Valdivia CR, Makielski JC, Towbin JA. Postmortem molecular analysis of SCN5A defects in sudden infant death syndrome. *JAMA*. 2001;286:2264–2269.
24. Brugada R, Hong K, Dumaine R, Cordeiro J, Gaita F, Borggrefe M, Menendez TM, Brugada J, Pollevick GD, Wolpert C, Burashnikov E, Matsuo K, Wu YS, Guerchicoff A, Bianchi F, Giustetto C, Schimpf R, Brugada P, Antzelevitch C. Sudden death associated with short-QT syndrome linked to mutations in HERG. *Circulation*. 2004;109:30–35.
25. Rhodes TE, Abraham RA, Welch RC, Vanoye CG, Crotti L, Arnestad M, Insolia R, Pedrazzini M, Ferrandi C, Vege A, Rognum T, Roden DM, Schwartz PJ, George AL Jr. Cardiac potassium channel dysfunction in sudden infant death syndrome. *J Mol Cell Cardiol*. 2007;44:571–581.
26. Tester DJ, Spoon DB, Valdivia HH, Makielski JC, Ackerman MJ. Targeted mutational analysis of the RyR2-encoded cardiac ryanodine receptor in sudden unexplained death: a molecular autopsy of 49 medical examiner/coroner's cases. *Mayo Clin Proc*. 2004;79:1380–1384.
27. Roden DM. Clinical practice. Long-QT syndrome. *N Engl J Med*. 2008;358:169–176.
28. Schulze-Bahr E, Fenge H, Etzrodt D, Haverkamp W, Monnig G, Wedekind H, Breithardt G, Kehl HG. Long QT syndrome and life threatening arrhythmia in a newborn: molecular diagnosis and treatment response. *Heart*. 2004;90:13–16.
29. Bankston JR, Yue M, Chung W, Spyres M, Pass RH, Silver E, Sampson KJ, Kass RS. A novel and lethal de novo LQT-3 mutation in a newborn with distinct molecular pharmacology and therapeutic response. *PLoS ONE*. 2007;2:e1258.
30. Yamagishi H, Furutani M, Kamisago M, Morikawa Y, Kojima Y, Hino Y, Furutani Y, Kimura M, Imamura S, Takao A, Momma K, Matsuoka R. A de novo missense mutation (R1623Q) of the SCN5A gene in a Japanese girl with sporadic long QT sydrome. *Hum Mutat*. 1998;11:481.
31. Kambouris NG, Nuss HB, Johns DC, Tomaselli GF, Marban E, Balser JR. Phenotypic characterization of a novel long-QT syndrome mutation (R1623Q) in the cardiac sodium channel. *Circulation*. 1998;97:640–644.
32. Schwartz PJ, Priori SG, Locati EH, Napolitano C, Cantu F, Towbin JA, Keating MT, Hammoude H, Brown AM, Chen LS. Long QT syndrome patients with mutations of the SCN5A and HERG genes have differential

responses to Na^+ channel blockade and to increases in heart rate. Implications for gene-specific therapy. *Circulation*. 1995;92:3381–3386.
33. Benhorin J, Taub R, Goldmit M, Kerem B, Kass RS, Windman I, Medina A. Effects of flecainide in patients with new SCN5A mutation: mutation-specific therapy for long-QT syndrome? *Circulation*. 2000;101:1698–1706.
34. Windle JR, Geletka RC, Moss AJ, Zareba W, Atkins DL. Normalization of ventricular repolarization with flecainide in long QT syndrome patients with SCN5A:8KPQ mutation. *Ann Noninvasive Electrocardiol*. 2001;6:153–158.
35. Wang DW, Yazawa K, Makita N, George AL Jr, Bennett PB. Pharmacological targeting of long QT mutant sodium channels. *J Clin Invest*. 1997;99:1714–1720.
36. Shimizu W, Aiba T, Antzelevitch C. Specific therapy based on the genotype and cellular mechanism in inherited cardiac arrhythmias: Long QT syndrome and Brugada syndrome. *Curr Pharm Des*. 2005;11:1561–1572.
37. 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.
38. Ruan Y, Liu N, Bloise R, Napolitano C, Priori SG. Gating properties of SCN5A mutations and the response to mexiletine in long-QT syndrome type 3 patients. *Circulation*. 2007;116:1137–1144.
39. Moss AJ, Zareba W, Hall WJ, Schwartz PJ, Crampton RS, Benhorin J, Vincent GM, Locati EH, Priori SG, Napolitano C, Medina A, Zhang L, Robinson JL, Timothy K, Towbin JA, Andrews ML. Effectiveness and limitations of β-blocker therapy in congenital long-QT syndrome. *Circulation*. 2000;101:616–623.
40. Priori SG, Napolitano C, Schwartz PJ, Grillo M, Bloise R, Ronchetti E, Moncalvo C, Tulipani C, Veia A, Bottelli G, Nastoli J. Association of long QT syndrome loci and cardiac events among patients treated with β-blockers. *JAMA*. 2004;292:1341–1344.

CLINICAL PERSPECTIVE

Mutations in *SCN5A* encoding the cardiac voltage-gated sodium channel have been associated with a spectrum of increased sudden death risk extending from fetal life to adulthood. We studied the functional and pharmacological properties of a novel de novo *SCN5A* mutation associated with an extremely severe perinatal presentation of congenital long-QT syndrome, characterized by late third trimester intrauterine fetal heart rhythm disturbances and life-threatening ventricular arrhythmia occurring within hours of emergency cesarean birth. The same mutation (G1631D), which was discovered in two subjects of different ethnic backgrounds with the same clinical presentation, caused a profound degree of sodium channel dysfunction that was more severe than that observed for any previous *SCN5A* variant. Despite the extreme nature of the mutation and the associated dire clinical scenario, the subjects survived owing to prompt therapeutic interventions, including treatment with the combination of mexiletine and propranolol, two drugs that exhibited enhanced and additive activity against the mutant allele. These observations illustrate the role of severe sodium channel mutations in a malignant perinatal variant of long-QT syndrome and successful use of combination pharmacotherapy to prevent perinatal mortality in this setting. Our data also illustrate the potential therapeutic benefits of a propranolol block of mutant sodium channels.

Phenotypic Variability in Caucasian and Japanese Patients with Matched LQT1 Mutations

Judy F. Liu, M.S.,* Ilan Goldenberg, M.D.,* Arthur J. Moss, M.D.,* Wataru Shimizu, M.D., Ph.D.,† Arthur A. Wilde, M.D., Ph.D.,‡ Nynke Hofman, M.Sc.,‡ Scott McNitt, M.S.,* Wojciech Zareba, M.D., Ph.D.,* Yoshihiro Miyamato, M.D., Ph.D.,† Jennifer L. Robinson, M.S.,* and Mark L. Andrews, B.B.A.*

*From the *Cardiology Division of the Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY; †the Division of Cardiology, Department of Internal Medicine and Laboratory of Molecular Genetics, National Cardiovascular Center, Suita, Japan; and ‡the Experimental and Molecular Cardiology Group and the Department of Clinical Genetics, Academic Medical Center, AZ Amsterdam, The Netherlands*

Background: Ethnic differences may affect the phenotypic expression of genetic disorders. However, data regarding the effect of ethnicity on outcome in patients with genetic cardiac disorders are limited. We compared the clinical course of Caucasian and Japanese long QT type-1 (LQT1) patients who were matched for mutations in the KCNQ1 gene.

Methods: The study population comprised 62 Caucasian and 38 Japanese LQT1 patients from the International LQTS Registry who were identified as having six identical KCNQ1 mutations. The biophysical function of the mutations was categorized into dominant-negative (>50%) or haploinsufficiency (≤50%) reduction in cardiac repolarizing IKs potassium channel current. The primary end point of the study was the occurrence of a first cardiac event from birth through age 40 years.

Results: Japanese patients had a significantly higher cumulative rate of cardiac events (67%) than Caucasian patients (39%; $P = 0.01$). The respective frequencies of dominant negative mutations in the two ethnic groups were 63% and 28% ($P < 0.001$). In multivariate analysis, Japanese patients had an 81% increase in the risk of cardiac events ($P = 0.06$) as compared with Caucasians. However, when the biophysical function of the mutations was included in the multivariate model, the risk associated with Japanese ethnicity was no longer evident (HR = 1.05; $P = 0.89$). Harboring a dominant negative mutation was shown to be the most powerful and significant predictor of outcome (HR = 3.78; $P < 0.001$).

Conclusions: Our data indicate that ethnic differences in the clinical expression of LQTS can be attributed to the differences in frequencies of the specific mutations within the two populations.

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long-QT syndrome; genetics; ethnicity

The congenital Long QT Syndrome (LQTS) is caused by mutations in eleven defined genes that encode channels that regulate sodium, potassium, and calcium currents, and by mutations that affect trafficking of ion channels, resulting in prolonged ventricular repolarization and an increased risk for sustained ventricular tachyarrhythmias.[1–4] Mutations in the KCNQ1 gene, also known as KvLQT1, cause the more common long QT type-1 (LQT1) genotype by creating faulty alpha subunits that are part of the delayed rectifier repolarizing potassium (IKs) channel.[5] LQT1 mutations are associated with two distinct biophysical mechanisms: (1) coassembly or cellular trafficking defects of the mutant subunits that allow only normal, wild-type, subunits to be successfully transported to the cell

Address for reprints: Ilan Goldenberg, M.D., Heart Research Follow-Up Program, Box 653, University of Rochester Medical Center, Rochester, NY 14642. Fax: 585-273-5283; E-mail: Ilan.Goldenberg@heart.rochester.edu

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membrane, resulting in reduced channel function by ≤50%; (haploinsufficiency); and (2) formation of defective channels involving mutant subunits with the altered channel protein transported to the cell membrane, resulting in a dysfunctional channel having >50% reduction in channel current (dominant-negative effect).[6] We have recently shown that the degree of ion channel dysfunction caused by the mutations is a significant predictor of outcome in LQT1 patients.[7]

Studies reporting the detection of single nucleotide polymorphisms (SNPs) within different populations have shown that there is a large variability in the LQTS genes across countries and ethnic groups.[8–15] However, the effect of ethnicity on the phenotypic expression of LQTS has not been studied. To assess possible mechanisms by which ethnicity influences the risk of cardiac events in LQTS patients, we compared the clinical course of Japanese and Caucasian LQT1 patients from the International LQTS Registry who shared identical mutations in the KCNQ1 gene.

METHODS

Study Population

Study patients were drawn from a population of 600 subjects with genetically confirmed KCNQ1 mutations derived from 101 proband-identified LQT1 families. Patients were enrolled in the U.S. portion of the International LQTS Registry (n = 425), The Netherlands' LQTS Registry (n = 93), and the Japanese LQTS Registry (n = 82), ethnicity was self reported. In our comparison study, Caucasian subjects from the U.S. and The Netherlands' LQTS Registries were matched with Japanese subjects harboring the same mutations. This analysis identified six identical LQT1 mutations in the two ethnic groups, resulting in a final study population of 100 patients (62 Caucasians and 38 Japanese). Patients with homozygous KCNQ1 mutations characteristic of the Jervell-Lange Nielsen syndrome, or with a second mutation in another LQTS ion-channel gene were excluded from the study. All subjects or their guardians provided informed consent for the genetic and clinical studies.

Data Collection and Management

For each patient, data on personal and family history, cardiac events, and therapy were systematically recorded at each visit or medical contact on prospectively designed forms. Clinical data included patient and family history, demographics, electrocardiographic (ECG), therapeutic, and cardiac event information. Upon enrollment in the LQTS Registry, a 12-lead ECG was obtained from each patient. From this first recorded ECG, the duration of the QT interval was assessed from lead II (or lead I or III if the QT interval could not be measured from lead II) and corrected for heart rate (QTc) using Bazett's formula. Data common to all three LQTS registries involving genetically identified LQT1 patients were electronically merged into a common database.

Genotype Characterization

The KCNQ1 mutations were identified using standard genetic tests performed in academic molecular-genetic laboratories. Genetic alterations of the amino acid sequence were characterized by location in the channel protein, the type of mutation (missense, splice site, in-frame insertions/deletions, nonsense [stop codon], and frameshift), and the biophysical functional effect of the mutation (dominant negative or haploinsufficiency; Table 1). The biophysical function was classified based on studies reported in literature;[12,16–18] mutations that have not yet been characterized were assumed haploinsufficiency if identified as splice site, nonsense, in-frame deletion, or frameshift mutations. The transmembrane region of the KCNQ1-encoded channel included 6 membrane-spanning segments [S1–S6], and was defined as the coding sequence involving amino acid residues from 120 through 355 (comprising two cytoplasmic loops between S2–S3 and S4–S5, and the S5-pore–S6 region), with the N-terminus region defined before residue 120, and the C-terminus region after residue 355.

End Points

The end point of the study was the first occurrence of an LQTS-related cardiac event (syncope, aborted cardiac arrest [ACA] or LQTS-related death). In the primary analysis we compared the outcome by ethnicity, with and without adjustment for biophysical function (e.g., dominant negative vs haploinsufficiency). In a secondary exploratory analysis, the ethnic group comparison was further stratified by gender.

Table 1. KCNQ1 Mutations in Study Patients

Mutation	Caucasian N = 62	Japanese N = 38	Position	Exon	Type	Biophysical Functional Effect
A341V	4 (6%)	16 (42%)*	S6	7	Missense	Dominant negative[12]
G314S	4 (6%)	4 (11%)	Pore loop	7	Missense	Dominant negative[16]
R243C	9 (15%)	4 (11%)	S4/S5	5	Missense	Dominant negative[17]
delF340	4 (6%)	3 (8%)	S6	7	In-frame deletion	Haploinsufficiency†
A344A/sp	20 (32%)	7 (18%)	S6	7	Splice site	Haploinsufficiency†
G269S	21 (34%)	4 (11%)*	S5	6	Missense	Loss of function[18]

*P value for the comparison between Caucasian and Japanese patients < 0.05.
†Splice site and in-frame deletion mutation-types were assumed haploinsufficiency.

Statistical Analysis

The clinical characteristics of Caucasian and Japanese subjects were compared using the chi-square test, and the Fisher's exact test, as appropriate. The Kaplan-Meier life-table method was used to assess the time to a first LQTS-related cardiac event and the cumulative event rates for each ethnic group and mutation-type. The results were compared using the log-rank statistic.

Multivariate Cox proportional hazards regression modeling was carried out to determine the significant and independent contribution of each factor as a predictor for a first cardiac event during follow-up. Prespecified factors included gender, ethnicity, QTc interval duration >500 ms, the biophysical functional effect of the mutation (e.g., dominant negative vs haploinsufficiency), and time-dependent beta-blocker therapy. Data from the International LQTS Registry have shown that an implanted cardioverter defibrillator (ICD) is very effective in preventing fatal LQTS-related cardiac events.[19] Therefore, to validate the consistency of the results the non-ICD population, all analyses were repeated with censoring of patients upon implantation of an ICD.

The statistical software used for the analyses was SAS version 9.13 (SAS, Cary, NC, USA). A 2-sided probability value < 0.05 was used for declaring statistical significance.

RESULTS

Clinical and ECG characteristics of Caucasian and Japanese study patients are shown in Table 2, panel A. Gender distribution and baseline QTc duration were similar between the two ethnic groups. However, when the baseline QTc was analyzed by gender, Japanese male patients exhibited a significantly longer QTc duration as compared with their Caucasian counterparts. β-blocker therapy was administered to a similar proportion of Japanese and Caucasian patients, whereas ICD implantation occurred only in Caucasian LQT1 subjects.

The two ethnic groups exhibited significant differences in the frequencies of individual mutations (Table 1) and their biophysical functional effects, with the higher-risk dominant negative mutations being significantly more frequent among Japanese patients than among Caucasians (Table 2, panel A).

Cardiac Events During Follow-Up

A comparison of the frequency of cardiac events experienced between the two populations revealed a higher event rate among Japanese patients (Table 2, panel B). Notably, the frequency of presentation with ACA as a first cardiac event was significantly higher in Japanese patients as compared with Caucasians. Accordingly, the cumulative probability of a first cardiac event from birth through age 40 years was significantly higher among Japanese patients (67%) than among Caucasians (39%, P = 0.007; Fig. 1). Furthermore, cardiac events occurred at a significantly earlier age in the former ethnic group. Thus, at age 10 years Japanese and Caucasians exhibited a respective cardiac event rate of 43% and 18% (Fig. 1).

Comparison of event rates for the individual mutations showed a lower rate for mutations resulting in haploinsufficiency (G269S, A344A/sp, and delF340), and an increasing event rate for mutations with dominant negative ion current effects (R243C, G314S, and A341V; Fig. 2). Accordingly, dominant negative mutation carriers exhibited a significantly greater probability of a first cardiac event than those with haploinsufficiency (Fig. 3), and this finding was consistent in both the Japanese (82% vs 38%, respectively; P = 0.001) and

Table 2. Characteristics of Caucasian and Japanese LQT1 Patients

	Caucasian (n = 62)	Japanese (n = 38)	P-Value
A. Characteristics			
Female, n (%)	38 (61)	21 (55)	0.55
QTc, ms	487 ± 53	497 ± 35	0.30
Female, ms	498 ± 59	496 ± 39	0.92
Male, ms	468 ± 38	499 ± 32	0.02
Beta-blocker use, n (%)	28 (45)	14 (36)	0.41
ICD, n	10	0	
Pacemaker, n (%)	5 (8)	1 (3)	0.27
Sympathectomy, n	0	0	
Mutation characteristics			
Missense mutation, n (%)	38 (61)	28 (74)	0.20
Dominant negative, n (%)	17 (28)	24 (63)	<0.001
B. Cardiac Events			
Any cardiac event, n (%)*	25 (40)	22 (58)	0.09
Syncope, n (%)	21 (34)	17 (45)	0.28
Aborted cardiac arrest, n (%)	4 (7)	6 (16)	0.13
Sudden cardiac death, n	5 (8)	2 (5)	0.59
Other Death, n (%)	3 (5)	0 (0)	0.17
Age at first cardiac event, y	11	5	0.12
Syncope as a first cardiac event	21 (34)	16 (42)	0.41
Aborted cardiac arrest as a first cardiac event	1 (2)	5 (13)	0.02
Sudden cardiac death as first a cardiac event	3 (5)	1 (3)	0.59

Plus-minus values are means ± SD.
*Denotes the number of patients who experienced at least one cardiac event during follow-up. Mean QTc values include 87 cases; 13 cases were missing: 6 males and 7 females.
ICD = implantable cardioverter defibrillator .

Caucasian (71% vs 29%, respectively; P = 0.001) groups.

Multivariate Analysis

Cox proportional hazards regression modeling demonstrated that Japanese ethnicity was associated with a marginally significant 81% increase in the risk of cardiac events as compared to Caucasian ethnicity, after adjustment for gender, QTc duration, and time-dependent beta-blocker therapy (Table 3, panel A). However, the risk associated with ethnicity was no longer evident when the biophysical functional effect of the mutation was added to the multivariate model (Table 3, panel B). In this model, a dominant negative mutation effect was shown to be the most powerful and significant predictor of outcome in study patients, and was associated with nearly a fourfold increase in the risk of cardiac events during follow-up. The risk associated with dominant negative mutations was consistent for both Japanese and Caucasian patients (P value for ethnicity × mutation effect interaction = 0.54).

Gender Differences within Ethnic Groups

We have previously shown that in LQT1 patients the risk of cardiac events is affected by age and gender.[20] We have therefore carried out a further exploratory analysis, in which the clinical course of males and females was compared and related to the biophysical functional effect of the mutations in the 2 ethnic groups. Overall, gender did not contribute significantly to outcome in study patients after adjustment for ethnicity or mutation effect (Table 3, panel A and panel B, respectively). However, when gender was related to the biophysical functional effect of the LQT1 mutations, dominant negative mutations were shown to exhibit a different gender effect in the two ethnic groups. The risk of cardiac events among Japanese males with dominant negative mutations was significantly higher than among Japanese females with the same mutations (HR = 3.46 [95% CI 1.06–11.27]; P = 0.03), whereas male and female Caucasians with dominant negative mutations exhibited a similar risk of cardiac events during follow-up (HR = 0.79 [95% CI 0.13–4.65]; P = 0.80).

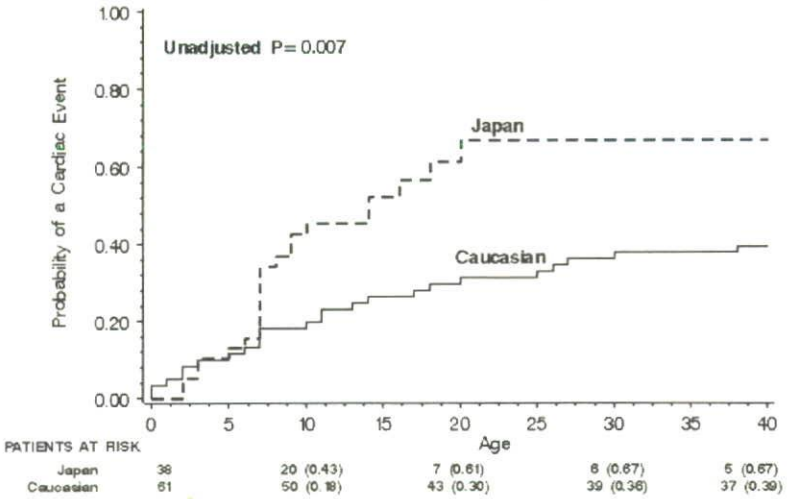

Figure 1. Kaplan-Meier estimates of the probability of a first cardiac event from birth through age 40 years by ethnicity.

DISCUSSION

In the present study, we sought to explore the extent of phenotypic variation that occurs across two ethnic groups that possess the same LQT1 mutations. Our findings suggest that the clinical severity of LQTS in different ethnic groups is largely determined by the frequency distribution of the LQT1 mutations in the population.

Ethnicity and Single Nucleotide Polymorphisms

Genotype-phenotype relationships and risk factors for cardiac events in LQTS patients have been the topic of much investigation.[20–25] However, previous studies that have analyzed the variability in disease expression and phenotype severity did not assess the effect of ethnicity on outcome in LQTS

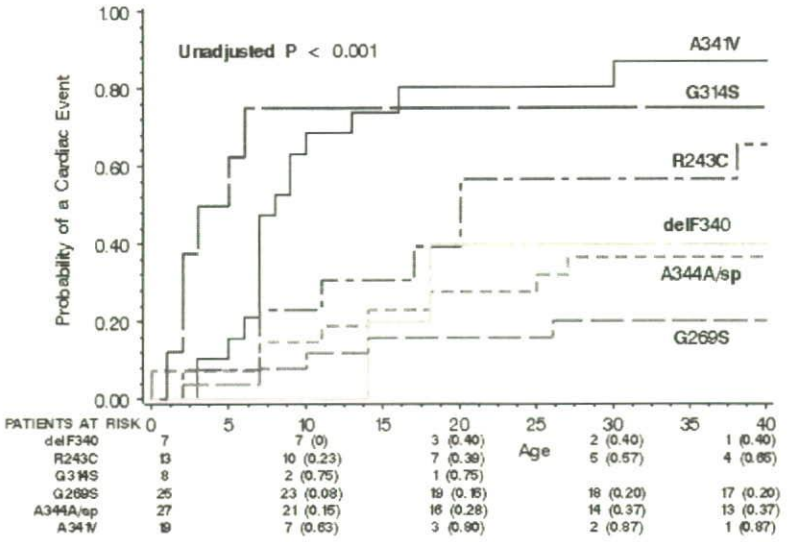

Figure 2. Kaplan-Meier estimates of the probability of a first cardiac event from birth through age 40 years for the six individual KCNQ1 mutations included in the study.

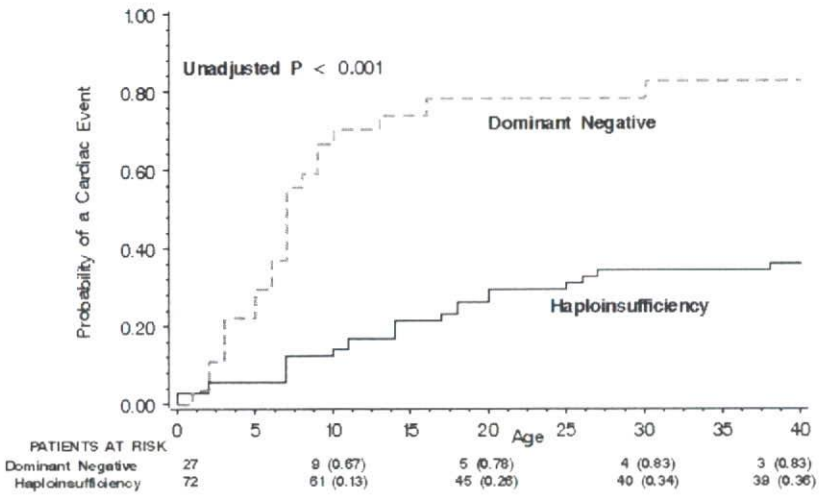

Figure 3. Kaplan-Meier estimates of the probability of a first cardiac event from birth through age 40 years by the biophysical functional effect of the mutations.

patients. Modifier genes and/or environmental factors may differ among ethnic groups and may explain why a given genotype confers variable susceptibility to cardiac arrhythmias in different ethnic groups. Thus, the identification of risk factors among LQTS populations with similar mutations may be important for risk-stratification in this genetic disorder. Ethnic-specific polymorphisms on the LQTS-related genes have been described in previous reports.[8–15,25–27] Notably, Ackerman et al.[8] studied 744 healthy black, white, Asian and Hispanic subjects and reported 49 distinct amino acid-altering variants of four LQTS-related potassium channel genes. These findings reflect the genetic diversity that may be a source of phenotype variation among different ethnic populations.

The evidence that certain polymorphisms on the KCNQ1 gene may increase susceptibility to cardiac

Table 3. Multivariate Analysis*

Variable	Hazard Ratio	95% Confidence Interval	P Value
A. Risk of a first cardiac event by ethnicity after adjustment for, gender, QTc and time-dependent beta-blocker therapy			
Japan:Caucasian	1.81	0.96–3.40	0.06
Female:Male	0.72	0.37–1.38	0.32
QTc>500:QTc≤500 ms	1.71	0.90–3.24	0.10
Beta-blockers no beta-blockers†	0.31	0.07–1.32	0.11
B. Risk of a first cardiac event by ethnicity after further adjustment for the biophysical function of the mutations			
Japan:Caucasian	1.05	0.53–2.10	0.89
Dominant negative:haploinsufficiency	3.78	1.90–7.51	<0.001
Female:Male	0.76	0.39–1.49	0.43
QTc>500:QTc≤500 ms	1.50	0.78–2.90	0.22
Beta-blockers No beta-blockers†	0.33	0.08–1.40	0.13

*The analysis involved 87 cases; 13 cases were omitted due to missing QTc values; virtually identical results were obtained when analysis were repeating with censoring of patients upon implantation of an ICD.
†Beta-blocker therapy was analyzed as a time-dependent variable in the multivariate model.

events has given credence to the concept of modifier genes. Kubota et al.[13] identified six patients that exhibited mild signs of LQTS as carriers of the G643S polymorphism, which is found in approximately 11% of the Japanese population. Through patch-clamp tests, it was found that this polymorphism, coexpressed with wild-type KCNQ1 and KCNE1, conferred approximately a 30% reduction in current. This same polymorphism has also been associated with QT intervals that vary with gender and age in Japanese subjects.[26] In addition, ethnic-specific SNPs have been recognized as important in the correct diagnosis and clinical management of LQTS suspected patients. In one report, a previously established KCNQ1 mutation, P488R, was found to be a common polymorphism in 14% of healthy Chinese volunteers after a healthy child of a proband was mistakenly diagnosed with LQTS while his undiagnosed brother was later found to have a novel HERG mutation.[27] Therefore, diagnosis based on genotyping requires correct discrimination of genetic polymorphisms from pathologic mutations, which may be confounded by ethnic genetic variability.

It has been previously suggested that the observed phenotypic differences between Caucasian and Japanese LQTS patients may be attributed to the effect of ethnic-specific common polymorphisms.[25] Evidence from the current study suggests that the clinical course of individual patients is dependent on the biophysical function of their mutations and is independent of ethnic origin. Japanese patients possessed a larger proportion of dominant negative mutations that are powerful and significant predictors of outcome in both ethnic groups, whereas the frequency of the lower-risk, haploinsufficiency mutations, was significantly higher among Caucasians. Accordingly, the risk of cardiac events among the Japanese patients was higher than among the Caucasians patients without adjustment for mutation effect, whereas a similar ethnic risk was shown when the biophysical functional effect of the mutations was incorporated into the multivariate model.

Notably, 42% of Japanese patients harbored the dominant negative A341V mutation compared with only 6% of Caucasian patients. The A341V mutation has been associated with a severe clinical course and early onset disease.[12] Consistently, in the current study we have shown that Japanese and Caucasian patients who carried the A341V mutation experienced the highest rate of cardiac events, and at an earlier age, compared to the other five shared mutations (Fig. 2). Eighty percent of the patients who were identified as carriers of the A341V mutation had a cardiac event prior to age 20 years. Therefore, our findings suggest that identification of the functional effect of KCNQ1 mutations should comprise a primary component in the risk stratification and clinical management of LQT1 patients, regardless of their ethnic origin.

Possible Gender-Related Phenotype Variability in Japanese and Caucasian Patients

A previous study from the International LQTS Registry demonstrated an age-gender interaction in LQT1 patients, in which the risk of cardiac events is higher in males than females before adolescence, with risk-reversal in the postadolescence period.[20] Possibly due to sample size limitations, we did not show an age-gender interaction in the two ethnic groups in the current study population. However, our secondary exploratory analysis suggests a possible gender-related difference in the functional effect of LQT1 mutation between the two ethnic groups. Japanese males who possessed dominant negative mutations had a significantly higher risk of cardiac events than Japanese females with the same mutations, whereas the effect of the dominant negative mutations did not show a significant gender-related difference in the Caucasian population. These findings may be due to the fact that Japanese patients with dominant negative mutations experienced a relatively high rate of cardiac events during childhood, a time-period that has been shown to be associated with a higher risk among LQT1 males.[20] It is also possible that modifier genes affect gender differences in risk in the two ethnic groups.

Limitations

The Japanese, United States, and the Netherlands' LQTS Registries have similar enrollment criteria. However, it is possible that higher risk patients were preferentially enrolled in the Japanese registry, leading to an unbalanced mutation distribution in the two ethnic groups. Therefore, the frequencies of individual KCNQ1 mutations in different ethnic groups need to be further assessed in large population studies. Nevertheless, our findings suggest that the risk associated with the biophysical

function of a mutation in an individual is more important than the risk associated with his/her ethnic origin.

The findings regarding gender differences in the risk between the two ethnic groups should be regarded as secondary and preliminary due to sample size limitations, and therefore need to be further validated in future studies.

Conclusions and Clinical Implications

The growing data regarding identification of gene defects in congenital LQTS has revolutionized our understanding of the basic mechanisms underlying QT prolongation and cardiac arrhythmias, and raises the possibility of mutation-specific therapeutic intervention. The LQT1 genotype accounts for approximately 50% of genotyped LQTS patients, and has shown to be associated with variable clinical expression, incomplete penetrance,[1] and ethnic-specific phenotypic variation.[25] Our findings suggest that, regardless of ethnic origin, risk-assessment in LQT1 patients should consider the biophysical functional effect of the mutation in an individual as an important determinant of outcome.

REFERENCES

1. 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.
2. Wang Q, Shen J, Splawski I, et al. SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. Cell 1995;80:805-811.
3. Sanguinetti MC, Jiang C, Curran ME, et al. A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the IKr potassium channel. Cell 1995;81:299-307.
4. Cronk LB, Ye B, Kaku T, et al. Novel mechanism for sudden infant death syndrome: Persistent late sodium current secondary to mutations in caveolin-3. Heart Rhythm 2007;4:161-156.
5. Wang Q, Curran ME, Splawski I, et al. Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. Nat Genet 1996;12:17-23.
6. Wang Z, Tristani-Firouzi M, Xu Q, et al. Functional effects of mutations in KvLQT1 that cause long QT syndrome. J Cardiovasc Electrophysiol 1999;10:817-826.
7. Moss AJ, Shimizu W, Wilde AA, et al. Clinical aspects of type-1 long-QT syndrome by location, coding type, and biophysical function of mutations involving the KCNQ1 gene. Circulation 2007;115:2481-2489.
8. Ackerman MJ, Tester DJ, Jones GS, et al. Ethnic differences in cardiac potassium channel variants: Implications for genetic susceptibility to sudden cardiac death and genetic testing for congenital long QT syndrome. Mayo Clin Proc 2003;78:1479-1487.
9. Modell SM, Lehmann MH. The long QT syndrome family of cardiac ion channelopathies: A HuGE review. Genet Med 2006;8:143-155.
10. Niu DM, Hwang B, Hwang HW, et al. A common SCN5A polymorphism attenuates a severe cardiac phenotype caused by a nonsense SCN5A mutation in a Chinese family with an inherited cardiac conduction defect. J Med Genet 2006;43:817-21.
11. Chen J, Xie X, Zhu J, et al. Single-nucleotide polymorphisms in SCN5A gene in Chinese Han population and their correlation with cardiac arrhythmias. Genet Med 2004;6:159.
12. Brink PA, Crotti L, Corfield V, et al. Phenotypic variability and unusual clinical severity of congenital long-QT syndrome in a founder population. Circulation 2005;112:2602-2610.
13. Kubota T, Horie M, Takano M, et al. Evidence for a single nucleotide polymorphism in the KCNQ1 potassium channel that underlies susceptibility to life-threatening arrhythmias. J Cardiovasc Electrophysiol 2001;12:1223-1229.
14. Hiraoka M. Inherited arrhythmic disorders in Japan. J Cardiovasc Electrophysiol 2003;14:431-434.
15. Fodstad H, Bendahhou S, Rougier JS, et al. Molecular characterization of two founder mutations causing long QT syndrome and identification of compound heterozygous patients. Ann Med 2006;38:294-304.
16. Kobori A, Sarai N, Shimizu W, et al. Additional gene variants reduce effectiveness of beta-blockers in the LQT1 form of long QT syndrome. J Cardiovasc Electrophysiol 2004;15:190-199.
17. Franqueza L, Lin M, Shen J, et al. Long QT syndrome-associated mutations in the S4-S5 linker of KvLQT1 potassium channels modify gating and interaction with minK subunits. J Biol Chem 1999;274:21063-21070.
18. Murray A, Potet F, Bellocq C, et al. Mutation in KCNQ1 that has both recessive and dominant characteristics. J Med Genet 2002;39:681-685.
19. Zareba W, Moss AJ, Daubert JP, et al. Implantable cardioverter defibrillator in high-risk long QT syndrome patients. J Cardiovasc Electrophysiol 2003;14:337-341.
20. Zareba W, Moss AJ, Locati EH, et al. International Long QT Syndrome Registry. Modulating effects of age and gender on the clinical course of long QT syndrome by genotype. J Am Coll Cardiol 2003;42:103-109.
21. Moss AJ, Schwartz PJ, Crampton RS, et al. The long QT syndrome. Prospective longitudinal study of 328 families. Circulation 1991;84:1136-1144.
22. Priori SG, Napolitano C, Schwartz PJ, et al. Association of long QT syndrome loci and cardiac events among patients treated with beta-blockers. JAMA 2004;292:1341-1344.
23. Zareba W, Moss AJ, Schwartz PJ, et al. Influence of genotype on the clinical course of the long-QT syndrome. International Long-QT Syndrome Registry Research Group. N Engl J Med 1998;339:960-965.
24. Priori SG, Schwartz PJ, Napolitano C, et al. Risk stratification in the long-QT syndrome. N Engl J Med 2003;348:1866-1874.
25. Shimizu W. The long QT syndrome: Therapeutic implications of a genetic diagnosis. Cardiovasc Res 2005;67:347-56.
26. Ozawa T, Ito M, Tamaki S, et al. Gender and age effects on ventricular repolarization abnormality in Japanese general carriers of a G643S common single nucleotide polymorphism for the KCNQ1 gene. Circ J 2006;70:645-650.
27. Sharma D, Glatter KA, Timofeyev V, et al. Characterization of a KCNQ1/KVLQT1 polymorphism in Asian families with LQT2: Implications for genetic testing. J Mol Cell Cardiol 2004;37:79-89.

Seasonal and circadian distributions of ventricular fibrillation in patients with Brugada syndrome

Masateru Takigawa, MD, Takashi Noda, MD, PhD, Wataru Shimizu, MD, PhD, Koji Miyamoto, MD, Hideo Okamura, MD, Kazuhiro Satomi, MD, PhD, Kazuhiro Suyama, MD, PhD, Naohiko Aihara, MD, Shiro Kamakura, MD, PhD, Takashi Kurita, MD, PhD

From the Division of Cardiology, Department of Internal Medicine, National Cardiovascular Center, Suita, Japan.

BACKGROUND It is well-known that the incidence of ventricular tachyarrhythmias is the highest in winter and during the daytime in patients with structural heart disease. However, little is known about the seasonal and circadian distributions of ventricular fibrillation (VF) in patients with Brugada syndrome.

OBJECTIVE The aim of this study was to investigate seasonal and circadian distributions of VF in patients with Brugada syndrome.

METHODS We analyzed the data of appropriate shock episodes for VF recorded by an implantable cardioverter-defibrillator (ICD) in patients with Brugada syndrome.

RESULTS Among 62 consecutive Brugada syndrome patients with an ICD (48 ± 14 years, 58 males), 19 patients had at least one episode of an appropriate ICD shock due to VF during a mean follow-up of 70 ± 36 months, and 98 episodes were evaluated as isolated VF. There was a significant peak between March and June ($P = .03$). As for the circadian variation, significantly more VF occurred from midnight to 6:00 ($P < .0001$). Electrical storms of VF occurred in seven patients. The seasonal and circadian variations of electrical storms were similar to those of the isolated VF episodes.

CONCLUSIONS In patients with Brugada syndrome, there was a significant seasonal peak from spring to early summer and a significant circadian peak from midnight to early morning in terms of the occurrences of VF.

KEYWORDS Brugada syndrome; Ventricular fibrillation; Implantable cardioverter-defibrillator; Electrical storm; Distribution

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Introduction

Brugada syndrome is an arrhythmogenic disease characterized by a particular electrocardiogram (ECG) pattern with a coved-type ST-segment elevation in the right precordial leads and an increased risk for sudden cardiac death due to ventricular tachyarrhythmias.[1–6] It is well-known that the incidence of ventricular tachyarrhythmias, including ventricular tachycardia (VT) and ventricular fibrillation (VF), is the highest in the winter and during the daytime in patients with structural heart disease.[7–12] In patients with Brugada syndrome, however, the seasonal patterns of the development of malignant ventricular tachyarrhythmias remain unknown, and the circadian patterns have been reported in only a small population.[4,13] Identification of the distribution of VF seems to contribute to a therapy option and the elucidation of the underlying pathophysiology of Brugada syndrome. Therefore, we investigated the seasonal and circadian patterns of VF by analyzing the data stored in an implantable cardioverter-defibrillator (ICD) of patients with Brugada syndrome.

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Methods

Study population

The study population consisted of 62 consecutive patients with Brugada syndrome (58 men and four women, mean age 48 ± 14 years) who received an ICD between July 1992 and October 2005 at National Cardiovascular Center, Suita, Japan. The following devices were implanted: Medtronic 7217CX, 7221CX, 7223CX, 7227CX, 7228CX, 7229CX, 7230CX, 7036CX, and 7274CX; Guidant 1859, 1860, 1861, and 1790; and CPI 1000, 1600, 1718, and 1742. Brugada syndrome was diagnosed when J-wave amplitude was over 0.2 mV and coved-type ST segment elevation was observed in the right precordial leads (V1–V3) in the presence or absence of a sodium channel blocker and in conjunction with one of the following: (1) documented VF or polymorphic VT, (2) a family history of sudden cardiac death at an age younger than 45 years, (3) a type 1 ECG in family members, (4) electrophysiological inducibility of VF or polymorphic VT with programmed electrical stimulation, or (5) history of aborted cardiac arrest (CA) with or without VF, syncope of unknown origin, or nocturnal agonal respiration.[3] There were no abnormal findings on the physical examination, chest radiography, or echocardiography suggesting the presence of organic heart diseases in any of the patients.

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