Congenital Long-QT Syndrome Caused by a Novel Mutation in a Conserved Acidic Domain of the Cardiac Na⁺ Channel

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Background—Congenital long-QT syndrome (LQTS) is an inherited condition of abnormal cardiac excitability characterized clinically by an increased risk of ventricular tachyarrhythmias. One form, LQT3, is caused by mutations in the cardiac voltage–dependent sodium channel gene, SCN5A. Only 5 SCN5A mutations have been associated with LQTS, and more work is needed to improve correlations between SCN5A genotypes and associated clinical syndromes.

Methods and Results—We researched a 3-generation white family with autosomal dominant LQTS who exhibited a wide clinical spectrum from mild bradycardia to sudden death. Molecular genetic studies revealed a single nucleotide substitution in SCN5A exon 28 that caused the substitution of Glu1784 by Lys (E1784K). The mutation occurs in a highly conserved domain within the C-terminus of the cardiac sodium channel containing multiple, negatively charged amino acids. Two-electrode voltage-clamp recordings of a recombinant E1784K mutant channel expressed in Xenopus oocytes revealed a defect in fast inactivation characterized by a small, persistent current during long membrane depolarizations. Coexpression of the mutant with the human sodium channel β1-subunit did not affect the persistent current, even though we did observe shifts in the voltage dependence of steady-state inactivation. Neutralizing multiple, negatively charged residues in the same region of the sodium channel C-terminus did not cause a more severe functional defect.

Conclusions—We characterized the genetics and molecular pathophysiology of a novel SCN5A sodium channel mutation, E1784K. The functional defect exhibited by the mutant channel causes delayed myocardial repolarization, and our data on the effects of multiple charge neutralizations in this region of the C-terminus suggest that the molecular mechanism of channel dysfunction involves an allosteric rather than a direct effect on channel gating. (Circulation. 1999;99:3165-3171.)

Key Words: long-QT syndrome ▪ sodium channel ▪ SCN5A ▪ genes ▪ heart defects, congenital

Congenital long-QT syndrome (LQTS) is an inherited condition of abnormal cardiac excitability characterized clinically by an increased risk of potentially fatal ventricular tachyarrhythmias, especially torsades de pointes.1–3 The syndrome is most often transmitted in families as an autosomal-dominant trait (Romano-Ward syndrome) and less commonly as a recessive illness combined with congenital deafness. LQTS derives its name from the characteristic prolongation of the QT interval on surface electrocardiograms of affected individuals, correlating with an increased duration of the ventricular action potential caused by an increase in the time required for myocardial cell repolarization. Congenital LQTS is an important disease model for understanding cardiac electrophysiology and the basic mechanisms underlying cardiac arrhythmias, including drug-induced LQTS, a major therapeutic obstacle to the safe use of many antiarrhythmic agents.4 Therefore, a more thorough understanding of the molecular basis of LQTS may lead to the development of more effective therapeutic strategies to prevent the widespread problem of sudden cardiac death.

Recent work on the molecular genetics of LQTS has demonstrated that it is an inherited disorder of cardiac ion channels.1–3 The majority of LQTS subjects seem to harbor mutations in 2 cardiac potassium channel genes (HERG and KvLQT1),5,6 but additional cases are caused by mutations in genes encoding a potassium channel regulatory subunit (KCNE1),7 a cardiac voltage–dependent sodium channel α-subunit (SCN5A),8 and other unidentified gene products.9 Preliminary genotype-phenotype correlations have suggested that sodium channel mutations may produce distinct clinical features in LQTS patients, including bradycardia, exercise-induced QT shortening, and mexiletine responsiveness.3,10 To
date, there have been only 5 identified SCN5A mutations associated with LQTS,4–13 and further work is needed to bolster those preliminary observations regarding the clinical and molecular aspects of the syndrome.

We report here the identification of a novel SCN5A mutation in autosomal-dominant LQTS. The mutation occurs in a highly conserved acidic domain within the carboxy terminus of the cardiac sodium channel and produces subtle gating abnormalities that are the likely cause of the disease. Results from additional mutagenesis experiments in this domain suggest that the biophysical mechanism may be allosteric rather than caused by direct interference with channel-gating processes. Our results provide additional molecular genetic and pathophysiological observations regarding LQTS and normal sodium channel physiology and contribute to our understanding of sudden cardiac death.

Methods

Patients

A 3-generation white family with autosomal-dominant LQTS was identified after the witnessed sudden death of the proband, a 13-year-old girl with no prior illnesses (Figure 1). The proband died while at rest. An autopsy demonstrated no evidence of structural heart disease. Two siblings and both parents were available for clinical evaluation of the immediate family. The father experienced occasional light-headedness and had 1 episode of syncope 9 years before evaluation, but he denied palpitations or recent syncpe. The proband’s mother and both siblings were asymptomatic. A paternal aunt had died suddenly at the age of 42 years of an unknown cause. Proband’s mother and both siblings were asymptomatic. Both individuals exhibited sinus bradycardia and QT prolongation. Other members of the extended family were either unavailable or had normal QT intervals.

Molecular Genetics

Informed consent was obtained from participating family members using a method approved by the Vanderbilt University Institutional Review Board. Genomic DNA was isolated from peripheral blood leukocytes using standard methods.14 Selected SCN5A exons were screened for the presence of nucleotide sequence polymorphisms by single-strand conformation analysis. Oligonucleotide primer pairs, as described by Wang et al,15 were used to amplify SCN5A exons 16 through 28 using polymerase chain reaction (PCR). Primers used to amplify the SCN5A-coding region containing nucleotides 5229 to 5538 were 5'-GAGCCCAAGCCGTCGCGCATCTC-3' (forward primer) and 5'-GTCCCCACTACCATAGGCCCCAG-3' (reverse primer). Amplification reactions were carried out using 200 ng of genomic DNA, 0.5 μmol/L of primers, 0.2 mmol/L dNTPs, and Taq polymerase. Single-strand conformation analysis was performed on 0.5× MDE gels that were electrophoresed overnight at 4 W and stained with silver nitrate. Abnormal conformers were excised from dried gels, eluted into sterile water, reamplified using the original primers, and sequenced using dye-terminator chemistry.

The presence of the specific mutation (E1784K) reported in this study was confirmed independently on PCR-amplified genomic DNA using an allele-specific oligonucleotide hybridization assay with a 32P end-labeled primer (5'-AGAGCACCAGCCGCCCTG-3').

Site-Directed Mutagenesis

Mutagenesis of human cardiac sodium channel α-subunit cDNA (hH1)16 using a 1-step recombinant PCR strategy was performed to create the E1784K mutation. A forward primer (5'-GTGTTCAACATGTACATTGCCCATCTCGGAACATCTCAGCCGACCCAGGGAGAGGACGA-3') spanning nucleotides 5287 to 5369 and the reverse primer described above were used to create the mutation (the changed codon is underlined) and incorporate natural restriction sites for BsrGI (nucleotide 5298) and AspEI (nucleotide 5461) in the final 252-bp product. Amplifications (20 cycles) were performed using 20 ng of hH1 cDNA as a template and Taq DNA polymerase. Final products were purified by spin-column chromatography (Qiagen) and digested with BsrGI and AspEI; the resulting 164-bp fragment was ligated into the corresponding sites in the plasmid pSP64T-hH1. The amplified region was sequenced entirely in the final construct to verify the mutation and to exclude polymerase errors.

Additional hH1 mutations were constructed using a similar approach. One mutation (Q-1) consisting of 4 Gin substitutions for Glu at positions 1773, 1780, 1781, and 1784 was constructed using the

![Figure 1](https://example.com/figure1.png)
forward primer 5'-GTGGTCAACATGTACATGGCCCATCATCCTGGCAAGACCTTGAGTGAGGACGA-3' (changed codons are underlined) and the previously described reverse primer. A second, multiple charge-neutralizing mutation (Q-II) was constructed in which Gln substitutions were made for negatively charged amino acids at positions 1788, 1789, 1790, and 1792 using the mutagenic forward primer 5'-AGCACCGAGCCCCTGAGTCACACAGCATCTATGAGATCTGG-3' (changed codons are underlined). Subcloning of Q-I and Q-II mutant fragments was performed as described for E1784K.

Electrophysiology
All cDNAs encoding wild-type and mutant hH1 were transcribed in vitro from pSP64T constructs using SP6 RNA polymerase, and the resultant cRNAs were microinjected into Xenopus oocytes. In most experiments, coexpression with a recombinant human β1-subunit (hβ1) was achieved by coinjecting oocytes with cRNA derived from the plasmid pSP64T-hβ1. Sodium channel expression was examined by 2-microelectrode voltage-clamp recording, as previously described. All measurements were made using the pCLAMP suite of programs (Axon Instruments) and analyzed as previously described. For measurements of tetrodotoxin (TTX)-sensitive current, recordings were made during repetitive 200-ms test pulses to -20 mV from a holding potential of -120 mV (interpulse duration was 5 s) in the absence and presence of 30 μmol/L of TTX. The average current was obtained 20 to 30 records after achieving steady state, and the difference between the presence and absence of TTX was used as the TTX-sensitive current component. TTX-sensitive currents were recorded from 4 to 6 oocytes for each mutation.

Results
Molecular Genetics
Because of the clinical findings of bradycardia and exercise-induced QT-interval shortening observed in the LQTS family, we chose to screen selected regions of the SCN5A coding
region for nucleotide sequence variants. Initially, exon segments containing previously identified mutations were evaluated by single-strand conformational analysis, but they were normal. We next initiated a systematic screen of additional SCN5A exons, beginning first with regions encoding the fourth domain (D4) and carboxy terminus. Examination of the coding region between the D4/S6 segment and the early C-terminus demonstrated an aberrant single-strand conformer in affected individuals from this family but not in unaffected members (Figure 2). Nucleotide sequencing of the aberrant product revealed a G\to A transition associated with a codon change (GAG to AAG), predicting the substitution of Glu1784 by Lys (designated E1784K). This nucleotide change was independently confirmed in all affected, but none of the unaffected, members of the family using an allele-specific oligonucleotide hybridization assay. The mutation was also not detected in 100 normal control DNA samples using this same assay. The cosegregation of the allele with the disease phenotype, its absence in the general population, and the resulting nonconservative amino acid substitution are consistent with a disease-producing mutation.

The affected residue (Glu1784) is located within a highly conserved acidic domain immediately following the D4/S6 segment. Glu or, rarely, Asp is found at this corresponding location in most voltage-gated sodium channel sequences in most animals, including invertebrates (Figure 3). Further inspection reveals that this region of the early C-terminus has clusters of negatively charged amino acid residues. A similar motif is also found in the corresponding region of L-type calcium channels, and it has a remote homology to an EF-hand Ca^{2+} binding motif.22,23 The functional importance of this region in sodium channels has not been explored.

Functional Characterization of E1784K

We used the Xenopus oocyte expression system and 2-electrode voltage-clamp recording to evaluate the functional consequences of the E1784K mutation engineered into a recombinant human cardiac sodium channel a-subunit (hH1). Expression of wild-type (WT) and mutant hH1 in oocytes gave rise to sodium currents of similar magnitude. Initial inspection of raw current traces obtained in response to depolarizing test potentials seemed similar between WT-hH1 and E1784K. However, when TTX-sensitive sodium currents were analyzed, a small (2% to 4%) residual current was observed in oocytes expressing E1784K but not WT-hH1. Coexpression of h\beta1 had no effect on the presence or magnitude of this residual current (Figure 4). This is best illustrated with an expanded current scale, as shown in Figure 4, C and D. The occurrence of a noninactivating steady-state...
The apparent defect in channel inactivation was further quantified by fitting the decay phase of both WT-hH1 and E1784K current traces with double exponential functions. Time constants for the fast phase of inactivation were quantified by fitting the decay phase of both WT-hH1 and E1784K. A, Steady-state inactivation curves obtained using pulse protocol shown in inset. Midpoint of steady-state inactivation ($V_{1/2}$) is as follows: WT-hH1, $-74.1 \pm 0.9$ mV (n=7); WT-hH1+$h\beta_4$, $-63.2 \pm 1.3$ mV (n=10); E1784K, $-79.3 \pm 0.5$ mV (n=20); and E1784K+$h\beta_4$, $-75.3 \pm 0.9$ mV (n=11). Significant differences exist between WT-hH1 and WT-hH1+$h\beta_4$ ($P<0.0001$), between E1784K and E1784K+$h\beta_4$ ($P<0.001$), between WT-hH1 and E1784K+$h\beta_4$ ($P<0.01$), and between WT-hH1+$h\beta_4$ and E1784K+$h\beta_4$ ($P<0.0001$). B, Time course of recovery from inactivation using 2-pulse protocol shown in inset. Each curve was fit with 2-exponential function. Time constants ($\tau_f$, $\tau_s$) for recovery from inactivation are as follows (amplitude of each component expressed as a percentage of total current given in parentheses): WT-hH1+$h\beta_4$ (n=10), $\tau_f=2.2 \pm 0.2$ ms (84%); $\tau_f=166.9 \pm 2.2$ ms (16%); E1784K+$h\beta_4$ (n=11), $\tau_f=1.8 \pm 0.2$ ms (76%); $\tau_f=86.5 \pm 7.4$ ms (24%); $P<0.005$ for $\tau_f$ only. Error bars represent SEM, which in some cases are smaller than data symbols.

The apparent defect in channel inactivation was further quantified by fitting the decay phase of both WT-hH1 and E1784K current traces with double exponential functions. Time constants for the fast phase of inactivation were indistinguishable ($\tau_{fast}$, WT-hH1=1.3±0.06, n=10, versus E1784K=1.3±0.06, n=23), but the time constant of the slow component was significantly larger in the mutant ($\tau_{slow}$, WT-hH1=12.1±0.33 versus E1784K=22.4±1.55; $P<0.001$). Coexpression with $h\beta_4$ produced currents that differed significantly in the fast time constant of inactivation, largely because of an effect on E1784K ($\tau_{fast}$, WT-hH1=1.2±0.03, n=31, versus E1784K=0.94±0.03, n=39; $P<0.0001$). Steady-state inactivation (Figure 5A) was also altered in the mutant, with a significant shift in the midpoint of steady-state inactivation ($V_{1/2}$) toward more negative potentials in the absence of $h\beta_4$ ($V_{1/2}$, WT-hH1=−74.0±0.9 mV, n=7, versus E1784K=−79.3±0.5 mV, n=20; $P<0.001$). Coexpression with $h\beta_4$ exaggerated this negative shift in $V_{1/2}$ ($V_{1/2}$, WT-hH1+$h\beta_4$=−63.2±1.3 mV, n=10, versus E1784K+$h\beta_4$=−75.3±0.9 mV, n=11; $P<0.01$). We also observed a small but significant change in the time course of recovery from inactivation elicited by a 500-ms prepulse (Figure 5B). The functional disturbances observed with oocytes expressing E1784K are similar to previously observed functional defects in 3 other LQTS SCN5A mutations 24–26 and are consistent with a molecular lesion being responsible for the disease.

Structure-Function Relationships
As discussed above, the highly conserved acidic domain within the early portion of the hH1 carboxy terminus has no known function. Because mutations in this region cause disease-producing channel dysfunction, we hypothesized that this structure might participate in channel gating. To evaluate this hypothesis, we neutralized clusters of acidic residues surrounding Glu1784 and studied their functional behavior in oocytes. Figure 6 illustrates the strategy used to make 2-charge neutralizing mutants and their corresponding whole cell recordings. The first mutant construct, Q-I, consists of 4 Gln substitutions for Glu at positions 1773, 1780, 1781, and 1782. Figure 6 demonstrates the functional consequences of these changes. Specifically, Q-I exhibits a small, noninactivating current component during long test depolarizations (Figure 6) and a negative shift in steady-state inactivation but no difference in the time course of recovery from inactivation (Figure 7). Additional Gln substitutions of the C-terminal to Glu1784 (mutant Q-II), including substitutions at positions 1788 through 1790 and 1792, produced a similar phenotype. The absence of more severe gating disturbances associated with the multiple-charge neutralizations strongly suggest that residues within

Figure 5. Voltage dependence of steady-state inactivation and time course of recovery from inactivation for WT-hH1 and E1784K. A, Steady-state inactivation curves obtained using pulse protocol shown in inset. Midpoint of steady-state inactivation ($V_{1/2}$) is as follows: WT-hH1, $-74.1 \pm 0.9$ mV (n=7); WT-hH1+$h\beta_4$, $-63.2 \pm 1.3$ mV (n=10); E1784K, $-79.3 \pm 0.5$ mV (n=20); and E1784K+$h\beta_4$, $-75.3 \pm 0.9$ mV (n=11). Significant differences exist between WT-hH1 and WT-hH1+$h\beta_4$ ($P<0.0001$), between E1784K and E1784K+$h\beta_4$ ($P<0.001$), between WT-hH1 and E1784K+$h\beta_4$ ($P<0.01$), and between WT-hH1+$h\beta_4$ and E1784K+$h\beta_4$ ($P<0.0001$). B, Time course of recovery from inactivation using 2-pulse protocol shown in inset. Each curve was fit with 2-exponential function. Time constants ($\tau_f$, $\tau_s$) for recovery from inactivation are as follows (amplitude of each component expressed as a percentage of total current given in parentheses): WT-hH1+$h\beta_4$ (n=10), $\tau_f=2.2 \pm 0.2$ ms (84%); $\tau_f=166.9 \pm 2.2$ ms (16%); E1784K+$h\beta_4$ (n=11), $\tau_f=1.8 \pm 0.2$ ms (76%); $\tau_f=86.5 \pm 7.4$ ms (24%); $P<0.005$ for $\tau_f$ only. Error bars represent SEM, which in some cases are smaller than data symbols.
this highly conserved acidic domain do not participate directly in the gating process.

Discussion

In this article, we describe a novel mutation in the human cardiac sodium channel \(\alpha\)-subunit gene (SCN5A) that causes autosomal-dominant congenital LQTS. Relatively few SCN5A mutations have been identified compared with the striking allelic heterogeneity found in the 2 cardiac potassium channel genes (HERG and KvLQT1) responsible for the majority of congenital LQTS cases. Although SCN5A is a much larger gene than either of the 2 cardiac potassium channel genes, the coding sequences of HERG and KvLQT1 are rich in CpG dinucleotides that are potential hot spots for mutations. It is important to make correlations between molecular genotype and the associated clinical syndrome to develop predictive markers for disease and help guide the most appropriate therapy.

The family we described has certain characteristics that have been associated with other SCN5A-linked forms of congenital LQTS, including bradycardia, exercise-induced QT-interval shortening, and isoelectric ST-T wave segments. The other important clinical observation in this family, as well as in many other families with LQTS, is the surprising lack of symptoms and objective manifestations of the disease in certain ECG- and genotype-positive individuals. This observation raises the prospect of gene-environmental interactions as important disease precipitants or the involvement of modifier genes in causing the phenotype. Clearly, more work is required to elucidate the basis for disease latency in such individuals.

The mutation we describe in this article (E1784K) occurs in a highly conserved acidic domain located in the early carboxy terminal segment of the sodium channel protein. This region has received little attention from sodium channel investigators, although there are now 3 known naturally occurring allelic variants within this domain in 2 distinct sodium channel isoforms. The first variant, described by Wang et al., occurs in the skeletal muscle sodium \(\alpha\)-subunit gene (SCN4A) and is associated with autosomal-dominant hyperkalemic periodic paralysis in a large Yugoslavian family. The allelic variant causes substitution of Lys for Glu1606 in SCN4A, only a few amino acids away from the corresponding residues in SCN5A that cause LQTS when substituted (E1784K, D1790G). The functional characterization of the skeletal-muscle sodium channel allele has not yet been reported. The functional characterization of the SCN5A allele reported by An et al. (Asp1790→Gly, or D1790G) has recently been described. This mutation causes little overt gating disturbances in recombiant cardiac sodium channels expressed in a cultured mammalian cell line. Rather, this mutation exhibits subtle differences in the voltage dependence of inactivation that are accentuated by coexpression with the accessory \(\beta\)-subunit. It is less clear how this pattern of dysfunction leads to delayed myocardial repolarization and LQTS, and it is difficult to reconcile with the work of several groups demonstrating that the cytoplasmic domain of the \(\beta\)-subunit is not required for its functional effects on mammalian sodium channels. Nonetheless, the accentuation of the functional defect by the \(\beta_1\) subunit is also apparent in our work on E1784K.

The functional characteristics of E1784K closely resemble the gating disturbances observed for many other SCN5A LQTS mutations. This defect is characterized by a small, persistent, inward current during long depolarizations that is expected to contribute to the delay in myocardial repolarization. The molecular basis for this channel dysfunction could involve direct interference with closure of the inactivation gate in the channel or involve indirect mechanisms that disrupt the critical conformational changes necessary for complete inactivation. However, it is not possible to assign a molecular mechanism solely on the basis of the results of a single-point mutation. Therefore, we tested the effects of additional structural changes in the highly conserved acidic domain to explore whether this structure participates directly in sodium channel gating. Our results suggest that neutralizing up to 4 negatively charged groups in this domain has little or no additional effect on the gating disturbance observed with the single-charge reversal mutation. These data argue in favor of an allosteric mechanism for channel dysfunction produced by both the disease-associated mutant and the multiply charged neutralization mutations.
In summary, we report the role of a novel SCN5A missense mutation in causing congenital LQTS and provide evidence that the functional basis for this disease-producing allele involves an allosteric effect on sodium channel gating. This work further emphasizes the importance of performing additional structure-function analyses before making conclusions about the direct involvement of mutated structures in channel behavior.

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