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Sodium Channel Abnormalities Are Infrequent in Patients With Long QT Syndrome: Identification of Two Novel *SCN5A* Mutations

Duangrurdee Wattanasirichaigoon,[1,3] Mark R. Vesely,[2] Priya Duggal,[2] Jami C. Levine,[2,3] Elizabeth D. Blume,[2,3] Grace S. Wolff,[4] Sam B. Edwards,[5] and Alan H. Beggs[1]*

[1]*Division of Genetics, Children's Hospital, Boston, Massachusetts*
[2]*Department of Cardiology, Children's Hospital, Boston, Massachusetts*
[3]*Department of Pediatrics, Harvard Medical School, Boston, Massachusetts*
[4]*Department of Cardiology, University of Miami, Jackson Memorial Medical Center, Miami, Florida*
[5]*Department of Cardiology, Children's Hospital of Southwest Florida, Fort Myers, Florida*

Long QT syndrome (LQTS) is a heterogeneous disorder caused by mutations of at least five different loci. Three of these, *LQT1, LQT2,* and *LQT5,* encode potassium channel subunits. *LQT3* encodes the cardiac-specific sodium channel, *SCN5A.* Previously reported LQTS-associated mutations of *SCN5A* include a recurring three amino acid deletion (ΔKPQ1505–1507) in four different families, and four different missense mutations. We have examined the *SCN5A* gene in 88 index cases with LQTS, including four with Jervell and Lange-Nielsen syndrome and the remainder with Romano-Ward syndrome. Screening portions of DIII–DIV, where mutations have previously been found, showed that none of these patients has the three amino acid deletion, ΔKPQ1505–1507, or the other four known mutations. We identified a novel missense mutation, T1645M, in the DIV; S4 voltage sensor immediately adjacent to the previously reported mutation R1644H. We also examined all of the additional pore-forming regions and voltage-sensing regions and discovered another novel mutation, T1304M, at the voltage-sensing region DIII; S4. Neither T1645M nor T1304M were seen in a panel of unaffected control individuals. Five of six T1304M gene carriers were symptomatic. In contrast to previous studies, $QT_{onset\text{-}c}$ was not a sensitive indicator of *SCN5A*-associated LQTS, at least in this family. These data suggest that mutations of *SCN5A* are responsible for only a small proportion of LQTS cases. Am. J. Med. Genet. 86:470–476, 1999. © 1999 Wiley-Liss, Inc.

KEY WORDS: arrhythmia; long QT syndrome; sodium channel; gene mutations

INTRODUCTION

Long QT syndrome (LQTS) is an inherited disorder of ventricular repolarization characterized by a prolonged QT interval on electrocardiogram (ECG), syncope, arrhythmias, and sudden death [Roden et al., 1996]. Romano-Ward syndrome is the more common autosomal dominant presentation while Jervell and Lange-Nielsen syndrome is an autosomal recessive variant associated with sensorineural deafness. Recently, five genetic loci, *LQT1-5,* four of which encode cardiac ion channel subunits, have been identified for the Romano-Ward and Jervell and Lange-Nielsen syndromes [Curran et al., 1995; Schott et al., 1995; Wang et al., 1995a, 1996a; Neyroud et al., 1997; Schulze-Bahr et al., 1997; Splawski et al., 1997; Duggal et al., 1998]. A number of different pathogenic mutations in the *KCNQ1* (formerly named *KVLQT1*) and *KCNH2* (formerly named *HERG*) potassium channel genes have been reported in patients with LQT1 and LQT2 respectively. Preliminary results suggested that *KCNQ1* mutations may account for up to half of LQTS cases [Wang et al., 1996a; Donger et al., 1997; Li et al., 1998] while mutations of *KCNH2* may be responsible for another 20–25% [Tanaka et al., 1997; Vesely et al., 1997; Itoh et al., 1998].

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*Correspondence to: Dr. Alan H. Beggs, Division of Genetics, Children's Hospital, 300 Longwood Ave, Boston, MA 02115. E-mail: beggs@rascal.med.harvard.edu

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LQT3 is associated with the persistence of an inward Na+ current throughout depolarization caused by mutations of the *SCN5A* gene [Roden et al., 1996]. This results in prolongation of the action potential leading to arrhythmias such as torsades de pointes and clinical symptoms of LQTS. *SCN5A* encodes the 240-kDa cardiac-specific Na channel α-subunit which is organized into four homologous domains, DI–DIV. Each domain is comprised of six transmembrane segments of which the S4 segments are thought to function as voltage sensors.

Given the complexity and relatively large size of *SCN5A,* it has been least studied of the known LQTS genes. In 1995, Wang et al. [1995a] described a three amino acid deletion (ΔKPQ1505–1507) in the DIII–DIV interdomain linker of SCN5A in four unrelated *LQT3*-linked families. An additional four missense mutations (Table I) have since been reported [Wang et al., 1995b; Benhorin et al., 1997; Matsuoka et al., 1997]. Interestingly, functional studies of these five *SCN5A* mutations demonstrated different pathogenic mechanisms and differential responses to antiarrhythmic drugs [Dumaine et al., 1996; Wang et al., 1996b, 1997; An et al., 1998; Kambouris et al., 1998; Makita et al., 1998]. Mutations of *SCN5A* have also recently been reported in patients with another separate autosomal dominant disorder, idiopathic ventricular fibrillation, demonstrating the variable nature of clinical presentations associated with abnormalities of the inward sodium current [Chen et al., 1998].

SCN5A mutations are thought to be associated with particularly prolonged QT_c intervals and more severe clinical presentations [Moss et al., 1995; Wang et al., 1995a; Benhorin et al., 1997]. Fortunately, they may respond to treatment with mexiletine, lidocaine, or other drugs [Schwartz et al., 1995; Wang et al., 1997; An et al., 1998; Kambouris et al., 1998]. Identification of the subset of LQTS patients with *SCN5A* mutations is therefore a clinically important issue. In the present study, the entire region containing previously reported mutations and an additional segment of the gene have been examined. Since the S4 voltage sensors are very sensitive to small changes in the membrane electrical field, mutations of these structures are likely to have a significant effect on function of the channel [Fozzard and Hank, 1996]. There have also been a number of mutations reported in the *KCNH2* pore region [Curran et al., 1995; Tanaka et al., 1997; Satler et al., 1998]. We therefore extended our study to cover all four voltage sensor segments, as well as the four pore-forming regions between S5 and S6.

MATERIALS AND METHODS

Identification of Patients

Inclusion criteria for this study were a provisional or confirmed clinical diagnosis of LQTS as determined by referring cardiologists at Children's Hospital, Boston and 45 other medical centers in North America. Each subject (index cases and relatives) provided informed consent approved by the Children's Hospital Institutional Review Board. Clinical histories were obtained both retrospectively and prospectively including syncope, palpitations, chest pain, seizures, hearing deficit, past medical history, medications, and previous ECG test results. Scores were assigned to each subject using the 1993 LQTS Diagnostic Criteria described by Schwartz [1993](a score ≥4 suggests high probability of LQTS). $QT_{onset\text{-}c}$ and QT_c were calculated using the Bazett formula as described ($parameter_c$ = measured parameter/√RR)[Moss et al., 1995]; they have units of seconds [Molnar et al., 1995]. To avoid interobserver bias, all electrophysiological measurements were performed by a single author (J.C.L.) who was blinded to additional clinical and molecular data.

Mutation Analysis

Peripheral blood was obtained and DNA extracted as described elsewhere [Duggal et al., 1998; Satler et al., 1998]. Primer sequences for amplifying exons 6, 9, 15, 16, 22, 23, 24, 25, 26, 27, and part of exon 28 (carboxy terminal) were published by Wang et al. [1996c]. Annealing temperatures were 65°C (for exons 26 and 28), 62°C (for exons 9, 22, 25, and 27), 60°C (for exon 16), 56°C (for exons 15 and 23), or 54°C (for exons 6 and 24). Polymerase chain reactions (PCRs), single-strand conformation polymorphism (SSCP) analysis, and DNA sequencing were performed as described previously [Duggal et al., 1998; Satler et al., 1998]. In addition, the three amino acid (ΔKPQ) deletion in exon 26 was as-

TABLE I. Summary of LQTS *SCN5A* Mutations and Nonpathogenic Variants

Nucleotide change	Coding effect	Amino acid change	Region affected	Exon[a]	References[b]
Δ4661–4669	In-frame deletion	Δ KPQ1505-1507	IDIII–IV	26	Wang et al., 1995a, 1995b
G5081A	Missense	R1644H	DIV; S4	28	Wang et al., 1995b
A4124G	Missense	N1325S	DIII; S4–5	23	Wang et al., 1995b
G5018A	Missense	R1623Q	DIV; S4	28	Matsuoka et al., 1997
A5519G	Missense	D1790G	C-terminus	28	Benhorin et al., 1997
C4062T	Missense	T1304M	DIII; S4	22	This study
C5084T	Missense	T1645M	DIV; S4	28	This study
G4650T	Missense[c]	K1500N	IDIII–IV	26	This study
C1167T	Silent[c]	Y339Y	DI; S5–6	9	This study
G4023A	Silent[c]	L1291L	DIII; S3	22	This study
C4659T	Silent[c]	S1503S	IDIII–IV	26	This study
C5604T	Silent[c]	A1818A	C-terminus	28	This study
C5607T	Silent[c]	D1819D	C-terminus	28	This study

[a]Exons numbered according to Wang et al. [1996b].
[b]Literature reference or pedigree number (this study) for LQTS-associated mutations.
[c]Nonpathogenic change.

sayed directly by separating radiolabeled PCR products on a 6% denaturing DNA-sequencing gel. Mutation analyses of normal controls and all available family members related to individuals with proven mutations was accomplished by repeating the SSCP analysis using the gel conditions that produced the most easily distinguished aberrant conformer. The Genbank accession number of *SCN5A* mRNA sequence used in this study is M77235.

RESULTS

Eighty-eight unrelated LQTS families including four families with Jervell and Lange-Nielsen syndrome and the remainder with Romano-Ward syndrome were enrolled in the study. Analysis of the *KCNH2* and *KCNE1* (Isk) genes in this population has been previously reported [Duggal et al., 1998; Vesely et al., 1997].

Identification of Two SCN5A Mutations in the S4 Segments of DIII and DIV and Associated Clinical Phenotypes

To identify previously described mutations of *SCN5A,* the primers originally described by Wang et al. [1995a] were used. None of these known mutations was found in the present group of patients. However, a C-to-T transition at nucleotide 5084 was identified in the propositus from family LQTS102 (Fig. 1). The change causes an amino acid substitution from an uncharged polar amino acid, threonine, to a nonpolar amino acid, methionine, at codon 1645 (T1645M). This mutation was not found in 81 normal control DNAs (Fig. 1A).

The study was extended to examine the three remaining voltage-sensing (S4) segments as well as the P-loops. The screen of exon 22 showed an aberrant conformer in one patient from family LQTS024 (Fig. 2). DNA sequence analysis demonstrated that this individual is heterozygous for a C-to-T transition at nucleotide 4062, resulting in substitution of the amino acid threonine to methionine, T1304M, in the DIII; S4 segment (Fig. 3). This change was not detected in the panel of 87 normal individuals (data not shown). Analysis of nine additional relatives of kindred LQTS024 documented a total of six T1304M mutation carriers in this family (Fig. 2A).

In family LQTS102, the propositus carrying the T1645M mutation is a 15-year-old boy who presented to the cardiologist following the sudden cardiac death of his 18-year-old sister. The sister had a history of multiple recurrent episodes of seizure and sudden loss of consciousness during her first two years of life. Her electroencephalogram (EEG) was unremarkable. She had been doing well on phenobarbital, which was discontinued at the age of 4, and subsequently remained asymptomatic and seizure-free until her death at age 18 years. There were no ECGs in her records. Both the propositus' father and paternal aunt had QT_c's of 0.38 sec but required ventricular pacemakers in their early forties for bradycardia, recurrent syncope, and either atrial standstill or very fine atrial fibrillation. The propositus had a past medical history of one syncopal episode that occurred immediately following an immunization; otherwise medical history was noncontributory.

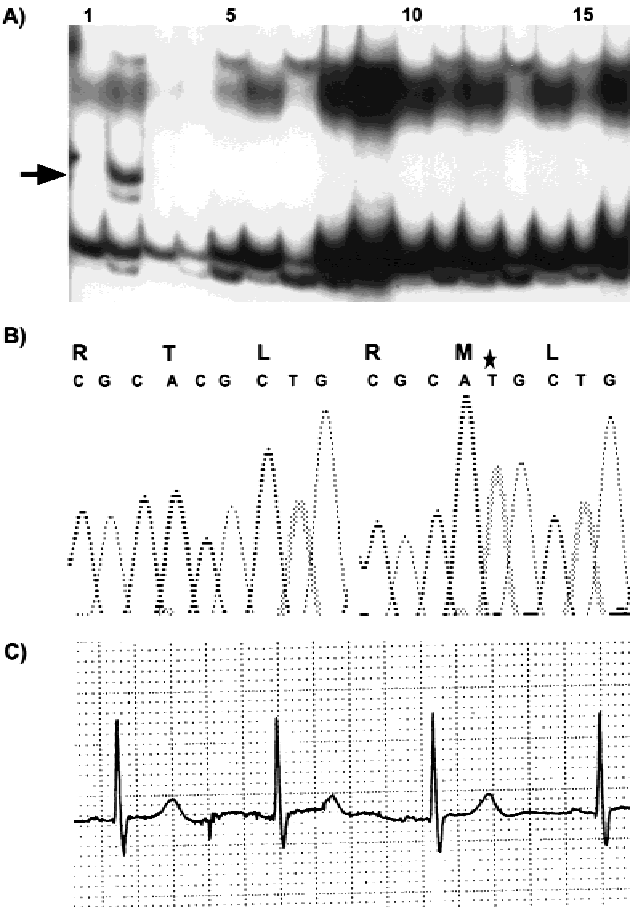

Fig. 1. Analysis of family LQTS102: **A:** SSCP analysis of patient LQTS102-001 (lane 2) and normal control DNAs illustrating the aberrant conformer (arrow) on a 7.5% SSCP nondenaturing polyacrylamide gel. **B:** DNA sequence chromatograms showing a comparison of the wild type (left) and mutant (right) *SCN5A* sequence. A C-to-T change creates a substitution of methionine for threonine at codon 1645 (star). **C:** Representative ECG tracing (lead II) of patient LQTS102-001.

Echocardiogram showed possible mitral valve prolapse but no other abnormalities. ECG at rest documented a marginally prolonged QT_c of 0.450 sec (Fig. 1C) and Holter study demonstrated a maximal QT_c of 0.551 sec (at 103 beats per minute). He had borderline delayed onset of repolarization ($QT_{onset\text{-}c}$ = 0.276 sec). DNA from other relatives was unavailable.

Family LQTS024 came to our attention because the propositus, a 33-yr-old previously healthy woman, was diagnosed as having LQTS after a syncopal episode at 26 weeks into pregnancy. Her QT_c measured 0.51 sec at rest, and she had exercise-induced ventricular ectopy. Clinical and electrophysiologic phenotypes in mutation-positive relatives were variable, with several having borderline positive findings of LQTS (Table II). Of note, individual 024-032 represents an apparently asymptomatic gene carrier. Affected relatives experienced syncopal episodes both following adrenergic stimulation and at rest.

Nonpathogenic Missense Mutations (Rare Variants)

One apparently nonpathogenic missense mutation was identified in family LQTS019 (Table I). It was a

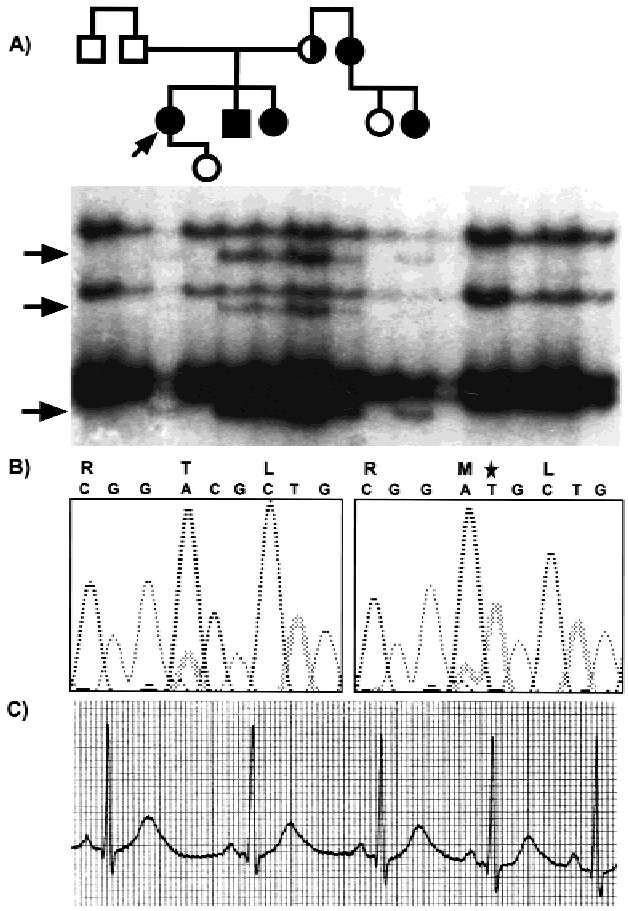

Fig. 2. Analysis of family LQTS024. **A:** Pedigree structure (above) and genotype data (below) on family LQTS024 (open circles, unaffected females; open squares, unaffected males; filled circles, affected females; filled square, affected male; half-filled circle, clinically unaffected mutation carrier). Arrow indicates proband. SSCP analyses using an MDE gel to analyze PCR products of primer pair *SCN5A* 21-22 (exon 22) are shown below the pedigree of LQTS024. Aberrant SSCP conformers (arrows) cosegregate with the disease in the family. Five lanes at right illustrate some of the normal controls screened on the same gel. **B:** DNA sequence chromatograms showing a wild type sequence (left) and the C-to-T transition resulting in a threonine-to-methionine substitution at codon 1304 (right) from LQTS proband 024-036. **C:** Representative resting ECG tracing, lead II of patient LQTS024-037.

single base pair change from G to T at nucleotide 4650, resulting in a lysine-to-asparagine amino acid substitution at codon 1500 (K1500N). The propositus is a 40-year-old woman with a history of multiple episodes of seizures and syncope since the age of 4 years. Her QT_c measured 0.55 sec and her LQT diagnostic score was 7.5. The propositus' 9-year-old affected daughter has experienced multiple episodes of sudden loss of conscious precipitated by physical stress. The daughter's QT_c measured 0.48 sec with LQT diagnostic score of 6.5. Molecular analysis showed that while the mother is heterozygous for the K1500N allele, her affected daughter did not inherit this variant. Of 76 normal controls, we identified one individual who was homozygous for the K1500N SSCP conformer and this was confirmed by DNA sequencing of the relevant band (data not shown). This particular individual has been in good health with no evidence for cardiac disorders of any kind. These data suggest that K1500N is a rare nonpathogenic variant with an estimated allele frequency of 0.9% (3 in 328 chromosomes). Further support for this is the fact that we subsequently identified an unrelated *KCNH2* mutation in the two affected members of family LQTS019 (M. R. Vesely et al., unpublished data, 1998).

Silent Mutations

In addition to the nonpathogenic missense change described above, we also found five silent DNA changes that do not alter the encoded amino acids. They are 1) C to T at position 1167; 2) G to A at nucleotide position 4023; 3) C to T at position 4659; 4) C to T at position 5604; and 5) C to T position 5607 (Table I). The first three of these are rare genetic variants as they were seen only in a single LQTS patient and were not detected in our normal control populations (frequency $\leq$ 0.3%). C5604T and C5607T are clearly benign polymorphisms as they were seen in both affected and control populations with frequencies of 1.3% (2 in 154 chromosomes), and 12.3% (32 of 260 chromosomes) respectively.

DISCUSSION

After screening one third of the *SCN5A* coding sequence in 88 unrelated individuals with LQTS, we identified only two probable *SCN5A* mutations. Together with reports from other groups, we conclude that mutations of *SCN5A* appear to be relatively infrequent in patients with LQTS. To our surprise, the reoccurring three amino acid deletion, ΔKPQ1505–1507, was not seen in our population, which, like that of Wang et al. [1995a, 1995b], was drawn from North American cardiology clinics. It is possible that this represents a bias of ascertainment since the ΔKPQ1505–1507 pedigrees were all selected for linkage analysis based on their large families whereas this study's inclusion criteria required only a single affected individual. However, pedigree 024 did contain six gene carriers, five of whom were symptomatic, and would have been a good family for linkage studies. Furthermore, none of the 45 Japanese LQTS families studied by Tanaka et al. [1997] were found to have the ΔKPQ1505–1507 deletion. Thus, the initial data suggesting that ΔKPQ1505–1507 might be a frequent cause of LQTS3 appear to be unsupported and may simply be a result of the initially small sample size.

T1304M and T1645M are nonconservative changes resulting in substitution of a nonpolar residue for an uncharged polar amino acid. Both these positions are completely evolutionarily conserved among all available sodium channel α-subunits whose sequence is known including: *Homo sapiens* (brain, skeletal muscle), *Rattus norvegicus* (rat cardiac, brain, and skeletal muscle), *Equus caballus* (horse skeletal muscle), *Oryctolagus cuniculus* (rabbit brain), *Mus musculus* (mouse brain and skeletal muscle), *Fugu rubripes* (pufferfish), *Loligo bleekeri* (squid), *Drosophila melanogaster,* and more (data not shown). Thus, these threonine residues at positions 1304 and 1645 in the wild type sodium channels are likely critical for proper sodium channel function. The association of T1304M

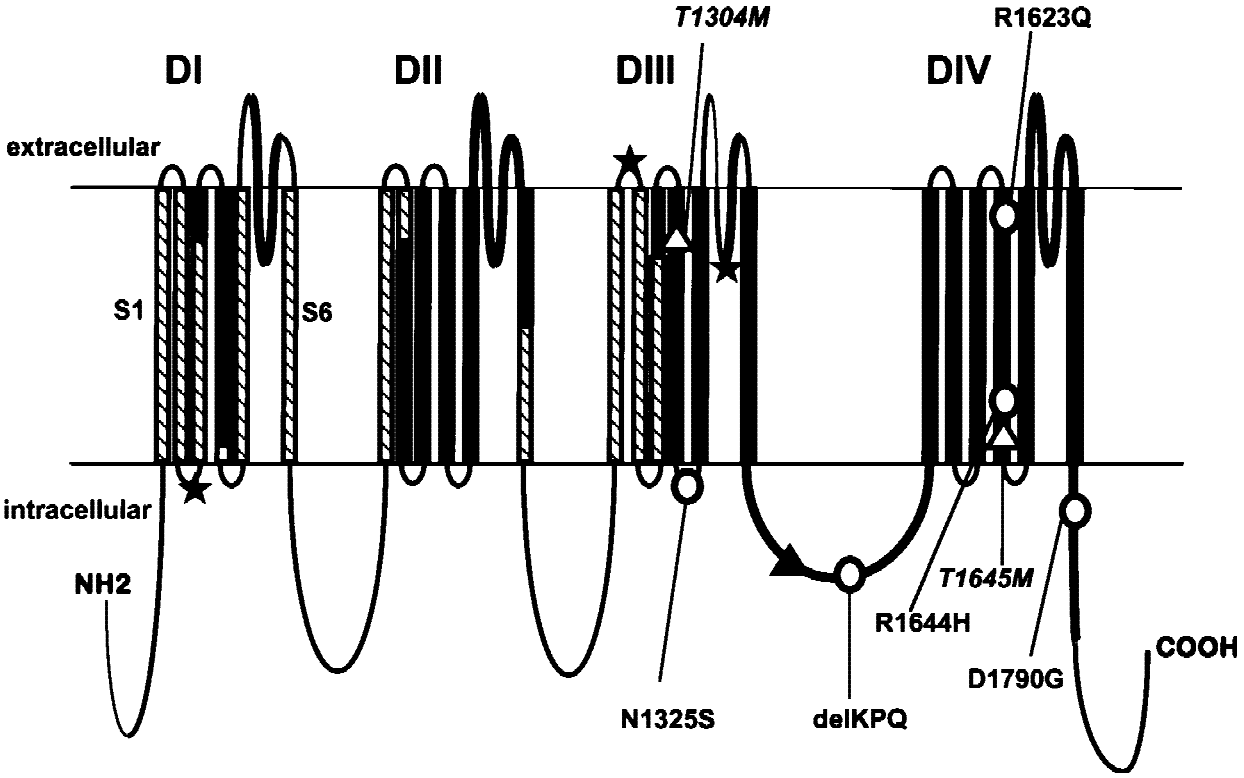

Fig. 3. Schematic diagram of *SCN5A* protein and all known *SCN5A* mutations and sequence variants. *SCN5A* has four homologous domains (DI–DIV), each containing six transmembrane segments (S1–S6). Bold/filled areas represent regions screened in this study. Open triangles in DIII and DIV represent the two novel mutations, T1304M and T1645M, respectively. Filled triangle indicates the K1500N variant. Open circles represent previously reported *SCN5A* LQTS mutations. Note that four out of seven LQTS mutations are located in the S4 segments. Stars indicate three *SCN5A* mutations identified in a separate disorder, idiopathic ventricular fibrillation [Chen et al., 1998].

and T1645M with symptoms of abnormal cardiac repolarization in our patients, and the absence of these changes among the normal control population, support the notion that these are likely pathogenic mutations and probably alter a function or functions of the cardiac sodium channels. Further functional studies will be needed to confirm this conclusion and delineate the pathogenic mechanism or mechanisms of these two mutations.

The nonpathogenic missense polymorphism, K1500N, illustrates the importance of caution in inferring the pathogenic nature of any given sequence change found in studies such as this one. Supporting evidence, such as its absence in a reasonable number of normal control individuals and presence in all affected family members, is essential and, as above, further functional studies should be required before final conclusions are drawn.

Regarding the three silent changes that were detected only in single LQTS patients, one of these,

TABLE II. Clinical Phenotypes and Genotypes in Families LQTS024 and LQTS102

Individual LQTS numbers	Genotype[a]	Sex/age (years)	Schwartz score[b]	Resting lead II QT_c (sec)	Resting lead II $QT_{onset\text{-}c}$ (sec)	Longest lead II QT_c (post exercise or on Holter) (sec)	Symptoms of LQTS, other medical complications and treatment
102–001	+/–T1645M	M/15	3.5	0.450	0.276	0.551	Syncope following immunization; inderal
024–037	+/–T1304M	M/32	7	0.567	0.218	0.567	Syncope ×2 following exercise
024–032	+/–T1304M	F/57	1	0.428	0.216	0.435	Asymptomatic
024–033	+/–T1304M	F/59	5	0.468[c]	NA[d]	NA	Syncope ×2 with emotional stress, LBBB
024–036	+/–T1304M	F/33	6	0.510[c]	NA	NA	Syncope ×2 with physical stress
024–039	+/–T1304M	F/30	3	0.420	0.224	0.464	Ventricular pacing, Lopressor syncope ×2 with physical stress
024–055	+/–T1304M	F/25	3	0.379	0.211	0.395	Syncope ×1 at rest
024–030	–/–	M/65	2	0.427	0.212	0.450	Diabetes, complicated arrhythmia, pernicious anemia, renal insufficiency
024–031	–/–	M/59	3	0.468	0.285	0.440	Inderal hypertension with ventricular ectopy
024–042	–/–	F/34	3	0.387	0.231	0.400	Syncope ×1 upon standing
024–100	–/–	F/0.3	1	0.443	0.181	0.455	Asymptomatic

[a]+/– Denotes heterozygotes for indicated amino acid change, –/– denotes homozygotes for wild type allele.
[b]Calculated accodring to Schwartz et al. [1993] using resting lead II QT_c values.
[c]Historical value from medical record prior to onset of LBBB or pacemaker implantation.
[d]NA, not available.

G4023A, does create an AG dinucleotide that may potentially serve as an alternative 3′ splice site, possibly causing production of an abnormal protein product. The change was not found in 87 normal individuals. The mutant sequence GGCCAACACCCTAG/G has a 3′ splice site consensus score of 67.85 using the scoring system of Shapiro and Senapathy [1987], whereas the wild type sequence at the same site is GGCCAACACCCTGG/G with a consensus score 51.6. However, the natural adjacent 3′ splice site with the sequence TTTGCCTCCCCCAG/G has the highest score at 100.0. Thus it seems unlikely that G4023A alters the normal splicing pattern. The other two changes, C1167T and C4659T, do not create new alternative splice sites and were not identified in 57 and 76 normal individuals respectively. In addition, the family with C4659T was subsequently found to carry a *KCNH2* mutation (M.R. Vesely et al., unpublished data). In the absence of myocardial biopsies as a source of cardiac mRNA, it is impossible to confirm that these do not alter splicing of the *SCN5A* mRNA, but we think it is unlikely that any of these three changes represent pathogenic mutations.

Interestingly, four out of seven known *SCN5A* mutations are in the S4 segments that are thought to play a role in voltage sensing (Fig. 3) [Fozzard and Hank, 1996]. This finding supports earlier evidence that each S4 segment is critical for the function of cardiac Na+ channel α-subunits [Yang et al., 1996]. Functional studies of the ΔKPQ1505–1507, R1644H, and N1325S mutations all resulted in sustained inward sodium currents secondary to defective channel inactivation and this defect was suppressed by low concentrations of mexilitine, a sodium channel blocker [Dumaine et al., 1996; Wang et al., 1996b, 1997]. In contrast, the R1623Q mutant created long openings and early reopenings of the channel, producing a markedly slow current decay (three-fold prolongation of channel inactivation) that was corrected by lidocaine [Kambouris et al., 1998; Makita et al., 1998]. Finally, a recent study by An et al. [1998] has shown that the *SCN5A* mutant, D1790G, possessed a novel pathogenic mechanism (no sustained inward current but, instead, a negative shift in steady state inactivation). Neither mexiletine nor lidocaine seemed to have therapeutic effects on the abnormality produced by the D1790G mutation. T1645M is located at the cytoplasmic side of DIV; S4, adjacent to the previously characterized R1644H mutation. Similarly, T1304M is at the extracellular border of DIII; S4 in a location analogous to that of the R1623Q mutation within DIV; S4. Functional studies will be important to determine whether or not the T1645M and T1304M mutants mimic the mechanisms of the R1644H and R1623Q mutants, respectively.

In studies of two large families segregating the ΔKPQ1505–1507 deletion, Moss et al. [1995] observed that the onset of repolarization (quantified as $QT_{onset\text{-}c}$) was significantly delayed in mutation carriers. In the present study, patient LQTS024-037 exhibited long duration T waves with normal QT_{onset} but markedly prolonged QT intervals (Fig. 2C). The $QT_{onset\text{-}c}$ values for four T1304M mutation carriers (0.217 ± 0.01 sec; e.g., Fig. 2C) were similar to control values obtained from unaffected relatives (0.227 ± 0.04 sec, n = 4), and none of the $QT_{onset\text{-}c}$ in the T1304M mutation carriers were within two standard deviations of the values recorded for ΔKPQ1505–1507 mutation carriers [Moss et al., 1995]. The single T1645M mutation carrier had a borderline prolonged $QT_{onset\text{-}c}$ of 0.276 sec. Thus, although delayed $QT_{onset\text{-}c}$ values may be specific, they may not be very sensitive indicators for at least a subset of *SCN5A* mutations.

Age and gender are important predictors of maximal QT_c values, both for the general population and for LQTS patients [Roden et al., 1996]. Following puberty, males tend to have shorter QT intervals, probably mediated by hormonal levels. However, Lehmann et al. [1997] have reported that, among carriers of *SCN5A* mutations, this trend is reversed with affected males having significantly longer QT intervals than their *SCN5A* mutation–carrying female relatives. Although the numbers are small, our data support this observation as the two affected males (ages 32 and 15) had maximal QT_c's greater than 0.5 second (0.567 and 0.551) while the five mutation carrying females had mean maximal QT_c's of 0.454 ± .04 sec (range 0.395–0.510) (Table II).

For counseling purposes, it is important to note that the incidence of symptoms in our *SCN5A* mutation carriers is 86% (six out of seven gene carriers), similar to previously published incidences of 83–100% among individuals with *SCN5A* mutations [Wang et al., 1995a, 1995b; Benhorin et al., 1997]. Therefore, *SCN5A* mutation carriers may be at higher risk for clinical manifestations than carriers of *KCNQ1* mutations in which the incidence of syncopal events was reported to be 40–79% [Vincent et al., 1992; de Jager et al., 1996; Saarinen et al., 1998]. However, several individuals in the present study had only minimal signs and symptoms of LQTS. The phenotype of patient LQTS102-001 was particularly mild and might not have been appreciated if not for the sudden cardiac death of his sister.

In summary, we have screened one-third (683 out of 2,016 amino acids) of the *SCN5A* coding sequence including the inactivation region (IDIII–IV), all S4 segments, and the P-loop in 88 unrelated LQTS patients. We have found two probable pathogenic mutations, one missense variant, and five silent variants. Taken together with the paucity of other reported *SCN5A* mutations in LQTS, it seems likely that mutations of *SCN5A* are responsible for only a small proportion of LQTS cases. However, studies on additional patient populations and analysis of the remainder of the *SCN5A* gene will be required before we can truly estimate the incidence of *SCN5A* abnormalities in the LQTS population. Given that there may be effective specific therapies for LQTS patients with *SCN5A* mutations, complete ascertainment of this population remains an important goal.

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