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SCN5A Mutations Associated with an Inherited Cardiac Arrhythmia, Long QT Syndrome

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Summary

Long QT syndrome (LQT) is an inherited disorder that causes sudden death from cardiac arrhythmias, specifically torsade de pointes and ventricular fibrillation. We previously mapped three LQT loci: *LQT1* on chromosome 11p15.5, *LQT2* on 7q35-36, and *LQT3* on 3p21-24. Here we report genetic linkage between *LQT3* and polymorphisms within *SCN5A*, the cardiac sodium channel gene. Single strand conformation polymorphism and DNA sequence analyses reveal identical intragenic deletions of *SCN5A* in affected members of two unrelated LQT families. The deleted sequences reside in a region that is important for channel inactivation. These data suggest that mutations in *SCN5A* cause chromosome 3-linked LQT and indicate a likely cellular mechanism for this disorder.

Introduction

Although sudden death from cardiac arrhythmias is thought to account for 11% of all natural deaths, the mechanisms underlying arrhythmias are poorly understood (Kannel et al., 1987; Willich et al., 1987). Long QT syndrome (LQT) is an inherited cardiac arrhythmia that causes abrupt loss of consciousness, seizures, and sudden death from ventricular tachyarrhythmias, specifically torsade de pointes and ventricular fibrillation (Ward, 1964; Romano, 1965; Schwartz et al., 1975; Moss et al., 1991). Autosomal dominant and autosomal recessive forms of this disorder have been reported. Autosomal recessive LQT (also known as Jervell–Lange-Nielson syndrome) has been associated with congenital neural deafness; this form of LQT is rare (Jervell and Lange-Nielson, 1957). Autosomal dominant LQT (Romano–Ward syndrome) is more common and is not associated with other phenotypic abnormalities. A disorder very similar to inherited LQT can also be acquired, usually as a result of pharmacologic therapy (Schwartz et al., 1975; Zipes, 1987).

We have used two strategies to identify LQT genes, a candidate gene approach and positional cloning. Positional information is now available for three LQT loci, as we have mapped *LQT1* to chromosome 11p15.5 (Keating et al., 1991a, 1991b), *LQT2* to 7q35-36, and *LQT3* to 3p21-24 (Jiang et al., 1994). The candidate gene approach relies on likely mechanistic hypotheses based on physiology. Although little is known about the physiology of LQT, the disorder is associated with prolongation of the QT interval on electrocardiograms, a sign of abnormal cardiac repolarization. This association suggests that genes encoding ion channels (or their modulators) are reasonable candidates for LQT. This hypothesis is now supported by our recent discovery that chromosome 7–linked LQT results from mutations in the human *ether-a-go-go*-related gene (*HERG*), a putative cardiac potassium channel gene (Curran et al., 1995 [this issue of *Cell*]). A neuroendocrine calcium channel gene (*CACNL1A2*; Chin et al., 1991; Seino et al., 1992) and a gene encoding a GTP-binding protein that modulates potassium channels (*GNAI2*; Weinstein et al., 1988; Magovcevic et al., 1992) became candidates for *LQT3* based on their chromosomal location. Subsequent linkage analyses, however, have excluded these genes (Q. W. and M. T. K., unpublished data).

In theory, mutations in a cardiac sodium channel gene could cause LQT. Voltage-gated sodium channels mediate rapid depolarization in ventricular myocytes and also conduct a small current during the plateau phase of the action potential (Attwell et al., 1979). Subtle abnormalities of sodium channel function (e.g., delayed sodium channel inactivation or altered voltage dependence of channel inactivation) could delay cardiac repolarization, leading to QT prolongation and arrhythmias. A few years ago, Gellens et al. (1992) cloned and characterized a cardiac sodium channel gene, *SCN5A*. The structure of this gene was similar to previously characterized sodium channels, encoding a large protein of 2016 amino acids. These channel proteins contain four homologous domains (DI–DIV), each of which contains six putative membrane-spanning segments (S1–S6). *SCN5A* was recently mapped to chromosome 3p21, making it an excellent candidate gene for *LQT3* (George et al., 1995).

In this study, we provide evidence suggesting that *SCN5A* is *LQT3*. We used genotypic analyses to show that *SCN5A* was tightly linked to *LQT3* in three unrelated families. We then identified the same intragenic deletion of *SCN5A* in affected members of two of these families. These deletions disrupted sequences within a region of known importance for sodium channel inactivation, suggesting a likely cellular mechanism for chromosome 3–linked LQT.

Table 1. PCR Primers Used to Define *SCN5A* Polymorphisms and Mutations

Primer	Sequence	Region Amplified	Exon
1L	GCCTGTCTGATCTCCCTGTGTGA	DIII/S6; IDIII–IV	21
2R	ACCCAGCCCAGTGGGGAGCTGGT		
3L	CCATGCTGGGGCCTCTGAGAAC	IDIII–IV	22
4R	GGCTCTGATGGCTGGCCATGTG		
5L	CCCAGCGAGCACTTTCCATTTG	IDIII–IV; DIV/S1–S3	23
6R	GCTTCTCCGTCCAGCTGACTTGTA		
7L	GAGCCCAGCCGTGGGCATCCT	DIV/S6	24
8R	GTCCCCACTCACCATGGGCAG		

All primers are shown in 5′ to 3′ direction. Sense strand oligonucleotides are indicated with an L and antisense oligonucleotides are indicated with an R. D, domain; ID, interdomain; S, membrane-spanning segment.

Results

Genetic Linkage of *SCN5A* and *LQT3*

In 1995, *SCN5A* was mapped to chromosome 3p21 (George et al., 1995). To test the candidacy of *SCN5A* for LQT, we used single strand conformation polymorphism (SSCP) analyses to identify polymorphisms within this gene and then performed linkage analyses in chromosome 3–linked families. Since the genomic structure of *SCN5A* was unknown, we designed oligonucleotide primer pairs from published *SCN5A* cDNA sequences (Gellens et al., 1992), based on the assumption that the genomic structure of *SCN5A* would be similar to the known structure of *SCN4A*, the skeletal muscle sodium channel gene (McClatchey et al., 1992a). Primer pairs that gave appropriately sized products from genomic DNA were used to screen DNA samples from patients. An aberrant SSCP conformer was identified using primer pair *7–8* (Table 1). The normal and aberrant bands were cloned and sequenced. The aberrant conformer resulted from a C to T substitution at position 3 of codon 1819 (cDNA nucleotide 5607; Gellens et al., 1992). This substitution did not affect the predicted amino acid sequence of the product of *SCN5A*. The observed heterozygosity for this polymorphism was <0.50. The *SCN5A* polymorphism was used for genotypic analyses in chromosome 3–linked families (Figure 1). No recombination events between *SCN5A* and LQT were identified in any of these families. The maximum combined two-point lod score for all chromosome 3–linked families was 2.74 at a recombination fraction of 0.0 (Table 2).

No additional SSCP anomalies were identified using

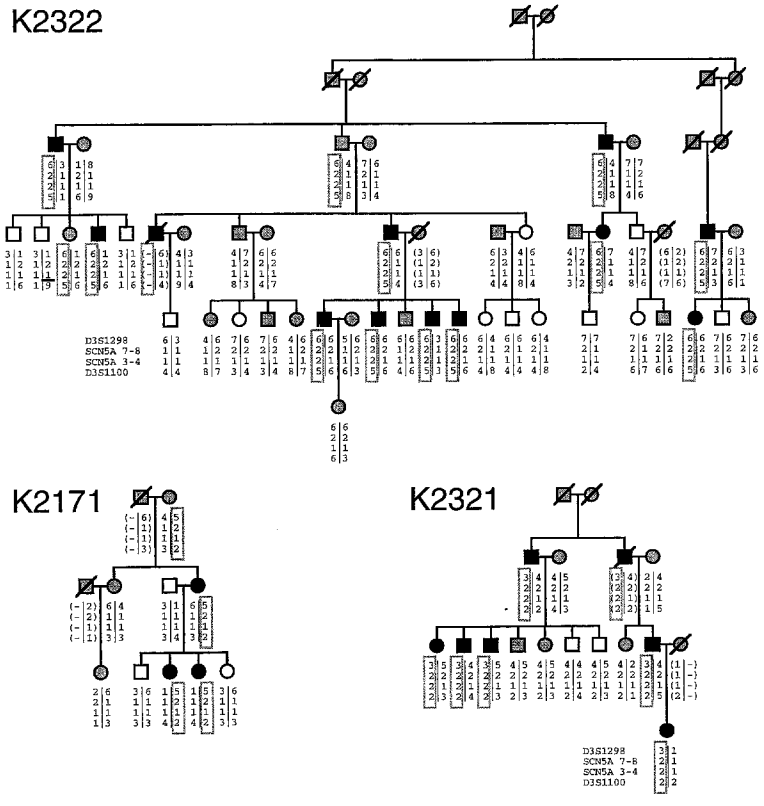

Figure 1. Genetic Linkage of *SCN5A* and *LQT3*

Pedigree structure and genotypic analyses of LQT kindreds 2171, 2321, and 2322. Individuals with the characteristic features of LQT, including prolongation of the QT interval on electrocardiogram and a history of syncope or aborted sudden death, are indicated by closed circles (females) or closed squares (males). Unaffected individuals are indicated by open symbols, and individuals with an equivocal phenotype are shown as stippled. Deceased individuals are indicated by a slash. The results of genotypic analyses are shown below each symbol. Genotypes for the following *LQT3*-linked polymorphic markers are shown (telomere to centromere): *D3S1298*, *SCN5A 7–8*, *SCN5A 3–4*, and *D3S1100*. Haplotypes cosegregating with the disease are indicated by a box. Recombination events are indicated by a horizontal black line. Haplotype analyses indicate that *LQT3* and *SCN5A* are tightly linked in all chromosome 3–linked families.

Table 2. Pairwise Lod Scores and Recombination Fractions for Linkage of *LQT3* with *SCN5A*

		Recombination Fractions						
Marker	Kindred	0.001	0.01	0.05	0.10	0.20	0.30	0.40
SCN5A 3–4	K2171	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	K2321	1.50	1.47	1.35	1.20	0.87	0.53	0.18
	K2322	4.03	3.95	3.62	3.20	2.34	1.47	0.61
	Total[a]	5.53	5.43	4.97	4.40	3.22	2.00	0.79
SCN5A 7–8	K2171	0.82	0.80	0.74	0.65	0.46	0.26	0.08
	K2321	0.12	0.12	0.10	0.08	0.04	0.02	0.00
	K2322	1.80	1.78	1.66	1.48	1.05	0.60	0.20
	Total[b]	2.74	2.70	2.50	2.20	1.55	0.87	0.28

Lod scores were calculated assuming autosomal dominant inheritance with a penetrance of 0.90 for all kindreds, as indicated by segregation analysis of these and other LQT kindreds. We assumed a disease allele frequency of 0.001 and that female and male recombination frequencies were equal. When penetrance was varied from 70%–100%, the maximum combined two-point lod score for linkage between *SCN5A 3–4* and *LQT3* ranged from 4.85 to 5.91, respectively. The results for *SCN5A 7–8* varied from 2.26 (70%) to 3.01 (100%).

[a] $Z_{max} = 5.54$, $\theta = 0.00$; Z_{max} indicates maximum lod score. θ indicates estimated recombination fraction at Z_{max}.

[b] $Z_{max} = 2.74$, $\theta = 0.00$.

cDNA sequences. To facilitate this work, we isolated and partially characterized two genomic P1 clones. These clones spanned the entire gene and were used to begin determining the genomic structure of *SCN5A*. We hypothesized that LQT-causing mutations would be subtle (e.g., missense mutations or small in-frame deletions) and might affect delayed inactivation of encoded sodium channels or alter the voltage dependence of channel inactivation. We focused, therefore, on regions of known importance for channel inactivation. Primers based on sequences predicted to encode the cytoplasmic region between DIII and DIV were synthesized. This region corresponds to exons 21–23 of *SCN4A* and is known to be critical for channel inactivation. These primers were used to characterize the flanking introns by cycle sequencing. Additional primers were designed to these intronic sequences (see Table 1).

When primer pair *3–4* was used in SSCP analyses, an anomalous conformer was identified in DNA samples from affected members of kindreds 2321 and 2322 (Figure 1). By contrast, only the normal conformer was seen in DNA samples from unaffected members of these families. The combined two-point lod score for linkage between this anomaly and *LQT3* was 5.54 (Table 2). Again, no recombination was observed between *SCN5A* and *LQT3*, indicating that these loci are tightly linked.

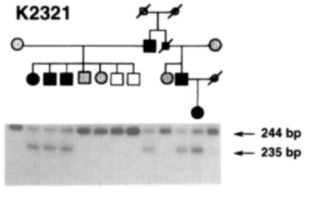

Figure 2. *SCN5A* Intragenic Deletion Cosegregates with the Disease in Kindred 2321

The pedigree structure for kindred 2321 is shown. The results of polymerase chain reaction (PCR) analyses using primer pair *3–4* and denaturing polyacrylamide gel electrophoresis are shown below each symbol. Note that the 235 bp allele cosegregates with the disease in this family, indicating the presence of a disease-associated intragenic deletion of *SCN5A*. To avoid phenotypic misclassification, the phenotypic criteria used in this study were stringent, and many individuals were classified as having an uncertain phenotype. Individuals with a QTc of 0.47 s or greater were classified as affected, whereas individuals with a QTc of 0.41 s or less were considered unaffected. All other individuals were classified as uncertain. If typical criteria were used (individuals with a QTc of 0.44 s or greater considered affected and individuals with a QTc less than 0.44 s classified as normal), all affected members of kindreds 2321 and 2322 would carry the *SCN5A* deletion and all unaffected individuals would carry only the normal allele.

Identical *SCN5A* Intragenic Deletions Associated with LQT in Two Unrelated Families

The mobility shift between the aberrant and normal SSCP conformers identified in kindreds 2321 and 2322 was large, suggesting the possibility of a small deletion. To test this hypothesis, the conformers were separated by electrophoresis on denaturing polyacrylamide gels (Figures 2 and 3). These data demonstrated the presence of two products of 235 bp and 244 bp. The 235 bp product was only seen in affected individuals. Furthermore, this aberrant conformer was not observed in more than 500 control individuals (data not shown). These data indicate that an intragenic deletion of *SCN5A* is the likely cause of LQT in these families.

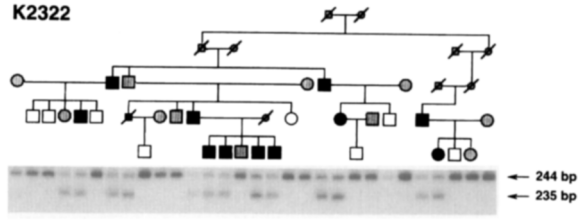

Figure 3. *SCN5A* Intragenic Deletion in Kindred 2322

The results of PCR analyses using primer pair *3–4* and denaturing polyacrylamide gel electrophoresis are shown below each symbol. Note that the 235 bp allele cosegregates with the disease in this family, indicating the presence of a disease-associated intragenic deletion. The individuals represented in lanes 4 and 8 carried the deletion but were phenotypically classified as uncertain. These individuals had a QTc of 0.46 s and with less stringent phenotypic criteria would be considered affected.

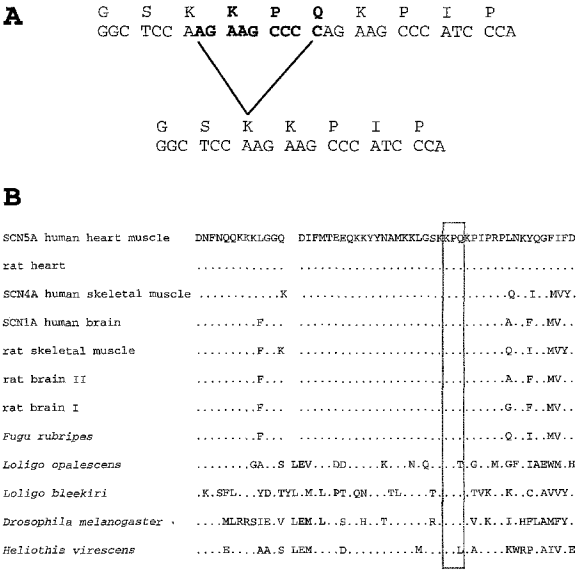

Figure 4. DNA and Amino Acid Sequence of the *SCN5A* Intragenic Deletion Associated with LQT

(A) DNA sequence analysis of the *SCN5A* intragenic deletion identified in kindred 2322. DNA sequence of normal and aberrant PCR products defines a 9 bp deletion. This mutation causes a deletion of three amino acids (KPQ) in the cytoplasmic region between DIII and DIV. Deleted sequences are indicated.

(B) Amino acid sequence homology of the cytoplasmic region between DIII and DIV of sodium channels. Sequences were obtained from GenBank.

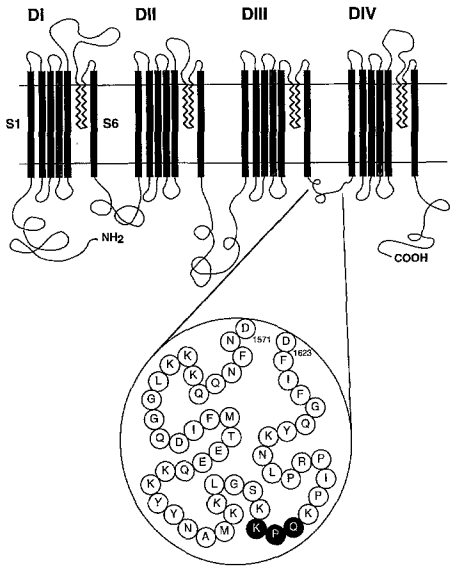

Figure 5. Schematic Representation of the Predicted Topology of the Protein Encoded by *SCN5A* and of the Location of the LQT-Associated Deletion

To determine the effect of the LQT-associated deletion on *SCN5A* structure, we amplified the normal and aberrant SSCP conformers and performed cycle sequencing. These experiments revealed the presence of a 9 bp deletion beginning at nucleotide 4661 of the cDNA (Figure 4A). We also cloned and sequenced the aberrant and normal SSCP conformers. These experiments confirmed the size and location of the deletion. This deletion disrupts the coding sequence, resulting in a deletion of three conserved amino acids, Lys-1505–Pro-1506–Gln-1507 (KPQ), in the cytoplasmic linker between DIII and DIV (Figures 4B and 5).

DNA sequence analyses of the aberrant conformer in kindred 2321 indicated that the intragenic deletion was identical to that found in kindred 2322 (data not shown). One possible explanation for the identical deletions in these two kindreds is that they are distantly related. Both families were North American of European descent, one German and the other English. Examination of a genealogy data base failed to reveal a relationship between these families for more than eight generations. Furthermore, genotypic analyses of these kindreds indicated different haplotypes on the disease chromosome (see Figure 1). The presence of identical deletions in two apparently unrelated LQT families strongly suggests that *SCN5A* is *LQT3*.

Discussion

We conclude that mutations in the cardiac sodium channel gene *SCN5A* are the likely cause of chromosome 3–linked LQT. Several lines of evidence support this conclusion. First, we identified genetic linkage between *SCN5A* and *LQT3* in three unrelated families. No recombination was identified between these loci. Second, we identified identical intragenic deletions of *SCN5A* in affected members of two LQT families. This deletion was not identified in more than 500 control individuals. Third, *SCN5A* is the cardiac sodium channel gene. Subtle mutations of this gene would be expected to cause an LQT phenotype. Finally, the type (an in-frame deletion of three amino acids) and location (a region of known importance for sodium channel inactivation) of the deletions support the conclusion that *SCN5A* is *LQT3*.

Additional evidence supports a role for *SCN5A* in the pathogenesis of LQT. Pharmacologic data, for example, suggest that abnormal cardiac sodium channel function could cause LQT. Toxins and drugs that slow the rate of sodium channel inactivation or that shift the voltage dependence of channel activation or inactivation can prolong cardiac action potential duration and induce arrhythmias (Honerjager, 1982). Molecular genetic studies also support a link between sodium channel inactivation and clinical electrophysiologic abnormalities. Studies of hyperkalemic periodic paralysis and paramyotonia congenita indicate that missense mutations in the skeletal muscle sodium channel gene (*SCN4A*) cause myotonia (Fontaine et al., 1990; Ptacek et al., 1991, 1992; Rojas et al., 1991; McClatchey et al., 1992b, 1992c; Ebers et al., 1991; Rudolph et al., 1992; Lerche et al., 1993). Physiologic data show that these mutations affect sodium channel inactivation, leading to repetitive depolarizations, consistent with the myotonic phenotype (Yang et al., 1994). By analogy, similar mutations in the cardiac sodium channel gene could cause a phenotype like LQT.

We have also demonstrated that mutations in *HERG*, a

putative cardiac potassium channel gene, cause the chromosome 7–linked form of LQT (Curran et al., 1995). The mutations that we identified in *HERG*, along with the biophysics of potassium channel α subunits, suggest that chromosome 7–linked LQT results from dominant negative mutations and a resultant reduction in functional channels. In chromosome 3–linked LQT, by contrast, the LQT-associated deletions identified in *SCN5A* are likely to result in functional cardiac sodium channels with altered properties, such as delayed inactivation or altered voltage dependence of channel inactivation. Delayed sodium channel inactivation would increase inward sodium current, depolarizing the membrane. This effect is similar to the altered membrane potential expected from *HERG* mutations, in which outward potassium current is decreased. It is unlikely that more deleterious mutations of *SCN5A* would cause LQT. A reduction of the total number of cardiac sodium channels, for example, would be expected to reduce action potential duration, a phenotype opposite that of LQT. The mutations described in this study cause the deletion of three amino acids, KPQ, in the cytoplasmic linker between DIII and DIV. The KPQ sequence is highly conserved, suggesting the presence of genetic pressure for its conservation during evolution (Figure 4B). The cytoplasmic peptide segment that links DIII and DIV is the region responsible for fast inactivation (West et al., 1992). Heterologous expression of neural sodium channels in the form of two polypeptides lacking this DIII–DIV linker results in a sodium current with greatly slowed inactivation (Stuhmer et al., 1989). Site-directed mutagenesis studies of this region in *SCN4A* have focused on another amino acid triplet, Ile-1488–Phe-1489–Met-1490 (IFM). Mutations of these amino acids to glutamine residues eliminate sodium channel fast inactivation, but leave slow inactivation intact (West et al., 1992). It will be of interest to determine whether the KPQ deletion has a similar functional effect on cardiac sodium channel inactivation.

Our data predict a likely cellular mechanism for chromosome 3–linked LQT. Delayed myocellular sodium channel inactivation would prolong action potential duration and the QT interval. Excessive prolongation could result in reactivation of L-type calcium or sodium channels, thereby leading to secondary depolarizations, a likely mechanism of torsades de pointes (Antzelevitch and Sicouri, 1994).

We have not yet identified a mutation in *SCN5A* in kindred 2171, the third chromosome 3–linked LQT family. Although the disease phenotype in this kindred appears to be linked to *SCN5A*, SSCP analyses of sequences encoding the putative inactivation region failed to show a deletion or other anomalies. Presumably, the disease in this family results from *SCN5A* mutations in another region of this approximately 35 kb gene. Mutational analyses of hyperkalemic periodic paralysis and paramyotonia congenita families have demonstrated several other regions of *SCN4A* that are important for channel inactivation (Fontaine et al., 1990; Ptacek et al., 1991, 1992; Rojas et al., 1991; McClatchey et al., 1992b, 1992c; Ebers et al., 1991; Rudolph et al., 1992; Lerche et al., 1993). Once we have determined the genomic structure of *SCN5A*, we will screen for additional LQT-causing mutations.

Although LQT kindreds 2321 and 2322 appear unrelated, both had the same KPQ deletion. The haplotype of the affected chromosome in each family was distinct, but this does not exclude the possibility of a distant relationship. Continued genotypic analysis of sequences near *SCN5A* may help determine whether these deletions are distinct.

Presymptomatic diagnosis of LQT has depended on identification of QT prolongation on electrocardiograms. Unfortunately, electrocardiograms are rarely performed in young, healthy individuals. In addition, many LQT gene carriers have relatively normal QT intervals, and the first sign of disease can be a fatal cardiac arrhythmia (Vincent et al., 1992). Now that two LQT genes have been identified, we can begin to contemplate genetic testing for this disorder. This will require continued mutational analyses and identification of additional LQT genes. With more detailed phenotypic analyses, we may also discover phenotypic differences among the varied forms of LQT. These differences may be useful for diagnosis and treatment.

In summary, we have identified a gene, *SCN5A*, that is likely to cause LQT. Along with our finding that mutations in *HERG* cause the chromosome 7–linked form of LQT, this discovery further supports the hypothesis that LQT results from mutations in cardiac ion channels.

Experimental Procedures

LQT Kindreds

This investigation was performed on three previously described LQT kindreds (Jiang et al., 1994). Phenotypic criteria were identical to those used in our previous studies (Keating et al., 1991a, 1991b; Keating, 1992). Individuals were evaluated for LQT based on the QT interval corrected for heart rate (QTc; Bazette, 1920) and on the presence of syncope, seizures, and aborted sudden death. Informed consent was obtained from individuals or their guardians, in accordance with local institutional review board guidelines. Phenotypic data were interpreted without knowledge of genotype.

Linkage Analyses

Pairwise linkage analysis was performed using MLINK in LINKAGE version 5.1 (Lathrop et al., 1985). As in our previous studies, we assumed a penetrance of 0.90 and an LQT gene frequency of 0.001 (Keating et al., 1991a, 1991b). Gene frequency was assumed to be equal between males and females.

Isolation of *SCN5A* Genomic Clones and Partial Characterization of Genomic Structure

SCN5A probes were generated using the products of PCRs with human genomic DNA and primer pairs based on *SCN5A* cDNA sequences. One primer pair, 5′-ACTTTCATCGTACTGAATAAAGGCAA-3′ and 5′-GAGTGAACCAGAATCTTCACAGC-3′, was designed from 5′ coding sequences and yielded the predicted product of 118 bp. The second primer pair, 5′-GGACCGTGAGTCCATCGTGTGA-3′ and 5′-AGCCCATTCACAACATATACAGTCT-3′, was derived from the 3′ noncoding sequences and yielded a product of 336 bp. The third primer pair, 5′-AGCAACTTCATCCCAGCTGCTGAG-3′ and 5′-CTCCCAGCATCTCAGGTCAAGTG-3′, was based on 3′ noncoding sequences and yielded a product of 297 bp. These PCR products were purified from 2% agarose gels, radiolabeled to high specific activity, and used to screen a human genomic P1 library (Sternberg, 1990).

To characterize the genomic structure of *SCN5A* exons encoding the cytoplasmic linker between DIII–DIV, we designed sequencing primers based on cDNA sequences and predicted genomic structure (McClatchey et al., 1992a; Gellens et al., 1992). The primer pair for presumed exon 21 (based on the structure of *SCN4A*) was 5′-TATGAAGAGCAGCCTCAGTGGGAA-3′ and 5′-CTTTTTCTTCTGTTGGTT-

GAAGTTG-3′. Primers for presumed exon 22 were 5′-TTAGGGG-GCCAGGACATCTTC-3′ and 5′-CAGGGGCCGTGGGATGGGCTTC-TGG-3′. Primers for presumed exon 23 were 5′-CACCATATTCAAGCA-GATCAG-3′ and 5′-CTGCGCCACTACTACTTCACC-3′. These primers were used to determine intronic sequences from *SCN5A* clones as described previously (Wang and Keating, 1994).

SSCP Analyses

Genomic DNA samples were amplified by PCR and used in SSCP analyses as described previously (Orita et al., 1989). Primer pairs used for this study are shown in Table 1. Annealing temperature was 62°C for all PCRs. Reactions (10 μl) were diluted with 50 μl of 0.1% SDS and 1 mM EDTA and 50 μl of 95% formamide dye. Diluted products were denatured by heating at 94°C for 10 min, and 5 μl of each sample was separated by electrophoresis on 10% nondenaturing polyacrylamide gels (50:1 acrylamide:bisacrylamide) at 4°C. Electrophoresis was carried out at 50 W for 2–5 hr. Gels were transferred to 3MM filter paper, dried, and exposed to X-ray film at −80°C for 12 hr.

Sequence Analyses of SSCP Conformers

Normal and aberrant SSCP conformers were cut directly from dried gels and eluted in 100 μl of distilled water at 65°C for 30 min. The eluted DNA (10 μl) was used as template for a second PCR using the original primer pair. Products were fractionated in 2% low melting temperature agarose gels (FMC Corporation), and DNA fragments were purified and sequenced directly by cycle sequencing (Wang and Keating, 1994). Alternatively, purified PCR products were cloned into pBluescript II SK(+) (Stratagene) using the T-vector method as described previously (Marchuk et al., 1990). Plasmid DNA samples were purified and sequenced by the dideoxy chain termination method using SequiTherm polymerase (Epicentre Technologies).

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