KVLQT1 mutations in three families with familial or sporadic long QT syndrome

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Congenital long QT syndrome (LQTS) is a heterogeneous group of heritable disorders characterized by prolongation of the QT interval on the electrocardiogram, ventricular arrhythmias and sudden death. At least four genes can, when mutated, produce this phenotype. Of these genes, the recently identified KVLQT1 potassium channel is thought to be the one most commonly responsible. In this study, we used single strand conformational polymorphism (SSCP) analysis to screen two large and nine small LQTS families for mutations of the KVLQT1 potassium channel gene. We identified a novel missense mutation in two unrelated families which substitutes a serine for a conserved glycine in the putative pore region of the KVLQT1 channel. In a third family, a new alanine to valine mutation at a CpG dinucleotide resulted in the spontaneous occurrence of the long QT syndrome in monozygotic twin offspring of unaffected parents. Mutations at this same nucleotide have been observed in eight of the 19 LQTS families determined to have KVLQT1 mutations, suggesting this is a mutational hot spot. Both of these mutations alter the amino acid sequence in, or adjacent to, the pore of the channel and may diminish the channel’s ability to conduct a repolarizing potassium current. To date, all KVLQT1 mutations determined to cause the LQTS are missense mutations. These data confirm the role of KVLQT1 in the LQTS and suggest that mutant KVLQT1 proteins may exert a dominant negative effect on repolarizing potassium currents by forming multimers with normal potassium channel protein subunits, dramatically reducing the number of fully-functional KVLQT1 channels.

INTRODUCTION

Abnormalities of myocardial repolarization resulting in prolongation of the QT interval can cause ventricular arrhythmias, syncope and sudden death, often in otherwise healthy individuals (1). The management and treatment of patients with repolarization abnormalities has been somewhat limited by the diversity of physiologic defects underlying the common clinical manifestations, and by the difficulty of presymptomatic diagnosis. Studies of patients with autosomal dominant congenital QT prolongation, the Romano-Ward syndrome (LQTS) (2,3), have determined that at least four genes, when mutated, can cause prolongation of the QT interval, ventricular arrhythmias and sudden death. Three of these genes have now been isolated using candidate gene and positional cloning approaches. The α subunit of the voltage-gated cardiac sodium channel gene (SCN5A) on chromosome 3p21 (4), the HERG potassium channel gene on 7q35 (5) and, most recently, the KVLQT1 potassium channel gene on 11p15.5 (6) have been determined to be responsible for LQT3-, LQT2- and LQT1-linked congenital long QT syndrome, respectively.

LQT1-linked LQTS has been determined to be the most common of the congenital disorders with mutations of the KVLQT1 gene estimated to be responsible for more than 50% of the cases of LQTS (6). Although the predicted KVLQT1 protein is similar to the Drosophila Shaker potassium channel DMSHAKE1, functional characteristics of the KVLQT1 potassium channel have yet to be delineated. Further characterization of this channel may increase our understanding of the repolarization process and may ultimately benefit the diagnosis, management and treatment of many patients with repolarization abnormalities.

In this study, we screened two multigenerational, LQT1-linked LQTS families (7), three small families and six sporadic LQTS cases for KVLQT1 mutations. In one of the large families, a novel missense mutation was identified which would be predicted to change the amino acid sequence in the pore region of the protein. Examination of nine small families and sporadic cases with LQTS revealed missense mutations in two families. One of these small families had the same mutation as the large LQT1-linked family but on a different haplotype. The other case was the sporadic occurrence of a previously-described (6) missense mutation in the sixth transmembrane domain (S6) of the KVLQT1 protein.

RESULTS

Missense mutation in the KVLQT1 pore region associated with LQTS in two families

Of the LQTS families in the study, families RWS84 and RWS21 had previously demonstrated linkage of the LQTS to marker D11S932 on chromosome 11p15.5 with LOD scores of 3.14 and 2.66, respectively (7). SSCP analysis, using the KVLQT1 PCR

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primer sets described by Wang et al. (6) was performed on DNA from these two LQT1-linked families and from nine small families or sporadic cases of LQTS in whom linkage to chromosome 11p15.5 could not be excluded. Variant SSCP bands were noted in affected and unaffected individuals with primer sets 1–2, 7–8, 9–10, and 11–12. Some of these variant bands were sequenced and determined to be intronic polymorphisms. The remainder were presumed to be polymorphisms due to high rates of occurrence in unaffected individuals (data not shown). However, with primer set 9–10, which amplifies an exon coding for the pore and S6 regions of the KVLQT1 potassium channel protein, certain variant SSCP bands were noted only in the affected individuals from three unrelated families. Two of these families, RWS84 and SQT02, shared a variant SSCP band that was not noted in more than 180 unrelated individuals (Fig. 1).

This band was cut from the SSCP gel, reamplified and directly sequenced. Sequencing of the variant band from both families revealed a G to A transition that would be predicted to change a glycine to a serine in the pore region of the protein (Fig. 1). This glycine is conserved in the Drosophila homologue, DMSHAK1 gene (8).

**Occurrence of the G185S mutation on different haplotypes**

These two families were genotyped for five markers on chromosome 11p15.5 (Fig. 2) to determine if these two families shared a common KVLQT1 region haplotype. Marker data from these loci showed that the G185S mutation in these two families resides on a different haplotype suggesting that these represent two independent mutation events.
This particular nucleotide may represent a mutationary ‘hot spot’ in the KVLQT1 gene. Not surprisingly, this site involves a CpG dinucleotide, a dinucleotide that has frequently been implicated in mutagenesis (9). However, until the entire KVLQT1 gene has been screened for mutations, the relative frequency of this particular mutation cannot be accurately assessed.

The other mutation encountered during this study also occurred at a CpG dinucleotide with a resultant G→A transition on the coding strand. This mutation would be predicted to change a conserved glycine to a serine in the pore region of the KVLQT1 channel. This glycine is part of the potassium channel signature sequence, a stretch of eight amino acids (TXXTXYG) that is shared by the Shaker channels (Fig. 3) and all other major classes of potassium channels (10). In a study by Heginbotham et al. (10), oligonucleotide mutagenesis of the Drosophila Shaker potassium channel determined that the two glycines in this signature sequence confer potassium selectivity to the channel. Amino acid substitutions replacing either of these glycines, including serine substituted for the first glycine, resulted in non-selective monovalent cation channels which were equally permeant to sodium and potassium ions (10). Furthermore, deletion of one glycine and the tyrosine from the GYG sequence resulted in a non-selective channel with a slow rate of potassium current activation (11). Therefore, based on these studies, this mutation, G185S, would be expected to encode for a potassium channel with altered conductance properties, potentially causing QT prolongation either by enhanced sodium inward current or diminished potassium outward current during myocardial repolarization.

While additional functional studies will be required to determine the effect this amino acid substitution has on the function of the KVLQT1 channel, its presence in only affected individuals from two unrelated LQTS families, coupled with the absolute conservation of this glycine residue in all major classes of potassium channels, suggests that this mutation is likely to be responsible for the LQTS in these two families. A dominant negative effect of these mutant proteins might cause greater repolarization abnormalities than would occur if a genetic mutation caused the loss of protein production from one copy of the KVLQT1 gene. Haploinsufficiency for KVLQT1 may not cause clinically detectable QT prolongation under normal circumstances, since it would only reduce the number of fully-functional KVLQT1 channels by half. An alternative explanation, that mutations not producing a mutant protein cause such a severe phenotype that the patients die before they can be diagnosed, will need to be examined by further studies.

Both of the mutations described in this study were in, or adjacent to, the pore region of the channel. While mutations in these regions appear to be a relatively frequent cause of KVLQT1-linked LQTS, the remainder of the KVLQT1 gene needs more exhaustive study. Mutations in as yet uncharacterized regions of the KVLQT1 gene may be important in causing the long QT syndrome. In our study we were unable to identify a KVLQT1 mutation in one of our LQT1-linked families and only found mutations in two of the nine small families. When more of the KVLQT1 genomic structure is characterized and suitable for

DISCUSSION

The mutations described here, particularly the occurrence of a KVLQT1 mutation in the sporadic LQTS family (SQT03), support the recent observation (6) that the KVLQT1 gene is responsible for the LQTI-linked LQTS. The mutation in the SQT03 family involved the replacement of alanine-212 by a valine (A212V). This relatively conservative amino acid substitution appears to be a frequent cause of KVLQT1-linked LQTS. Of the 19 unrelated LQTS families that have been determined to have KVLQT1 mutations, two additional families demonstrated a transversion (C→T) transition that would be predicted to change an alanine to a valine in the S6 region of the protein (Fig. 1). In a prior study, Wang et al. (6) reported the same mutation in five unrelated LQTS families. In that study, two additional families demonstrated a transversion (C→A) mutation at the same nucleotide. To verify paternity, this family was genotyped for 24 polymorphic markers (heterozygosity – 0.80) on five different chromosomes (data not shown). Based on these markers there was no evidence of misattributed paternity, indicating that this sequence change represents a new mutation in this family and is associated with the development of the LQTS phenotype in the offspring.

New mutation noted in monozygotic twin offspring of unaffected parents

A variant band for primer set 9–10 was also noted in the twin offspring of two unaffected parents. Neither parent nor more than 180 unrelated individuals demonstrated the variant band. In this case, sequencing of the variant band revealed a C→T transition that would be predicted to change an alanine to a valine in the S6 region of the protein (Fig. 1). In a prior study, Wang et al. (6) reported the same mutation in five unrelated LQTS families. In that study, two additional families demonstrated a transversion (C→A) mutation at the same nucleotide. To verify paternity, this family was genotyped for 24 polymorphic markers (heterozygosity – 0.80) on five different chromosomes (data not shown). Based on these markers there was no evidence of misattributed paternity, indicating that this sequence change represents a new mutation in this family and is associated with the development of the LQTS phenotype in the offspring.

Figure 2. Affected 11p15.5 haplotype comparison of family RWS84 and SQT02. Both families were genotyped for the markers D11S922, tyrosine hydroxylase (TH), D11S1318, D11S860 and D11S932. The marker order of telomere-D11S922-[THID11S1318]-KVLQT1-D11S860-D11S932-centromere was obtained from references 7 and 23. The position of marker D11S1318 relative to the TH gene has not been established. The affected haplotype is enclosed in an open box. KVLQT1 alleles are as follows: + indicates the normal SSCP band, – indicates the variant SSCP band.
SSCP analysis, additional *KVLQT1* mutations will likely be identified in these families. While SSCP analysis may not identify all mutations in a given region, SSCP analysis using amplification products of less than 200 bp electrophoresed under two different conditions (e.g. at room temperature and at 4°C) would be predicted to identify greater than 90% of single base pair changes in the regions amplified (12).

In addition to revealing important characteristics of the *KVLQT1* gene, testing for *KVLQT1* mutations in LQTS patients may become an important aspect of diagnosis and management of patients with this disorder. In family SQT02, an asymptomatic individual with QTc measurement of 0.42 s and no family history of the LQTS possessed a mutation of the *KVLQT1* gene which was inherited by his severely affected daughter. While the risk of sudden death remains to be determined in individuals with a *KVLQT1* mutation but a relatively normal QTc measurement, it is clear that they can transmit the mutant gene to their offspring who may exhibit a much more severe phenotype, perhaps due to the influence of other genes which modify the expression of a given *KVLQT1* mutation. Genetic testing of all individuals in a LQTS family may be indicated both to determine the risk of sudden death and to identify individuals at risk for transmitting the mutation. Genetic counseling will be a critical component of the appropriate management of families with this disorder.

Furthermore, LQTS patients with defects in different LQT genes may require different therapeutic interventions (13,14). Patients with *KVLQT1* defects have a unique clinical phenotype (15) which may be more effectively treated by therapy directed at correcting the pathophysiologic defect responsible for QT prolongation in these patients. The pathophysiology of a particular *KVLQT1* mutation may depend on whether or not it alters the selectivity of the channel in addition to diminishing the channel’s ability to conduct a potassium current. Further characterization of normal and mutant *KVLQT1* channels may enable the development of more specific therapies for patients with *KVLQT1* defects. As clinical trials progress, genetic testing in all patients with the LQTS may become the cornerstone of optimal patient management, helping reduce the mortality of this often fatal disorder.

**MATERIALS AND METHODS**

**Phenotyping of LQTS patients**

Patients with the long QT syndrome were identified at a number of clinical centers including the University of Michigan, Atlanta Children’s Heart Center, and Children’s National Medical Center. Informed consent or assent was obtained from each individual in the study (and/or their guardian) according to the specifications of the Institutional Review Board for that center. For each individual, a medical history questionnaire was completed which recorded the incidence and number of syncopal episodes, any history of documented *tortolades de pointes* (corroborated by medical chart review), the age at onset of symptoms, and a record of any medications taken at (i) the time of the study and (ii) prior to any of the syncopal episodes. A 12 lead electrocardiogram, recorded at a paper speed of 50 mm/s, and a blood sample was obtained from each individual in the study. The QT interval, measured in lead II or V5, was corrected for heart rate using Bazett’s formula [QT/(preceding R-R interval)]1/2. Based on prior LQTS studies, affected individuals were defined those patients with a corrected QT interval (QTc) of ≥0.470 s in males, ≥0.480 s in females, or ≥0.440 s in either sex with evidence of symptomatology (i.e., syncpe, *tortolades de pointes*, or sudden death). Unaffected individuals were identified as those patients with a QTc interval of ≤0.410 s. Individuals that could not be classified as affected or unaffected based on these criteria were categorized as indeterminant for the LQTS. Similar criteria for the diagnosis of the LQTS have been used in prior studies (4–7,15).

**Genomic DNA samples**

DNA was isolated from peripheral blood lymphocytes using standard methods (16) or using the Puregene DNA isolation kit as directed by the manufacturer (Genta System, Inc.). From each blood sample, an Epstein-Barr virus transformed lymphoblastoid cell line was established as previously described (17). The control group of unrelated individuals is a subset of the group described in Struewing et al. (18).

**SSCP methods**

The PCR primers used for mutation analysis were those used by Wang et al. (6). Prior to PCR amplification, both primers were end-labelled with 32P ATP (1 mCi per patient sample) using T4 polynucleotide kinase (Promega) as previously described (19). PCR was performed using standard conditions using 1 µl (20 ng) of genomic DNA template, 2 µl of 10x PCR buffer (Promega), 0.4 µl of 10 mM dNTP mix (10 mM dATP, 10 mM dCTP, 10 mM dGTP, 10 mM dTTP), 0.5 µl of 10 µM kinased forward primer, 0.5 µl of 10 mM reverse primer, 0.1 µl PerfectMatch (Stratagene,
Inc.), and 0.2 μl of AmpliTaq DNA polymerase (5U/μl). The reactions were set up on ice and placed in a preheated (to 95°C) Perkin-Elmer 9600 thermal cycler. For amplification, each sample was denatured for 3 min at 94°C, and subjected to 35 rounds of amplification. Each amplification cycle consisted of 1 min of denaturation at 94°C, 30 s of annealing at 60°C, and 90 s of extension at 72°C. After 10 min of extension at 72°C, 80 μl of denaturing stop solution (95% formamide, 10 mM NaOH, 0.25% bromophenol blue) was added to each reaction. The samples were denatured for 8 min at 95°C and rapidly cooled on ice. The gels were prepared as previously described (18). Duplicate gels were run at 4°C and at room temperature. Two microclorites of each sample was loaded. The gels were run in 0.6× TBE buffer at 4 Watts for 12–14 h, dried under vacuum at 80°C, and exposed to autoradiography film at –80°C.

Sequencing of PCR products

Variant SSCP bands were cut from the dried gels after alignment with the autoradiograph, and placed directly into PCR reactions containing 85 μl of ddH2O, 10 μl 10× PCR buffer, 2 μl of 20 μM forward primer, 2 μl of 20 μM forward primer, 2 μl of 10 mM dNTPs, and 1 μl of AmpliTaq DNA polymerase. Amplification was performed as described above and 10 μl of the product was run on a 1.5% agarose gel to visualize yield. The amplified DNAs were purified using a Microcon-30 filter as directed by the manufacturer (Amicon, Inc.). The samples were eluted using an adaptation of the manufacturer’s instructions. The filter was soaked with 25 μl of 37°C ddH2O, the filter unit was inverted, and the sample was spun in a microcentrifuge at 14K r.p.m. for 5 s. Yield and purity were determined using the optical DENSITY of a 1:100 dilution of the sample at wavelengths of 260 and 280 nm.

The Taq Terminator reactions used 500 ng of the purified PCR product, 5 pmol unlabeled primer and 9.5 μl of Taq Terminator mix. After an initial denaturation at 93°C for 3 min, the following three temperature cycle was repeated 25 times on an MJ-PTC-100–96 thermal cycler (Watertown, MA): 94°C for 30 s, 50°C for 5 s, and 60°C for 3 min. The sequencing reactions were placed over Centri-Sep columns (Princeton Separations; Adelphia, N.J.) to remove excess dye terminators using the manufacturer’s protocol. The samples were then dried, resuspended in 4 μl of loading buffer (deionized formamide with 50 mM EDTA), and heated to 90°C for 1 min. The reactions were loaded completely on the Applied Biosystems 373A Sequencer and run under standard electrophoretic conditions. All of the fluorescent sequencing reagents were purchased from Applied Biosystems (Foster City, CA) and unlabeled primers were purchased from Genosys Inc. (The Woodlands, TX). At least two bands from separate PCR reactions were sequenced in both directions in order to confirm the identified mutations.

Genotype analysis

In order to establish linkage of the LQTS to one of the previously-described LQTI loci, each LQTS family was genotyped for markers D11S922, D11S932, D11S860, D3S1100, and D7S483 as previously described (20). Genotyping data for the RWS84 family was reported in a previous study (7). The MLINK subroutine of the LINKAGE program was used to calculate LOD scores (21). Those families with a LOD score of ≥2 to one or more markers on chromosome 11p15.5 were categorized as LQTI-linked families. Small families and sporadic cases in whom linkage to 11p15.5 could not be excluded were also included in the SSCP analysis of the KVLQTI gene. The members of the SQT03 family were genotyped for the above markers as well as 19 markers on chromosomes 12 and 13 (D12S578, D12S285, D12S285, D12S314, D12S353, D12S345, D12S346, D12S351, D12S366, D12S1294, D13S130, D13S170, D13S175, D13S218, D13S285, and D13S325) as previously described (22), in order to confirm correct paternity assignment. The SQT02 and QT84 families were genotyped for markers D11S922, D11S1318, tyrosine hydroxylase (23), D11S860, and D11S932 as previously described (20).

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