Mutation of the Gene for IsK Associated With Both Jervell and Lange–Nielsen and Romano–Ward Forms of Long-QT Syndrome

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Background—Long-QT syndrome (LQTS) is a disorder of ventricular repolarization characterized by a prolonged QT interval, syncope, seizures, and sudden death. Recently, three forms of LQTS have been shown to result from mutations in potassium or sodium ion channel genes: *KVLQT1* for LQT1, *HERG* for LQT2, and *SCN5A* for LQT3. IsK, an apparent potassium channel subunit encoded by *KCNE1* on chromosome 21, regulates both KVLQT1 and HERG. This relationship makes *KCNE1* a likely candidate gene, because mutations of these genes are known to cause both the autosomal dominant Romano-Ward and recessive Jervell and Lange-Nielsen (JLN) forms of LQTS.

Methods and Results—We screened 84 unrelated patients with Romano-Ward and 4 with JLN for possible mutations in *KCNE1*. We identified one homozygous mutation in a JLN patient that results in the nonconservative substitution of Asn for Asp at amino acid 76. The patient is congenitally deaf-mute, with recurrent syncopal events and a greatly prolonged QT_c interval. The proband's mother and half-sister are both heterozygous for this mutation. Remarkably, both these family members have prolonged QT_c intervals and would have been classified as Romano-Ward patients if not for the proband's diagnosis of JLN. This mutation was not identified in more than 100 control individuals.

Conclusions—These data provide strong evidence that *KCNE1* mutations represent a fifth LQTS locus (LQT5). Further functional analysis, as well as the identification of more LQTS patients with *KCNE1* mutations, will be important to confirm the role of IsK in LQTS. (***Circulation.*** **1998;97:142–146.**)

Key Words: arrhythmia ■ genes ■ molecular biology ■ long-QT syndrome ■ syncope

Long-QT syndrome (LQTS) is a rare cardiac disorder characterized by abnormal ventricular repolarization and a prolonged QT interval on the ECG. Clinically, two inherited forms of LQTS have been defined: autosomal dominant Romano-Ward syndrome[1,2] and autosomal recessive Jervell and Lange-Nielsen (JLN) syndrome.[3] Patients with both Romano-Ward and JLN syndrome are predisposed to syncope, seizures, and sudden death, typically due to polymorphic ventricular tachycardia (torsade de pointes). In addition, the JLN syndrome is associated with congenital bilateral deafness, and these patients often have a more prolonged QT_c on surface ECGs. Romano-Ward syndrome is genetically heterogeneous, with at least four different known loci.[4–7] Recently, homozygous mutations of one of these, *KVLQT1*, were reported to be responsible for JLN syndrome in three families.[8,9] However, it is also clear that the disease genes in certain JLN families are not linked to the *KVLQT1* locus.[10] Thus, JLN syndrome must also be a genetically heterogeneous group of clinically related disorders.

IsK, an apparent potassium channel regulatory subunit encoded by the *KCNE1* gene on chromosome 21, has recently been shown to coassemble with both K_VLQT1, to produce the slowly activating cardiac delayed rectifier K^+ current, I_{Ks},[11,12] and HERG, to regulate the rapidly activating cardiac delayed rectifier K^+ current, I_{Kr}.[13] This relationship makes *KCNE1* an attractive candidate gene for LQTS, because mutations of both *KVLQT1* and *HERG* are known to cause LQTS.[4,5,8,9] Originally cloned from rat kidney, the human *KCNE1* gene encodes a 129-amino-acid protein with a single putative transmembrane domain.[14] Thus far, IsK has no sequence homology with other cloned channel subunits. Like K_VLQT1, IsK is expressed in many tissues, including heart and inner ear.[8,11,15]

A recent study reported a knockout mouse strain with a disruption of the endogenous *Kcne1* coding sequence and absence of IsK protein.[15] Although heterozygous mice appear normal, homozygous null mutant mice are deaf and exhibit classic shaker/waltzer behavior. Both IsK knockout mice and
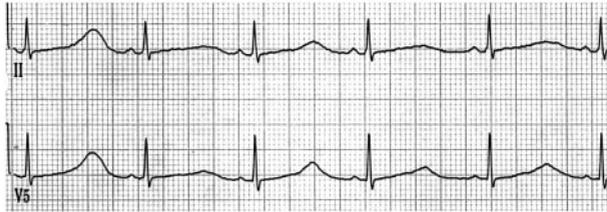

Figure 1. ECG tracing from proband (LQTS086–001) at 7 years of age just before surgery, illustrating prolonged QT intervals and beat-to-beat variation of T-wave morphology in leads II (above) and V_5 (below).

JLN patients appear to have similar developmental defects involving the strial marginal cells of the cochlea capsule, resulting in deficient endolymph production and congenital deafness.[16,17] These findings support the idea that mutations in IsK may be responsible for JLN syndrome. To test this hypothesis, 84 Romano-Ward and 4 JLN patients were examined for possible mutations of *KCNE1*.

Methods

Patient Population

Patients with clinical diagnoses of LQTS and their family members were referred from cardiology clinics in North America and represented diverse ethnic backgrounds. Peripheral blood for DNA extraction was collected after informed consent was obtained according to guidelines approved by the Children's Hospital Institutional Review Board. Additional data collected included clinical and family histories of syncope, palpitations, seizures, hearing loss/deficiency, and sudden cardiac death, as well as surface ECGs, exercise tests (if available), Holter studies (if available), and recent clinical notes from the referring physicians. All probands met established criteria for LQTS.[18] Rate-corrected QT intervals were calculated by Bazett's formula, where $QT_c = QT/\sqrt{RR}$ and have units of seconds$^{1/2}$.[19]

Clinical History of Family LQTS086

The proband (LQTS086–001) is an 8½-year-old girl who presented to the emergency room at age 3½ years after a syncopal spell while sliding into a pool. Syncope was preceded by a gasp and followed by near drowning after she lost consciousness. She had a history of recurrent syncopal events beginning at 2 years of age, including a similar event in the pool and a near-syncopal episode after tickling and playing hard. The patient was congenitally deaf-mute. ECG indicated a maximal QT_c of 0.66 in leads V_2 and V_3 at a heart rate of 67 bpm. While she was on propranolol, an initial Holter study revealed rare premature ventricular beats and a prolonged QT interval. On exercise testing, she achieved a maximal heart rate of 135 at 7 minutes, with a QT_c of 0.54 second.

There was a positive family history of syncope, seizures, and partial hearing loss of unclear cause (left ear) in her 33-year-old mother. The proband's mother and one 3-year-old maternal half sister also have prolonged QT_c values of 0.48 and 0.47 second, respectively. Paternal history is unknown and unavailable.

She continued on propranolol 2 mg · kg^{-1} · d^{-1} without symptoms until 3½ years later, when she presented with another syncopal episode after exertion. Her ECG showed a prolonged QT_c of 0.68 second, with abnormal T-wave morphology and a sinus rate of 64 bpm (Fig 1). At exercise testing, while she was still on propranolol, her maximal heart rate was only 110 bpm, with no ectopy. Holter recording revealed a single ventricular couplet and a maximal heart rate of 85 bpm. On the basis of these findings and her history of recurrent syncopal attacks despite β-blockade, it was recommended that the patient undergo surgery. She underwent a partial left stellate gangliectomy and dorsal sympathectomy and was discharged 3 days later, still on propranolol (2.5 mg · kg^{-1} · d^{-1}), with residual Horner's syndrome. Since surgery, she has generally been doing well, with QT_c

values ranging from 0.50 to 0.62, but recently she suffered another syncopal episode that was preceded by prodromal symptoms, including dizzy spells and a general feeling of malaise.

DNA Collection and *KCNE1* Mutation Analysis

Genomic DNA was prepared from peripheral blood lymphocytes with the Puregene DNA Isolation kit (Gentra Systems, Inc). Initial polymerase chain reaction (PCR) assays used primers 1F, 2R, 3F, and 4R of Tesson et al.[20] To amplify additional portions of the human *KCNE1* gene, primers 5F (5′-GCGCCTGCAGCAGTGGAAC CTT-3′) and 6R (5′-TTGAATGGGTCGTTCGAGTG-3′) were designed on the basis of a published full-length cDNA sequence (Genbank M26685; all base numbers in this report are based on this sequence) (Fig 2A). These primers amplify a product of 268 nucleotides containing 11 bp of 5′ untranslated region and codons 1 to 72. The primer 8R (5′-TTTAGCCAGTGGTGGGGGTT-3′) was also designed and paired with previously published primer 3F (5′-TA-CATCCGCTCCAAGAAG-3′).[20] These primers amplify a product of 216 nucleotides, including the final 57 codons (eg, 71 to 129) of *KCNE1*. The promoter and other transcriptional control elements are not included in these assays.

Single-strand conformational polymorphism (SSCP) analysis, band elution, and DNA sequencing were performed essentially as described.[21] Products were also analyzed on two types of 0.5X MDE gels (FMC Bioproducts), with and without 5% glycerol, run at room temperature at 8 W for 12 to 14 hours.

To confirm the presence of the D76N mutation, a restriction digest assay was designed using primers 9F (5′-CTCTACGTCCTCATG GTACTG-3′) and 10R (5′-CGATGTAGACGTTGAATGAGT-3′). These primers amplify a 115-bp product containing the mutated site. Primer 10R is mismatched at nucleotide 257, producing a T instead of a wild-type C three bases downstream from the mutation. This mismatch produces a *Hin*fI restriction site (GACTC) in the wild-type *KCNE1* gene at position 254. The D76N mutation (G254A) abolishes this restriction site. Wild-type PCR products are cleaved by *Hin*fI at position 184 and at 254 to give a predominant product of 71 bp, whereas aberrant products containing the D76N mutation will be cleaved only at position 184, generating a 91-bp product. Genomic DNA samples were amplified with primers 9F and 10R as described[21] at an annealing temperature of 58°C. A 5-μL volume containing 3 U *Hin*fI, 3× BSA, and 3× restriction enzyme buffer 2 (New England Biolabs) was added to each 10-μL reaction and incubated at 37°C for 3 hours. PCR products were separated on DNA sequencing gels and visualized with autoradiography.[21]

Direct sequencing of the *KCNE1* gene was performed on PCR products of primer pairs 5F/6R and 3F/8R on an ABI 377 DNA sequencer after purification with Wizard PCR preparation kits (Promega).

Results

To test the hypothesis that IsK may be mutated in patients with LQTS, we used SSCP analyses to screen *KCNE1* in a panel of affected individuals with Romano-Ward or JLN syndrome. Initial SSCP analyses using primers 1F/2R identified two previously reported polymorphisms[20,22] but no apparent LQTS-associated mutations. To allow inclusion of the translational start and stop sites, we designed three new primers (5F, 6R, 8R) that, together with 3F, amplify the entire *KCNE1* coding region in two PCR reactions. With these primers, 75 LQTS patients were scored for the S38G polymorphism[22]; 40% were homozygous for glycine, 24% were homozygous for serine, and 36% were heterozygous for the variation. Similarly, 71 patients were scored for the D85N polymorphism,[20] 94% were homozygous for aspartic acid, and 6% were heterozygous at this site. A third polymorphism was also identified in this population, a G-to-A transition at base 112, resulting in a previously unreported silent change at serine codon 28. Of 75
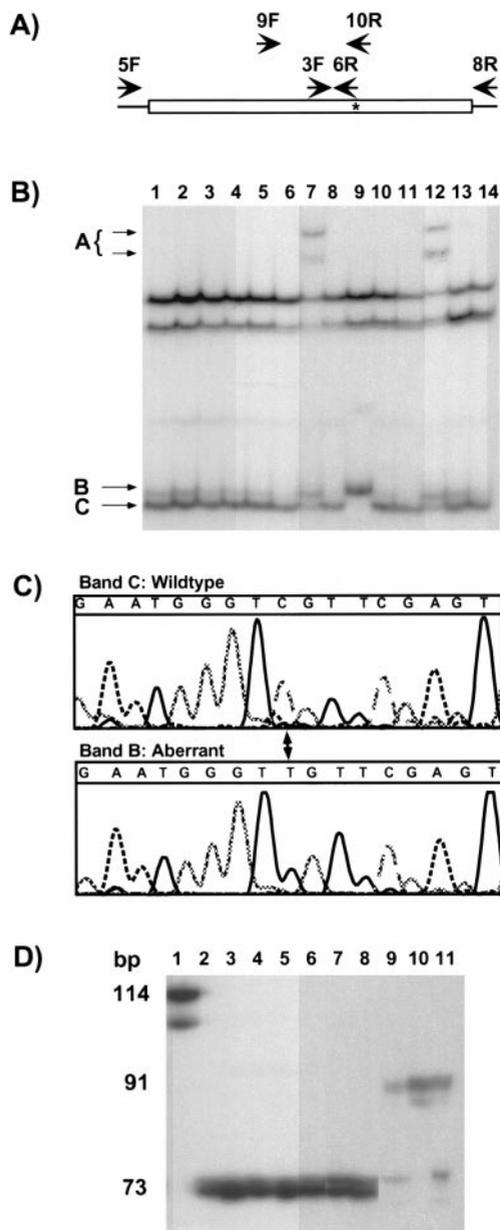
Figure 2. Identification of *KCNE1* mutation in patients with LQTS. A, Schematic of 436-bp contiguous *KCNE1* coding region, indicating relative locations of different primers used in this study. Box indicates protein coding portion of gene; *, location of mutated nucleotide 254. B, Autoradiograph of SSCP gel containing polymerase chain reaction (PCR) products of primers 3F and 8R from 14 unrelated patients with LQTS. Products from patient LQTS086–01 are in lane 9. Sequencing of bands labeled A in lanes 7 and 12 revealed that they reflect the D85N polymorphism. C, Partial DNA sequence of band B from lane 9, panel A, and a band C with sequencing primer 8R (ie, reverse complement). The two sequences differ at a single base (indicated by arrow), demonstrating a G-to-A transition at position 254 on the *KCNE1* mRNA (Genbank M26685). D, Mismatched primer, mutation-specific, restriction digestion assay for the G-to-A transition at base 254 on normal control DNAs (lanes 1 to 8) and the mother, proband, and half sister of family LQTS086 (lanes 9, 10, and 11, respectively). *Hin*fI digestion was omitted in lane 1 to illustrate full-length PCR products. Wild-type DNAs are digested at two sites to generate a major 73-bp fragment, whereas mutant DNAs are cut only once to create a 91-bp fragment.
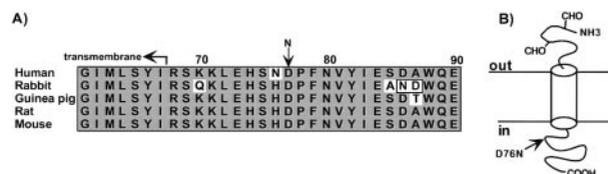

Figure 3. A, Lineup of IsK amino acid sequences showing high degree of evolutionary conservation in region around D76. Residue numbers (above) are based on rabbit and rat sequences, which contain one additional amino acid, at position 30, relative to other genes. Approximate intracellular boundary of transmembrane domain is indicated above, as is location of D76N mutation identified in family LQTS086. B, Predicted molecular topology of IsK protein illustrating location of D76N mutation in intracellular domain. Amino (NH3) and carboxyl (COOH) terminal ends of peptide are indicated, as are two potential N-linked glycosylation sites (CHO).

individuals screened, 95% were homozygous for guanine-112 and 5% were heterozygous for the change.

A novel anomalous conformer was identified in one DNA sample (LQTS086−001) from the panel of LQTS patients (Fig 2B). The patient has a diagnosis of JLN as described above. The aberrant conformer was not observed in any of the 84 patients with Romano-Ward syndrome or the 3 other JLN patients. DNA sequence analysis of the aberrant conformer revealed a G-to-A transition at nucleotide 254 that is predicted to create a nonconservative missense mutation of aspartic acid to asparagine at codon 76 (D76N) (Fig 2C). The aspartic acid at position 76 is close to the putative transmembrane region in the intracellular carboxyl-terminal domain of IsK,[14] and this residue is completely conserved in mammalian IsK genes (Fig 3).

Because the SSCP shift was subtle and difficult to appreciate on gels containing the normal control PCR products, a PCR restriction digest assay was designed to allow unambiguous identification of the mutation. The D76N mutation was not detected in DNA samples from 104 North American control individuals with no clinical signs of LQTS (Fig 2D). The proband was homozygous for the change, whereas her mother and half sister were both heterozygous for this mutation. Additional family members were not available for study.

Because the sensitivity of SSCP analysis is well below 100%, we amplified by PCR and directly sequenced the *KCNE1* genes of all JLN probands to look for additional mutations that might have been missed in the initial screen. The D76N mutation was readily identified in both the homozygous patient and her heterozygous mother and half sister. None of the other three unrelated JLN patients had any identifiable mutations in their *KCNE1* genes.

Discussion

The identification of K_VLQT1 and HERG as major structural subunits of the I_{Ks} and I_{Kr} channels established that abnormalities of these currents were responsible for LQTS.[11,12,23] Therefore, it was logical to speculate that mutations of IsK, a probable regulatory subunit that associates with K_VLQT1[11,12] and HERG,[13] might also be involved in causing heritable defects in cardiac repolarization. The present data implicate the *KCNE1* gene as a fifth locus for LQTS (LQT5). A unique missense mutation, D76N, was found in a homozygous patient with JLN syndrome and was not detected in 104 normal

control individuals. The proband's DNA was also included in screens of the other three known LQTS genes, *KVLQT1*,[4] *HERG*,[5] and *SCN5A*,[6] and no mutations were identified (data not shown). The patient's clinical presentation included congenital deafness, extremely prolonged QT_c intervals, and recurrent syncopal events that were refractory to β-blockade, eventually necessitating surgical intervention. It may be that this relatively severe clinical picture is related to the fact that IsK most likely regulates both the I_{Ks} and I_{Kr} currents. The proband's mother and half sister were both heterozygous for the D76N mutation and had clinical histories that included syncope, seizures, partial hearing loss, and/or prolonged QT_c intervals. These findings are consistent with previous reports of JLN pedigrees in which homozygous individuals have severe cardiac disease and complete hearing loss, whereas obligate heterozygotes may have a milder phenotype more closely resembling the Romano-Ward form of LQTS.[8,9,24]

A previous study did not find evidence for involvement of IsK in JLN syndrome in four autosomal recessive JLN families,[20] and subsequently, these workers did identify a frame shift mutation in the carboxyl terminus of K_VLQT1 for two of their four JLN families.[8] However, linkage data for both *KCNE1* and *KVLQT1* were inconclusive in the two remaining families. Although our initial screen failed to detect any aberrant conformers, a slight change in the location of primer 4R (eg, 8R) resulted in increased sensitivity for the G-to-A transition at nucleotide 254, leading to our identification of this mutation. This illustrates how the sensitivity of SSCP analysis can be altered in unpredictable ways by apparently subtle changes to the assay conditions. To date, we have found a homozygous *KVLQT1* mutation in one of the remaining three JLN families (unpublished data, 1997), but the apparent absence of mutations in the other two suggests the possibility of additional genetic heterogeneity in this disorder.

There have been numerous previous studies of IsK structure and function; however, many of these are difficult to interpret because they were performed before the appreciation that IsK is probably a regulatory subunit and not a primary (alpha) channel subunit.[11–13] Nevertheless, studies performed in *Xenopus* oocytes recorded currents that result from the coassembly of IsK and an endogenous K_VLQT1-like peptide and probably reflect true functions of IsK. Fortuitously, the rat equivalent of the D76N mutation reported here (D77N, since the rat IsK gene includes one additional residue at position 30) has been previously created and functionally studied in *Xenopus* oocytes.[25,26] Rat D77N IsK proteins were expressed and incorporated efficiently in the plasma membrane, yet these peptides exhibited a drastic reduction in channel activity resulting in virtually undetectable potassium currents.[25] Coinjection of equal amounts of D77N and wild-type mRNAs resulted in only a small fraction of the current seen with wild-type protein alone, suggesting that incorporation of a single mutant IsK subunit was sufficient to disrupt channel function and possibly explaining the dominant phenotype seen in family LQTS086.[26] Functional studies of D76N mutant human IsK coexpressed with human K_VLQT1 will be important to determine the molecular mechanism of this relationship as well as to prove that human IsK-D76N has a

pathological effect on I_{Ks} currents. Identification of additional *KCNE1* mutations in LQTS patients will also confirm this relationship and provide new insights into the structure and function of IsK.

In the absence of information on the proband, the mother and half sister of family LQTS086 would probably have been given a diagnosis of Romano-Ward syndrome. Therefore, as is the case for *KVLQT1*, mutations of IsK should be looked for in patients with either the Romano-Ward or the JLN forms of LQTS. We suggest that the *KCNE1* gene, which encodes the IsK protein, be designated as a fifth LQTS locus, LQT5.

Note Added in Proof

KCNE1 mutations in patients with LQTS have also recently been reported by Schultze-Bahr et al. and Splawski et al. (*Nat Genet*. 1997;17:267–268 and 338–340, respectively).

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