

Homozygous Deletion in *KVLQT1* Associated With Jervell and Lange-Nielsen Syndrome

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Background—Long-QT (LQT) syndrome is a cardiac disorder that causes syncope, seizures, and sudden death from ventricular arrhythmias, specifically torsade de pointes. Both autosomal dominant LQT (Romano-Ward syndrome) and autosomal recessive LQT (Jervell and Lange-Nielsen syndrome, JLNS) have been reported. Heterozygous mutations in 3 potassium channel genes, *KVLQT1*, *KCNE1* (*minK*), and *HERG*, and the cardiac sodium channel gene *SCN5A* cause autosomal dominant LQT. Autosomal recessive LQT, which is associated with deafness, has been found to occur with homozygous mutations in *KVLQT1* and *KCNE1* in JLNS families in which QTc prolongation was inherited as a dominant trait.

Methods and Results—An Amish family with clinical evidence of JLNS was analyzed for mutations by use of single-strand conformation polymorphism and DNA sequencing analyses for mutations in all known LQT genes. A novel homozygous 2-bp deletion in the S2 transmembrane segment of *KVLQT1* was identified in affected members of this Amish family in which both QTc prolongation and deafness were inherited as recessive traits. This deletion represents a new JLNS-associated mutation in *KVLQT1* and has deleterious effects on the *KVLQT1* potassium channel, causing a frameshift and the truncation of the *KVLQT1* protein. In contrast to previous reports in which LQT was inherited as a clear dominant trait, 2 parents in the JLNS family described here have normal QTc intervals (0.43 and 0.44 seconds, respectively).

Conclusions—A novel homozygous *KVLQT1* mutation causes JLNS in an Amish family with deafness that is inherited as an autosomal recessive trait. (*Circulation*. 1999;99:1344-1347.)

Key Words: long-QT syndrome ■ deafness ■ Jervell and Lange-Nielsen syndrome ■ potassium channel

The inherited long-QT syndrome (LQT) is characterized by prolonged QT interval on ECGs, syncope (sudden loss of consciousness), seizures, and sudden death from ventricular arrhythmias, specifically torsade de pointes.^{1,2} Both autosomal dominant (Romano-Ward syndrome, RWS)^{3,4} and autosomal recessive (Jervell and Lange-Nielsen syndrome, JLNS)⁵ forms of LQT have been reported. Four genes for RWS have been mapped to chromosomes 11p15.5 (*LQT1*),⁶⁻⁸ 7q35-36 (*LQT2*),⁹ 3p21-24 (*LQT3*),⁹ and 4q25-27 (*LQT4*),¹⁰ and 3 LQT genes (*LQT1*, *LQT2*, and *LQT3*) were subsequently identified,¹¹⁻¹³ including the potassium channel genes *KVLQT1* (*LQT1*)¹¹ and *HERG* (*LQT2*)¹² and the cardiac sodium channel gene *SCN5A* (*LQT3*).¹³ Recently, mutations in *KCNE1* (*minK*)¹⁴ were also found to cause RWS.

JLNS is rare and is associated with congenital sensorineural deafness. It was first described in 1957 by Jervell and Lange-Nielsen⁵ in a Norwegian family in which 4 of the 6 children were affected by both LQT and congenital sensorineural deafness but the parents appeared normal. Three of the affected children died suddenly at the ages of 9, 5, and 4 years. Since then, several other cases of autosomal recessive LQT have been reported.¹⁵⁻¹⁷ In some cases, the heterozygous parents had moderate QTc prolongation despite the fact that the syndrome was inherited as a recessive trait. In 1997, Neyroud et al¹⁸ and Splawski et al¹⁹ reported the identification of homozygous mutations of *KVLQT1* in JLNS. In both studies, LQT appeared to be inherited in an autosomal dominant fashion (ie, at least 1 of the parents was affected by LQT), and deafness was inherited in an autosomal recessive

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fashion. Recently, mutations in *KCNE1* (*minK*) also have been found to cause JLNS, establishing *minK* as a new gene for JLNS.²⁰

In this article, we describe a homozygous deletion of 2 bp within the second transmembrane domain of *KVLQT1* (S2) in a family with 2 individuals affected with LQT and deafness. This deletion causes a frameshift and premature termination and leads to a nonfunctional *KVLQT1* potassium channel.

Methods

JLNS Patient Evaluation

The JLNS family described here was identified in an Amish community and referred for molecular genetic studies. Only the nuclear family allowed evaluation. Consanguinity was denied by the parents. Informed consent was obtained from all participants or their guardians in accordance with standards established by local institutional review boards. Phenotypic characterization was performed as described previously,² with the Bazett QT correction for heart rate (ie, QTc).²¹

Single-Strand Conformation Polymorphism and DNA Sequence Analysis

Genomic DNA was prepared from peripheral blood lymphocytes or lymphoblastoid cell lines derived from Epstein-Barr virus–transformed lymphocytes from the JLNS family and from 120 control individuals.²² Polymerase chain reaction (PCR) for single-strand conformation polymorphism (SSCP) analysis was performed as previously described.¹¹

Normal and abnormal SSCP conformers were cut directly from dried gels, eluted in 100 μ L of distilled water at 55°C, and reamplified. PCR products were fractionated in 1.5% FMC NuSieve low-melting-temperature agarose gel and purified. Purified PCR products (200 fmol) were sequenced directly by cycle sequencing with the Cyclist™ Exo-Pfu DNA Sequencing Kit (Stratagene). For each sequencing reaction, 20 μ Ci of α -³⁵S-dATP and 2.5 U of the Exo-Pfu enzyme were used. PCRs were carried out in a Perkin-Elmer System-9600 thermocycler with the following profiles: 1 cycle at 95°C for 5 minutes, 45 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute.

Restriction Fragment Length Polymorphism Analysis

Genomic DNA was PCR amplified to yield a 200-bp fragment. One of the primers was end labeled with [γ -³²P]ATP with T₄ polynucleotide kinase (NEB) under standard conditions and included in a 20- μ L PCR reaction as described for SSCP. At the end of the PCR reaction, 10 μ L of the reaction mixture was digested in a 50- μ L reaction under standard conditions with either *Bgl*II (which is unique to the wild-type PCR fragment) or *Msp*I (which is unique to the mutant PCR). After incubation at 37°C for 1.5 hours, 25 μ L of formamide buffer was added. The mixture was heated at 95°C for 3 to 5 minutes and cooled immediately on ice, and 3 μ L was loaded onto 6% urea-denaturing polyacrylamide gels (acrylamide:bisacrylamide=19:1). The gels were run in 1 \times tris borate EDTA buffer at 65 W, dried on filter paper (Schleicher and Schuell), and exposed to x-ray film (Kodak).

Results

Phenotypic Characterization

Phenotypic analysis of this Amish family revealed deafness in both children but normal hearing in the parents (as defined by clinical evaluation only), indicating that the deafness in this family is inherited as an autosomal recessive trait. ECG analysis of this family revealed that the deaf male and female children are both affected by LQT, having QTc intervals of

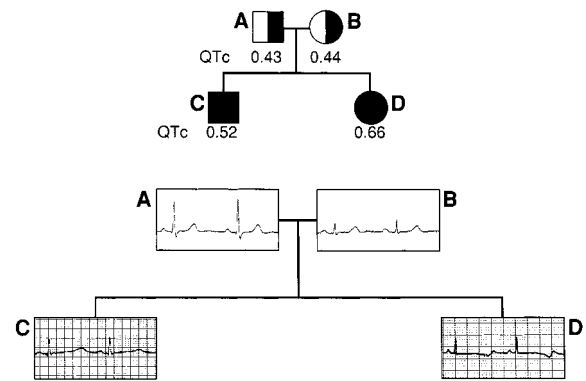


Figure 1. Pedigree structure of Amish family affected by JLNS and associated ECGs of each individual. Affected female individuals are indicated by filled circles; male individuals, by filled squares; and individuals with borderline QTc interval, by half-filled circles and half-filled squares. QTc values are given in seconds under pedigree symbols. Note that parents (individuals A and B) have normal QTc intervals, whereas offspring (individuals C and D) have definitely abnormal QTc intervals, consistent with autosomal recessive inheritance of LQT (and deafness).

0.52 and 0.66 second, respectively (Figure 1). No ventricular tachycardia was documented in these children. The father and mother, however, have borderline QTc intervals of 0.43 and 0.44 second, respectively. No provocative testing on the parents (ie, electrophysiology study with pharmacological provocation, stress testing) was performed, however. Therefore, with respect to the clinical expression of QTc prolongation in this family, there is autosomal recessive inheritance with subclinical manifestations in the heterozygous parents. With respect to the ECG findings, however, the trait is inherited as an incomplete dominant. These studies are consistent with the diagnosis of JLNS in this family as well.

Mutational Analysis

Screening for mutations in *KVLQT1* with SSCP and DNA sequencing analysis identified an abnormal SSCP conformer in the male patient of this Amish family (proband, Figure 2A) but not from >100 control individuals. Sequence analysis of the abnormal SSCP conformer revealed a 2-bp deletion in the S2 transmembrane domain of *KVLQT1* (Figure 2B). This mutation results in a frameshift and premature termination of *KVLQT1*.

Cosegregation of a Homozygous Deletion in *KVLQT1* With JLNS

To determine whether the 2-bp deletion was homozygous in the affected individuals, the DNA segment that was amplified from genomic DNA of each affected person was sequenced. The sequencing patterns, including the deletion of the 2 nucleotides AA from both affected individuals, were identical to that of the SSCP abnormal band (data not shown), indicating that both affected individuals are homozygous for the 2-bp deletion. The sequencing patterns of both parents are identical to that of the SSCP abnormal band flanking the 2-bp deletion but are a mixture of 2 overlapping sequencing panels within the 2-bp deletion region (data not shown). These data suggest that the parents are heterozygous for the 2-bp deletion

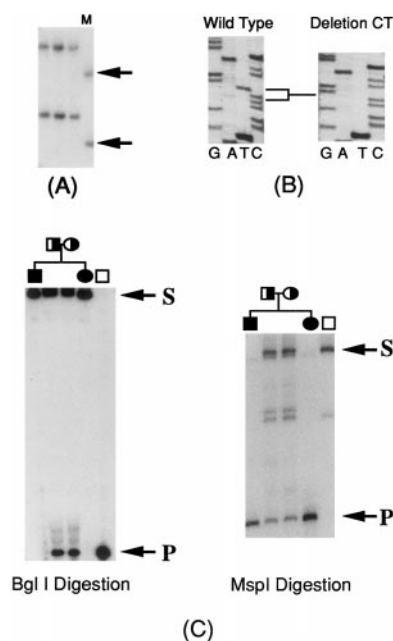


Figure 2. Identification of 2-bp deletion in *KVLQT1* in proband of Amish family. A, SSCP analysis showing abnormal conformer in proband, lane M, as indicated by arrows. B, DNA sequence analyses of normal (wild-type) and abnormal (SSCP) conformers revealed a 2-bp (CT) homozygous deletion in proband. Deletion occurs in S2 transmembrane segment of *KVLQT1*, leading to frameshift and premature termination of *KVLQT1*. C, Restriction fragment length polymorphism analysis showing homozygous 2-bp deletion in *KVLQT1* cosegregating with JLNS in Amish family. The 2-bp deletion in S2 transmembrane segment of *KVLQT1* leads to change in susceptibility of DNA to *MspI* (right) instead of *BglI* (left). Pedigree of Amish family is shown above each digestion panel. Empty square indicates unrelated normal individual; S indicates substrate from PCR used for restriction digestion; P, products generated after restriction digestion.

and that homozygous deletion of the 2 bp in *KVLQT1* cosegregates with JLNS.

To further confirm that the affected individuals are homozygous and that the parents are heterozygous for the 2-bp deletion, restriction fragment length polymorphism analysis was performed. In the analysis, genomic DNA of all family members was used to produce the end-labeled DNA fragments by PCR (see Methods). The DNA fragments were then digested with an allele-specific restriction digestion enzyme, *BglI* or *MspI*. *BglI* cuts only the wild-type allele, whereas *MspI* cuts only the mutant allele. As shown in Figure 2C, DNA fragments from both affected individuals were completely cut by *MspI* but remained intact after exposure to *BglI*, confirming that affected individuals in the family are homozygous for the 2-bp deletion. In contrast, DNA fragments from both parents are partially cut by either *BglI* or *MspI*, further suggesting that they are heterozygous for the 2-bp deletion.

Discussion

In this study, we identified a new homozygous deletion in *KVLQT1* cosegregating with the patients of an Amish family affected by both LQT and deafness. The deletion is located in the S2 transmembrane domain of *KVLQT1*, resulting in a frameshift of the predicted amino acid sequence and prema-

ture stop of *KVLQT1*. Because the mutation leads to a frameshift of *KVLQT1* starting from the middle of domain S2, it is expected to produce a nonfunctional potassium channel because most transmembrane domains and the pore region of *KVLQT1* are deleted. The 2-bp deletion may also result in instability of the mutant RNA or lead to a truncated (ie, shortened) protein that is unable to be incorporated into the membrane to form a functional channel. Together with the recent identification of 2 other *KVLQT1* mutations in patients with JLNS,^{18,19} these data further confirm that homozygous mutations in *KVLQT1* result in JLNS.

The S2 transmembrane domain of *KVLQT1* has been reported to contain mutations by previous authors. Splawski et al¹⁹ identified a homozygous insertion mutation in S2 that caused a frameshift, disrupting the coding sequence of *KVLQT1* after S2 and leading to a premature stop codon and a truncated protein lacking the pore region in a family with JLNS. Others, such as Chouabe et al²³ and Tanaka et al,²⁴ reported missense mutations in S2, with the clinical phenotype resulting in a variable phenotype, ranging from mild to moderate to severe RWS. In addition, Donger et al²⁵ identified S2 mutations with widely varying clinical findings in 3 families with RWS, including several gene carriers with borderline QTc. Hence, the clinical phenotype seen with mutations in the S2 transmembrane domain is heterogeneous but, in most cases, appears to be mild.²⁶ Chouabe et al²³ performed biophysical analysis of a variety of *KVLQT1* mutants, including mutations in S2, and found that no matter where the mutation occurred, the general rule is that the only discernable effect is a reduction in current density, corresponding to a dominant-negative suppression of *KVLQT1* function. JLNS mutations studied also produced a dominant-negative effect, but the extent of the inhibition was lower than in RWS patients. Hence, JLNS mutations produce no functional channels and have little effect on expression of wild-type subunits with the relatively normal findings in heterozygotes. Therefore, depending on the severity of the dominant-negative effect of the different mutations, the disease is either dominant or recessive. In the latter case, the reduction in the current normally carried by the *KVLQT1* subunit is so high that the defect becomes apparent in other tissues expressing this protein, the inner ear being most evident.

Tyson et al²⁶ identified a family with normal hearing and normal QTc in the parents (400 and 430 ms) of a child with JLNS, whereas in 2 other families, the same mutation caused QT prolongation (470 ms) in 1 parent. It is likely that modification of the clinical phenotype occurs because of other genetic influences (ie, modifier genes) and environmental influences and that, in some cases, QTc depends on these influences. As noted previously by Vincent et al,²⁷ QTc values in *KVLQT1* mutation carriers may range from normal (420 to 440 ms) to severely abnormal, and this may vary even within families.

In contrast to the previous reports by Neyroud et al¹⁸ and Splawski et al¹⁹ in which at least 1 parent had clearly prolonged QTc (0.484 and 0.480 second, respectively), both parents in this study have normal or borderline QTc intervals of 0.430 to 0.440 second. Thus, the clinical expression of LQT in this family is inherited as an autosomal recessive trait

with subclinical manifestations in heterozygotes (ie, the parents), which is closely related to the original descriptions of JLNS by Jervell and Lange-Nielsen,⁵ Levine and Woodworth,¹⁵ and Fraser et al.^{16,17} With respect to the ECG findings, however, the trait is inherited as an incomplete dominant trait, whereas deafness appears to be inherited as an autosomal recessive trait. This finding further extends our understanding of the clinical, genetic, and molecular genetic aspects of LQT.

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