Genomic Organization of the KCNQ1 K⁺ Channel Gene and Identification of C-Terminal Mutations in the Long-QT Syndrome

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Abstract—The voltage-gated K⁺ channel KVLQT1 is essential for the repolarization phase of the cardiac action potential and for K⁺ homeostasis in the inner ear. Mutations in the human KCNQ1 gene encoding the α subunit of the KVLQT1 channel cause the long-QT syndrome (LQTS). The autosomal dominant form of this cardiac disease, the Romano-Ward syndrome, is characterized by a prolongation of the QT interval, ventricular arrhythmias, and sudden death. The autosomal recessive form, the Jervell and Lange-Nielsen syndrome, also includes bilateral deafness. In the present study, we report the entire genomic structure of KCNQ1, which consists of 19 exons spanning 400 kb on chromosome 11p15.5. We describe the sequences of exon-intron boundaries and oligonucleotide primers that allow polymerase chain reaction (PCR) amplification of exons from genomic DNA. Two new (CA)n repeat microsatellites were found in introns 10 and 14. The present study provides helpful tools for the linkage analysis and mutation screening of the complete KCNQ1 gene. By use of these tools, five novel mutations were identified in LQTS patients by PCR–single-strand conformational polymorphism (SSCP) analysis in the C-terminal part of KCNQ1: two missense mutations, a 20-bp and 1-bp deletions, and a 1-bp insertion. Such mutations in the C-terminal domain of the gene may be more frequent than previously expected, because this region has not been analyzed so far. This could explain the low percentage of mutations found in large LQTS cohorts. (Circ Res. 1999;84:290-297.)

Key Words: KCNQ1 ■ KVLQT1 ■ K⁺ channel ■ long-QT syndrome

Several K⁺ currents are responsible for the repolarization that terminates the plateau phase of the cardiac action potential. Among them, the very slowly activating delayed rectifier K⁺ current, Iₛ, is important in tuning the adaptation of the action potential duration to the heart rate. The same Iₛ current is also essential in maintaining the endolymph K⁺ homeostasis in the inner ear. The channel that underlies Iₛ is formed via the assembly of two transmembrane proteins, KVLQT1 and MinK, encoded by KCNQ1 and KCNE1 genes, respectively. Northern blot analyses and in situ hybridization indicate that KCNQ1 is expressed in kidney, pancreas, lung, placenta, inner ear, and in heart with the highest level of expression. This gene is located on 11p15.5, in a large domain of contiguous genes abnormally imprinted in cancer and the Beckwith-Wiedemann syndrome. The human KCNQ1 gene shows tissue-specific imprinting in several fetal tissues with the exception of the heart. Nevertheless, a developmentally regulated loss of imprinting has been observed in mice resulting in a complete biallelism in adult animals. Mutations in KCNQ1 are the most frequent cause of the long-QT syndrome (LQTS), which is an inherited cardiac disorder that predisposes individuals to syncope, seizures, and sudden cardiac death from ventricular tachyarrhythmias. KCNQ1 is implicated in the two different entities underlain by this disease: the Romano-Ward (RW) syndrome, transmitted as an autosomal-dominant trait, and the Jervell and Lange-Nielsen (JLN) syndrome, transmitted as a recessive trait, which associates bilateral deafness with the cardiac abnormalities.

KCNQ1 genomic organization has been partially described by Lee et al. without reporting intronic sequences flanking the exons. Analysis of the gene demonstrated that at least 6 alternative splice variants can exist that differ depending on...
the use of the several exons located in the 5' part of the gene.3,4,7,15,16 Two major 5' splice variants are present in human heart. A full-length isoform, isoform 1, which contains an open reading frame encoding a 676-amino acid polypeptide and a truncated isoform, isoform 2, which encodes a 549-amino acid protein lacking the cytoplasmic N-terminus and the initial part of the first transmembrane domain S1.7,15,17 Both isoforms have been expressed by transfection in COS or HEK cells, but only isoform 1 produced the $I_{Ks}$ current associated with MinK.3,15 However, isoform 2 has a dominant-negative effect on the $I_{Ks}$ current when coexpressed with isoform 1 and MinK.18,19

A better knowledge of the KCNQ1 gene structure and intronic sequences flanking the exon boundaries is essential for identifying LQTS-associated mutations and their pathophysiological consequences. In the present study, we have determined the complete genomic organization and sequence of the human KCNQ1 gene by virtual screening of public sequence databases. KCNQ1 contains 19 exons and exceeds 400 000 bp, suggesting a possibility of intragenic recombinations. We localized the (CA)$_n$ microsatellite D11S4088 in 400 000 bp, suggesting a possibility of intragenic recombin- 

The availability of tools to screen the entire KCNQ1 gene will be helpful for the linkage analysis. Further, we identified five novel mutations in the C-terminal part of the gene, which has not been analyzed so far from genomic DNA. The availability of tools to screen the entire KCNQ1 gene should increase the percentage of mutations found in large LQTS cohorts.

### Materials and Methods

#### Genomic Organization and Sequence of KCNQ1

The organization and sequence of the entire human KCNQ1 gene was established by used of cyberscreening the EMBL database with the Basic Local Alignment Search Tool (BLAST) program at the National Center for Biotechnology Information (NCBI) World Wide Web site (http://www.ncbi.nlm.nih.gov). The nucleotide sequence versus nucleotide sequence database (BLASTN) option was used for the screening of EMBL with the complete KCNQ1 cDNA sequence.15,17

#### Characterization of Three (CA)$_n$ Repeat Polymorphisms in KCNQ1

The BLASTN program was used to search for (CA)$_n$ repeat polymorphisms and to localize known microsatellites in the KCNQ1 introns. Polymerase chain reaction (PCR) amplification and analysis of microsatellite markers obtained were carried out as previously described.6

#### Subjects

Ninety-three LQTS families (84 RW and 9 JLN families) were enrolled in a large genetic study. LQTS mutations have previously been identified in KCNQ1 and reported for some of the families.6,17,21,22 In the remaining 53 families, clinical evaluation was made and blood samples were collected after written informed consent was obtained in accordance with the guidelines established by the Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale du Groupe Hospitalier de la Pitié-Salpêtrière (Paris, France). All subjects underwent clinical and cardiovascular examinations, including a 12-lead ECG. Subjects were considered as being affected by the RW syndrome when they presented with (1) syncopes or documented torsades de pointes, (2) a QTc interval (QT interval measured in lead II and corrected for heart rate by the Bazett formula) >460 ms, or (3) a QTc >440 ms associated with bradycardia or abnormal T-wave pattern.21 Subjects were considered as being affected by the JLN syndrome when they presented with a QTc >460 ms associated with syncopes and congenital bilateral deafness.6

In RW family 11356 (Figure 2), the proband died suddenly at the age of 30 years under treatment for an abscessed tonsillitis. At the age of 66 years, his affected mother began to experience several syncopes at rest. In RW family E-973, the proband experienced several syncopes triggered by emotion since the age of 6 years, whereas her affected mother was asymptomatic. In RW family 11232, the proband experienced several stress-induced syncopes since the age of 3 years, but her affected father was asymptomatic. In JLN family 12664, the JLN patient was deaf and experienced his first syncope at the age of 2 years. In JLN family 9976, the JLN patient was deaf and experienced his first syncope at 13 months, but his heterozygous father was asymptomatic.

### Single-Strand Conformational Polymorphism (SSCP) Analysis and Direct Sequencing of the PCR Products

The primers were constructed on the basis of flanking intron sequences and were used to amplify each exon (Table 1). The touchdown PCRs were performed from LQTS patient genomic DNA by decreasing the annealing temperature in the first 10 cycles according to the conditions reported in Table 1. For SSCP, the PCR products were denatured for 5 minutes at 96°C in a low ionic strength solution, which generates stable single-stranded DNA,21 kept on ice for 5 minutes, loaded onto a 10% polyacrylamide gel, and run at 8 mA, at 25°C and 7°C, in a Hoefer apparatus. The bands were visualized after silver staining of the gels (Bio-Rad). The PCR products were sequenced by the dideoxynucleotide chain termination method with fluorescent dideoxynucleotides on an ABI-Prism 377 DNA sequencer (Perkin-Elmer/Applied Biosystems).

### Results

#### Genomic Organization and Sequence of KCNQ1

The BLASTN sequence alignment program was used to screen the EMBL database with the complete KCNQ1 cDNA sequence.15,17 All of the coding sequence was found to be distributed (94% to 100% homology) in six overlapping human genomic clones mapped on chromosome 11p15.5, containing a total of 850 kb. The accession numbers of these clones are AC003693, AC00377, AC002403, U90095, AC003675, and AC001228 (G.A. Evans et al, unpublished data, April through December, 1997). We organized the different clones in a contig of 400 kb from exon 1a to 15. The complete sequence has been deposited in the EMBL database under the accession number AJ006345.

The schematic organization of the human KCNQ1 gene and the alignment of exons with structural domains of the protein are shown in Figure 1. The gene spans more than 400 kb and contains 19 exons. We identified an additional exon in the 3' part of KCNQ1, thus completing the partial genomic organization published by Lee et al.7 The region previously identified on the cDNA sequence as exon 11 corresponds, in fact, to exons 11 and 12, which are separated by an intron of 7 kb on genomic DNA. Moreover, we determined the full-length exon 1a. This exon (386 bp excluding 5'- untranslated region [UTR]) comprises the initiation codon for KVLQT1 isoform 1. Fifteen exons (1 to 15 with the exception of exon 2a) are common to isoforms 1 and 2. The N-terminal part of isoform 2, which has a truncated S1 transmembrane...
The segment, is encoded by exon 1b (5 bp excluding 5'-UTR). The exon named 1a encodes the full-length S1 transmembrane segment preceded by the 128-amino acid residue N-terminal domain of isoform 1. Exons 1c and 2a belong to isoforms 3 and 4 described by Lee et al. The sizes of exons and introns are summarized in Table 2. The exon sizes, excluding the 5'- and 3'-UTRs, vary from 5 to 386 bp and intron sizes from 570 bp to 107 kb.

### Table 1. Oligonucleotide Primers and PCR Conditions for Detection of Mutations in the Human KCNQ1 Gene

<table>
<thead>
<tr>
<th>Exon</th>
<th>Size, bp</th>
<th>PCR Conditions</th>
<th>Forward Primer Sequence (5'→3')</th>
<th>Reverse Primer Sequence (5'→3')</th>
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<td>1a</td>
<td>510</td>
<td>TD70–60 +10% DMSO</td>
<td>GCGCCTTGCGCTGAGCT</td>
<td>TCCCCACACCAAGCTCTCTAG</td>
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<td>1b</td>
<td>209</td>
<td>TD65–55</td>
<td>CTCCTGCTACCCACCAAA</td>
<td>TCTAACGCAACATCAACTAAT</td>
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<tr>
<td>1</td>
<td>310</td>
<td>TD70–60</td>
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<td>GTGGGGGCAAAGGCTGAGGAG</td>
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<tr>
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<td>435</td>
<td>TD70–60</td>
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<td>GTGGCGTGAGGCTGAGGAG</td>
</tr>
<tr>
<td>3</td>
<td>330</td>
<td>TD70–60</td>
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<tr>
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Primers are given for genomic amplification of isoforms 1 and 2 of KCNQ1. Exon 15 is amplified by 5 overlapping primer sets. TD indicates touchdown temperatures.

**Figure 1.** Genomic structure of the human KCNQ1 gene. Schematic structure of the gene, with location of exons shown by boxes and introns shown by the horizontal line. Because we found an additional exon in the gene, the first 15 exons were numbered according to Lee et al. but numbering of the last 4 was shifted one place. Alternatively, spliced exons (1a, 1b, 1c, and 2a) are symbolized in gray. Exons encoding putative transmembrane domains (S1 to S6) are numbered in bold. The initiation (ATG) and the termination (TGA) codons are indicated. The 3 intragenic microsatellites and 2 microsatellites flanking the gene are indicated by arrows. Accession numbers are given for the 6 clones, which permitted the identification of the KCNQ1 genomic structure (solid lines) as well as the resulting clone submitted to the EMBL database (shaded gray line). Scale is shown in kilobases (kb).
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Three (CA)$_n$ Repeat Microsatellites in KCNQ1
We localized the (CA)$_n$ microsatellite D11S4088 in intron 10 of KCNQ1 using the BLASTN program. We also report two new microsatellites in introns 10 and 14 with EMBL accession numbers AJ006724 and AJ006725. The primer sets used for their amplification are as follows: 095L, 5’-TCACAGC-CCTCAGCCTAT-3’; 095R, 5’-GACACAAATGCCAAACAGA-3’ and 1228L, 5’-TCCCTGGGGTTCTCAGT-3’; 1228R, 5’-CTTGCTGTACCACTGCCT-3’, respectively. By analyzing DNA samples from 80 unrelated Caucasians, we detected three alleles for each microsatellite. Allele frequencies were 0.11, 0.22, and 0.67 for AJ006724 and 0.13, 0.42, and 0.45 for AJ006725. Their heterozygosity indexes were 0.11 and 0.35, respectively.

Identification of Mutations in KCNQ1 Associated With LQTS
Exons 1 to 15 and corresponding exon-intron boundaries were analyzed by PCR-SSCP according to the conditions described in Table 1. In 40 LQTS families, 27 different mutations have previously been found in the transmembrane segments (References 21 and 22 and data not shown) and 3 in the C-terminal domain of KCNQ1. 6,17,21 Five new mutations, reported in the present study, were identified in the C-terminal domain of KCNQ1 in five unrelated LQTS families (Figure 2). Mutations are numbered from the ATG codon of the full-length isoform 1.15,17

A 1-bp deletion was identified in exon 9 of KCNQ1. This deletion of a C at position 1343 (1343delC) was found in RW families. Both JLN patients are compound heterozygotes. The C-terminal JLN mutations are indicated by half-filled symbols and the other KCNQ1 mutations by hachured symbols. The mutations are as follows: T587M is a missense mutation in exon 14, and 1892del20 is a frameshift mutation in exon 15. In family JLN12664, T587M is a de novo mutation occurring on the paternal chromosome. Paternity was tested and confirmed. The second mutation is a splice mutation in intron 1 inherited from the mother. In family JLN9976, the frameshift mutation was inherited from the father, and the other mutation is still unidentified.
family 11356. It introduces a frameshift that leads to a premature stop codon 17 amino acids later. Two missense mutations were identified in exon 14. One is a C to T transition at position 1760 (T587M) identified in JLN family 12664 that changes a threonine at position 587 to a methionine. The second is a G to A transition at position 1772 (R591H) found in RW family E-973 that changes an arginine at position 591 to a histidine. Two frameshift mutations were found in exon 15. One is a 20-bp deletion (CCA-GAGAGGCGGKGCCAC) at position 1892 (1892del20) identified in JLN family 9976. It introduces a frameshift leading to a premature stop codon 13 amino acids later. The second is an insertion of a C at position 1893 (1893insC) found in RW family 11232. This 1-bp insertion introduces a frameshift that leads to a premature stop codon 19 amino acids later. Both mutations 1892del20 and 1893insC are predicted to produce truncated proteins with a stop signal close to the end of the normal protein. In contrast, mutation 1343delIC is predicted to produce a protein lacking 212 amino acids in its C-terminal domain. None of the SSCP patterns corresponding to each mutation has been found in 100 healthy individuals.

Discussion
In the present study, we report the genomic organization of the entire human K\(^+\) channel gene KCNQ1, which is responsible for the chromosome-11-linked LQTS. The gene spans more than 400 kb and contains 19 exons. We synthesized oligonucleotide primers on the basis of flanking intronic sequences for all exons and established conditions for the PCR amplification of each exon from genomic DNA.

Six alternative splice variants have been reported in KCNQ1 that differ depending on the use of the exons located in the 5′ part of the gene.\(^3,\(^4,\)7,\(^4,\)5,\(^6,\)15,\(^6,\)16\) All isoforms have in common the coding sequence from exons 2 to 15 (Figure 1). Isoforms 0 and 1 share the 3′ part of exon 1a and the full exon 1 but are divergent in the 5′ part of exon 1a.\(^4,\)15,\(^6,\)17 The genomic sequence analysis cannot definitely exclude that the difference in the 5′ part of exon 1a in isoform 0 is due to a cryptic splice site. Nevertheless, isoform 0 is more probably a cloning artifact, given that the 5′ part of the sequence specific to this isoform (33 bp) is invert-homolog to another part of the gene (position 246 to 278). However, it appears that the divergent N-terminus does not prevent isoform 0 expression, because it has been found to be functional in Chinese hamster ovary cells.\(^4\) Isoforms 1 and 2 comprise exons 1a and 1b, respectively, followed by exon 1 and are the two major KCNQ1 splice variants present in the heart.\(^3,\)^7,\(^15\) Isoform 3 contains exons 1b, 1c, and 1, and isoform 4 comprises exons 1b, 1c, 1, and 2a. These two isoforms probably represent untranslated transcripts, because exon 1c introduces a stop codon in the KCNQ1 sequence.\(^7\) Isoform 5 contains the 3′ part of exon 1a and exons 1c and 1,\(^1,\)6 The first transmembrane domain of isoform 5 is changed when compared with isoforms 0 and 1, and isoform 5 did not yield novel currents when expressed in Xenopus oocytes. However, because there is no in-frame 5′ stop codon, an initiation site further upstream leading to a longer coding sequence of KCNQ1 cannot be excluded. The physiological role of isoforms 3, 4, and 5 remains enigmatic, whereas isoforms 1 and 2 associate with MinK to form a functional K\(^+\) channel in the heart underlying the I\(_{Ks}\) current.\(^1,\)8,\)19 KCNQ1 mutations are numbered according to isoform 0\(^1,\)11,\)16,\)24,\)25 or to isoform 1.\(^6,\)17,21,26,\)27\) The identification of the full-length exon 1a from genomic DNA suggests that a common nomenclature should be used for numbering KCNQ1 mutations according to isoform 1 sequence, which has been independently determined by two groups.\(^1,\)15,\)17

The knowledge of exon-intron boundaries allowed us to identify five new mutations in the C-terminal domain of KCNQ1. At present, 40 different mutations have been identified in KCNQ1 in LQTS,\(^5,\)6,\)11,\)16,\)17,21,22,24–33\) but only three have been reported by our group in the C-terminal region of the gene.\(^6,\)17,21 Indeed, most of known KCNQ1 mutations are localized in the transmembrane domains of the protein, but the C-terminal domain has not been analyzed in most of the studies. The three mutations already described in this part of the gene are a deletion-insertion (1630–7+8) observed in a JLN family\(^6\) and two missense mutations (R539W and R555C) identified in RW families.\(^1,\)21 One of the two new C-terminal missense mutations reported in the present study affects also an arginine (R591H) in an RW family. This arginine at position 591 has been conserved throughout evolution, from Caenorhabditis elegans to Homo sapiens. KCNQ1 belongs to a family of voltage-gated K\(^+\) channel genes, which contains KCNQ2 and KCNQ3, both expressed in brain and involved in epilepsy.\(^3,\)4,\)35 These three genes show high sequence homology in the S1-to-S6 transmembrane domains but a lower similarity in their C-terminal region,\(^4,\)35 suggesting that this part may be related to a specific function of these channels in different tissues. Indeed, KCNQ1 showed a highly positively charged C-terminal subregion containing numerous arginines where we have already found two mutated arginines in RW patients (R539W and R555C).\(^1,\)17 These arginines have been well conserved throughout species. The R555C mutation was clearly associated with a forme fruste of the RW syndrome.\(^2\) For the R591H and R539W mutations, we could not determine the severity of the disease because of the small size of the families. A 1-bp deletion and a 1-bp insertion were also identified in two small RW families. Only one sudden death was reported in all families, which occurred in a patient treated for an abscessed tonsillitis (family 11356, patient II-2, mutation 1343delIC).

In two nonconsanguineous JLN families, we identified two mutations in the C-terminal domain of KCNQ1. One is a missense mutation (T587M) and the other is a frameshift mutation. The mutated threonine at position 587 is conserved in Squalus acanthias, Mus musculus, and H sapiens but not in C elegans. It is the second missense mutation that causes the JLN syndrome; the first has been identified in the pore.\(^2\) With the 20-bp deletion that we identified in the present study, four of the six known mutations causing the JLN syndrome induced a putative premature truncation of the subunit,\(^5,\)11,\)32 suggesting that frameshift mutations, especially
in the C-terminal domain of the KVLQT1 channel, could be responsible for most of the JLN cases. Thus, analysis of the whole KCNQ1 gene will assist in genetic diagnosis of asymptomatic mutation carriers at risk for ventricular arrhythmias.

We suggest that the five new mutations reported in the present study cause LQTS, because they affect conserved amino acids of the protein or lead to putative truncated proteins and they are not present in control subjects. However, functional studies would be required to absolutely confirm the pathogenic nature of these changes. In our series, systematic screening of exons 1 to 15 of KCNQ1 by PCR-SSCP identified 28 different mutations in KCNQ1 in 38 of 84 RW families (45%), 5 of them occurring in the C-terminal domain (18%). Seven KCNQ1 mutations including three in the C-terminal domain (43%) were identified in JLN families. One mutation in a compound heterozygous JLN patient was not identified, although linkage analysis supports the hypothesis that this second mutation is in KCNQ1, and no genetic defect in KCNE1 has been evidenced. Because exons 1a and 1b were not screened in our study, we can hypothesize that nonidentified mutations are located in this region of the gene. Nevertheless, it is well established that SSCP is not 100% effective in the detection of mutations, and we could have missed them.

Intragenic microsatellite markers are of great help for the linkage analysis. Hence, the entire genomic sequence of the human K⁺ channel KCNQ1 gene has been analyzed for the presence of such DNA markers. Two new intragenic microsatellites were found and the previously known marker D11S4088 was localized in intron 10. Mutations in parts of the gene other than transmembrane domains are probably more numerous than initially expected. The presence of mutations in the C-terminal domain, and even in the N-terminus, which has not been analyzed so far, may explain the low percentage of mutations identified in large LQTS cohorts. In conclusion, this work provides helpful tools for linkage analysis and systematic mutation screening in the KCNQ1 gene.

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References


Genomic Organization of the \textit{KCNQ1} \(K^+\) Channel Gene and Identification of C-Terminal Mutations in the Long-QT Syndrome

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