A Founder Mutation of the Potassium Channel KCNQ1 in Long QT Syndrome
Implications for Estimation of Disease Prevalence and Molecular Diagnostics

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OBJECTIVES
We took advantage of the genetic isolate of Finns to characterize a common long QT syndrome (LQTS) mutation, and to estimate the prevalence of LQTS.

BACKGROUND
The LQTS is caused by mutations in different ion channel genes, which vary in their molecular nature from family to family.

METHODS
The potassium channel gene KCNQ1 was sequenced in two unrelated Finnish patients with Jervell and Lange-Nielsen syndrome (JLNS), followed by genotyping of 114 LQTS probands and their available family members. The functional properties of the mutation were studied using a whole-cell patch-clamp technique.

RESULTS
We identified a novel missense mutation (G589D or KCNQ1-Fin) in the C-terminus of the KCNQ1 subunit. The voltage threshold of activation for the KCNQ1-Fin channel was markedly increased compared to the wild-type channel. This mutation was present in homozygous form in two siblings with JLNS, and in heterozygous form in 34 of 114 probands with Romano-Ward syndrome (RWS) and 282 family members. The mean (± SD) rate-corrected QT intervals of the heterozygous subjects (n = 316) and noncarriers (n = 423) were 460 ± 40 ms and 410 ± 20 ms (p < 0.001), respectively.

CONCLUSIONS
A single missense mutation of the KCNQ1 gene accounts for 30% of Finnish cases with LQTS, and it may be associated with both the RWS and JLNS phenotypes of the syndrome. The relative enrichment of this mutation most likely represents a founder gene effect. These circumstances provide an excellent opportunity to examine how genetic and nongenetic factors modify the LQTS phenotype. (J Am Coll Cardiol 2001;37:562–8) © 2001 by the American College of Cardiology

The long QT syndrome (LQTS) is an inherited disorder of cardiac repolarization, characterized by electrocardiographic (ECG) abnormalities, syncopal attacks and risk of sudden death due to ventricular tachyarrhythmias such as torsade de pointes (1,2). The LQTS may occur as an autosomally dominantly inherited form (Romano-Ward syndrome, RWS) (3,4), or as a part of the autosomally recessively inherited Jervell and Lange-Nielsen syndrome (JLNS) (5) in which prolongation of the QT interval is associated with sensorineural deafness. The exact prevalence of LQTS remains to be determined, although an estimate of 1 in 10,000 has been suggested (6). The LQTS is associated with significant morbidity and mortality, with estimated annual rates of 5% and 1% for syncope and death, respectively (2).

Until now, mutations of five different genes, including those encoding the cardiac potassium channel alpha-subunits KCNQ1 (former KVLQT1) (7) and HERG (8), beta-subunits minK (KCNE1) (9) and MiRP1 (KCNE2) (10) as well as the sodium channel SCN5A (11) have been found to underlie LQTS. The most prevalent form of LQTS appears to be that caused by mutations of the potassium channel KCNQ1 (LQT1), accounting for some 50% of the cases with RWS (7). Both KCNQ1 and minK proteins assemble to form the slowly activating component of the delayed rectifier potassium current (IKs) present in heart and inner ear (12,13), and mutations of either of them may be the underlying molecular pathology in the JLNS when present in a homozygous form (14,15).

The molecular nature of the causative mutation typically varies from family to family with LQTS. However, in Finland a founder point mutation of the KCNQ1 gene, located relatively close to the cytoplasmic tail of the ion channel subunit, was identified in a large number of LQTS probands.

METHODS
Characterization of patients and controls. Three children (two brothers and a nonrelated boy) with prolonged corrected QT (QTc) interval and congenital deafness, compatible with the diagnosis of JLNS, were initially investigated (Fig. 1). Clinical records, ECGs, and deoxyribonucleic acid (DNA) samples from 114 apparently unrelated probands...
(80 females, 34 males; mean age (± SD) 38 ± 18, range 2 to 79 years) from families with RWS were subsequently examined. Diagnostic criteria included prolonged rate-adjusted QT (QTc) interval (>440 ms) and at least one symptomatic episode (syncope or tachyarrhythmia) or a family history of LQTS. The QTc interval was measured from standard 12-lead ECG using lead II.

All index cases were of Finnish origin. The birthplaces of the oldest obligate mutation carriers in each family were traced using local parish registers. The study was approved by the Ethical Review Committee of the Department of Medicine and was in accordance with the Helsinki Declaration. All subjects gave their informed consent. Control DNA samples were obtained from 200 unrelated healthy adult individuals (men and women equally).

**Genetic analysis.** All exons of the KCNQ1 gene were amplified by polymerase chain reaction (PCR) with primers published previously (7) or with novel primers designed according to Lee et al. (16). A forward primer 5’-GGATCCTAATGCCCAGGATGATC-3’ and reverse primer 5’-GTCGACTTCTCATGGGAAGGCTTC-3’ were used to amplify the whole coding sequence of the minK gene. The resulting amplicons were sequenced using ABI Prism 377 DNA sequencer (PE Biosystems, Foster City, California). For specific detection of the G589D mutation, primers 5’-GCAAAAGAGCAAGGATGGCG-3’ and 5’-CTTGTCTTCTACTCGGTTCAG-3’ were used to amplify a 64-bp fragment of exon 14. After PCR, the samples were digested with the enzyme HhaI (New England Biolabs, Beverly, Massachusetts), followed by electrophoresis on a 12% polyacrylamide gel. The normal

**Figure 1.** Pedigrees of families 1012 and 1034. Squares represent males, circles are females and symbols with slash mark represent deceased subjects. Half-filled symbols indicate heterozygous KCNQ1-Fin carriers, filled symbols homozygous KCNQ1-Fin carriers and half-striped symbols are heterozygous Y171X carriers. Open symbols denote unaffected individuals verified by deoxyribonucleic acid (DNA) assay. Subjects IV/6 and IV/8 in family 1012 and III/7 in family 1034 have Jervell and Lange-Nielsen syndrome (JLNS). The QTc interval (ms) is shown under each symbol; those marked with an asterisk have been recorded during medication.
allele is cleaved into two fragments (38 and 26 bp in size), whereas the allele corresponding to the G589D mutation remains uncleaved (64 bp).

Three highly polymorphic microsatellite markers (D11S860, D11S1318 and TH) were studied to construct possible disease-associated haplotypes. Genetic distances and order of markers were determined by combining the data in a genetic map and in a physical map (17) of the corresponding area.

**In vitro mutagenesis and electrophysiological studies.** Complementary DNAs (cDNAs) for KCNQ1 (AF000571) and minK (L33815) in the pIRE-CD8 vector were used in these studies. The G589D mutation was introduced into the KCNQ1 cDNA using the QuickChange site-directed mutagenesis kit according to the manufacturer’s instructions (Stratagene, La Jolla, California). Transfections for transient in vitro expression studies in COS cells were made using the diethylaminoethyl-dextran precipitate method. Whole-cell membrane currents were measured and analyzed using a Biologic RK 400x patch-clamp amplifier and pCLAMP software (Axon Instruments, Foster City, California) essentially as described previously (18).

**Statistical analysis.** The mean QTc intervals in different groups were compared to each other using the Student t test. For comparison of dichotomous variables a standard chi-square test was used. A p value <0.05 was considered statistically significant.

**RESULTS**

**Identification of a common cause for LQTS in Finland.** The entire coding regions of the minK and KCNQ1 genes were screened for mutations in two unrelated JLNS probands (Fig. 1). Survey of the minK gene disclosed no mutations. When the KCNQ1 gene was sequenced, two different mutations were identified: a change from G to A at nucleotide 1876 of exon 14, predicted to result in a substitution of aspartic acid for glycine at position 589 (G589D), and a C to G substitution at nucleotide 623 in exon 2, resulting in a premature stop codon at position 171 (Y171X) and predicted synthesis of a truncated protein. The G589D mutation is located in the cytoplasmic carboxy-terminal portion of the ion channel subunit, whereas the Y171X truncation takes place right after the second transmembrane domain S2. The two brothers with JLNS (family 1012) were homozygous for the G589D substitution, whereas the JLNS patient of family 1034 was a compound heterozygote, carrying both the G589D and Y171X mutations. The older of them had a syncopal spell while swimming, despite ongoing beta-antiadrenergic medication. After subsequent left cardiac sympathetic denervation (LCSD) he has remained symptom-free for 10 years. Although the younger brother was treated with beta-antiadrenergic medication from birth on, he died at the age of nine in his first cardiac event. The homozygous parents of the siblings had prolonged QTc intervals (522 ms and 517 ms) but were asymptomatic. The QTc value of the compound heterozygote, carrying both the KCNQ1-Fin and Y171X mutation, was 566 ms (Fig. 1).

**Clinical findings in homozygous and heterozygous individuals.** The QTc values of the two KCNQ1-Fin homozygotes were 661 ms and 592 ms (Fig. 1). The older of them had a syncopal spell while swimming, despite ongoing beta-antiadrenergic medication. After subsequent left cardiac sympathetic denervation (LCSD) he has remained symptom-free for 10 years. Although the younger brother was treated with beta-antiadrenergic medication from birth on, he died at the age of nine in his first cardiac event. The homozygous parents of the siblings had prolonged QTc intervals (522 ms and 517 ms) but were asymptomatic. The QTc value of the compound heterozygote, carrying both the KCNQ1-Fin and Y171X mutation, was 566 ms (Fig. 1). He was likewise treated with beta-antiadrenergic medication from birth on and subsequently with a cardiac pacemaker, yet he experienced a syncopal spell while swimming at the age of two years. The LCSD was performed at the age of 10 years, thereafter he has remained symptom-free (follow-up 2.5 years). All three children were deaf since birth.

The mean QTc interval of the heterozygous KCNQ1-Fin carriers was significantly longer than that of the non-

**Functional expression of KCNQ1-Fin.** When coexpressed with minK, the KCNQ1-Fin construct produced functional channels in COS cells, but the currents were much smaller than those observed for the normal (KCNQ1-wt) cDNA, and there was a pronounced rightward shift in the voltage of activation, with mean (± SE) half-activation voltages of 11.0 ± 2.2 mV and 41.3 ± 2.9 mV for KCNQ1-wt and KCNQ1-Fin, respectively (Fig. 2). Moreover, detectable KCNQ1-Fin currents were present in only 4 of 23 cells expressing the CD8 reporter, whereas all 21 CD8-positive cells transfected with KCNQ1-wt, or, simultaneously, with KCNQ1-wt and KCNQ1-Fin in a 1:1 ratio, displayed robust currents. No significant differences were seen in the mean current amplitudes, activation voltages or activation time constants between cells expressing KCNQ1-wt alone or KCNQ1-wt together with KCNQ1-Fin (data not shown), suggesting that KCNQ1-Fin does not contribute substantially to the currents recorded when the KCNQ1-wt and KCNQ1-Fin constructs are coexpressed. The mean (± SE) deactivation time constants observed at −40 mV were similar for KCNQ1-wt (993 ± 56 ms, n = 7) and KCNQ1-Fin (965 ± 54 ms, n = 4).
carriers (Table 1). The mean (± SD) QTc of the 34 heterozygous index cases (490 ± 40 ms) was longer than the mean (± SD) QTc of the remaining family members positive for KCNQ1-Fin (450 ± 30, p < 0.001). Using the same QTc interval length 440 ms applied to choose pro-bands, a diagnostic specificity of 86% and sensitivity of 68% were obtained. Among the KCNQ1-Fin heterozygotes, women had on average a longer QTc (470 ± 32 ms) than did men (446 ± 38 ms, p < 0.001) (Fig. 3). There were altogether 83 (26%) symptomatic heterozygous carriers comprising 52 females and 31 males. The percentage of the symptomatic of all heterozygous individuals was not significantly different between adults (>16 years; 28%) and children (≤16 years; 19%). The mean (± SD) QTc interval

Table 1. Clinical Characteristics of Heterozygous Carriers and Noncarriers of the KCNQ1-Fin (G589D) Mutation in 34 Families

<table>
<thead>
<tr>
<th>Variable</th>
<th>Carriers (n = 316)</th>
<th>Noncarriers (n = 423)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males/Females (n)</td>
<td>142/174</td>
<td>201/222</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>37 ± 21</td>
<td>37 ± 12</td>
</tr>
<tr>
<td>Range of ages (yrs)</td>
<td>2–87</td>
<td>1–91</td>
</tr>
<tr>
<td>QTc (ms)</td>
<td>460 ± 40</td>
<td>410 ± 20†</td>
</tr>
<tr>
<td>Range of QTc (ms)</td>
<td>390–580</td>
<td>350–490</td>
</tr>
<tr>
<td>Number (%) of symptomatic individuals</td>
<td>83 (26%)</td>
<td>ND</td>
</tr>
<tr>
<td>Triggers of symptoms‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swimming</td>
<td>17 (20%)</td>
<td></td>
</tr>
<tr>
<td>Other physical effort</td>
<td>15 (18%)</td>
<td></td>
</tr>
<tr>
<td>Anxiety</td>
<td>16 (19%)</td>
<td></td>
</tr>
<tr>
<td>Medication§</td>
<td>7 (8%)</td>
<td></td>
</tr>
<tr>
<td>Sleep</td>
<td>4 (5%)</td>
<td></td>
</tr>
<tr>
<td>Undefined¶</td>
<td>38 (46%)</td>
<td></td>
</tr>
</tbody>
</table>

*Figures indicate the mean ± SD; †p < 0.001 between carriers and noncarriers; ‡Different causes may have triggered symptoms in the same individual; §Two patients received terfenadine alone; in addition, terfenadine was used concomitantly with chinin hydrochloride in one case and with ketoconazol in another. One patient used thioridazine and another a combination of haloperidol and chlorprotixen hydrochloride. In addition, hydrochlorotiazide was used in combination with cephprol and lisinopril in one patient; ¶During normal daily activities. QTc = QT interval corrected for heart rate according to Bazett’s formula; ND = not determined.
of symptomatic mutation carriers (470 ± 30 ms) was significantly longer than that of the asymptomatic ones (450 ± 30 ms, p < 0.001), and there was an increasing likelihood of occurrence of symptoms by increasing QTc value (Fig. 3). In 48 cases (58%) the triggering factor for syncope could unequivocally be related to physical or psychological stress, whereas seven patients (8%) had had a cardiac event during various types of medication (Table 1).

**A case of proposed sudden infant death syndrome.**

Detailed questionnaires to the families identified a baby girl who died at the age of three months while sleeping. As the autopsy did not disclose any abnormalities and no family data suggesting LQTS were available then, the case was originally classified to represent a sudden infant death syndrome (SIDS) of unknown origin. During the present survey, one of the index carriers of the KCNQ1-Fin mutation proved to be a second cousin of the proposed SIDS case. The DNA extracted from paraaffin-embedded tissue samples of the deceased baby demonstrated heterozygosity for the KCNQ1-Fin mutation.

**Existence of a founder gene.** The occurrence of KCNQ1-Fin in one-third of LQTS patients suggested a founder gene effect. A common haplotype of 1500 kb between markers D11S860 and D11S1318 could indeed be detected in 25 of 26 individuals in which analysis was feasible. When family data were traced back to the level of the oldest mutation carrier in each family, the birthplaces of the affected ancestors were found to cluster in a belt-like fashion from the eastern North Karelia (city of Joensuu) area to the northwestern region of the city of Oulu (Fig. 4).

**DISCUSSION**

The present study demonstrates that a specific and potentially lethal gene mutation underlying severe arrhythmic propensity can show relative enrichment at the population level. Although presence of mutational hot spots of the genes encoding cardiac potassium (19) and sodium (11,20) channels has been suggested, there are no previous reports on the incidence of arrhythmia founder genes widely in a specific population. The common occurrence of the KCNQ1-Fin mutation in the Finnish LQTS probands may be attributed to the relatively benign nature of the mutation itself as well as the population history of the Finns.

**KCNQ1-Fin mutation is located in a conserved region possibly responsible for subunit assembly.** The KCNQ1-Fin mutation affects the codon 589, located in the cytoplasmic tail downstream of the transmembrane domains of the protein. There are previous reports on identification of C-terminal mutations of the KCNQ1 subunit (14,21–25), but the G589D mutation appears to represent the most distal one of those whose electrophysiological characteristics have been documented. The glycine 589 is conserved in the KCNQ1 genes of *Mus musculus* (U70068), *Rattus norvegicus* (AJ133685) and *Squalus acanthias* (AJ223714), suggesting that the C-terminus may represent a unique regulatory region for the KCNQ1 channels (22).

Recently, Schmitt et al. (26) described a domain between residues 589 and 620 that may be critically important for subunit assembly; they proposed that JLNS mutations are characterized by their defective ability to subunit interaction. Our functional studies with KCNQ1-Fin support this assumption. When expressed in vitro, the KCNQ1-Fin construct was able to form functional channels to some extent as a homomer. However, the expression efficiency of KCNQ1-Fin in COS cells was subnormal as less than 20% of the transfected cells displayed detectable KCNQ1-Fin-mediated currents. In agreement with low functional expression efficiency, no dominant negative effect was observed with KCNQ1-Fin in vitro. These data suggest that the KCNQ1-Fin allele is unable to produce functional tetramers efficiently because of a dysfunctional assembly domain (26).
The mild phenotype of the heterozygotes may have enabled enrichment of the KCNQ1-Fin mutation. Our data confirm that the same KCNQ1 mutation can cause JLNS in homozygous and RWS in heterozygous form. Approximately one-fourth of the individuals heterozygous for the KCNQ1-Fin mutation were symptomatic (Table 1), a proportion somewhat lower than that proposed earlier (27,28). There is also previous evidence that LQT1 (LQT1 type of long QT syndrome) patients with the C-terminal mutations have milder phenotypes than do those with mutations of the transmembrane domains of the same channel (24,25,29). The occurrence of symptoms increased with increasing QTc value: among all subjects with QTc <440 ms, only 16% were symptomatic, whereas 50% were symptomatic in the group of QTc >500 ms (Fig. 3). However, these data also demonstrate that mutation carriers are at risk of cardiac events even when the QTc interval is normal. In accordance with earlier findings among LQT1 patients (30,31), swimming appeared to be an important risk factor for KCNQ1-Fin carriers, triggering symptoms in 20% of all instances.

In addition to its relatively benign nature, the population history of the Finns may provide another prerequisite for the prevalence of the KCNQ1-Fin mutation. The Finnish population represents a genetic isolate due to effective geographical and linguistic separation within the historical period (32). The geographical distribution of the birthplaces for the oldest KCNQ1-Fin carriers closely matches the internal migration wave commenced from the southeast in the sixteenth century (Fig. 4). The founder gene phenomenon is further supported by the common haplotype associated with the disease in virtually all affected individuals examined. The affected haplotype common to all but one of the probands was relatively short, comprising only 1500 kb. Along with the distribution of ancestral birthplaces, this suggests that the KCNQ1-Fin mutation was introduced into the population some 500 to 750 years or 25 to 35 generations ago (33). Previously, a founder KCNQ1 mutation has been suggested to occur in five South African families based on identical haplotype around the disease locus (34).

Prevalence of LQTS in Finland. Very recently, we identified several Finnish LQTS families with a specific HERG channel mutation (L552S), and we provided genetic and genealogical evidence for its role as a founder gene (18). Today, a combination of two simple PCR tests, one for the KCNQ1-Fin and another for the HERG L552S mutation, correctly identifies 35% of Finnish LQTS patients and is used as an adjunct to clinical diagnostics. Until now, LQTS-causing mutations have been detected in half (56/114) of the Finnish index families with altogether 472 molecularly defined carriers ((18,27,35), unpublished observations, 2000), suggesting a minimum prevalence of 1:5000 for LQTS in the Finnish population of five million people. We are not aware of any reports suggesting a higher prevalence of LQTS in other populations.

Conclusions and clinical implications. We have demonstrated that LQTS founder genes may occur in the population. It remains to be examined whether the KCNQ1-Fin mutation occurs in other populations as well. The LQTS founder genes should provide a useful model for studies in which genetic or nongenetic factors, such as common gene polymorphisms, life style factors, endocrine and metabolic determinants as well as pharmaceutical substances, modify the course and prognosis of the arrhythmic tendency.

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