C-terminal HERG Mutations
The Role of Hypokalemia and a KCNQ1-Associated Mutation in Cardiac Event Occurrence

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Background—The long-QT syndrome (LQTS) is a genetically heterogeneous disease in which 4 genes encoding ion-channel subunits have been identified. Most of the mutations have been determined in the transmembrane domains of the cardiac potassium channel genes KCNQ1 and HERG. In this study, we investigated the 3’ part of HERG for mutations.

Methods and Results—New specific primers allowed the amplification of the 3’ part of HERG, the identification of 2 missense mutations, S818L and V822M, in the putative cyclic nucleotide binding domain, and a 1-bp insertion, 3108+1G. Hypokalemia was a triggering factor for torsade de pointes in 2 of the probands of these families. Lastly, in a large family, a maternally inherited G to A transition was found in the splicing donor consensus site of HERG, 2592+1G-A, and a paternally inherited mutation, A341E, was identified in KCNQ1. The 2 more severely affected sisters bore both mutations.

Conclusions—The discovery of mutations in the C-terminal part of HERG emphasizes that this region plays a significant role in cardiac repolarization. Clinical data suggests that these mutations may be less malignant than mutations occurring in the pore region, but they can become clinically significant in cases of hypokalemia. The first description of 2 patients with double heterozygosity associated with a dramatic malignant phenotype implies that genetic analysis of severely affected young patients should include an investigation for >1 mutation in the LQT genes. (Circulation. 1999;99:1464-1470.)

Key Words: long-QT syndrome ▪ torsade de pointes ▪ hypokalemia ▪ LQT1 ▪ LQT2

The long-QT syndrome (LQTS) is an inherited cardiac disease that can cause sudden death in young, otherwise healthy subjects. Five loci have been mapped to chromosome 11p15.5 (LQT1), 7q35-36 (LQT2), 3p21-24 (LQT3), 4q25-27 (LQT4), and 21q22-23 (LQT5). The genes responsible for LQT1, LQT2, and LQT5 have been identified as cardiac potassium channel subunit genes (KVLQT1, HERG, KCNE1), respectively, and for LQT3 as a cardiac sodium channel gene (SCN5A).9

Mutations in KVLQT1, recently renamed KCNQ1,10 caused half of the LQTS5,11,12 in all the previous cases studied. Nevertheless, screening for mutations in KCNQ1 and HERG in large cohorts of probands has led to detection of a percentage lower than that suggested by linkage analyses.2,11 Moreover, only 13 missense mutations in KCNQ1 (out of 191 families) and 5 in HERG (out of 32 families) were identified.13–15 In these studies, only the membrane-spanning domains have been investigated. Thus, the inability to identify the underlying mutations may be owing to many reasons, eg, the presence of a mutation in an unexamined part of the gene and/or failure of the SSCP assay to detect a particular type of mutation.

Indeed, some mutations have already been identified in the cytoplasmic C-terminal parts of both KCNQ1 and HERG. In KCNQ1, 2 missense mutations, R539W and R555C, were found in Romano-Ward families,16,17 in addition to an insertion-deletion in Jervell and Lange-Nielsen patients.18 In HERG, a splicing mutation,4 a missense mutation, V822M, in the putative splice donor site,19 and a 31-bp duplication were identified in the 3’ part of the gene.20 This suggests that the C-terminal parts of these genes should also be analyzed for disease-causing mutations.

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We therefore undertook the study of the genomic organization of the 3' part of HERG, paralleling the work of Itoh and coworkers, who recently published its complete structure. In this part of the gene, we identified 4 mutations, including 3 new ones: a missense mutation affecting a serine at position 818, a splice mutation, and a 1-bp insertion in the terminal part of the gene; this suggests that this region of the protein plays a significant role in cardiac repolarization. We also report the first description of 2 severely affected sisters both carrying a splice mutation in HERG and a missense mutation in KCNQ1.

Materials and Methods

Genomic Organization of the 3' Region of HERG
Genomic organization of the 3' part of the gene was established by sequenced polymerase chain reaction (PCR) product on control genomic DNA. Oligonucleotide primers were determined in the cDNA sequence (Genbank U04270). PCR annealing temperatures were chosen as indicated in the Table. Sequencing of PCR fragments was performed by the dideoxynucleotide chain termination method with fluorescent dideoxynucleotides on an ABIprism DNA sequencer (PE Applied-Biosystem).

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Primers used for genomic amplification of HERG exons, SSCP analysis, and direct sequencing. TD indicates touchdown temperature. *These primers have been reported previously.

Haplotype Analysis
Genomic DNA was extracted from blood samples by standard procedure. (CA)n microsatellite markers (D3S3521, D3S1298, D4S1564, D4S407, D4S1612, D7S2461, D7S636, D7S483, D11S1318, D11S4088, D11S4146, D11S1758) encompassing each LQT locus were provided by the Genethon human genetic linkage map. PCR amplifications were performed as previously described.

Single Strand Conformational Polymorphism (SSCP) Analysis and Direct Sequencing of PCR Products
Two previously published primer pairs and 12 pairs determined in the present study were used to amplify the transmembrane segments and the C-terminal domain from genomic DNA. SSCP analysis at 2 temperatures, 7°C and 25°C, was performed as previously described. For each abnormal SSCP pattern, the cosegregation with the disease was studied in the family. Sequencing of the PCR products was performed as described above. An additional primer pair (5'-TACCCGTAGTCTCCGACCAC-3', 5'-AAAGGGGCGAGCCACAGCAGAC-3') was designed to amplify a 121-bp fragment flanking the 2592+1G→A mutation (Family 5969), which could not be detected by SSCP with the primers designed to amplify the whole exon.

The sequences of the 2 KCNQ1 primers used to detect the second mutation of Family 5969 in exon 6 were 5'-GGTGGTTGGAATTGCAGTTGG-3' and 5'-AAAGGGGCGAGCCACAGCAGAC-3'.

Results
Genomic Structure of the C-terminal Part of HERG
We hypothesized that genomic structure of the human gene would be similar to the genomic structure of the mouse HERG channel gene. Primers chosen in the predicted exon sequences were used for intron and exon/intron boundary PCR amplification and sequencing (not shown). We identified 7 exons in the 3' part of HERG and determined their splice junctions (which are identical to those recently published by Itoh et al and Splawski et al). Primers were
designed to sequence these exons numbered 9 to 15. New primers were also designed to amplify the exons 6 and 8 for S1 to S3 domains and the terminal part of the S6 domain, respectively (Table).

**HERG Missense Mutations in the Putative Cyclic Nucleotide Binding Domain Associated with LQTS in 2 Families**

In Family 10025, the proband was a 27-year-old woman who experienced her first syncope with seizure at 25 with documented torsade de pointes. She was treated by nadolol and a pacemaker. Her 2-year-old daughter had a QTc interval of 472 ms, but her parents had normal QTc values and were asymptomatic.

The analysis of exon 10 PCR product by SSCP revealed an aberrant pattern for the proband (Figure 1a). This pattern was not observed in DNA samples from 100 control subjects (data not shown). The sequence analysis revealed a transition (C to T) resulting in a replacement of the serine 818 by a leucine within the putative cyclic nucleotide binding domain (Figure 1b). Linkage analysis with 5 polymorphic markers confirmed the paternity. Thus, the proband is a de novo case.
of LQTS and has transmitted the mutation to her daughter, who is asymptomatic at 2 years.

In Family 10626, the proband, a 45-year-old woman, experienced several syncopes during emotional stress at 43, occurring after a period of diet. A test evidenced hypokalemia (2.8 meq/L). None of the children aged 22, 18, and 9 years were symptomatic, but their QTc values were 492, 433, and 457 ms, respectively. In exon 10 of HERG, aberrant conformers were identified by SSCP in the proband in her 3 children (Figure 1). Direct sequencing identified a missense mutation, corresponding to a single base transition (G to A) at position 2464 (Figure 1). This mutation results in substitution of the valine 822 by a methionine.

**An Intragenic Insertion in the Exon 13 of HERG in a LQTS Family**

In Family 7092, the proband, a 32-year-old woman with anorexia nervosa, experienced several syncopes, some of them triggered by a ringing telephone. The diagnosis was made at 26 years in a context of severe hypokalemia (2.1 meq/L to 2.7 meq/L on several occasions). After potassium supplement and nadolol therapy, she was asymptomatic. Nevertheless, there were 2 recurrences, one under erythromycin associated with nadolol interruption, and the other during nocturnal arousal while on low dose of nadolol (40 mg). Her father and her paternal grandmother were asymptomatic [at the ages of 45 and 87 years], although they had QTc values of 466 and 510 ms, respectively. Her daughter was diagnosed at birth with a QTc interval of 454 ms.

Linkage analysis was performed on Family 7092 with LQT1 to LQT4 loci microsatellite markers. Only LQT2 marker analysis revealed cosegregation of a haplotype with the disease, suggesting that a mutation in HERG caused LQTS in Family 7092 (Figure 2a). KCNE1 (LQT5) was excluded by direct sequencing. No abnormal conformer was detected in all HERG PCR fragments by SSCP analysis; direct sequencing of each PCR product was thus undertaken. An insertion of a guanine at position 3108 was identified in exon 13 (Figure 2b). This 1bp-insertion resulted in a frameshift leading to the truncation of the protein at amino acid position 1117. This mutation created a new site for the restriction enzyme Bgl I (Figure 2a).

**LQTS Associated With the Presence of a HERG Splicing Mutation and a KCNQ1 Missense Mutation in the Same Patients**

In Family 5969, 3 girls (II-7, II-11, and II-12) experienced their first syncopes at the ages of 2.5, 4, and 7, respectively. Two of them, II-7 and II-11, had particularly long QTc values of 770 and 640 ms, respectively (Figure 3a). Their mother had died suddenly at age 20, and one of her nieces (I-6) had a syncope at 31 and a QTc value of 494 ms; her own mother had a QTc value of 492 ms; one of her sisters died suddenly at 46 after interruption of a beta blocking treatment given for hypertension.

The family members were genotyped with 11 microsatellite markers corresponding to LQT1 to LQT4. Haplotypes were analyzed, taking into account that the 2 parents were affected. The results suggested that the father could be a carrier of a KCNQ1 mutation transmitted to 2 of his daughters (II-7 and II-11) and the mother of a HERG mutation transmitted to 3 of her daughters (II-7, II-11, II-12) and to her grandchild (III-1). In the 2 unaffected siblings (II-9 and II-10), neither of the 2 at-risk haplotypes was found (Figure 3b). The 2 more severely affected sisters (II-7 and II-11) were used as probands for LQT1 and LQT2 screening.

SSCP analysis of KCNQ1 revealed aberrant bands for exon 6 in all the LQT1-linked individuals (I-5, II-7, and II-11, data not shown). Sequence analysis of PCR products revealed a transversion at position 1022 (C to A) resulting in the replacement of the alanine 341 by a glutamate within the S6 transmembrane domain (Figure 3c). The loss of a restriction site for Hha I induces the appearance of an uncut band of 247 bp in all LQT1-linked individuals (Figure 3b).

To determine the HERG mutation, direct sequencing was undertaken. A G to A transition was found in the splicing

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**Figure 2. Detection of an intragenic insertion in Family 7092.** a, Haplotype of chromosome 7q35-36 microsatellite markers are shown below each individual. The proband of the family is indicated by an arrow. The migration on agarose 2% after enzymatic digestion with Bgl I of the genomic PCR-fragments corresponding to exon 13 is presented below. The control and unaffected individuals present a fragment of 284 bp and mutation carriers present 2 additional fragments of 184 and 101 bp. The molecular weight marker used is a 100-bp ladder. b, Partial HERG sequence of a mutation carrier showing an insertion of 1 base at position 3108. This mutation causes the appearance of a stop codon at position 1057.
This mutation induced no restriction modification. An abnormal conformer was revealed by SSCP at 20°C using a new primer pair (see Methods; Figure 3d). This mutation was absent in 100 unrelated controls.

The 2 more severely affected sisters (II-7 and II-11) turned out to be carriers of both the LQT1 and the LQT2 mutations. They were severely symptomatic and experienced stress-induced and rest-triggered syncopes. Because seizure occurring during syncopes was the initial presentation, LQTS was not diagnosed and propranolol (120 mg) was not started before age 16. However, the treatment did not prevent torsade de pointes recurrence, and a left cardiac sympathectomy was then performed. However, torsade de pointes persisted even after pacemaker implantation. Propranolol was then changed to nadolol (240 mg/d) and produced an important decrease in the number of syncopes. There was no recurrence for 2 years, until a gastrointestinal infection occurred with diarrhea and vomiting. Verapamil (120 mg/d) was then combined to nadolol (160 mg/d) and the pacing rate was increased to 85 bpm with good results. The sister (II-11) received propranolol (120 mg/d) at age 10 and remained asymptomatic until age 16 when syncopes resulting from documented torsade de pointes recurred. Propranolol was changed to nadolol (160 mg/d) combined with pacemaker implantation with a pacing rate of 80 bpm. Since then, she has been asymptomatic.

**Discussion**

The present work describes investigations of the 3’ part of HERG in an attempt to identify new mutations. This domain contains 7 exons in accordance with recent reports.19,24 A series of new primer pairs is now available to analyze these exons by SSCP or sequencing. In this part of the gene, only 3 mutations have been previously described, a splice mutation in the donor site of intron 9,4 a missense mutation,19 and a 31-bp duplication.20 Here we describe 4 mutations, 3 of which are new.

We identified 2 missense mutations, the mutation V822 M, previously described by Satler and coworkers, in a large Irish family,19 and another missense mutation very close to the previous one, S818L. Interestingly, whereas the valine 822 was found in brain and olfactory cyclic nucleotide-gated channels and in most of the members of the eag family, the serine 818 is conserved only among the members of the eag family. The discovery of these 2 mutated, highly conserved residues suggests that this region plays a significant role in regulating
membrane excitability, which is probably not a direct result of the cyclic nucleotide action.

In a third family, a 1-bp insertion at position 3108 predicted to form a truncated subunit shorter of 103 amino acids with normal membrane spanning and putative cyclic nucleotide binding domains.

In the 3 families mentioned above, among the 10 affected individuals who were genotyped, only the 3 probands were symptomatic. Their first syncpe occurred in adulthood between ages 25 and 45. For 2 of them, syncopes occurred while serum potassium levels were low. HERG encodes subunits that form the human IKr potassium current, and a hallmark feature of this current is its modulation by external potassium.\(^{25,26}\) In oocytes bathed by K\(^+\) concentrations ranging from 0.5 to 20 mmol/L, HERG current amplitude varied as a linear function of [K\(^+\)].\(^{27}\) Unlike most other K\(^+\) currents, the magnitude of outward HERG current is paradoxically reduced on removal of extracellular K\(^+\), thus allowing the increased action potential and prolonged ventricular repolarization in hypokalemic patients. In addition, an increase in serum potassium corrects or reduces part of the abnormalities of repolarization in patients with chromosome 7-linked LQT.\(^{28}\) which was the case in our patients. Yang et al showed that the IKr blocking effects of quinidine and dofetilide, 2 antiarrhythmic agents, are increased by low serum potassium levels.\(^{29}\)

Thus, in individuals receiving IKr blockers, as with HERG mutation patients, hypokalemia would be expected to result in a disproportionate action potential prolongation favoring torsade de points. Our data are in favor of a critical role played by hypokalemia in the occurrence of ventricular arrhythmias in genetically affected subjects with HERG mutations.

In the large 4 generation Irish family with the V822 M mutation (described by Satler and coworkers), 16 members were considered clinically affected, on the basis of electrocardiographic and clinical criteria, but 26 were found to be genetically affected by linkage and SSCP analysis.\(^{19}\) No history of sudden death was reported in this family. Syncope events were noted in 7 individuals. The majority of the V822 M carriers was asymptomatic (19 of 26, 73%).

In 3 families mentioned above and the Irish family, no stress-induced syncpe occurred before age 10, and no history of sudden death was reported. This suggests that these mutations occurring in the C-terminal domain may be less malignant than mutations occurring in the pore region. Electrophysiological characterization of these HERG mutations would shed light on their molecular mechanisms and may allow prediction of clinical outcome, as has been attempted for the KCNQ1 C-terminal mutation, R555C.\(^{16,17}\)

In contrast, in Family 5969, the fourth HERG mutation we identified, 2592+1G→A, is a substitution which disrupts the splice-donor sequence of intron 10 and appears more severe because 2 sudden deaths occurred at the ages of 9 and 20, and one of the 2 living carriers had syncopes at 4 years with a 2-day coma. The paternally inherited mutation, A341E, was the most frequently identified KCNQ1 mutation. In this family, 2 of the sisters were carriers of the LQT1 and LQT2 mutations. They were severely symptomatic and experienced stress-induced and rest-triggered syncopes starting in childhood. Only high doses of nadolol (160 mg/d) combined with permanent pacing allowed control of symptom recurrence. This is the first description of patients carrying 2 mutations in 2 different LQT genes. This suggests that these malignant forms are nevertheless compatible with life. Parents of severely affected probands, and, when possible, other members of the families, should be carefully examined on a clinical and electrocardiographic basis to orient the genetic analysis strategy.\(^{30,31}\)

In the present study, we describe 4 families with a C-terminal mutation in HERG and 2 sisters presenting a HERG mutation associated with a second mutation in KCNQ1. We would like to draw several conclusions. First, the whole HERG gene has to be screened for mutation to identify all the LQT2 mutations and to evaluate in larger groups the phenotypic expression of these various mutations, either occurring in the C-terminal domain or in the transmembrane domains. Secondly, this is the first description of 2 mutations occurring in 2 different LQT2 genes in the same patients, responsible for a malignant phenotype with syncopes due to ventricular arrhythmias, and finally prevented by the combination of nadolol and pacing. Thus, in order to identify such cases, it seems necessary to carefully examine both parents of probands, even if they are asymptomatic.

Thirdly, hypokalemia induced by diet, anorexia nervosa, diarrhea, and, of course, diuretic treatment, can be a major trigger for the clinical expression of the disease. The importance of preventing hypokalemia, particularly in women (who may diet to lose weight), should be clearly explained to the untreated carriers, and be part of the counseling strategy. This should be in addition to issuing a contra-indicated drug list to prevent ventricular arrhythmias and syncopes. To more fully evaluate the role of mutations in the 3’ part of HERG and potential interactions of these mutations with hypokalemia, larger studies in unselected populations are needed.

Acknowledgments

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References


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