A Molecular Basis for Cardiac Arrhythmia: HERG Mutations Cause Long QT Syndrome

Mark E. Curran,*† Igor Splawski,*†
Katherine W. Timothy,‡ G. Michael Vincent,‡§
Eric D. Green,# and Mark T. Keating*†‡∥
*Department of Human Genetics
†Eccles Program in Human Molecular Biology
  and Genetics
‡Cardiology Division
∥Howard Hughes Medical Institute
University of Utah Health Sciences Center
Salt Lake City, Utah 84112
§Department of Medicine
Latter Day Saints Hospital
Salt Lake City, Utah 84037
#National Center for Human Genome Research
National Institutes of Health
Bethesda, Maryland 20892

Summary

To identify genes involved in cardiac arrhythmia, we investigated patients with long QT syndrome (LQT), an inherited disorder causing sudden death from a ventricular tachyarrhythmia, torsade de pointes. We previously mapped LQT loci on chromosomes 11 (LQT1), 7 (LQT2), and 3 (LQT3). Here, linkage and physical mapping place LQT2 and a putative potassium channel gene, HERG, on chromosome 7q35-36. Single strand conformation polymorphism and DNA sequence analyses reveal HERG mutations in six LQT families, including two intragenic deletions, one splice-donor mutation, and three missense mutations. In one kindred, the mutation arose de novo. Northern blot analyses show that HERG is strongly expressed in the heart. These data indicate that HERG is LQT2 and suggest a likely cellular mechanism for torsade de pointes.

Introduction

Long QT syndrome (LQT) is an inherited cardiac disorder that causes syncope, seizures, and sudden death, usually in young, otherwise healthy individuals (Ward, 1964; Romano, 1965; Schwartz et al., 1975). Most LQT gene carriers manifest prolongation of the QT interval on electrocardiograms, a sign of abnormal cardiac repolarization (Vincent et al., 1992). The clinical features of LQT result from episodic cardiac arrhythmias, specifically torsade de pointes, named for the characteristic undulating nature of the electrocardiogram in this arrhythmia. Torsade de pointes may degenerate into ventricular fibrillation, a particularly lethal arrhythmia. Although LQT is not a common diagnosis, ventricular arrhythmias are very common; more than 300,000 Americans die suddenly every year (Kannel et al., 1987; Willich et al., 1987), and in many cases the underlying mechanism may be aberrant cardiac repolarization. LQT,

therefore, provides a unique opportunity to study life-threatening cardiac arrhythmias at the molecular level.

A molecular basis for LQT was not previously known. In 1991, we discovered tight linkage between autosomal dominant LQT and a polymorphism at H-RAS (Keating et al., 1991a). This discovery localized an LQT gene (LQT1) to chromosome 11p15.5 and made genetic testing possible in some families. Autosomal dominant LQT was previously thought to be genetically homogeneous, and the first seven families that we studied were linked to 11p15.5 (Keating et al., 1991b). In 1993, however, several laboratories, including our group, identified families that were not linked to chromosome 11p15.5 (Benhorin et al., 1993; Curran et al., 1993a; Towbin et al., 1994). In 1994, we identified two additional LQT loci, LQT2 on chromosome 7q35-36 (nine families) and LQT3 on chromosome 3p21-24 (three families) (Jiang et al., 1994). Since three families in our study remained unlinked, at least one more LQT locus exists. This degree of heterogeneity suggests that distinct LQT genes may encode proteins that interact to modulate cardiac repolarization and arrhythmia risk.

Since LQT is associated with abnormal cardiac repolarization, genes that encode ion channels (or their modulators) are reasonable candidates. H-RAS, which was localized to chromosome 11p15.5, was excluded as a candidate for LQT1 based on direct DNA sequence analyses (unpublished data) and by linkage analyses (Roy et al., 1994). A skeletal muscle chloride channel (CLCN1; Koch et al., 1992) and a cardiac muscarinic–acetylcholine receptor (CHRM2; Bonner et al., 1987) became candidates for LQT2 based on their chromosome 7q35-36 location, but subsequent linkage analyses have excluded these genes (Wang et al., submitted).

Warmke and Ganetzky (1994) identified a novel human cDNA, the human ether-a-go-go-related gene (HERG). HERG was localized to human chromosome 7 by polymerase chain reaction (PCR) analysis of a somatic cell hybrid panel (Warmke and Ganetzky, 1994). The function of the protein encoded by HERG is not known, but it has predicted amino acid sequence homology to potassium channels. HERG was isolated from a hippocampal cDNA library by homology to the Drosophila ether-a-go-go (eag) gene, which encodes a Ca²⁺-modulated potassium channel (Bruggeman et al., 1993). HERG is not the human homolog of eag, however, their products sharing only ~50% amino acid sequence homology.

In this study, we provide evidence indicating that HERG is LQT2. First, we identified and characterized new LQT families and showed that all were linked to markers on chromosome 7q35-36, confirming the location of LQT2. Second, we mapped HERG to chromosome 7q35-36, making HERG a candidate gene for LQT2. Third, we demonstrated that HERG is strongly expressed in the heart. Finally, we identified six HERG mutations associated with LQT; one of these mutations arose de novo.
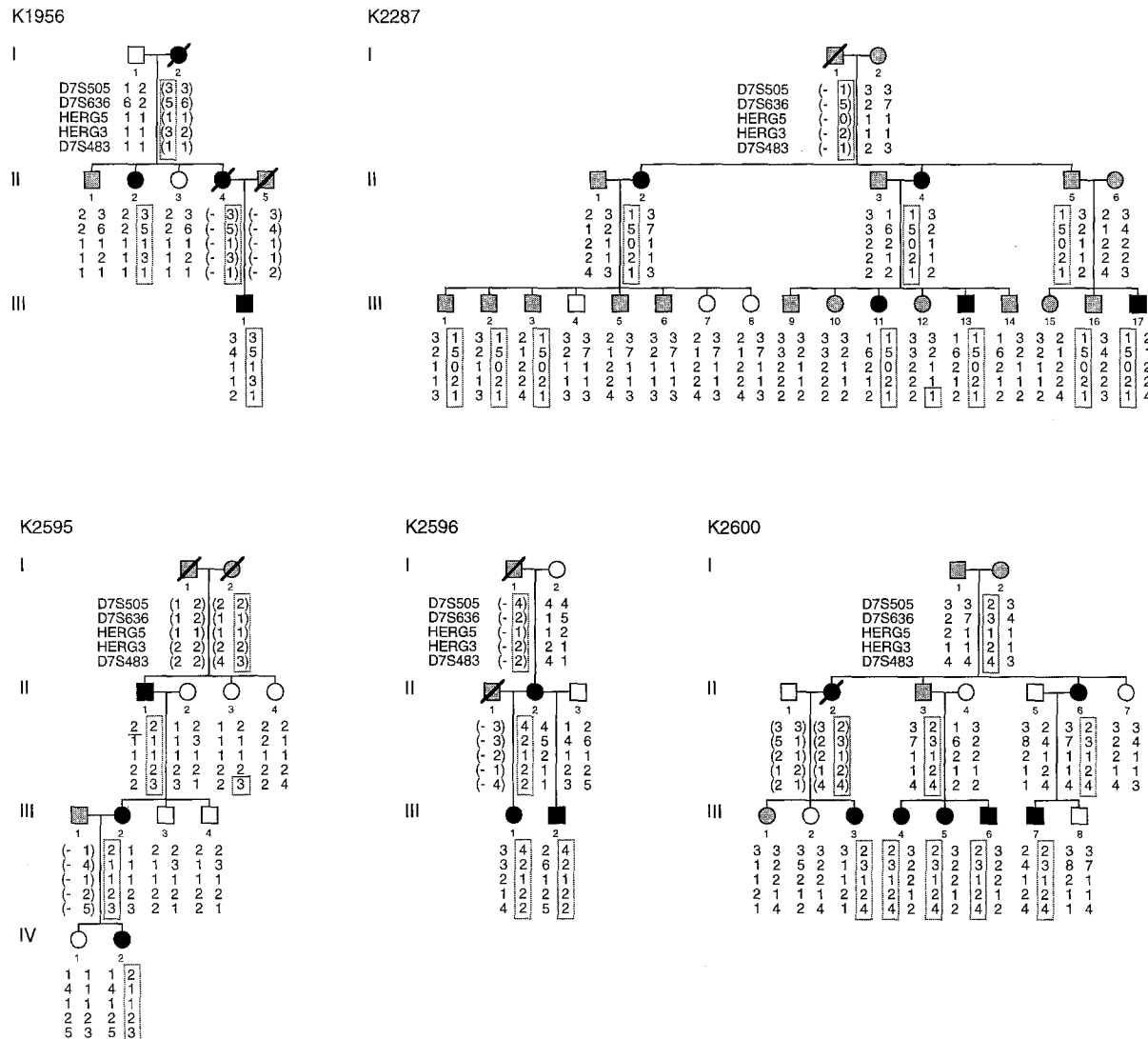
Figure 1. Pedigree Structure and Genotypic Analyses of Five LQT Families

Individuals showing the characteristic features of LQT, including prolongation of the QT interval and history of syncope, seizures, or aborted sudden death, are indicated by closed circles (females) or closed squares (males). Unaffected individuals are indicated by open circles or open squares. Individuals with an equivocal phenotype or those for whom phenotypic data are unavailable are shown as stippled. Circles or squares with a slash denote deceased individuals. Haplotypes for polymorphic markers linked to *LQT2* are shown under each individual. These markers include (centromere to telomere) *D7S505*, *D7S636*, *HERG 5-11*, *HERG 3-8*, and *D7S483* (Gyapay et al., 1994; Wang et al., submitted). Haplotypes cosegregating with the disease phenotype are indicated by a box. Recombination events are indicated with a horizontal black line. Informed consent was obtained from all individuals or from their guardians in accordance with local institutional review board guidelines. Haplotype analyses indicate that the LQT phenotype in these kindreds is linked to markers on chromosome 7q35-36.

Results

LQT2 Is Linked to Markers on Chromosome 7q35-36

To determine the relative frequency of the three known LQT loci (*LQT1*, *LQT2*, and *LQT3*), we performed linkage analyses in families with this disorder. Five LQT families were identified and phenotypically characterized (Figure 1). These families were unrelated and of varying descent, including Mexican (Spanish), German, English, and Danish. In each case, an autosomal dominant pattern of inheritance was suggested by inspection of the pedigree. Af-

fected individuals were identified by the presence of QT prolongation on electrocardiograms and, in some cases, a history of syncope or aborted sudden death. No patients had signs of congenital neural hearing loss, a finding associated with the rare autosomal recessive form of LQT, or other phenotypic abnormalities. Genotype analyses with polymorphic markers linked to the known LQT loci suggested that the disease phenotype in these families was linked to polymorphic markers on chromosome 7q35-36 (Figure 1). The maximum combined two-point lod score for these five families was 5.13 at *D7S636* (θ = 0.0; Table 1). When combined with our previous studies (Jiang et al.,

Table 1. Maximum Pairwise Lod Scores, Recombination Fractions for Linkage of *LQT2* with *HERG*, and Polymorphic Markers on Chromosome 7

	Families from Present Study		Families Studied to Date	
Locus	Z_{max}	θ	Z_{max}	θ
D7S505	4.40	0.0	22.91	0.009
D7S636	5.13	0.0	26.14	0.00
HERG 3-8	0.11	0.0	6.34	0.00
HERG 5-11	3.55	0.0	9.64	0.00
D7S483	2.48	0.0	22.42	0.00

Markers are shown in chromosomal order (centromere to telomere) (Gyapay et al., 1994). The first column (families from present study) indicates combined lod scores for the five families described in this study. The second column (families studied to date) indicates combined lod scores from the five families studied here, as well as the nine families from our previous study (Jiang et al., 1994). Z_{max} indicates maximum lod score; θ indicates estimated recombination fraction at Z_{max}.

Figure 2. Partial Genomic Structure of *HERG* and Location of PCR Primers Used in This Study

Regions encoding predicted membrane-spanning domains (S1–S6), the pore domain, and the nucleotide-binding domain (NBD) are indicated. The DNA sequences for the intron–exon boundaries are these: intron I, 5'-AGGAGgtgggg...ccccagCTGATC-3'; intron II, 5'-TGG-CTgtgagt...ccccagCCCTC-3'; intron III, 5'-CCTGGgtatgg...ctccagG-GAAG-3'.

1994; Wang et al., submitted), the maximum combined two-point lod score for the 14 chromosome 7-linked families was 26.14, also at *D7S636* (θ = 0.0; Table 1). Haplotype analyses were consistent with our previous studies, placing *LQT2* between *D7S505* and *D7S483* (Figure 1; Wang et al., submitted), localizing this gene to chromosome 7q35-36.

HERG Maps to Chromosome 7q35-36

HERG was previously mapped to chromosome 7 (Warmke and Ganetzky, 1994). To test the candidacy of this gene, we refined the localization of *HERG* using two physical mapping techniques. First, we mapped *HERG* on a set of yeast artificial chromosome (YAC) contigs constructed for chromosome 7 (Green et al., 1994). *HERG* was localized to the same YAC as *D7S505*, a polymorphic marker that was tightly linked to *LQT2* (Table 1). Second, we mapped *HERG* to chromosome 7q35-36 using fluorescent in situ hybridization (FISH) with a P1 genomic clone containing *HERG* (data not shown).

To determine whether *HERG* was genetically linked to the LQT locus, we used single strand conformation polymorphism (SSCP) analyses to identify polymorphisms within *HERG* and performed linkage analyses in the chromosome 7-linked families. Two aberrant SSCP conformers were identified in DNA samples from patients and controls using primer pairs 5-11 and 3-8 (Figure 2). These conformers were cloned and sequenced. One abnormal conformer resulted from a C to T substitution at position 3 of codon 489 (cDNA nucleotide 1467; observed heterozygosity of 0.37). The second abnormal conformer resulted from an A to G substitution at position 3 of codon 564 (cDNA nucleotide 1692; observed heterozygosity of 0.44). Neither substitution affected the predicted amino acid sequence of HERG. *HERG* polymorphisms were used for genotypic analyses in chromosome 7-linked families (see Figure 1; data not shown). No recombination events between *HERG* and LQT were identified in any of these families. The maximum combined lod score for the 14
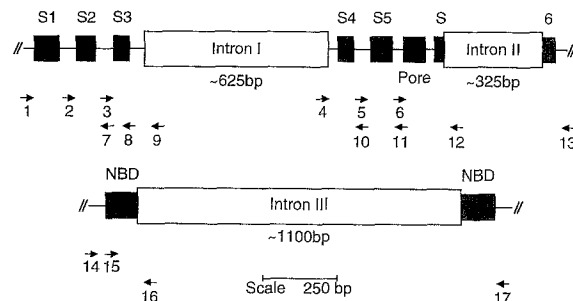
families was 9.64 (θ = 0.0; Table 1). These data indicate that *HERG* is completely linked to *LQT2*.

HERG Intragenic Deletions Associated with LQT in Two Families

To test the hypothesis that *HERG* is *LQT2*, we used SSCP analyses to screen for mutations in affected individuals. Since the genomic structure of *HERG* was unknown, oligonucleotide primer pairs were designed from published (Warmke and Ganetzky, 1994) *HERG* cDNA sequences (Figure 2; Table 2). In most cases, single products of expected size were generated. For primer pairs 1-10, 6-13, and 15-17, however, products of greater than expected size were obtained, suggesting the presence of intronic sequences. To examine this possibility, we cloned and sequenced these larger products. DNA sequence analyses identified three introns at positions 1557/1558, 1945/

Table 2. *HERG* PCR Primers

Name	Position	Sequence
1 L	1147–1166	GACGTGCTGCCTGAGTACAA
2 L	1291–1312	TTCCTGCTGAAGGAGACGGAAG
3 L	1417–1437	ACCACCTACGTCAATGCCAAC
4 L	Intron I	TGCCCCATCAACGGAATGTGC
5 L	1618–1636	GATCGCTACTCAGAGTACG
6 L	1802–1823	GCCTGGGCGGCCCCTCCATCAA
7 R	1446–1426	CACCTCCTCGTTGGCATTGAC
8 R	1527–1503	GTCGAAGGGGATGGCGGCCACCATG
9 R	Intron I	TACACCACCTGCCTCCTTGCTGA
10 R	1643–1623	GCCGCGCCGTACTCTGAGTAG
11 R	1758–1736	CAGCCAGCCGATGCGTGAGTCCA
12 R	Intron II	GCCCGCCCCTGGGCACACTCA
13 R	2034–2016	CAGCATCTGTGTGTGGTAG
16 R	Intron III	GGCATTTCCAGTCCAGTGC
15 L	2259–2278	CCTGGCCATGAAGTTCAAGA
14 L	2214–2233	GCACTGCAAACCCTTCCGAG
17 R	2550–2529	GTCGGAGAACTCAGGGTACATG

All primers are shown in 5' to 3' direction. Sense strand oligonucleotides are indicated with an L and antisense oligonucleotides are indicated with an R. cDNA sequence was obtained from the GenBank data base; nucleotide numbering begins with the initiator methionine.
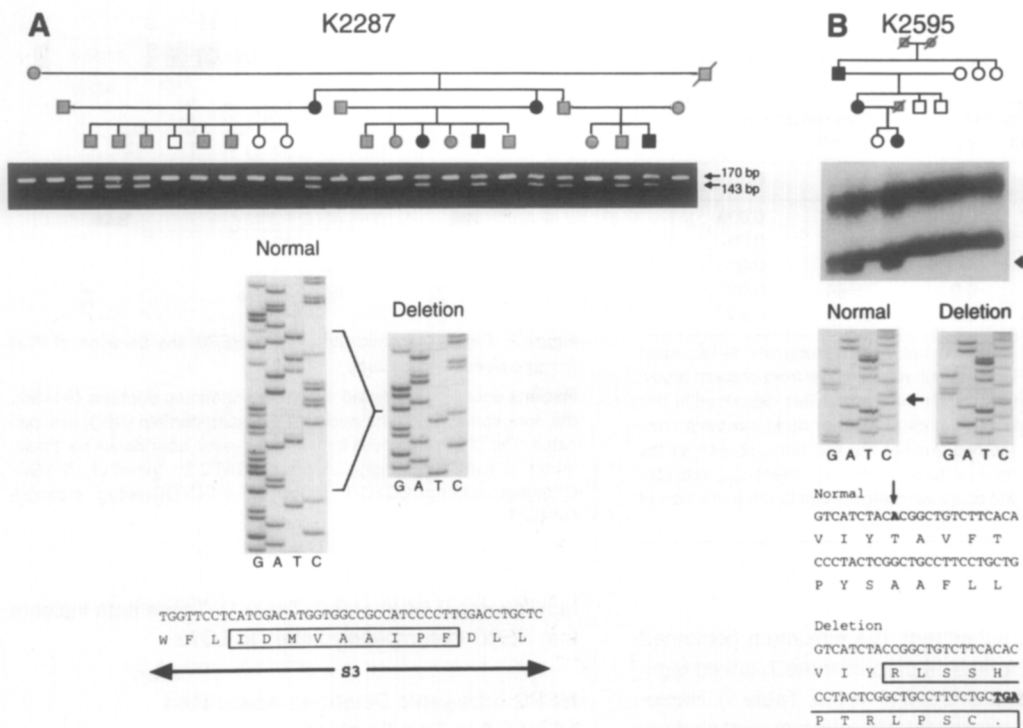
A K2287

B K2595

←170 bp
←143 bp

Normal

Deletion

G A T C

G A T C

Normal   Deletion

G A T C   G A T C

Normal ↓
GTCATCTACACGGCTGTCTTCACA
V   I   Y   T   A   V   F   T
CCCTACTCGGCTGCCTTCCTGCTG
P   Y   S   A   A   F   L   L

Deletion
GTCATCTACCGGCTGTCTTCACAC
V   I   Y   R   L   S   S   H
CCTACTCGGCTGCCTTCCTGCTGA
P   T   R   L   P   S   C   .

TGGTTCCTCATCGACATGGTGGCCGCCATCCCCTTCGACCTGCTC
W   F   L   I   D   M   V   A   A   I   P   F   D   L   L

←———————————— S3 ————————————→

Figure 3. *HERG* Intragenic Deletions Associated with LQT in Two Families

(A) Pedigree structure of K2287, results of PCR amplification using primer pair 3-9, and the effect of the deletion on the predicted structure of HERG protein are shown. Note that an aberrant fragment of 143 bp is observed in affected members of this kindred, indicating the presence of a disease-associated intragenic deletion. DNA sequence of normal and aberrant PCR products defines a 27 bp deletion (ΔI500-F508). This mutation causes an in-frame deletion of nine amino acids in the third membrane-spanning domain (S3). Deleted sequences are indicated.

(B) Pedigree structure of K2595 is shown. Deceased individuals are indicated by a slash. The results of SSCP analyses using primer pair 1-9 are shown beneath each individual. Note that an aberrant SSCP conformer cosegregates with the disease in this family. DNA sequence shows a single base pair deletion (Δ1261). This deletion results in a frameshift followed by a stop codon 12 amino acids downstream. The deleted nucleotide is indicated with an arrow.

1946, and 2398/2399 of the cDNA sequence (Figure 2). These boundaries were confirmed by direct DNA sequencing of *HERG* genomic clones containing *HERG* (data not shown). To facilitate SSCP analyses, we designed additional primers to intronic sequences.

As indicated previously, SSCP analyses using primer pair 3-8 identified an A to G polymorphism within *HERG* (cDNA nucleotide 1692). Analysis of kindred 2287 (K2287) using this SSCP defined a pattern of genotypes consistent with a null allele (see Figure 1). Possible explanations for these findings included multiple misinheritances, a possibility not supported by our previous genotypic analyses, DNA sample errors, base pair substitutions, or a deletion. To test the hypothesis that the genotypic data were due to a small deletion, we repeated PCR analyses of K2287 using a new primer pair (3-9) flanking the previous set of primers. These experiments identified two products of 170 bp and 143 bp in affected members of K2287 (Figure 3A). By contrast, only a single product of 170 bp was observed in unaffected members of this kindred. Furthermore, only the 170 bp band was seen in DNA samples from more than 200 unaffected individuals (data not shown). The 143 bp and 170 bp products were cloned from affected individual II-2. Direct sequence analyses of the aberrant PCR product revealed the presence of a 27 bp deletion begin-

ning at position 1498 (ΔI500-F508). This deletion disrupts the third membrane-spanning domain (S3) of HERG.

To test further the hypothesis that *HERG* is *LQT2*, we continued SSCP analyses in additional kindreds. SSCP using the primer pair 1-9 identified an aberrant conformer in affected individuals of K2595 (Figure 3B). Analyses of more than 200 unaffected individuals failed to show this anomaly (data not shown). The normal and aberrant conformers were cloned and sequenced, revealing a single base deletion at position 1261 (Δ1261). This deletion results in a frameshift in sequences encoding the first membrane-spanning domain (S1), leading to a new stop codon within 12 amino acids. The identification of intragenic deletions of *HERG* in two LQT families suggests that *HERG* mutations can cause LQT.

Three *HERG* Point Mutations Associated with LQT

To identify additional *HERG* mutations in *LQT2*, we continued SSCP analyses in linked kindreds and sporadic cases. Three aberrant SSCP conformers were identified in affected members of K1956, K2596, and K2015 (Figure 4). In each case, the normal and aberrant conformers were cloned and sequenced. In K1956, a C to T substitution at position 1682 was identified. This mutation results in substitution of valine for a highly conserved alanine at
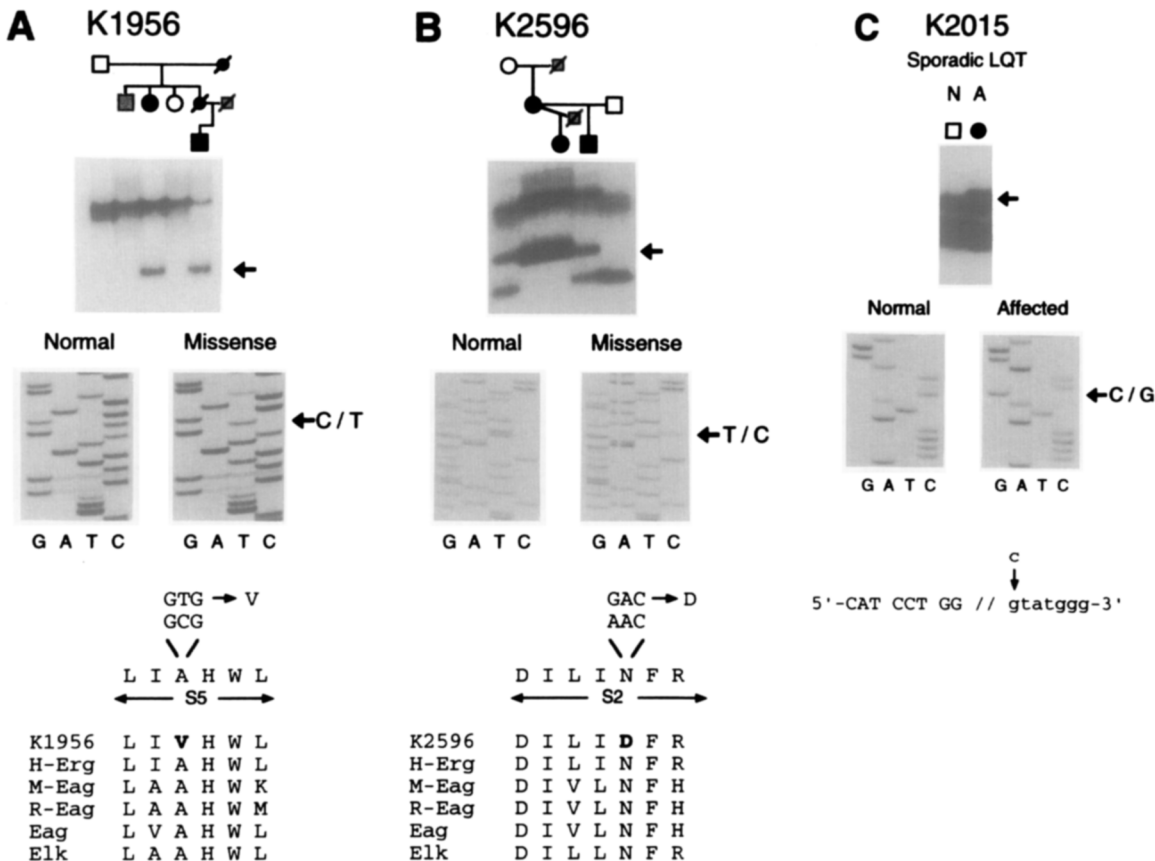
Figure 4. HERG Point Mutations Identified in Three LQT Kindreds

Pedigree structure of K1956 (A), K2596 (B), and K2015 (C) are shown. Below each pedigree, the results of SSCP analyses with primer pair 5-11 (K1956), primer pair 1-9 (K2596), and primer pair 14-16 (K2015) are shown. Aberrant SSCP conformers cosegregate with the disease in each kindred. DNA sequence analyses of the normal and aberrant conformers reveals a C to T substitution at position 1682 in K1956. This mutation results in substitution of valine for a highly conserved alanine residue at codon 561 (A561V). Analyses of K2596 reveals an A to G substitution at position 1408 (T to C substitution on the antisense strand is shown). This mutation results in substitution of aspartic acid for a conserved asparagine in the second transmembrane domain (N470D). Analyses of K2015 reveals a G to C substitution (C to G substitution on the antisense strand is shown). This mutation occurs in the splice-donor sequence of intron III. Coding sequences are shown in uppercase and intronic sequences are lowercase. Note that the G to C substitution disrupts the splice-donor site (HERG, M-eag, elk [Warmke and Ganetzky, 1994]; R-eag [Ludwig et al., 1994]).

codon 561 (A561V), altering the fifth membrane-spanning domain (S5) of the HERG protein (Figure 4A). In K2596, an A to G substitution was identified at position 1408. This mutation results in substitution of aspartic acid for a conserved asparagine at codon 470 (N470D), located in the second membrane-spanning domain (S2) (Figure 4B). In K2015, a G to C substitution was identified. This substitution disrupts the splice-donor sequence of intron III, affecting the cyclic nucleotide-binding domain (Figure 4C). None of the aberrant conformers were identified in DNA samples from more than 200 unaffected individuals (data not shown).

De Novo Mutation of HERG in a Sporadic Case of LQT

To substantiate that HERG mutations cause LQT, we used SSCP analysis to screen for mutations in sporadic cases. Primer pair 4-12 identified an aberrant conformer in affected individual II-1 of K2269 (Figure 5). This conformer was not identified in either parent or in more than 200

unaffected individuals. Direct DNA sequencing of the aberrant conformer identified a G to A substitution at position 1882. This mutation results in substitution of serine for a highly conserved glycine at codon 628 (G628S), altering the pore-forming domain. Genotype analysis of this kindred using nine informative short tandem repeat polymorphisms confirmed maternity and paternity. The identification of a de novo mutation in a sporadic case demonstrates that HERG is LQT2.

HERG Is Expressed in the Heart

HERG was originally identified from a hippocampal cDNA library (Warmke and Ganetzky, 1994). To determine the tissue distribution of HERG mRNA, we isolated partial cDNA clones and used them in Northern blot analyses. Northern blot analyses showed strongest hybridization to heart mRNAs, with faint signals in brain, liver, and pancreas (Figure 6). Nonspecific hybridization was also seen in lung, possibly due to genomic DNA contamination. The size of the bands observed in cardiac mRNA was consis-
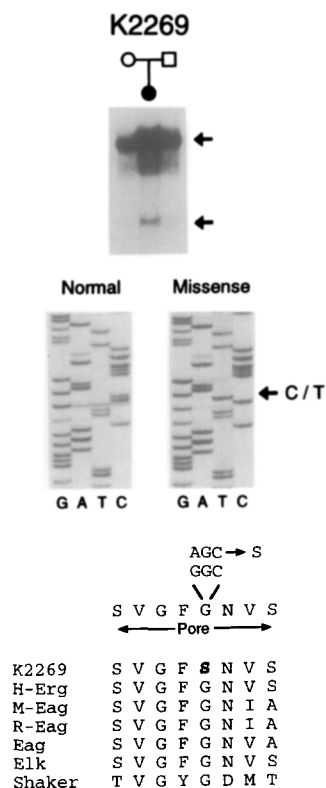
K2269

Normal   Missense

G A T C   G A T C

AGC → S
GGC
\/
S V G F G N V S
←——Pore——→

K2269	S V G F **S** N V S
H-Erg	S V G F G N V S
M-Eag	S V G F G N I A
R-Eag	S V G F G N I A
Eag	S V G F G N V A
Elk	S V G F G N V S
Shaker	T V G Y G D M T

Figure 5. De Novo Mutation of *HERG* in a Sporadic Case of LQT

Pedigree structure of K2269 and SSCP analyses (primer pair *14-16*) showing an aberrant conformer in a sporadic case of LQT. DNA sequence analyses identified a G to A substitution at position 1882 of the cDNA sequence (C to T substitution on the antisense strand is shown). Note that this mutation results in the substitution of a serine for a highly conserved glycine residue at codon 628 (G628S). This amino acid sequence is known to be critical for potassium ion selectivity.

Figure 6. Northern Blot Analysis of *HERG* mRNA Showing Strong Expression in the Heart

A Northern blot (poly[A]$^+$ RNA, 2 μg/lane; Clontech) was probed using an *HERG* cDNA containing nucleotides 679–2239 of the coding sequence. Two cardiac mRNAs of ~4.1 and 4.4 kb are indicated. Background in mRNA extracted from lung was high, but no specific bands were identified.

tent with the predicted size of *HERG*. Two bands of ~4.1 and 4.4 kb were identified, possibly due to alternative splicing or the presence of a second related mRNA. These data indicate that *HERG* is strongly expressed in the heart, consistent with its involvement in LQT.

Discussion

We conclude that mutations in *HERG* cause the chromosome 7–linked form of LQT. Several lines of evidence support this conclusion. First, we used linkage analyses to map an LQT locus (*LQT2*) to chromosome 7q35-36 in 14 families. Second, we used physical and genetic mapping to place *HERG* in the same chromosomal region as *LQT2*. Third, we demonstrated that *HERG* is expressed in the heart. Fourth, we identified intragenic deletions of *HERG* associated with LQT in two families. Fifth, we identified four *HERG* point mutations in LQT patients. Finally, one of the point mutations arose de novo and occurs within a highly conserved region encoding the potassium-selective pore domain.

Our data suggest a likely molecular mechanism for chromosome 7–linked LQT. Although the function of *HERG* is
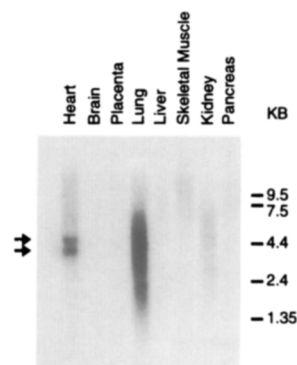
not yet known, analyses of its predicted amino acid sequence indicates that it encodes a potassium channel α subunit. Potassium channels are formed from four α subunits (MacKinnon, 1991), either as homo- or heterotetramers (Covarrubias et al., 1991). These biophysical observations suggest that combination of normal and mutant HERG α subunits could form abnormal HERG channels. This raises the possibility that *HERG* mutations have a dominant negative affect on potassium channel function.

The mutations that we identified are consistent with a dominant negative mechanism (Figure 7). Two mutations result in premature stop codons and truncated proteins (Δ1261 and the splice-donor mutation). In the first case, only the amino terminus and a portion of the first membrane-spanning domain (S1) remain. In the second, the carboxyl end of the protein is truncated, leaving all membrane-spanning domains intact. HERG contains a cyclic nucleotide-binding domain near the carboxyl terminus, and in both mutations this domain is deleted. In another mutation, an in-frame deletion of nine amino acids disrupts the third membrane-spanning domain (ΔI500-F508). Two missense mutations also affect membrane-spanning domains, A561V in the S5 domain and N470D in S2. Both mutations affect amino acids conserved in the eag family of potassium channels and likely alter the secondary structure of the protein. The de novo missense mutation G628S occurs in the pore-forming domain. This domain is highly conserved in all potassium channel α subunits. This mutation affects a conserved amino acid that is of known importance for ion selectivity. When this substitution was introduced into *Shaker* H4, potassium ion selectivity was lost (Heginbotham et al., 1994). As discussed above, these mutations could induce the loss of HERG function.

Our data have implications for the mechanism of arrhythmias in LQT. Two hypotheses for LQT have previously been proposed (Schwartz et al., 1995). One suggests that a predominance of left autonomic innervation causes abnormal cardiac repolarization and arrhythmias. This hypothesis is supported by the finding that arrhyth-
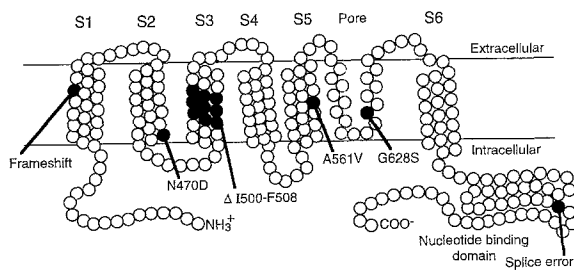
Figure 7. Schematic Representation of the Predicted Topology of the Protein Encoded by *HERG* and the Location of LQT-Associated Mutations

For simplicity, not all amino acids are shown. SSCP analyses identified *HERG* mutations in 4 of the 14 chromosome 7–linked kindreds.

mias can be induced in dogs by removal of the right stellate ganglion. In addition, anecdotal evidence suggests that some LQT patients are effectively treated by β-adrenergic blocking agents and by left stellate ganglionectomy (Schwartz et al., 1995). The second hypothesis for LQT-related arrhythmias suggests that mutations in cardiac-specific ion channel genes (or genes that modulate cardiac ion channels) cause delayed myocellular repolarization. Delayed myocellular repolarization could promote reactivation of L-type Ca^{2+} channels, resulting in secondary depolarizations (January and Riddle, 1989). These secondary depolarizations are the likely cellular mechanism of torsade de pointes arrhythmias (Surawicz, 1989). This hypothesis is supported by the observation that pharmacologic block of potassium channels can induce QT prolongation and repolarization-related arrhythmias in human and animal models (Antzelevitch and Sicouri, 1994). The discovery that one form of LQT results from mutations in a cardiac potassium channel gene supports the myocellular hypothesis.

The presence of a cyclic nucleotide-binding domain in *HERG* suggests a mechanism for the link between altered autonomic nervous activity and arrhythmias in LQT. β-Adrenergic receptor activation increases intracellular cAMP and enhances L-type Ca^{2+} channel function. Cyclic AMP may also activate HERG, thereby increasing net outward current and accelerating the rate of myocellular repolarization. Dominant negative mutations of HERG might interrupt the normal modulation of HERG function by cAMP, thereby permitting a predominant effect on L-type Ca^{2+} channel function. The resulting imbalance would increase the likelihood that enhanced sympathetic tone could induce Ca^{2+} channel-dependent secondary depolarizations, the probable cellular mechanism of torsades de pointes. β-Adrenergic blocking agents could act by interrupting the effect of cAMP on L-type Ca^{2+} channels, possibly explaining the beneficial effects of β blockers in some LQT patients.

The relative frequency of the three LQT loci is not yet known. In this study, we identified five new families with autosomal dominant LQT, and all were linked to chromosome 7. This brings the total number of chromosome 7–linked families to 14. To date, we have linked seven

families to chromosome 11 (*LQT1*), 14 families to chromosome 7 (*LQT2*), and three families to chromosome 3 (*LQT3*), and three families remain unlinked (Keating et al., 1991a, 1991b; Jiang et al., 1994). Although preliminary, these data suggest that *LQT2* is a common form of inherited LQT.

This work may have important clinical implications. Recently, presymptomatic diagnosis has been possible in large families using linkage analysis. Most cases of LQT are sporadic, and therefore genetic testing using linkage analysis is not feasible. Continued mutational analyses of *LQT2* will facilitate genetic testing for this form of LQT. Identification and characterization of genes responsible for other forms of LQT will be necessary for the development of generalized diagnostic tests. Improved diagnostic capacity may enable rational therapy. For example, chromosome 7–linked LQT patients may respond to potassium channel activators, like pinacidil.

In summary, our results provide the molecular mechanism of a life-threatening cardiac arrhythmia. Future experiments will define the specific function of HERG and its role in LQT. In addition, we will continue to search for LQT genes; these genes will provide further insight into cardiac repolarization and repolarization-related arrhythmias.

Experimental Procedures

Identification and Phenotyping of LQT Kindreds

LQT kindreds were ascertained from medical clinics throughout North America. Phenotypic criteria were identical to those used in our previous studies (Keating et al., 1991a, 1991b; Keating, 1992; Curran et al., 1993a; Jiang et al., 1994). Individuals were evaluated for LQT based on the QT interval corrected for heart rate (QTc; Bazette, 1920) and for the presence of syncope, presence of seizures, and aborted sudden death. Informed consent was obtained from all individuals or from their guardians in accordance with local institutional review board guidelines. Phenotypic data were interpreted without knowledge of genotype.

Genotypic and Linkage Analyses

Amplification and detection of microsatellite markers were performed as described previously (Curran et al., 1993a; Jiang et al., 1994). Polymorphic markers used in this study were *D7S483*, *D7S636*, and *D7S505* (Gyapay et al., 1994). Pairwise linkage analysis was performed using the MLINK program of the LINKAGE software package (Lathrop et al., 1985). In accordance with our previous studies, we assumed a penetrance of 0.90 and a disease gene frequency of 0.001. Gene frequency between males and females was assumed to be equal.

Isolation of *HERG* Genomic and cDNA Clones

HERG probes were generated using the products of PCRs with human genomic DNA and primer pairs *1-10*, *6-13*, and *15-17*. These products were cloned, radiolabeled to high specific activity, and used to screen a human genomic P1 library (Sternberg, 1990). Positive clones were purified, characterized, and used for FISH and DNA sequence analyses. To isolate *HERG* cDNA clones, genomic probes containing *HERG* coding sequences were used to probe 10^6 recombinants of a human hippocampal cDNA library (Stratagene). A single clone containing ~2.2 kb of *HERG* coding sequence was isolated and characterized.

YAC-Based Mapping of *HERG*

A PCR assay specific for the 3′ untranslated region of *HERG* (employing primers 5′-GCTGGGCCGCTCCCCTTGGA-3′ and 5′-GCATCTTCAT-TAATTATTCA-3′ and yielding a 309 bp product) was used to screen a collection of YAC clones highly enriched for human chromosome 7 (Green et al., 1995). Two positive YAC clones were identified (*yWSS2193* and *yWSS1759*), both contained within a larger contig that

includes YACs positive for the genetic marker *D7S505* (Green et al., 1994).

FISH

Metaphase chromosome spreads were prepared from normal cultured lymphocytes (46 X,Y) by standard procedures of colcimid arrest, hypotonic treatment, and acetic acid–methanol fixation. *HERG* P1 clone *16B4* was labeled by incorporation of biotin-14-dATP (BioNick System, GIBCO BRL), hybridized to metaphase spreads, and detected with streptavidin–Cy3 according to standard methods (Lichter et al., 1988). A digoxigenin-labeled centromere-specific α-satellite probe (Oncor) was cohybridized and detected with antidigoxigenin–FITC to identify chromosome 7. Chromosomes were counterstained with DAPI and visualized directly on the photomicroscope.

SSCP and DNA Sequence Analyses

Genomic DNA samples were amplified by PCR and used in SSCP analyses as described (Orita et al., 1989; Ptacek et al., 1991). Primer pairs used for this study are shown in Table 2. Annealing temperature was 58°C for all PCRs. Reactions (10 μl) were diluted with 40 μl of 0.1% SDS and 1 mM EDTA and with 30 μl of 95% formamide dye. Diluted products were denatured by heating at 100°C for 5 min, and 3 μl of each sample was electrophoresed on 7.5% nondenaturing polyacrylamide gels (49:1 polyacrylamide:bisacrylamide) at 4°C. Electrophoreses was carried out at 40 W for 2–5 hr. Gels were transferred to filter paper, dried, and exposed to X-ray film at −80°C for 12–36 hr.

Normal and aberrant SSCP conformers were cut directly from dried gels and eluted in 75 μl of distilled water at 37°C for 30 min. The eluted DNA (10 μl) was used as template for a second PCR using the original primer pair. Products were fractionated in 2% low melting temperature agarose gels (FMC Corporation), and bands were cloned directly into pBluescript II SK(+) (Stratagene) using the T vector method as described (Marchuk et al., 1990). Plasmid DNA samples were purified and sequenced as previously described (Curran et al., 1993b).

Northern Blot Analysis

A multiple tissue Northern blot containing ~2 μg/lane of poly(A)⁺ mRNA was purchased from Clontech (human MTN blot 1). A high specific activity (>1.5 × 10⁹ cpm/μg DNA) and radiolabeled *HERG* cDNA fragment containing nucleotides 679–2239 of the coding sequence was prepared by random hexamer priming as described (Feinberg and Vogelstein, 1983). Probe was added to the hybridization solution at a final concentration of 5 × 10⁶ cpm/ml. Hybridization was carried out at 42°C for 24 hr in 20 ml of Quickhyb solution (Stratagene). Final washes were carried out at 65°C for 30 min in a solution of 0.1% SDS, 0.1× SSC.

Acknowledgments

References

Antzelevitch, C., and Sicouri, S. (1994). Clinical relevance of cardiac arrhythmias generated by afterdepolarizations: role of M cells in the generation of U waves, triggered activity and *torsade de pointes*. J. Am. Coll. Cardiol. *23*, 259–277.

Bazette, H. C. (1920). An analysis of the time-relationships of electrocardiograms. Heart *7*, 353–370.

Benhorin, J., Kalman, Y. M., Madina, A., Towbin, J., Rave-Harel, N., Dyer, T. D., Blangero, J., MacCluer, J. W., and Kerem, B. (1993). Evidence of genetic heterogeneity in the long QT syndrome. Science *260*, 1960–1962.

Bonner, T. I., Buckley, N. J., Young, A. C., and Brann, M. R. (1987). Identification of a family of muscarinic acetylcholine receptor genes. Science *237*, 527–532.

Bruggeman, A., Pardo, L. A., Struhmer, W., and Pongs, O. (1993). *ether-a-go-go* encodes a voltage-gated channel permeable to K⁺ and Ca²⁺ and modulated by cAMP. Nature *365*, 445–448.

Covarrubias, M., Wei, A., and Salkoff, L. (1991). *Shaker, shal, shab,* and *shaw* express independent K⁺ current systems. Neuron *7*, 763–773.

Curran, M. E., Atkinson, D., Timothy, K., Vincent, G. M., Moss, A. J., Leppert, M., and Keating, M. T. (1993a). Locus heterogeneity of autosomal dominant long QT syndrome. J. Clin. Invest. *92*, 799–803.

Curran, M. E., Atkinson, D. L., Ewart, A. K., Morris, C. A., Leppert, M. F., and Keating, M. T. (1993b). The elastin gene is disrupted by a translocation associated with supravalvular aortic stenosis. Cell *73*, 159–168.

Feinberg, A. P., and Vogelstein, B. A. (1983). Techniques for radiolabeling DNA to high specific activity. Anal. Biochem. *132*, 6–13.

Green, E. D., Idol, J. R., Mohr-Tidwell, R. M., Branden, V. V., Peluso, D. C., Fulton, R. S., Massa, H. F., Magness, C. L., Wilson, A. M., Kimura, J., Weissenbach, J., and Trask, B. J. (1994). Integration of physical, genetic and cytogenetic maps of human chromosome 7: isolation and analysis of yeast artificial chromosomes for 117 mapped genetic markers. Hum. Mol. Genet. *3*, 489–501.

Green, E. D., Branden, V. V., Fulton, R. S., Lim, R., Ueltzen, M. S., Peluso, D. C., Mohr-Tidwell, R. M., Idol, J. R., Smith, L. M., Chumakov, I., LePaslier, D., Cohen, D., Featherstone, T., and Green, P. (1995). A human chromosome 7 yeast artificial chromosome (YAC) resource: construction, characterization, and screening. Genomics, in press.

Gyapay, G., Morissette, J., Vignal, A., Dib, C., Fizames, C., Millasseau, P., Marc, S., Bernardi, G., Lathrop, M., and Weissenbach, J. (1994). The 1993–1994 Genethon human genetic linkage map. Nature Genet. *7*, 246–339.

Heginbotham, L., Lu, Z., Abramson, T., and MacKinnon, R. (1994). Mutations in the K⁺ channel signature sequence. Biophys. J. *66*, 1061–1067.

January, C. T., and Riddle, J. M. (1989). Early afterdepolarizations: mechanism of induction and block. Circ. Res. *64*, 977–990.

Jiang, C., Atkinson, D., Towbin, J. A., Splawski, I., Lehmann, M. H., Li, H., Timothy, K., Taggart, R. T., Schwartz, P. J., Vincent, G. M., Moss, A. J., and Keating, M. T. (1994). Two long QT syndrome loci map to chromosomes 3 and 7 with evidence for further heterogeneity. Nature Genet. *8*, 141–147.

Kannel, W. B., Cupples, A., and D'Agostino, R. B. (1987). Sudden death risk in overt coronary heart diseases: the Framingham study. Am. Heart J. *113*, 799–804.

Keating, M. T. (1992). Linkage analysis and long QT syndrome: using genetics to study cardiovascular disease. Circulation *85*, 1973–1986.

Keating, M. T., Atkinson, D., Dunn, C., Timothy, K., Vincent, G. M., and Leppert, M. (1991a). Linkage of a cardiac arrhythmia, the long QT syndrome, and the Harvey *ras-1* gene. Science *252*, 704–706.

Keating, M. T., Atkinson, D., Dunn, C., Timothy, K., Vincent, G. M., and Leppert, M. (1991b). Consistant linkage of the long QT syndrome to the Harvey *ras-1* locus on chromosome 11. Am. J. Hum. Genet. *49*, 1335–1339.

Koch, M. C., Steinmeyer, K., Lorenz, C., Ricker, K., Wolf, F., Otto, M., Zoll, B., Lehmann-Horn, F., Grzeschik, K.-H., and Jentsch, T. J. (1992). The skeletal muscle chloride channel in dominant and recessive human myotonia. Science *257*, 797–800.

Lathrop, G. M., Lalouel, J.-M., Julier, C., and Ott, J. (1985). Multilocus linkage analysis in humans: detection of linkage and estimation of recombination. Am. J. Hum. Genet. *37*, 482–498.

Lichter, P., Cremer, T., Borden, J., Manuelidis, L., and Ward, D. C. (1988). Delineation of individual human chromosomes in metaphase and interphase cells by *in situ* suppression hybridization using recom-

binant DNA libraries. Hum. Genet. *80*, 224–234.

Ludwig, J., Terlau, H., Wunder, F., Bruggeman, A., Pardo, L. A., Marquardt, A., Strumer, W., and Pongs, O. (1994). Functional expression of a rat homologue of the voltage gated *ether a go-go* potassium channel reveals differences in selectivity and activation kinetics between the *Drosophila* channel and its mammalian counterpart. EMBO J. *13*, 4451–4458.

MacKinnon, R. (1991). Determination of the subunit stoichiometry of a voltage-activated potassium channel. Nature *350*, 232–235.

Marchuk, D., Drumm, M., Saulino, A., and Collins, F. S. (1990). Construction of T-vectors, a rapid and general system for direct cloning of unmodified PCR products. Nucl. Acids Res. *19*, 1154.

Orita, M., Iwahana, H., Kanazawa, H., and Sekiya, T. (1989). Detection of polymorphisms of human DNA by gel electrophoresis as single strand conformation polymorphisms. Proc. Natl. Acad. Sci. USA *86*, 2766–2770.

Ptacek, L. J., George, A. L., Griggs, R. C., Tawil, R., Kallen, R. G., Barchi, R. L., Robertson, M., and Leppert, M. F. (1991). Identification of a mutation in the gene causing hyperkalemic periodic paralysis. Cell *67*, 1021–1027.

Romano, C. (1965). Congenital cardiac arrhythmia. Lancet *1*, 658–659.

Roy, N., Kahlem, P., Dausse, E., Bennaceur, M., Faure, S., Weissenbach, J., Komajda, M., Denjoy, I., Coumel, P., Schwartz, K., and Guicheney, P. (1994). Exclusion of HRAS from long QT locus. Nature Genet. *8*, 113–114.

Schwartz, P. J., Periti, M., and Malliani, A. (1975). The long QT syndrome. Am. Heart J. *109*, 378–390.

Schwartz, P. J., Locati, E. H., Napolitano, C., and Priori, S. G. (1995). The long QT syndrome. In Cardiac Electrophysiology: From Cell to Bedside, D. P. Zipes and J. Jalife, eds. (Philadelphia: W. B. Sanders Company), pp. 788–811.

Sternberg, N. (1990). Bacteriophage P1 cloning system for the isolation, amplification, and recovery of DNA fragments as large as 100 kilobase pairs. Proc. Natl. Acad. Sci. USA *87*, 103–107.

Surawicz, B. (1989). Electrophysiologic substrate of *torsade de pointes*: dispersion of repolarization or early afterdepolarizations? J. Am. Coll. Cardiol. *14*, 172–184.

Towbin, J. A., Li, H., Taggart, T., Lehmann, M. H., Schwartz, P. J., Satler, C. A., Ayyagari, R., Robinson, J. L., Moss, A., and Hejtmancik, F. (1994). Evidence of genetic heterogeneity in Romano–Ward long QT syndrome (LQTs): analysis of 23 families. Circulation *90*, 2635–2644.

Vincent, G. M., Timothy, K. W., Leppert, M. F., and Keating, M. T. (1992). The spectrum of symptoms and QT intervals in carriers of the gene for the long QT syndrome. N. Engl. J. Med. *327*, 846–852.

Ward, O. C. (1964). A new familial cardiac syndrome in children. J. Ir. Med. Assoc. *54*, 103–106.

Warmke, J. E., and Ganetzky, B. (1994). A family of potassium channel genes related to *eag* in *Drosophila* and mammals. Proc. Natl. Acad. Sci. USA *91*, 3438–3442.

Willich, S. N., Levy, D., Rocco, M. B., Tofler, G. H., Stone, P. H., and Muller, J. O. E. (1987). Circadian variation in the incidence of sudden cardiac death in the Framingham heart study population. Am. J. Cardiol. *60*, 801–806.