Am. J. Hum. Genet. 57:1114–1122, 1995

Mapping of a Gene for Long QT Syndrome to Chromosome 4q25-27

Jean-Jacques Schott,[1,2,3] Flavien Charpentier,[3] Sophie Peltier,[4] Patrick Foley,[2] Emmanuel Drouin,[3] Jean-Brieuc Bouhour,[4] Patricia Donnelly,[2] Gilles Vergnaud,[1] Lucien Bachner,[2] Jean-Paul Moisan,[2] Hervé Le Marec,[3] and Olivier Pascal[2]

[1]Laboratoire de Physiopathologie et Pharmacologie Cellulaires et Moléculaires, [2]Laboratoire de Génétique Moléculaire, [3]Laboratoire de Génétique des Espèces, and [4]Clinique Cardiologique, URA CNRS 1340, CHU de Nantes, Nantes; and [5]Service de Bioinformatique, CNRS-INSERM GIS INFOBIOGEN, Villejuif

Summary

Long QT syndrome (LQTS) is a heterogeneous inherited disorder causing syncope and sudden death from ventricular arrhythmias. A first locus for this disorder was mapped to chromosome 11p15.5. However, locus heterogeneity has been demonstrated in several families, and two other loci have recently been located on chromosomes 7q35-36 and 3p21-24. We used linkage analysis to map the locus in a 65-member family in which LQTS was associated with more marked sinus bradycardia than usual, leading to sinus node dysfunction. Linkage to chromosome 11p15.5, 7q35-36, or 3p21-24 was excluded. Positive linkage was obtained for markers located on chromosome 4q25-27. A maximal LOD score of 7.05 was found for marker D4S402. The identification of a fourth locus for LQTS confirms its genetic heterogeneity. Locus 4q25-27 is associated with a peculiar phenotype within the LQTS entity.

Introduction

Idiopathic long QT syndrome (LQTS) is a clinically heterogeneous congenital disease characterized by a prolonged QT interval inducing syncope and risk of sudden death, particularly in association with emotional or physical stress. These stress-induced syncopal episodes are due to a peculiar form of ventricular tachyarrhythmia known as *torsades de pointes* (Moss et al. 1991). In most cases (Romano-Ward syndrome), it is inherited as an autosomal dominant trait. The phenotype is characterized by an obvious prolongation of the QT interval on the electrocardiogram, which shows a value >440 msec when corrected to heart rate (QTc) by Bazett's formula (1920). However, repolarization abnormalities may vary from one electrocardiographic (ECG) recording to another, and there is a large phenotypic spectrum in which delayed repolarization can be less marked. The T-wave morphology is often unusual, showing several different features (bifid or notched T waves, a prominent U wave, etc.) (Moss and Robinson 1992). Several other factors, such as a clinical history of stress-induced or emotional syncope, *torsades de pointes,* a family history of unexplained sudden death, and low heart rate for age are among the diagnostic criteria for LQTS. All these factors have been included in a scoring proposal (Schwartz et al. 1993) to evaluate the probability of LQTS.

Ventricular repolarization is a complex phenomenon resulting from the activation of several currents. Changes in one of these currents may lengthen or shorten repolarization. An increase in inward currents or a decrease in outward currents can prolong cellular repolarization, leading to an LQT phenotype. Such modifications could result from alterations of ionic channels as well as regulatory proteins or receptors, in which case idiopathic LQTS would show great genetic heterogeneity. A first locus was mapped to chromosome 11p15.5 (Keating et al. 1991*a,* 1991*b*), but several families have not shown linkage to this locus (Towbin et al. 1992, 1994; Benhorin et al. 1993; Curran et al. 1993; Dean et al. 1993; Pascal et al. 1993; Satler et al. 1992; Taggart et al. 1993). Two other genes for LQTS have been mapped to chromosomes 7q35-36 and 3p21-24 (Jiang et al. 1994). These three loci have been designated, respectively, as LQT1, LQT2, and LQT3. The genes of LQT2 and LQT3 have recently been identified. Curran et al. (1995) found mutations in the human ether-a-go-go related gene (HERG), which encodes the major subunit for the I_{kr} channel (Sanguinetti et al. 1995), responsible for LQT2, and Wang et al. (1995) found mutations in a sodium channel SCN5A responsible for LQT3. However, some families are not linked to any of these loci, which would indicate the existence of at least a fourth chromosomal locus for LQTS. We report a new localization for a gene implicated in a peculiar form of

Received March 6, 1995; accepted for publication August 9, 1995.

Address for correspondence and reprints: Dr. Olivier Pascal, Laboratoire de Génétique Moléculaire, Hôtel Dieu, BP 1005, 44035 Nantes Cedex, France.

0002-9297/95/5705-0018$02.00

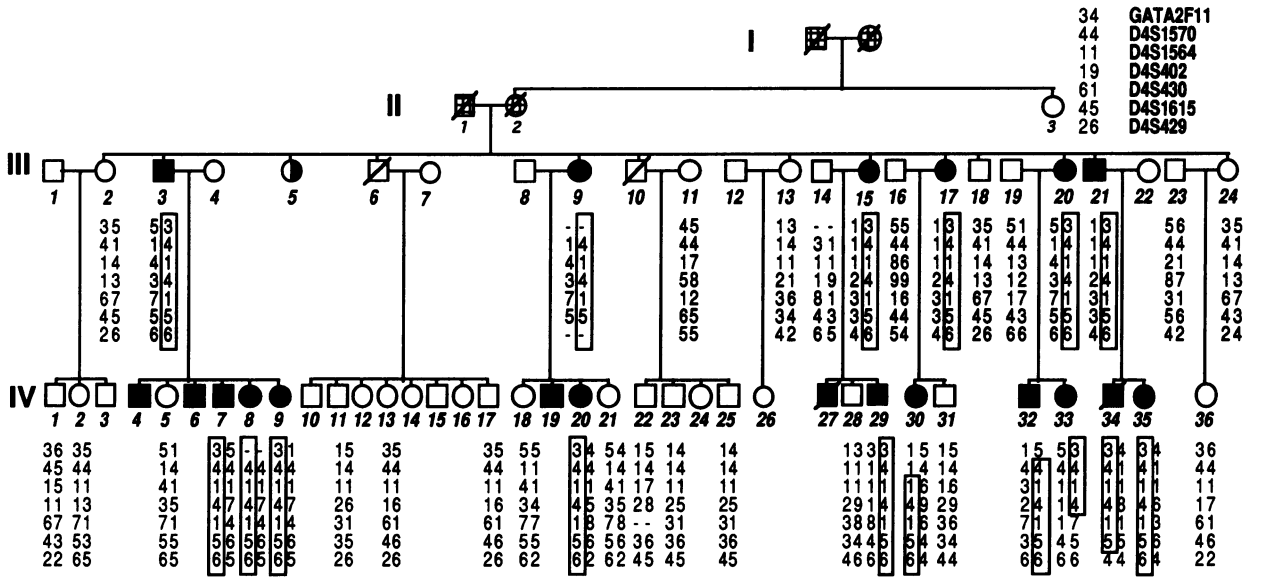

Figure 1 Pedigree and haplotypes of family S affected by LQTS. The markers shown are presented in the upper rigth part of the figure (see table 2).

LQTS in which repolarization abnormalities are associated with severe sinus node bradycardia.

Material and Methods

Phenotypic Analysis of the Family

The four-generation family (family S) described here (fig. 1) includes 65 members, 56 of whom are still alive. Two sudden deaths occurred in this family, the first in a 12-year-old patient after exercise at the top of a dune he had just climbed and the second in an 18-year-old boy on being awakened. The family was referred to us after the second death, and ventricular repolarization abnormalities were identified, leading us to suspect a LQTS.

Two methods were used to characterize the status of family members with respect to LQTS. First, the patients were classified according to the algorithm proposed by Schwartz et al. (1993) (table 1). However, as this algorithm may have limitations, we also applied a more conservative QTc approach based on a method used by Keating et al. (1991*a*, 1991*b*). In that case, the diagnosis of LQT was made on the basis of a QTc ±0.45 s in an individual with symptoms or a QTc ±0.47 s in the absence of symptoms. Disease status was classified as unknown when the QTc was between 0.42 and 0.46 s in asymptomatic patients. ECG parameters were measured on a 12-lead ECG performed at rest.

Based on the algorithm proposed by Schwartz et al. (1993), 21 members (all with uncommon repolarization abnormalities) had high probability scores for LQTS. The conservative approach diagnosed 19 as LQT and 2 as uncertain.

Ventricular repolarization abnormalities were present in 21 of 56 family members (fig. 2). Incorrect QT rate adaptation (QTc interval longer than 0.44 s as calculated by Bazett's formula) was identified by serial ECG. In affected patients, QTc was 0.49 ± 0.03 s (mean ± SD), and its prolongation was particularly marked during recovery from exercise when maximal QTc was 0.59 ± 0.08 s (n=15). In 35 of 56 patients, repolarization was normal and mean QTc was 0.38 ± 0.03 s, with normal adaptation during an exercise stress test and recovery.

The phenotype of this family differed from that common to idiopathic LQTS, since sinus node bradycardia was more severe than usual, atrial fibrillation occurred, and T-wave morphology was uncommon. In all 21 cases, the resting sinus rate was often (though inconsistently) slower than 50 beats per minute (bpm). However, bradycardia was rarely slower than 40 bpm. For each individual, normal sinus rhythm could alternate with sinus bradycardia or junction escape rhythm. Paroxysmal atrial fibrillation occurred in 12 patients. During the maximal exercise stress test, sinus rate rarely exceeded 130 bpm. Because of severe bradycardia during β-blocking therapy, nine affected patients were equipped with a rate-responsive atrial pacemaker. A major finding for the diagnosis of LQTS was that all these patients showed persistent LQT (fig. 3) despite normalization of their atrial rate. Although T-wave morphology could sometimes resemble the type 3 or 4 form (fig. 2) described by Moss and Robinson (1992), it was often very

Table 1

Scores of Family S Members Affected by the LQTS (n = 21), as Determined by the Algorithm of Schwartz et al. (1993)

		III-3	III-5	III-9	III-15	III-17	III-20	III-21	IV-4	IV-6	IV-7	IV-8	IV-9	IV-19	IV-20	IV-27	IV-29	IV-30	IV-32	IV-33	IV-34	IV-35
ECG findings:																						
QTc:																						
480 msec	3		3					3												3		
460–470 msec	2	2		2	2	2	2				2	2	2	2	2		2	2	2		2	2
450 msec$^{1/2}$ (in males)	1								1	1												
Torsade de pointes	2																					
T-wave alternans	1									1			1	1								
Notched T wave in 3 leads	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Low heart rate for ages	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5
Clinical history:																						
Syncope:																						
With stress	2															2					2	
Without stress	1						1	1														
Congenital deafness	.5																					
Family history:									1													
Family members with definite LQTS	1	1	1	1	1	1	1	1		1	1	1	1	1	1	1	1	1	1	1	1	1
Unexplained sudden cardiac death below age 30 among immediate family members	.5																					
Probability of LQTS:																						
Low	≤1																					
Intermediate	2–3																					
High	≥4	4.5	5.5	4.5	5.5	4.5	5.5	6.5	3.5	4.5	4.5	4.5	5.5	5.5	4.5	4.5	4.5	4.5	4.5	5.5	5.5	3.5

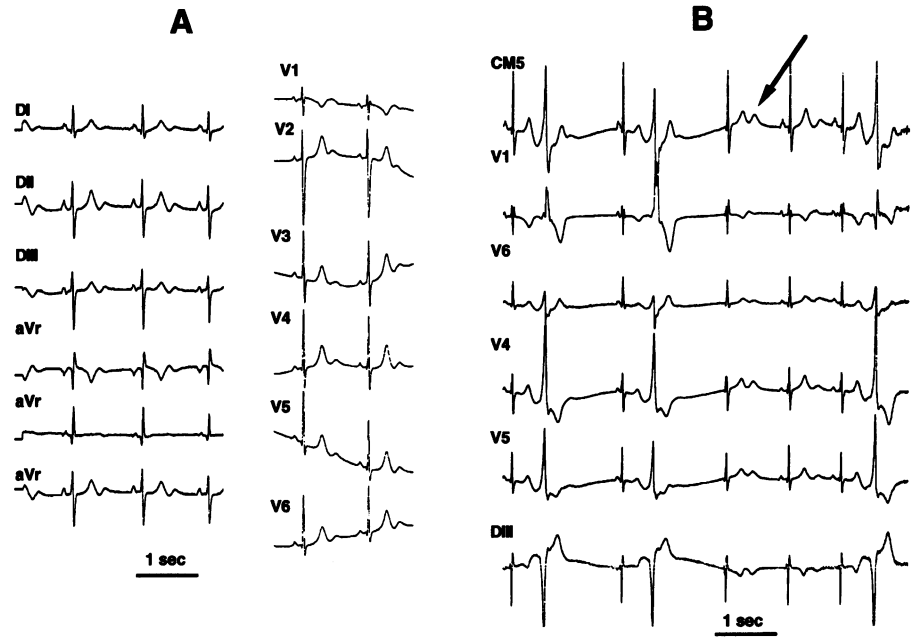

Figure 2 ECGs of a member of family S affected by the disease. ECG recorded at rest, with the patient lying down (*A*) or sitting on a bicycle (*B*). *A*, Sinus rhythm of 57 bpm, and repolarization was characterized by a sinusoidal TU wave well-defined in DII and DIII (QTc = 0.45 s, QTUc = 0.65 s). *B*, ECG showing a sequence of ventricular arrhythmias. Ventricular premature beats (beats 2, 4, 8) occurred at the end of the T wave. The repolarization of the following beat (arrow) was markedly modified with the occurrence of a bifid T wave.

uncommon and characterized by polyphasic features (fig. 3) not described elsewhere.

Determination of the Genotype

After informed consent was obtained, genomic DNA was extracted from blood samples, as described elsewhere (Grunenbaum et al. 1984). All genotyping was performed in 96-well plates on a Techne PHC3 thermal cycler in a final volume of 20 μl, as described elsewhere (James et al. 1994). Amplifications were carried out using the hot-start procedure. Conditions were 96°C for 4 min, followed by 35 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 10 s. After checking amplification products in agarose gels, 6 μl of each sample were loaded onto a 6% denaturing polyacrylamide gel in 1 × TBE buffer. A multiplexing strategy was adopted to reduce the number of gels; that is, several systems revealing different allele sizes were analyzed in the same gel. After capillary transfer to a positively charged nylon membrane (Pall Biodyne B), hybridization was performed using radiolabeled CA oligonucleotides or radiolabeled primers, depending on the multiplexing type.

Linkage Analysis

Linkage analysis was performed using the Linkage V5.2 program (Lathrop and Lalouel 1984) on a 5000/25 DEC station. On the basis of estimates derived elsewhere (Keating et al. 1991*a*), penetrance was set to .90, disease frequency was assumed to be .001, and female and male recombination equivalent. To avoid bias, all polymorphisms were scored without knowledge of phenotypic data.

Results

Exclusion of LQT1, LQT2, and LQT3 Loci

Family S was examined using Hras-1 clone pEJ6.6 (Lidereau et al. 1986), insulin locus (Egland et al. 1987) at 4% recombination from Hras-1 (Leppert et al. 1987), and anonymous markers (D11S899, D11S1338, D11S907, and CEB41) located on chromosome 11p15.5, as well as D7S483, D3S1766, and D3S1768 (Jiang et al. 1994). Negative LOD scores were obtained for all these markers, clearly indicating exclusion of linkage to LQT1, LQT2 and LQT3 (results not shown).

Linkage of the Disease to Chromosome 4

We selected 208 microsatellite markers located every 20 cM and having a reported high heterozygosity rate (Weissenbach et al. 1992). No significant linkage was obtained for 139 markers, leading to the exclusion of ~67% of the genome.

Positive results were obtained for markers located on chromosome 4q25-27. Table 2 shows that maximum LOD scores at $\theta = 0$ were obtained for markers D4S193 ($Z_{max} = 6.67$), D4S406 ($Z_{max} = 6.60$), D4S402 ($Z_{max} = 7.05$), D4S430 ($Z_{max} = 4.89$), and D4S1615 ($Z_{max} = 4.32$). Haplotype analysis showed a recombination

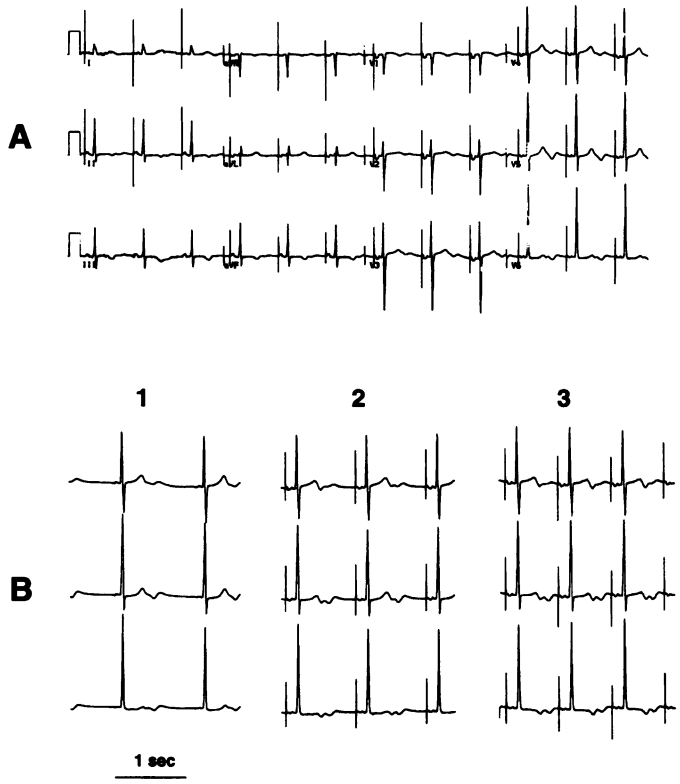

Figure 3 *A*, Electrocardiogram of an affected patient equipped with a rate-responsive atrial pacemaker. Despite normalization of atrial rhythm (70 bpm), repolarization remained prolonged (QTUc= 0.69 s) and abnormal. *B*, In the same patient, at rest, the pacemaker was programmed to different atrial rates from 51 (spontaneous atrial rate 1) to 60 (2) and 80 (3) bpm. Only leads V4–V6 are shown. The QTUc remained prolonged (0.69 s) at the different atrial rates.

event for D4S430 despite a maximum LOD score at 0% recombination (fig. 1). As probands III-19, III-20, and IV-33 had the same alleles 1 and 7, the maternal origin for proband IV-33 was impossible to assess. D4S1571 and D4S1564 were poorly informative. D4S1570 showed a recombination for proband IV-30. Thus, the candidate area was bordered by D4S1570 on the centromeric side and by D4S430 on the telomeric side, covering 18 cM (fig. 4).

Multipoint Data Analysis

Four-point lod scores between the disease and two sets of three markers have been computed with the program Linkmap from the Fastlink package (Cottingham et al. 1993; Schaffer et al. 1994). Computations have been done on a Sparc Server 1000 at the Infobiogen Computing Center. Results are in accordance with two-point lod scores (table 3). The lack of recombinants has prevented a more precise mapping of the disease.

Discussion

Although a single gene is often affected in genetic diseases, different genes may be involved in the same disease. This is the case for hypertrophic cardiomyopathy, in which four or more genes are implicated (Solomon et al. 1990; Thierfelder et al. 1994; Hengstenberg and Schwartz 1994), and is also apparently the case for LQTS. Several families, despite a first localization on chromosome 11p15.5 (Keating et al. 1991*b*), have shown no linkage to this locus, and two other loci have recently been located on chromosomes 7q35-36 and 3p21-24 (Jiang et al. 1994). It is not surprising that LQTS is a heterogeneous disorder, since cellular repolarization results from a cascade of events implicating several ionic currents. Modifications of one of these currents can lead to the same clinical entity (LQTS).

Our study was performed on a four-generation family affected by LQTS. Initially, no positive linkage was found using the technique of RFLP or that of VNTR revealed with radiolabeled probes. Subsequently, the use of Généthon markers (Weissenbach et al. 1992) and other highly polymorphic tetranucleotide repeat markers regularly spaced on the genome led us to exclude 11p15.5, 7q35-36, and 3p21-24, loci as well as ~67% of the genome. Positive linkage was obtained with five markers located on chromosome 4q25-q27 when LQTS pedigrees were used to describe the disease locus.

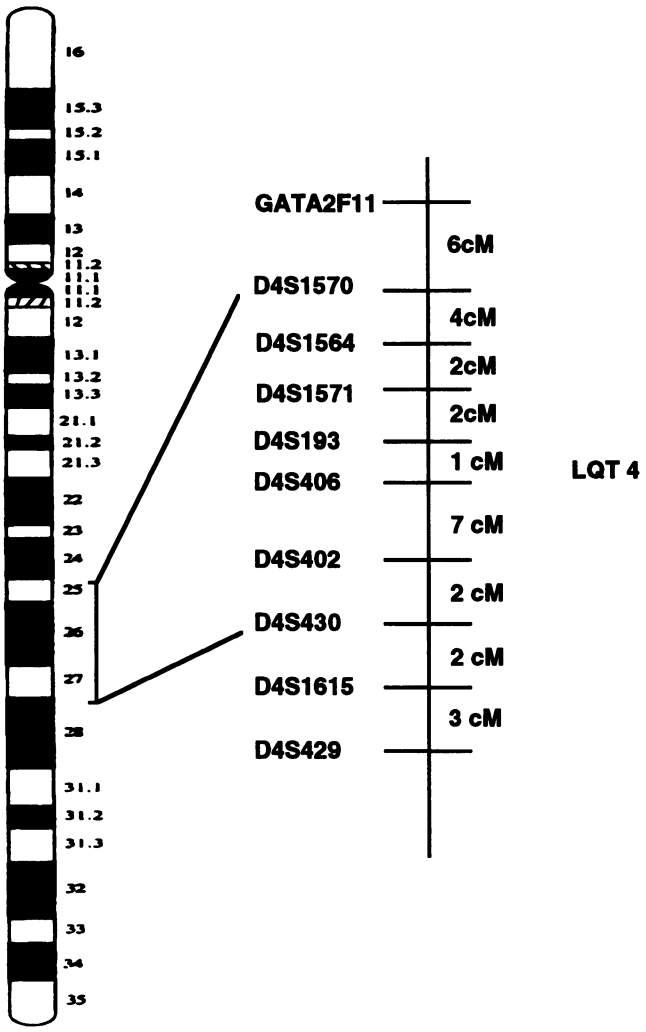

Figure 4 Ideogram and genetic map of chromosome 4 showing the location of the disease gene and the four linked markers at $\theta = 0$. The loci order and their genetic distance are those determined by linkage analysis in the CEPH families.

Identification of the phenotype is a crucial step when linkage analysis is used in genetic mapping. This is particularly true for LQTS, for which the identification of affected patients is difficult. However, during the past decade new insights into the clinical profile of LQTS have improved diagnostic accuracy for this syndrome. For instance, it has emerged that QTc (at the basis of the phenotype) is significantly longer in women than men and that QTc duration overlaps between normal and affected patients. Furthermore, QTc can be normal in some carriers of the LQTS gene (Vincent et al. 1992). It would also appear that the spectrum of clinical abnormalities observed in LQTS is larger than previously thought, and includes abnormal T-wave morphology and lower-than-normal heart rate, especially in children. On the basis of accumulated data, the diagnostic criteria for LQTS were reconsidered by Schwartz et al. (1993), who proposed a new scoring algorithm to improve LQTS diagnosis. Using these new criteria, we found that 21 members of family S had high probability scores for LQTS. The same scoring technique was applied to the other members of the family, but none reached a score of 2, which led to their exclusion as affected patients. However, the algorithm proposed by Schwartz et al. (1993) may have limitations, especially in families already identified, since 25% of the score is automatically attributed for a positive family history. For this reason, we also applied a more conservative approach to characterize the status of family members with respect to LQTS. With this approach, 19 members were found to be affected, and the statuses of 2 members were considered to be uncertain. Though the LOD score calculated for the two approaches was different, significant linkage to chromosome 4q25-27 was apparent for both.

In light of the result of the linkage study, 21 family members were considered to be affected by LQTS inherited as an autosomal dominant trait. However, the phenotype of this family differed from the common one for idiopathic LQTS, since sinus node bradycardia was more severe than usual, leading to sinus node dysfunction (SND), and the T wave was uncommon. It is thus hardly surprising that the disease concerned a locus other than the three described elsewhere.

This unusual phenotype raised several questions. The first was whether LQT in our family was a genetic disease or the result of SND. Repolarization abnormalities (acquired LQTS) leading to *torsades de pointes* have been described in patients with severe bradycardia, i.e., during complete AV block, but not in those with congenital SND (Lehman 1978; Kugler 1991). When β-blocking drugs were introduced in our family, the presence of severe sinus node bradycardia led to implantation of a rate-responsive atrial pacemaker in nine affected patients. The persistence of repolarization abnormalities in these patients, despite normalization of their atrial rate (fig. 2*B*), clearly shows that LQT did not result from sinus bradycardia.

The second question was whether bradycardia and LQT are related to the same genetic defect or result from two different genetic alterations. Although the two different loci responsible for these phenotypic features could have been associated in the same family, it is also possible that we were confronted with a monogenic disease affecting both automaticity and repolarization. This second hypothesis appeared more likely for several reasons. First, we observed a constant association of both abnormalities in the affected patients. Indeed, no family members with normal sinus node rhythm had repolarization abnormalities, and all members with LQTS had sinus bradycardia. If two genetic defects were involved, the two loci coding for them should have been so closely

Table 2

Markers Located on q24-q27: LOD Scores for Various Recombination Percentages

	LOD Scores[a] at θ =						
Markers	0	.01	.05	.1	.2	.3	.4
GATA2F11	−6.03 (−∞)	−2.03 (−2.68)	−.67 (−.77)	−.15 (−.11)	.18 (.28)	.17 (.25)	.02 (.02)
D4S1570	−1.73 (−∞)	−2.03 (.95)	−.67 (1.5)	−.15 (1.58)	.18 (1.35)	.17 (.91)	.02 (.32)
D4S1564	1.67 (1.70)	1.66 (1.69)	1.58 (1.63)	1.45 (1.51)	1.12 (1.18)	.71 (.75)	.22 (.24)
D4S1571	.90 (1.04)	.91 (1.04)	.93 (1.04)	.91 (.99)	.76 (.81)	.48 (.52)	.13 (.16)
D4S193	6.01 (6.67)	5.92 (6.57)	5.55 (6.14)	5.06 (5.58)	3.92 (4.33)	2.61 (2.90)	1.09 (1.25)
D4S406	6.08 (6.60)	5.99 (6.51)	5.63 (6.13)	5.13 (5.60)	4.00 (4.38)	2.67 (2.96)	1.13 (1.28)
D4S402	6.49 (7.05)	6.38 (6.93)	5.93 (6.46)	5.35 (5.83)	4.10 (4.49)	2.70 (2.99)	1.13 (1.28)
D4S430	5.37 (4.89)	5.30 (4.86)	4.99 (4.67)	4.56 (4.33)	3.55 (3.45)	2.35 (2.32)	.95 (.94)
D4S1615	3.61 (4.32)	3.56 (4.26)	3.34 (4.01)	3.04 (3.01)	2.33 (2.80)	1.48 (1.80)	.51 (.64)
D4S429	−1.14 (−∞)	.83 (1.25)	1.38 (1.78)	1.47 (1.83)	1.25 (1.53)	.79 (.97)	.22 (.27)

[a] LOD Scores calculated with the conservative approach are given. Those calculated with the algorithm of Schwartz et al. (1993) are given in parentheses.

related that recombination could not have occurred. The hypothesis of a monogenic disease is also supported by the fact that sinus bradycardia, with heart rates comparable to those observed in our family, is frequent in LQTS (Schwartz et al. 1993; Kugler 1991).

The third question concerns the morphology of the T wave, which appeared to be very uncommon, showing polymorphic features that were particularly marked for the precordial leads (fig. 3). To date, no studies have sought to associate a specific feature or a specific behavior of ventricular repolarization with a specific gene alteration. However, with the development of genotyping in LQTS and the identification of genes, it is quite likely that clinical studies will improve the phenotypic identification of LQTS.

From a theoretical point of view, multiple abnormalities could lead to a LQT phenotype. A lengthening of repolarization could result from alteration of membrane channels, whether responsible for an inward (depolarizing) or outward (repolarizing) current, or from alteration of regulatory proteins. Recently, Wang et al. (1995) found mutations in a sodium channel SCN5A causing LQT3 characterized by a deletion of three amino acids in a putative inactivation region. This mutation could lead to delayed inactivation of the Na inward current. In several families linked to LQT2, Curran et al. (1995) found different mutations in a HERG. This gene encodes the major subunit for the potassium channel I_{kr} (Sanguinetti et al. 1995), which is the target for arrhythmogenic effects of several drugs that prolong repolarization (acquired LQT). These mutations included deletion of the cyclic nucleotide-binding domain or alteration of the secondary structure of the protein, both of which could affect the process of repolarization of cardiac. The alteration of these two genes results in an increase in net inward current and prolonged repolarization. In case of an increase in beta-adrenergic tone, prolonged repolarization could promote reactivation of L-type Ca^{++} channels, thereby leading to early afterdepolarizations (January and Riddle 1989) which are thought to be the cause of *torsades de pointes*.

Table 3

Maximum Multipoint LOD Scores

Z_{max}	θ	Markers Map[a]
5.97	0	11=5-6-7
6.01	0	5-11=6-7
6.75	0	11=7-8-9
4.89	0	7-8=11-9
4.92	0	7-8-9=11

[a] An equals sign (=) represents a test interval. 5 = D4S103; 6 = D4S406; 7 = D4S402; 8 = D4S430; 9 = D4S1615; and 11 = the morbid locus. Recombination fraction for these markers are as follows: .01 between markers 5 and 6, .07 between 6 and 7, .02 between 7 and 8, and .02 between 8 and 9.

In our family, the presence of sinus bradycardia associated with a prolonged QT interval could have resulted from the alteration of a protein involved in both automaticity and repolarization. The candidate area without recombination is as large as 18 cM, and, up to now, there are no genes coding for ionic channels localized in this area. By PCR screening of a YAC contig of this area with oligonucleotides described by Kamb et al. (1989) we were unable to find sequences of genes coding for K^+ channels. Because of the role of the autonomic nervous system in modulating the repolarization, it could be hypothesized that a membrane receptor or a regulatory protein may be involved in this syndrome. A gene encod-

ing for an endothelin-A receptor has been assigned to chromosome 4 (Hosoda et al. 1992). It could have been a good candidate, as it plays a role in the regulation of repolarization and automaticity (Ono et al. 1994). However, we have excluded it from the candidate area by FISH mapping (authors' unpublished results). We assigned an expressed sequence tag (EST) (clone 81q10, EMBL F00219, from the "Genexpress cDNA program") to four overlapping YACs positive for the marker D4S1611, which is located between markers D4S406 and D4S402. This EST shows high homology with a gene coding for a rat δ isoform of a Ca^{++}/calmodulin-dependent protein kinase II (authors' unpublished results). This protein kinase II isoform is implicated in the phosphorylation (activation) of a delayed outward potassium current (Onozuka et al. 1991) as well as an anionic (chloride) channel (Fuller et al. 1994)—both of these being possibly involved in the repolarization process. The Ca^{++}/calmodulin-dependent protein kinase II is activated by an increase in intracellular calcium that can result, for example, from the activation of the adrenergic system. Genetic modifications of the Ca^{++}/calmodulin-dependent protein kinase II could alter the activation of repolarizing currents that could, in turn, result in a decrease in a net repolarizing current. Therefore, the Ca^{++}/calmodulin-dependent protein kinase II could become a good candidate for the genetic defect of this family.

Conclusion

We mapped a new gene associating LQTS with a more severe form of sinus bradycardia than usual. This genetic defect, as well as those of classical Romano-Ward syndrome, might cause sudden death. Inherited prolonged repolarization, regarded as idiopathic LQTS, would appear to be a final common entity in which several proteins are involved. To date, two genes coding for LQTS have been identified. Their identification is of considerable value for an understanding of abnormal ventricular repolarization, which is apparently an important predictive factor for the risk of arrhythmia and sudden cardiac death (Schwartz and Wolf 1978; Day et al. 1990; Barr et al. 1994).

Acknowledgments

This study was supported in part by grants from G.R.E.G. 77, AFM, Atlangene, EUROGEM, the Nantes University Hospital, and INSERM CRE 92-0412, France. We would like to thank Dr. C. Auffray, from the "Genexpress cDNA program," for giving us the ESTs, and Mr. J. Gray for correcting the manuscript.

References

Barr CS, Naas A, Freeman M, Lang CC, Struthet AD (1994) QT dispersion and sudden unexpected death in chronic heart failure. Lancet 343:327–329

Bazett HC (1920) An analysis of the time-relations of electrocardiograms. Heart 7:353–370

Benhorin J, Kalman YM, Medina A, Towbin J, Rave-Harel N, Dyer TD, Blangero J, et al (1993) Evidence of genetic heterogeneity in the long QT syndrome. Science 260:1960–1962

Cottingham RW Jr, Idury RM, Schäffer AA (1993) Faster sequential genetic linkage computations. Am J Hum Genet 53:252–263

Curran ME, Atkinson D, Timothy K, Vincent GM, Moss AJ, Leppert M, Keating MT (1993) Locus heterogeneity of autosomal dominant long QT syndrome. J Clin Invest 92:799–803

Curran ME, Splawski I, Timothy KW, Vincent GM, Green ED, Keating MT (1995) A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. Cell 80:795–803

Day CP, McComb JM, Cambell RWF (1990) QT dispersion: an indication of arrhythmia risk in patients with long QT intervals. Br Heart J 63:342–344

Dean JCS, Cross S, Jennings K (1993) Evidence of genetic and phenotypic heterogeneity in the Romano-Ward syndrome. J Med Genet 30:947–950

Egland J, Gerhard D, Pauls D, Sussex J, Kidd K, Allen C, Hostetter A, et al (1987) Bipolar affective disorders linked to DNA markers on chromosome 11. Nature 325:783–787

Fuller CM, Ismailov II, Keeton DA, Benos DJ (1994) Phosphorylation and activation of a bovine tracheal anion channel by Ca^{2+}/Calmodulin-dependent protein kinase II. J Biol Chem 269:26642–26649

Grunenbaum L, Cazenave JP, Camerino G, Kloepfer C, Mandel JL, Tolskoshev P, Jaye M, et al (1984) Carrier detection of hemophiliae B by using a restriction site polymorphism associated with the coagulation factor IX gene. J Clin Invest 73:1491

Hengstenberg C, Schwartz K (1994) Molecular genetics of familial hypertrophic cardiomyopathy. J Mol Cell Cardiol 26:3–10

Hosoda K, Nakao K, Tamura N, Arai H, Ogawa Y, Suga SI, Nakanishi S, et al (1992) Organization, structure, chomosomal assignment, and expression of the gene encoding the human endothelin-A receptor. J Biol Chem 287:18797–18804

James MR, Richard CW, Schott JJ, Yousry C, Clark K, Bell J, Terwilliger JD, et al (1994) A radiation hybrid map of 506 STS markers spanning human chromosome 11. Nat Genet 8:70–76

January CT, Riddle JM (1989) Early afterdepolarizations: mechanism of induction and block. Circ Res 64:977–990

Jiang C, Atkinson D, Towbin J, Splawski I, Lehmann M, Li H, Timothy K, et al (1994) Two long QT syndrome loci maps to chromosomes 3 and 7 with evidence for further heterogeneity. Nat Genet 8:141–147

Kamb A, Weir M, Rudy B, Warmus H, Kenyon C (1989) Identification of genes from pattern formation, tyrosine ki-

nase, and potassium channel families by DNA amplification. Proc Natl Acad Sci USA 86:4372–4376

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

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

Kugler JD (1991) Sinus nodal dysfunction in young patients with long QT syndrome. Am Heart J 121:1132–1136

Lathrop GM, Lalouel JM (1984) Easy calculation of Lod scores and genetic risks on small computers. Am J Hum Genet 36:460–465

Lehman H (1978). Familial sinus node dysfunction with autosomal dominant inheritance. Brit Heart J 40:1314–1316

Leppert M, O'Connel LP, Nakamura Y, Lathrop GM, Malsen C, Litt M, Cartwright P, et al. (1987) A partial primary genetic linkage map of chromosome 11. In: Karger S (ed) Human Gene Mapping 9. Postfach, Basel, p 648

Lidereau R, Escot C, Theillet C, Champeme MH, Brunet M, Gest J, Callahan R (1986) High frequency of rare alleles of the human Harvey ras 1 protooncogene in breast cancer patients. J Natl Cancer Inst 77:697–700

Moss AJ, Robinson J (1992) Clinical features of the idiopathic long QT syndrome. Circulation Suppl 85:I-140–I-144

Moss AJ, Schwartz PJ, Crampton RS, Tzivoni D, Locati EH, MacCluer J, Hall WJ, et al (1991) The long QT syndrome: prospective longitudinal study of 328 families. Circulation 84:1136–1144

Ono K, Tsujimoto G, Sakamoto A, Eto K, Masaki T, Ozaki Y, Satake M (1994) Endothelin-A receptor mediates cardiac inhibition by regulating calcium and potassium currents. Nature 370:301–304

Onozuka M, Furuichi H, Imai S, Fukami Y (1991) Evidence that Ca^{2+}/calmodulin-dependent protein phosphorylation is involved in the opening process of potassium channels in identified snail neurons. Neurosci Lett 124:35–38

Pascal O, Peltier S, Herbert O, Le Marec H, Moisan JP, Bouhour JB (1993) Genetic heterogeneity of cardiac diseases. J Cell Biochem 17 (suppl D): 202

Sanguinetti MC, Jiang C, Curran ME, Keating MT (1995) A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the Ikr potassium channel. Cell 81:299–307

Satler C, Walsh E, Korf B, Nadal-Ginard B (1992) Linkage analysis with Hras-1 probe identifies genetic heterogeneity of the long QT syndrome. Circulation Suppl 86:I-348

Schaffer AA, Gupta SK, Shriram K, Cottingham RW Jr (1994) Avoiding recomputation in genetic linkage analysis. Hum Hered 44:225–237

Schwartz PJ, Moss AJ, Vincent GM, Crampton RS (1993) Diagnostic criteria for the long QT syndrome: an update. Circulation 88:782–784

Schwartz PJ, Wolf S (1978) QT interval prolongation as predictor of sudden death in patients with myocardial infarction. Circulation 57:1074–1077

Solomon SD, Jarcho JA, McKenna W, Geisterfer-Lowrance A, Germain R, Salerni R, Seidman JG, et al (1990) Familial hypertrophic cardiomyopathy is a genetically heterogeneous disease. J Clin Invest 86:993–999

Taggart RT, Smith SD, Frankovitch P, O'Brien C, Fresard JA, Lehman MH (1993) Identification of Romano-Ward type long QT syndrome (LQTS) families that are not linked to chromosome 11p15.5. J Am Coll Cardiol 21:477A

Thierfelder L, Watkins H, MacRae C, Lamas R, McKenna W, Vosberg HP, Seidman JG, et al (1994) Alpha-tropomyosin and cardiac troponin T mutations cause familial hypertrophic cardiomyopathy: a disease of the sarcomere. Cell 77:701–712

Towbin JA, Li H, Taggart RT, Lehmann MH, Schwartz PJ, Satler CA, Ayyagari R, et al (1994) Evidence of genetic heterogeneity in Romano-Ward long QT syndrome. Circulation 90: 2635–2644

Towbin JA, Pagotto L, Siu B, Robinson JL, McCabe ERB, Hejtmancik JF (1992) Romano-Ward long QT syndrome (RWLQTS): evidence of genetic heterogeneity. Pediatr Res 31: 23A

Vincent GM, Timothy KW, Leppert BS, Keating M (1992) The spectrum of symptoms and QT intervals in carriers of the gene for the long QT syndrome. N Engl J Med 327:846–52

Wang Q, Shen J, Splawski I, Atkinson D, Li Z, Robinson JL, Moss AJ, et al (1995) SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. Cell 80:805–811

Weissenbach J, Gyapay G, Dib C, Vignal A, Morissette J, Milasseau P, Vaysseix G, et al (1992) A second generation linkage map of the human genome. Nature 359:794–801