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ORIGINAL INVESTIGATION

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The long QT syndrome: a novel missense mutation in the S6 region of the KVLQT1 gene

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Abstract The Romano Ward long QT syndrome (LQTS) has an autosomal dominant mode of inheritance. Patients suffer from syncopal attacks often resulting in sudden cardiac death. The main diagnostic parameter is a prolonged QT_c interval as judged by electro-cardiographic investigation. LQTS is a genetically heterogeneous disease with four loci having been identified to date: chromosome 11p15.5 (LQT1), 7q35–36 (LQT2), 3p21–24 (LQT3) and 4q25–26 (LQT4). The corresponding genes code for potassium channels KVLQT1 (LQT1) and HERG (LQT2) and the sodium channel SCN5A (LQT3). The KVLQT1 gene is characterized by six transmembrane domains (S1–S6), a pore region situated between the S5 and S6 domains and a C-terminal domain accounting for approximately 60% of the channel. This domain is thought to be co-associated with another protein, viz. minK (minimal potassium channel). We have studied a Romano Ward family with several affected individuals showing a severe LQTS phenotype (syncope and occurrence of sudden death). Most affected individuals had considerable pro-

longations of QT_c. By using haplotyping with a set of markers covering the four LQT loci, strong linkage was established to the LQT1 locus, whereas the other loci (LQT2, LQT3 and LQT4) could be excluded. Single-strand conformation polymorphism analysis and direct sequencing were used to screen the KVLQT1 gene for mutations in the S1–S6 region, including the pore domain. We identified a Gly-216-Arg substitution in the S6 transmembrane domain of KVLQT1. The mutation was present in all affected family members but absent in normal control individuals, providing evidence that the mutated KVLQT1-gene product indeed caused LQTS in this family. The mutated KVLQT1-gene product thus probably results in a dominant negative suppression of channel activity.

Introduction

Cardiac ventricular arrhythmias are the main cause of sudden death in the Western population. Most often structural abnormalities underlie the arrhythmia but, occasionally, abnormalities in cardiac action potential repolarization, visualized as a prolonged QT interval, are of importance. The long QT syndromes (LQTS) include acquired and inherited forms.

Two familial LQTS have been described: Jervell and Lange-Nielsen syndrome and Romano Ward syndrome. The rare Jervell Lange-Nielsen syndrome is an autosomal recessive hereditary cardiac arrhythmogenic disorder, associated with prolongation of the QT interval and congenital deafness (Jervell and Lange-Nielsen 1957). The more common Romano Ward syndrome, identified by Romano (1965) and Ward (1964), shows an autosomal dominant mode of inheritance and is not associated with congenital deafness. The prevalence is assumed to be 1:10 000 to 1:15 000 (Wang et al. 1995).

LQTS is associated with a high risk of sudden cardiac death caused by ventricular arrhythmia. Patients suffer from ventricular arrhythmia (tachycardia, torsade de pointes, leading to syncope) or sudden death. Diverse other ECG

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and clinical aspects have been observed, including T-wave alterations and sinus bradycardia.

The disease is genetically heterogeneous. Loci have been mapped to four chromosomal regions (Keating et al. 1991; Jiang et al. 1994; Schott et al. 1995): 11p15.5 (LQT1), 7q35–36 (LQT2), 3p21–24 (LQT3) and 4q23–24 (LQT4). Additional loci are indicated by the absence of linkage to the LQT1, 2, 3 and 4 loci in established LQTS families. Recently, the genes have been identified for the LQT1 (KVLQT1), LQT2 (HERG) and LQT3 (SCN5A) loci (Wang et al. 1995, 1996; Curran et al. 1995). In this study, we report an LQTS family with a novel mutation in the voltage-gated potassium channel gene, KVLQT1.

Materials and methods

Pedigree

This study was based on a four-generation family of Dutch origin; the pedigree is shown in Fig. 1. The sudden death of a boy at the age of 9 years prompted a family study for LQTS. Clinical features observed in several relatives indicated the Romano Ward syndrome. Therefore, clinical evaluation and genetic analysis of this pedigree was performed.

Clinical diagnostic tests and evaluation

Patients, their relatives at risk and their relatives by marriage were screened by 12-lead electrocardiography (ECG). The clinically defined (affection) status of individuals was obtained by combining the ECG and clinical evaluations according to the criteria of

Schwartz et al. (1993). Patients were screened by Holter monitoring and exercise stress tests.

Haplotyping

Genomic DNA was extracted from peripheral blood lymphocytes by the high salt extraction method (Müllerbach et al. 1989). DNA haplotyping for the four LQT loci was performed by using a set of 10 polymorphic microsatellite markers. The LQT1 locus was haplotyped by using markers D11S1318, TH01 and D11S860, the LQT2 locus with markers D7S483 and D7S636, the LQT3 locus with markers D3S1298 and D3S1100, and the LQT4 locus with markers D4S193, D4S406 and D4S402. The oligonucleotide sequences of the microsatellite markers were obtained from the Genome Database (GDB). DNA fragments were amplified in vitro by the polymerase chain reaction (PCR) according to standard procedures (Perkin and Elmer apparatus) and further analysed on an ABI-377 automatic sequencer using Gene-Scan software.

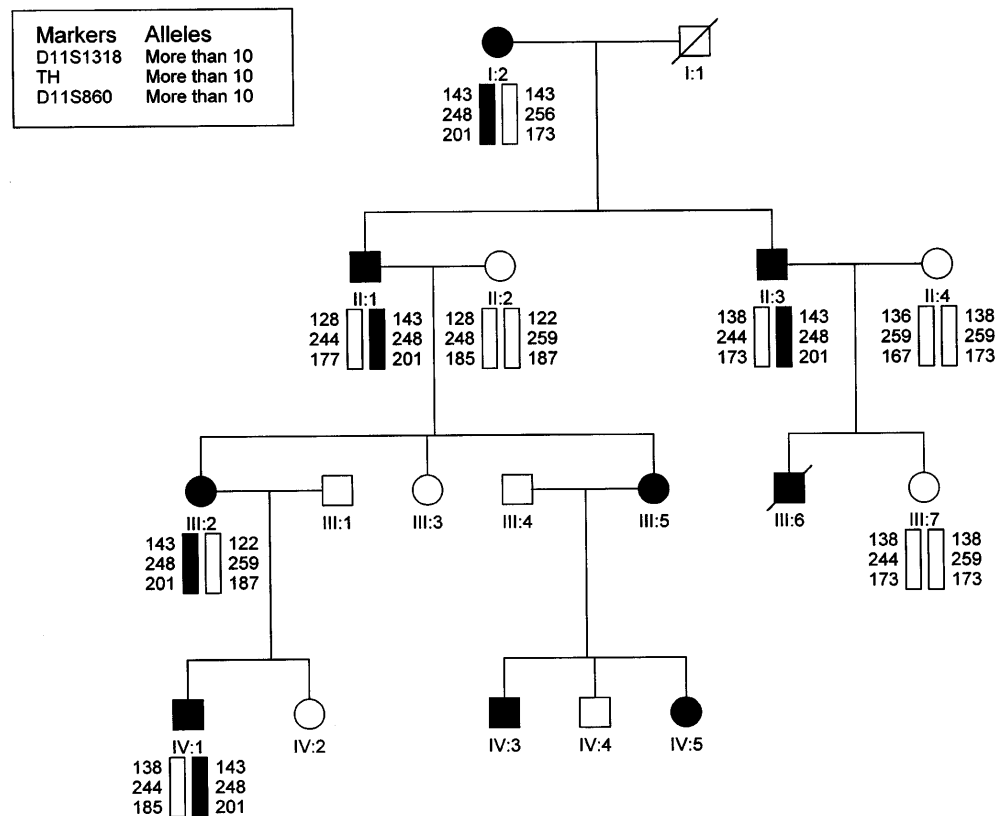
Linkage analysis

Two-point linkage analysis was performed with the Linkage v5.1 computer program (Lathrop et al. 1985). A disease penetrance of 0.9 and a gene frequency of LQTS of 0.001 were assumed (Keating et al. 1991). Allele frequencies were obtained from data provided by the depositor or publisher of the DNA markers (GDB; Weissenbach et al. 1992).

Mutation analysis

Genomic fragments coding for the S2–S6 transmembrane domains (including the pore region) were amplified by using the primer sets described by Wang et al. (1996). Fragments were amplified on a Perkin and Elmer 9600 PCR thermal cycler in the presence of 100

Fig. 1 Pedigree of the LQTS family. *Solid bar* Risk allele, *open bar* normal allele. The *bars* are flanked by the haplotype information of the LQT1 markers



ng forward and reverse primers, 0.2 mM deoxyribonucleotide, 1 U *Taq* DNA polymerase (BRL), 1.5 mM MgCl₂ and 100 ng genomic DNA (volume 50 µl). Subsequently, PCR fragments were screened for single-strand conformation polymorphism analysis (SSCP) by using the GenePhor System (Pharmacia Biotech). Gene Gel Exel 12.5/24 SSCP gels were run at 10°C according to standard procedures. Gels were silver-stained according to the instructions of the manufacturer.

The sequences of genomic fragments were analysed by using the dye terminator sequencing procedure and an ABI-377 automatic sequencer. In the sequence analysis, identical primers were used as in SSCP and primary PCR analysis.

Results

Clinical analysis

All family members at risk for LQTS were clinically evaluated as described above. The clinical evaluation of the patients participating in the study is described in Table 1. All but one (II.3) of the affected family members met the LQT criteria for high probability. Furthermore, the sex differences in QT values previously found in LQTS patients (Vincent et al. 1992) were also present in this family. The trigger for syncope and sudden death (one indi-

vidual) was most often exercise. The pedigree (Fig. 1) suggested an autosomal dominant inheritance pattern.

Linkage study

In order to identify the locus involved in LQTS, genetic analysis was performed by using the markers described above. Results of the haplotyping for the LQT1 locus are shown in Fig. 1. Haplotyping resulted in significant linkage to the LQT1 locus and exclusion of LQT2, 3 and 4 (Table 2). Our subsequent analysis of the KVLQT1 gene for mutations was based on the linkage data.

Mutation analysis

SSCP analysis revealed a mobility shift in the PCR fragment derived from the S6 transmembrane domain of KVLQT1 of affected individuals (Fig. 2). The normal and aberrant PCR fragments were sequenced and a substitution of G to C at position 617 (based on the Genbank: U40990 sequence) was identified in exon 7 (Fig. 3).

This mutation gives rise to a Gly→Arg substitution at amino acid 216 and also disrupts a *Bst*NI restriction site in genomic DNA (not in cDNA). Restriction enzyme analysis of the normal S6 PCR product gave rise to three fragments (26, 42 and 121 bp), whereas the mutant allele resulted in 26 and 163 bp fragments, respectively. The *Bst*NI digestion patterns of the S6 PCR fragments of patients, their relatives and controls are shown in Fig. 4. As predicted, both the 163 bp and the 121 bp fragments are visible in the patients, whereas no 163 bp fragment is observed in the controls and non-affected relatives (26 and 42 bp fragments are barely visible).

Discussion

By using haplotyping, linkage to the LQT1 locus has clearly been demonstrated in a family with LQTS, and loci LQT2, 3 and 4 have been excluded. The KVLQT1 gene has also been screened for mutations by SSCP and sequence analysis and a Gly-216-Arg mutation has been detected in the S6 domain of the gene. To eliminate the possibility that the observed mutation is a normal allelic

Table 1 Clinical evaluation of the family with LQT1

ID	Sex	QT _C	LQT criteria ^a	Trigger ^b
I.2	F	540	5.5	Undefined
II.1	M	450	4.5	Exercise
II.3	M	440	3.5	Exercise
III.2	F	520	7.5	Exercise & emotion
III.3	F	400	3	–
III.5	F	550	6.5	Exercise
III.6	M	480	6.5	Exercise, sudden death at age 9
III.7	M	420	1.5	–
IV.1	M	450	6.5	–
IV.2	F	440	1.5	–
IV.3	M	520	5.5	Exercise
IV.4	M	360	1.5	–
IV.5	F	510	5.5	–

^aCriteria according to Schwartz et al. (1983)

^bTrigger of syncope

Table 2 Two point linkage analysis of the LQT1, 2, 3, 4 loci

Thetas	D11S1318	TH	D11S860	D7S483	D7S636	D3S1298	D3S1100	D4S193	D4S402	D4S406
0.000	0.86	1.16	0.86	–5.10	–5.07	–0.44	–3.08	–0.22	–0.78	–4.61
0.001	0.86	1.16	0.86	–3.13	–2.14	–0.44	–3.08	–0.22	–0.77	–2.39
0.005	0.86	1.15	0.85	–2.42	–1.45	–0.42	–3.04	–0.22	–0.74	–1.70
0.01	0.85	1.14	0.84	–2.10	–1.15	–0.41	–2.94	–0.22	–0.70	–1.40
0.05	0.80	1.05	0.76	–1.32	–0.50	–0.32	–1.98	–0.23	–0.49	–0.72
0.1	0.73	0.94	0.66	–0.95	–0.27	–0.25	–1.34	–0.21	–0.33	–0.44
0.2	0.58	0.72	0.48	–0.55	–0.09	–0.16	–0.68	–0.13	–0.15	–0.19
0.3	0.42	0.48	0.31	–0.33	–0.03	–0.11	–0.32	–0.04	–0.06	–0.08
0.4	0.22	0.24	0.16	–0.16	–0.01	–0.06	–0.11	0.00	–0.01	–0.02

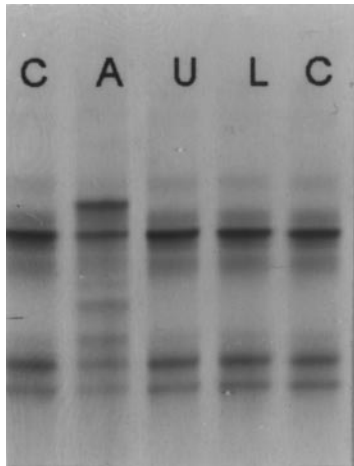


Fig. 2 SSCP analysis of genomic DNA from normal and affected individuals. C Unaffected control, A affected family member, U unaffected family member, L an unrelated LQTS patient of a family linked to the LQT1 locus

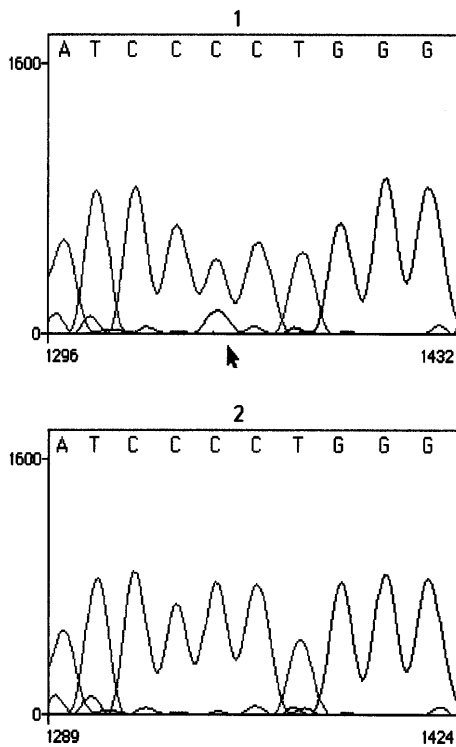


Fig. 3 Sequence analysis of the S6 region of KVLQT1. The S6 region of the KVLQT1 gene of an affected individual (II.1) and an unaffected individual was amplified by PCR. This PCR fragment was used for cycle sequencing. The reverse sequences of both the affected (1) and unaffected (2) individuals are shown. The sequences reveal a G at position 617 in the healthy individual but a G+C (arrow) in the affected person indicating a G to C substitution in one allele

variant not related to LQTS, the DNA of 50 normal individuals has been tested. The mutation is not observed in these individuals, nor in the unaffected family members of the LQTS family.

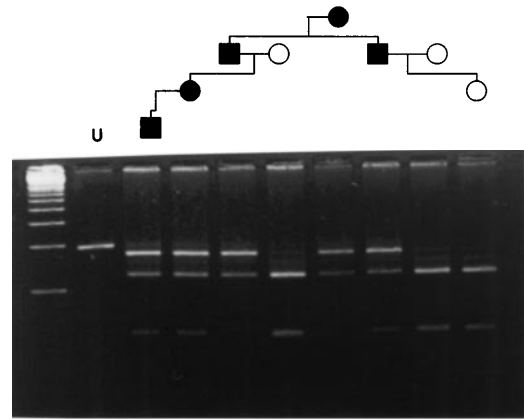


Fig. 4 *Bst*NI restriction enzyme analysis of the S6 fragment. The PCR fragment of the S6 region (see Fig. 2) of the affected individuals (lanes 2, 3, 4, 6, 7) and unaffected individuals (lanes 5, 8, 9) of the LQTS family were digested with *Bst*NI and subsequently electrophoresed. The undigested S6 fragment is shown in lane U

The genetic defect seems to be correlated to a severe phenotypic response (sudden death at an early age occurred in this family, whereas three other family members had observed but non-documented syncopal attacks). However, because of the large variation of the phenotype in LQT1-linked LQTS, this correlation is not absolute. The trigger for syncope and/or sudden death is exercise in this family, in agreement with the data from the ECG registry showing that exercise triggers arrhythmic events in the majority of LQT1 patients (P. J. Schwartz, unpublished data, presented at the American Heart Association meeting, New Orleans, 1996).

The LQT disease status observed in the affected individuals varies considerably from almost normal to severe (Table 1). However, it should be noted that $QT_{(c)}$ time variation is observed in the general population. This makes the clinical diagnosis in LQTS families sometimes difficult. Vincent et al. (1992) have reported that a normal $QT_{(c)}$ interval (< 0.44 s) is observed in 6% of the genetically established LQT carriers. A number of factors may modulate the $QT_{(c)}$ interval. For instance, specific HLA-DR haplotypes may be associated with either an increased or a decreased risk of QT prolongation (Weitkamp et al. 1994). In addition, effects of gender (females have longer $QT_{(c)}$ than males) and effects of low calory diet (hypoglycaemia gives rise to increased prolongation of the $QT_{(c)}$ interval) have been found (Vincent et al. 1992). Consequently, clinical diagnosis based only on QT interval prolongation is not always reliable. Linkage analysis can be hampered by misdiagnosis of the LQT affection status. Therefore, additional analysis of ECG morphology is required to confirm this status (Moss and Robinson 1992; Vincent et al. 1992; Schweitzer 1992).

The mutation Gly-216-Arg and several other described mutations (Wang et al. 1996) are located in the S6 region of the KVLQT1-gene product. Remarkably, a KVLQT1 mutation leading to substitution of the same amino acid (Gly-216-Glu) has been detected within another pedigree,

indicating a possible hot spot for mutations (Wang et al. 1996). All mutations (except for one 3-nucleotide in-frame deletion) known to date in the KVLQT1 gene are missense mutations (Russel 1996; Tanaka et al. 1997). The Gly-216-Arg mutation adds another missense mutation and increases the total number to 16 published to date.

Mutations in another potassium channel, viz. HERG, which is involved in LQT2, are thought to give rise to loss of function with or without a dominant negative suppression of wild-type HERG function (Sanguinetti et al. 1996a). Furthermore, functional studies of HERG mutants have provided evidence that truncated or mutants missing several amino acids in the S3 transmembrane region are not incorporated in the tetrameric potassium channel and do not affect the activity of wild-type HERG. In LQTS patients carrying these gene defects, the LQTS condition probably results from a decreased number of HERG membrane channels (Sanguinetti et al. 1996a). Missense mutations tested by Sanguinetti et al. (1996a) result in a loss of function and a negative effect on the activity of wild-type HERG. The residual activity and the suppression of wild-type HERG functioning depends on the position of the mutation (Sanguinetti et al. 1996a). Thus, since the mutation Gly-216-Arg in KVLQT1 is located in the S6 domain, both loss of activity and a dominant negative effect on wild-type KVLQT1 can be expected, by analogy with HERG.

KVLQT1 may co-assemble with minK (minimal potassium channel) and this complex might provide the inward potassium current I_{Ks} (Barhanin et al. 1996; Sanguinetti et al. 1996b). The KVLQT1 mutation described in our LQT family may therefore directly affect pore function. The KVLQT1 minK complex, which is required for normal channel activity, may also be affected.

Surprisingly, the KVLQT1 frameshift mutation observed in Jervell Lange-Nielsen syndrome does not lead to Romano Ward syndrome in unaffected (heterozygous) gene carriers (Neyroud et al. 1997). The most probable explanation of LQTS induction is thus not a gene-dose effect but a dominant negative effect of mutated KVLQT1. Missense mutations result in dominant negative suppression of potassium channel activity (Russel 1996), suggesting that LQT1 Romano Ward syndrome is exclusively associated with missense mutations.

Since the correct functioning of the KVLQT1 potassium channel depends on co-assembly with the minK protein, mutations affecting the binding of minK are expected to result in a deregulation of channel function (Barhanin et al. 1996; Sanguinetti et al. 1996b). However, these mutations can also influence channel function by directly affecting pore activity or the tetrameric configuration leading to channel disfunction.

Recently, the effects of specific drugs on LQT channel proteins have been reviewed. As the LQT genes and mechanistic involvement have been well characterized, gene-related therapy might be an achievable option in the future (Compton et al. 1996; Schwartz et al. 1995).

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