Evidence of a long QT founder gene with varying phenotypic expression in South African families

Tertia de Jager, Clive H Corbett, Jacobus C W Badenhorst, Paul A Brink, Valerie A Corfield

Abstract

We report five South African families of northern European descent (pedigrees 161, 162, 163, 164, and 166) in whom Romano-Ward long QT syndrome (LQT) segregates. The disease mapped to a group of linked markers on chromosome 11p15.5, with maximum combined two point lod scores, all generated at θ = 0, of 15.43 for the D11S922, 10.51 for the D11S1318, and 14.29 for the tyrosine hydroxylase (TH) loci. Recent studies have shown that LQT is caused by an Ala212Val mutation in a potassium channel gene (KVLQT1) in pedigrees 161 to 164. We report that the same mutation is responsible for the disease in pedigree 166. Haplotype construction showed that all the families shared a common haplotype, suggesting a founder gene effect. DNA based identification of gene carriers allowed assessment of the clinical spectrum of LQT. The QTc interval was significantly shorter in both carriers and non-carriers in pedigree 161 (0.48 s and 0.39 s, respectively) than the same two groups in pedigree 161 (0.52 s and 0.42 s, respectively). The spectrum of clinical symptoms appeared more severe in pedigree 162. The possible influence of modulating genetic factors, such as HLA status and sex of family members, on the expression of an LQT founder gene is discussed.

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Key words: LQT phenotype; founder gene.

Familial long QT syndrome (LQT) is characterised by recurrent syncope and early sudden death resulting from cardiac arrhythmias, with affected subjects frequently exhibiting prolongation of the QT interval on electrocardiograms. Cardiac events are often associated with periods of heightened autonomic tone, such as exercise, anxiety, or excitement. The first evidence of genetic heterogeneity, which has subsequently become a feature of this disease, was the early recognition of two familial forms of LQT. The more commonly occurring Romano–Ward syndrome is distinguished by an autosomal dominant mode of inheritance and normal hearing, in contrast to the autosomal recessive inheritance pattern and deafness associated with Jervell–Lange–Nielsen syndrome.

The existence of familial forms of LQT has provided the opportunity of identifying disease causing genes by linkage analysis and positional cloning techniques. Linkage analysis studies have shown locus heterogeneity in Romano-Ward LQT, with the disease linked to loci on chromosome 3 (LQT3), chromosome 7 (LQT2), chromosome 11 (LQT1), and chromosome 4 (LQT4). Recently, mutations in a cardiac sodium channel gene (SCN5A), the human ether-a-go-go-related gene (HERG), and a potassium channel gene (KVLQT1) have been implicated as causes of chromosome 3, chromosome 7, and chromosome 11 linked LQT respectively.

Identifying disease causing mutations at known loci allows unequivocal DNA based detection of gene carriers in affected families; this is significant in LQT where natural variation in the QT interval may complicate clinical diagnosis. In addition, evidence is accumulating that the spectrum of symptoms and precipitating factors leading to sudden death in LQT may vary depending on the specific causal genetic locus. As distinct from a major locus effect, there is also evidence that other loci, for example HLA, and other factors, such as sex and age, may affect the clinical spectrum. Further genotype-phenotype analyses in families in which LQT is segregating are necessary to validate these observations. We describe differences in the clinical spectrum in two large South African Afrikaner families of northern European origin, who both carry a KVLQT1 Ala212Val mutation. The presence of the same mutation and a shared haplotype in three smaller Afrikaner families suggests the presence of a founder effect in this population. The Afrikaners are a genetically distinct subgroup of South Africans of northern European descent.

Materials and methods

FAMILY STUDIES AND ASCERTAINMENT OF PHENOTYPE

Two large South African families (pedigrees 161 and 162) and three smaller families (pedigrees 163, 164, and 166), all of northern European Afrikaner descent, in whom LQT segregates were identified by referral. Following informed consent, or parental consent in the case of minors, pedigrees were extended, electrocardiograms and the history of syncope were obtained for people participating in the
Pedigree 161

Pedigree 162

Figure 1 Pedigree structure, phenotypic status, and genotypic analyses of LQT families. (A) Pedigrees 161 and 162, (B) pedigrees 163, 164, and 166. The QTc interval in seconds of 78 subjects studied by electrocardiogram is shown in bold beneath the genetic symbol or haplotype. Affected subjects had either symptoms of syncope associated with a QTc interval on electrocardiogram of ≥0.45 s or were asymptomatic with a QTc interval of ≥0.47 s. Clinically normal subjects had a QTc of <0.42 s. Family members with an equivocal electrocardiogram were asymptomatic with a QTc between 0.42 and 0.46 s. The haplotypes were constructed from the results of the combination of alleles inherited at the 7 HRAS1, D11S922, D11S1318, TH, KVLQ1, D11S860, D11S1323, loci in the order indicated, from telomere to centromere. At KVLQ1 the normal allele and the mutant allele were represented by 1 and 2 respectively. Pedigree structure was altered to protect confidentiality. — = not tested.

study, and the incidences and circumstances of sudden death were documented. Electrocardiograms were independently examined by two investigators (CHC and JCBW) and a mean QT ascertained. After correction of the QT interval for heart rate (QTc), according to
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B

Pedigree 163

I

II

III

Pedigree 164

I

II

III

IV

Figure 1B—continued

Bazett’s formula,18 a positive diagnosis of LQT was made based on the following criteria: (1) symptomatic people with a QTc of ≥0.45 s, and (2) asymptomatic people with a QTc of ≥0.47 s. Asymptomatic family members with a QTc of between 0.42 and 0.46 s were designated equivocal clinical status. Phenotypic criteria used were identical for males and females and corresponded to those used by Keating et al.9 10

DNA ANALYSIS
Genomic DNA was extracted from lymphocytes or Epstein–Barr virus transformed cell lines according to published procedures.15 Detection of the KVLQT1 Ala212Val mutation in pedigree 166 followed the previously described method, which was used to identify the same mutation in pedigrees 161 to 164.14 Briefly, this was based on the detection of a single strand conformation polymorphism (SSCP) in the S6 transmembrane domain of KVLQT1 on gel electrophoresis of polymerase chain reaction (PCR) products, amplified with primers I1 and 1214 following standard methods.20 Sequencing of both the normal and aberrant products detected by SSCP analysis in one affected subject showed a C to T transition at codon 212 of KVLQT1. All other members displaying the same SSCP were scored as positive for the mutation. Subjects were genotyped at six linked marker loci that spanned KVLQT1 on chromosome 11p15.5. The HRAS1 locus was analysed by Southern blotting11 using the probe pT24, which detects a variable number of tandem repeat (VNTR) polymorphisms.22 The microsatellite polymorphic repeat sequences at the D11S922,23 D11S1318,23 tyrosine hydroxylase (TH),24 D11S860,25 and D11S132322 loci were analysed by PCR based assays according to previously described standard methods,26 using primers purchased from Research Genetics (Huntsville, AL).
Comparison of QTc intervals in LQT carrier and non-carrier pedigrees 161 and 162. The mean of the QTc interval in seconds of all carriers and non-carriers (with the number of subjects assessed in brackets) is shown separately for each pedigree. Shaded bars = non-carriers; solid bars = carriers.

**Results**

**Ascertaining of Phenotype**

The five families identified, pedigrees 161 to 164 and pedigree 166 (fig 1), showed an autosomal dominant mode of inheritance, normal hearing, and characteristic features associated with the Romano–Ward form of LQT. The spectrum of clinical presentation is discussed below.

Electrocardiographic data were available for 78 family members, and blood samples for genotypic analysis were available for 100 people.

**Mutation Detection and Haplotype Construction**

Forty-eight of the total of 100 family members of the five pedigrees subjected to genotypic analysis at KVLQT1 carried the Ala212Val mutation (fig 1). These results incorporated data generated for some family members of pedigrees 161 to 164 genotyped in a previous study and subjects newly entered into the present study, as well as new information for pedigree 166.

The highest combined lod scores generated between LQT and marker loci were at D11S922 (Zmax 15.43), D11S1318 (Zmax 10.51), and TH (Zmax 14.29), all at \( \theta = 0 \). Subsequent studies of Wang et al isolated KVLQT1 from a 700 kb interval of chromosome 11p15.5 close to TH.

The construction of haplotypes showed that in pedigrees 161 and 162 carrying the Ala212Val mutation shared the same combination of alleles at the six linked marker loci tested, that is, 4432254, including the mutant allele, in the order shown in fig 1. These markers spanned a distance of around 8 cm. Assuming that the Ala212Val mutation in the other three pedigrees was identical by descent, recombination events had occurred in these families. The recombination breakpoints lay between KVLQT1 and D11S860 in pedigree 163, D11S860 and D11S1323 in pedigree 164, and D11S922 and D11S1318 in pedigree 166. The shared disease associated haplotype in all five pedigrees was therefore 322, in the order D11S1318, TH, KVLQT1 (fig 1).

**Clinical Spectrum of LQT1 Carriers and Non-carriers**

A total of 78 family members were examined clinically. Based on the strict criteria described in the Materials and methods section, 44 people were clinically affected, that is, possessing either a QTc of \( \geq 0.45 \) s in the presence of symptoms or \( \geq 0.47 \) s in their absence (fig 1). Carrier status was consistent with clinical assessment (fig 1). An electrocardiogram was not available for I-1 (pedigree 166), but she suffered syncope and was considered an obligate heterozygote as she had an affected child and the disease associated haplotype. Ten asymptomatic people showed QTc intervals in the equivocal range of between 0.42 to 0.47 s, as did a married in subject II-2 (pedigree 161).

In addition, in pedigree 161 two symptomatic
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people had an equivocal electrocardiogram, that is, ≤0.45 s (III-7 and III-11). Mutation analysis indicated that three of the five equivo
cal subjects in pedigree 161 were gene car
riers (II-4, III-7, and III-11). However, in pedigree 162, six of the seven equivocal people were non-carriers (II-1, III-20, III-25, IV-4, IV-7, and IV-8), while DNA of the seventh person (III-24) was not available for geno
typing. Consequently, the total number of gene carriers and non-carriers assessed in com
parative clinical studies was 48 and 30 re
spectively (37 from the direct line of descent and three spouses). Of the gene carriers, 21 were male (45%) and 26 were female (55%),
the monozygotic twins III-2 and III-4 in pedi
gree 162 being counted as a single genetic
event.

Syncopal episodes were suffered by 38/48
(79%) of the gene carriers in the five pedigrees.
When making comparisons of clinical features
in pedigrees 161 and 162 only, it was evident that the majority of the seven asymptomatic
 carriers in these two pedigrees were members
of pedigree 161 (six out of seven people). None
of the non-carrier family members had a history
of syncope. The age of the first reported episode
of symptoms in carriers also showed a differ
cence between the two pedigrees, with a range
of from 3 to 42 years (mean 11 years) in pedi
gree 161 and from 2 to 12 years (mean 6 years)
in pedigree 162. The average number of syncope episodes per person was lower in pedi
gree 161 (6-75 episodes) than in pedigree
162 (nine episodes).

When making a comparative analysis of the
clinical spectrum in the two larger pedigrees,
it was apparent that the range and mean of the
QTc interval was shorter in pedigree 161 than
in pedigree 162 in both carriers and non-car
riers (fig 2). In pedigree 161, QTc ranged from
0.44 s to 0.53 s (mean 0.48 s) in carriers and
from 0.35 s to 0.44 s (mean 0.39 s) in non-car
riers. In pedigree 162, QTc ranged from
0.45 s to 0.59 s (mean 0.52 s) in carriers and
from 0.37 s to 0.45 s (mean 0.42 s) in non-car
riers. An analysis of variance showed these
differences to be statistically significant, when
comparing the QTc of carriers (p=0.003) or
non-carriers (p=0.0143) in the two pedigrees.
This may mean that the “normal” QTc vari
ation existing within families may play an addi
tional contributory role in the length of the
pathological QTc interval.

The numbers of gene carriers in pedigrees
163, 164, and 165 were not large enough for
statistical comparisons of QTc interval, but
members of pedigree 164 appeared to be most
severely affected, with the QTc ranging from
0.49 s to 0.70 s (mean 0.56 s), accompanied by
frequent episodes of syncope and convulsions.
The QTc in three non-carriers from the direct
line of descent in pedigree 164 was 0.41 s (fig
1).

All episodes of syncope in the five pedigrees
were preceded by activity or excitement, with
a notably high occurrence (53%) associated
with swimming or participation in water related
exercise. Ten patients reported syncopal attacks
after emotional upset. In 11 patients the loss
of consciousness was followed by convulsions
(pseudoepileptic seizures) and the patients
were originally misdiagnosed as epileptics.
Twelve sudden deaths at an early age (10 to
18 years) were documented, with a male:female
ratio of 2:1 (table). In all cases the deaths were
exercise related. One person (II-4 in pedigree
166) was resuscitated from a cardiac arrest.

Seven of the 10 asymptomatic carriers were
receiving no treatment, while the remaining
three were given β blockade. Seven of the
symptomatic carriers were untreated and, of
these, II-9, II-15, (pedigree 161) II-2, II-5
(pedigree 162), and I-1 (pedigree 166), aged
68, 62, 71, 74, and 68 years respectively, had
been symptom free for 38 years or more. III-11
(pedigree 161), aged 16 years, had suffered
only one previous attack at the age of 10 years
and showed an equivocal ECG. II-2 (pedigree
164), with a QTc of 0.70 s, declined treatment
and had not suffered syncope or convulsions
for the past two years. Of the remaining 31
symptomatic carriers, 23 were on β blockade
alone, three had undergone left sympathec
tomy, and five were on β blockade therapy as
well as having undergone gan
glionectomy. All patients receiving treatment
were free of further syncope episodes, with
the exception of IV-13 (pedigree 162), III-7
(pedigree 164), and II-4 (pedigree 166). The
first patient seemed resistant to surgery and
the subsequent reintroduction of β blockade
and had suffered a further five episodes of syncope
despite treatment. Both his sibs (IV-11 and
IV-12), as well as his mother (II-14), were
severely affected and had undergone surgical
intervention, although in their cases treatment
had proved effective. The other two patients
had not undergone surgery, the 13 year old
III-7 continued to suffer syncopal episodes,
while II-4 had been symptom free for eight
years.

Discussion
In the five pedigrees described, LQT was
caused by an Ala212Val mutation in KVLQ
T1 on chromosome 11p15.5. A common haplo
type at markers linked to the mutant allele
indicated descent from a common ancestor.
Evidence to support the premise that this was
the disease causing mutation included identi
fication of an Ala212Val substitution in an

<table>
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<th>Pedigree</th>
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<tr>
<td>II-2</td>
<td>Male</td>
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unrelated family (K1807) of Italian origin and its absence in 200 unaffected controls reported in a previous study. In addition, an Ala-212Glu mutation in the same KVLQ1T1 codon has been implicated as a cause of LQT in two families. These mutations occur in the region of KVLQ1T1 showing cross species homology and thought to encode one of the functionally important transmembrane domains present in voltage gated potassium channels.

Previous studies have suggested evidence of a locus specific phenotype in LQT. In addition to the differences associated with hearing in Jervell–Lange–Neilsen and Romano–Ward forms of LQT, the factors precipitating sudden death in the latter syndrome may be distinguished. The sudden deaths in pedigree 161 and 162 all occurred at an early age (10 to 19 years) and were exercise related. Four members of two of the smaller families also died suddenly while playing or swimming, two at an early age, while the ages of the others who had died were unknown (table). This confirmed the observation of Curran et al that, in chromosome 11 linked LQT, arrhythmias and sudden death were frequently precipitated by exercise and anxiety. In contrast, in non-chromosome 11 linked LQT, sudden death occurred during sleep or on awakening. Similar comparisons have not been made for sudden deaths associated with the other LQT causal loci. The incidence of sudden death was 12% for pedigree 161 and 9% for pedigree 162, in contrast to the 4% incidence noted previously in three large chromosome 11 linked families.

DNA based identification of gene carriers allowed assessment of the clinical spectrum of LQT in the South African families. In addition to phenotypic variation owing to locus heterogeneity, there may be subtle differences of expression between families showing linkage to the same locus. This may reflect the underlying specific mutation as, for example, comparative studies of different familial hypertrophic cardiomyopathy causing mutations have shown. In the present study, in which all carriers bore the same mutation, phenotype-genotype correlations of carriers versus non-carriers showed a statistically significant difference in the QTc interval between carriers of the LQT gene in pedigrees 161 and 162. Furthermore, the difference extended to non-carriers in the two pedigrees, with the QTc interval significantly longer in both groups in pedigree 162. Additionally, although no statistical significance could be attached to the differences, the clinical spectrum of symptoms appeared more severe in pedigree 162, where there was an earlier age of appearance of the first symptoms, a higher average number of syncopal episodes, and only one asymptomatic carrier.

The five families shared a common disease associated haplotype of 322 at D11S1318, TH, KVLQ1T1 (fig 1). This indicated descent from a common ancestor, as this haplotype was absent from 18 married in family members and can be assumed to be uncommon in the general population. Continuing genealogical studies of pedigrees 161 and 162, extending back through nine generations to the early 18th century, showed two marriages between ancestors bearing each of the family surnames, suggesting an as yet unconfirmed link. The five kindreds are all of Afrikaner descent. As yet, no families in South Africa have been found in whom LQT is linked to the LQT2, LQT3, or LQT4 loci, nor has the disease been observed in any other population group. This evidence strongly suggests the existence of a founder LQT1 gene in the Afrikaner population of South Africa.

Founder gene effects have previously been reported for other cardiac associated disorders seen in the Afrikaner population of South Africa, namely progressive familial heart block and familial hypercholesterolaemia. In the latter disease, two separate "founder" mutations in the lipoprotein low density receptor shared a common haplotype.

Differences in the spectrum of clinical symptoms manifested in pedigrees 161 and 162 may reflect the influence of different modulating environmental or genetic backgrounds on expression of the same mutant allele. The more prolonged pathological QTc interval present in pedigrees 161 and 162 may be associated with the underlying longer QTc interval measured in non-carriers in this family. Additionally, Wettkamp et al have shown that expression of LQT genes may be influenced by HLA haplotypes, derived from both affected and unaffected parents, and the sex of the affected person. Posen et al noted that the penetrance and severity of the clinical picture of familial hypertrophic cardiomyopathy in carriers of the same β myosin heavy chain gene mutation decreased beyond the confines of the originally identified nuclear family. Further molecular analyses of these LQT affected South African pedigrees may help to dissect and identify the confounding factors that influence this genetically heterogeneous disease. Such information may have therapeutic implications. Currently, treatment of LQT is based on β blockade and sympathetic ganglionectionomy, following a course reviewed by Schwartz et al 20 years ago. This therapy is probably not directed at the underlying disease mechanisms and is not always effective.

In the present study, genotypic analysis allowed identification of asymptomatic gene carriers who were at risk of syncope and sudden death. Significantly, previous studies have shown that diagnosis of LQT is difficult and often missed by clinicians, resulting in people at risk not receiving appropriate treatment. Three of the nine clinically equivocal subjects, namely II-4, III-7, and III-11 (pedigree 161), had inherited the mutant allele. Consequently, in these at risk family members, treatment combined with the avoidance of precipitating factors, would be advised. The remaining clinically equivocal family members could be reassured that they were not gene carriers and be spared unnecessary treatment.
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