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 $\theta = 0$, 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.

(*J Med Genet* 1996;33:567–573)

Key words: LQT phenotype; founder gene.

University of Stellenbosch and Medical Research Council Centre for Molecular and Cellular Biology, Department of Medical Physiology and Biochemistry, University of Stellenbosch Medical School, PO Box 19063, Tygerberg 7505, South Africa
T de Jager
V A Corfield

Department of Internal Medicine, University of Stellenbosch Medical School and Tygerberg Hospital, South Africa
C H Corbett
P A Brink

Unitas Hospital, Verwoerdburg, South Africa
C W Badenhorst

Correspondence to:
Dr Corfield.

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.[1][2] Cardiac events are often associated with periods of heightened autonomic tone, such as exercise, anxiety, or excitement.[3] 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,[4][5] in contrast to the autosomal recessive inheritance pattern and deafness associated with Jervell–Lange–Nielsen syndrome.[6]

The existence of familial forms of LQT has provided the opportunity of identifying disease causing genes by linkage analysis and positional cloning techniques.[7] Linkage analysis studies have shown locus heterogeneity in Romano–Ward LQT, with the disease linked to loci on chromosome 3 (LQT3),[8] chromosome 7 (LQT2),[8] chromosome 11 (LQT1),[9][10] and chromosome 4 (LQT4).[11] 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.[12–14]

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.[15][16] 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.[17] Further genotype-phenotype analyses in families in whom 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
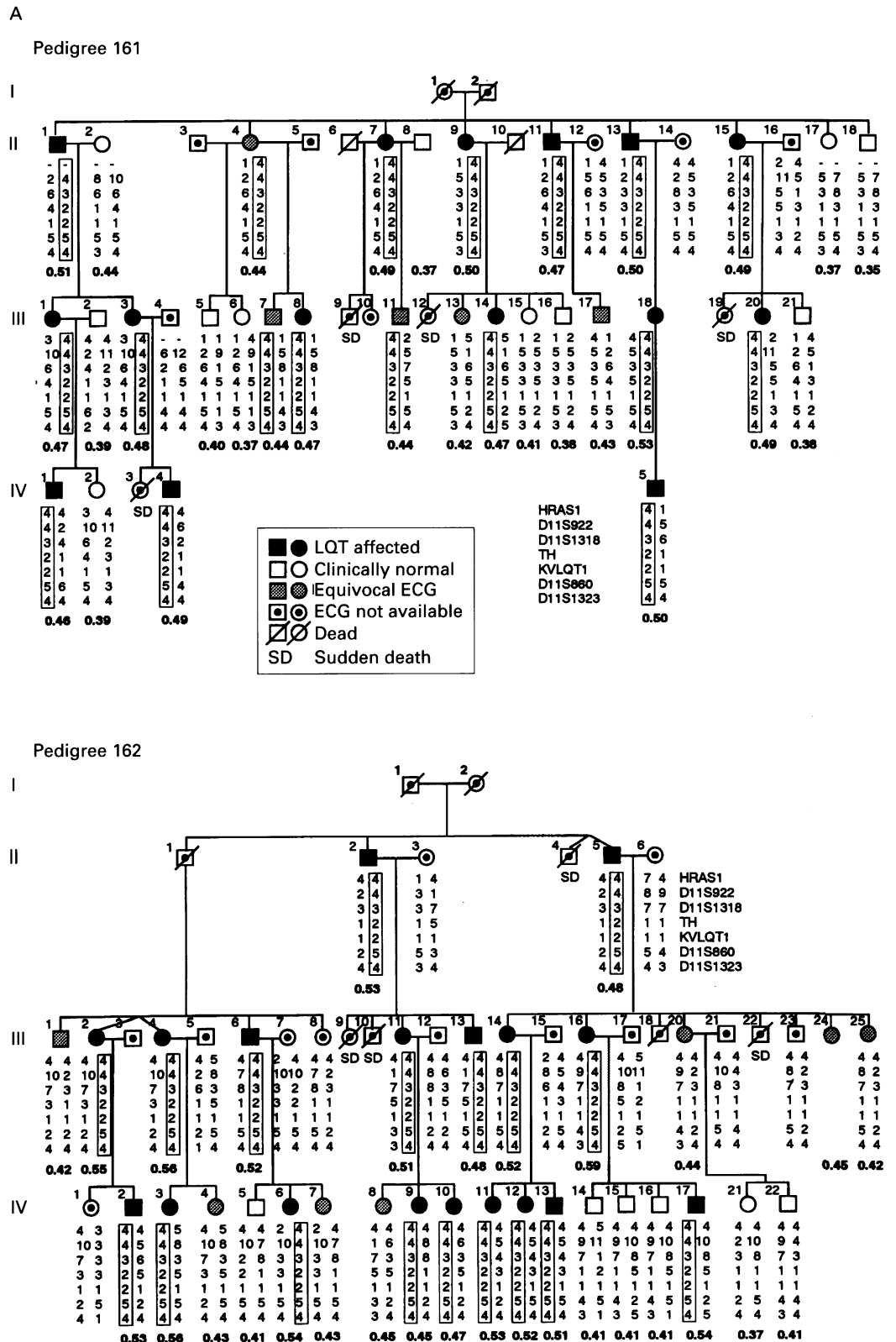
Figure 1   Pedigree structure, phenotypic status, and genotypic analyses of LQT families. (A) Pedigrees 161 and 162, (B) pedigrees 163, 164, and 166. The QTc interval in seconds of 78 subjects studied by electrocardiogram is shown in bold beneath the genetic symbol or haplotype. Affected subjects had 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 HRAS1, D11S922, D11S1318, TH, KVLQT1, D11S860, D11S1323, loci in the order indicated, from telomere to centromere. At KVLQT1 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 JCWB) and a mean QT ascertained. After correction of the QT interval for heart rate (QTc), according to
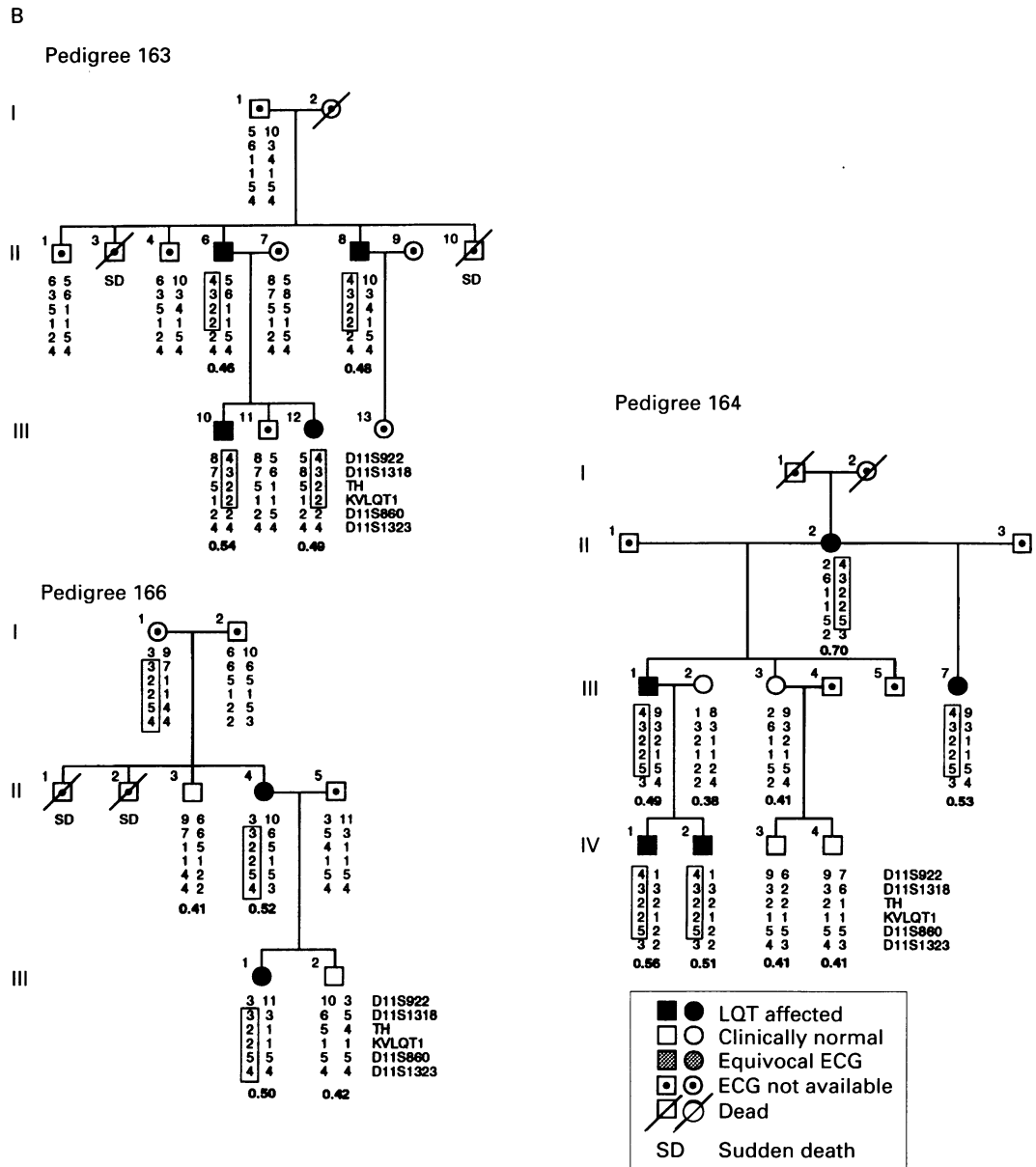
B

Pedigree 163

Pedigree 164

Pedigree 166

LQT affected
Clinically normal
Equivocal ECG
ECG not available
Dead

SD **Sudden death**

D11S922
D11S1318
TH
KVLQT1
D11S860
D11S1323

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.[19] 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 11 and 12[14] 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 blotting[21] 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 D11S1323[22] loci were analysed by PCR based assays according to previously described standard methods,[20] using primers purchased from Research Genetics (Huntsville, AL).

Mean QTc of carriers = 0·48 sec (n = 18)
Mean QTc of non-carriers = 0·39 sec (n = 13)

Mean QTc of carriers = 0·52 sec (n = 18)
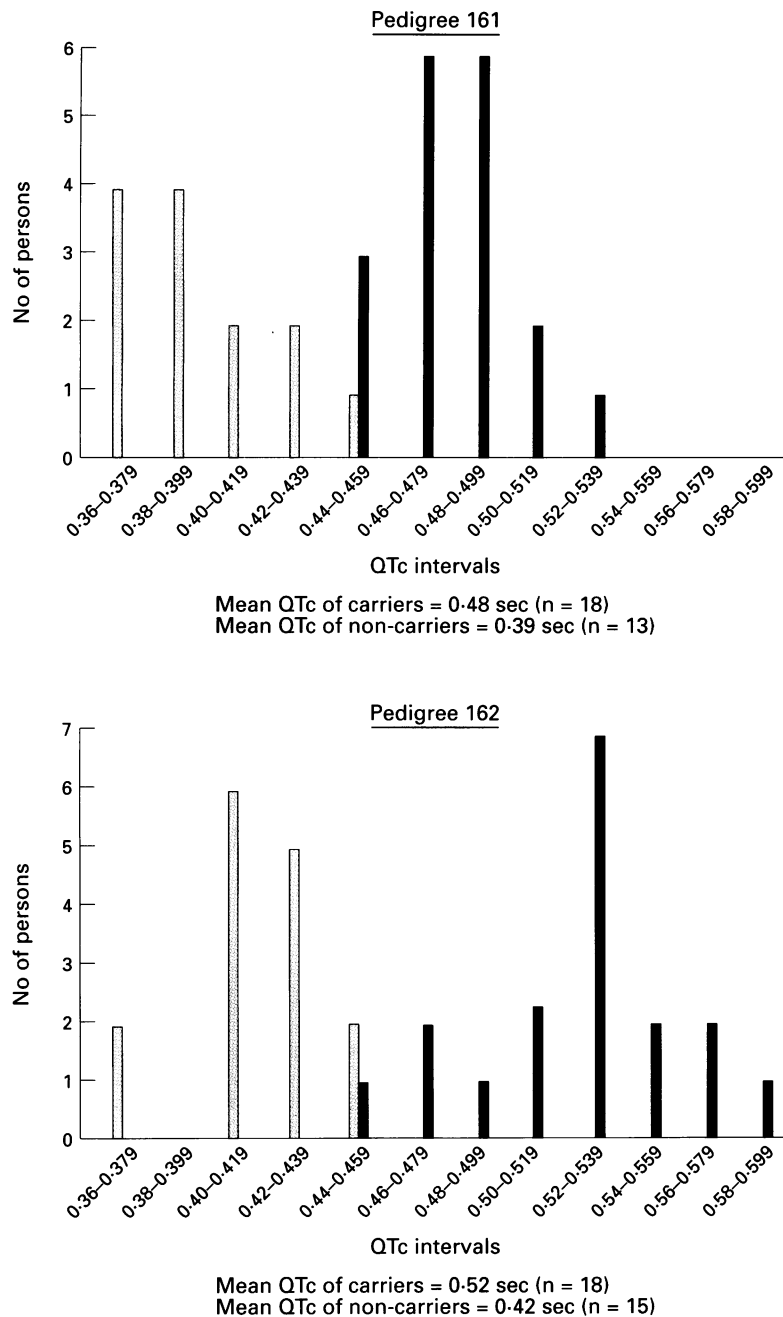Mean QTc of non-carriers = 0·42 sec (n = 15)

Figure 2   Comparison of QTc intervals in LQT carriers and non-carriers in 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.

STATISTICAL ANALYSIS
Two point lod scores were generated from marker allele, disease status, and pedigree position information of each person entered into the study using the LINKAGE computer programe.[26] Subjects of equivocal clinical status were coded as unknown in linkage calculations. A penetrance of 0·9, a frequency of the disease allele of 0·001, and equal male and female recombination fractions were used in linkage calculations. These parameters were identical to those defined in previous studies.[9 10 15] Equal allele frequencies were used at all marker loci except at HRAS1, where published frequencies were used.[22]

Results

ASCERTAINMENT 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[14] 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 θ=0. Subsequent studies of Wang *et al*[14] 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 people 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 about 8 cM.[23] 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 ≥0·45 s in the presence of symptoms or ≥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

people had an equivocal electrocardiogram, that is, ≤ 0.45 s (III·7 and III·11). Mutation analysis indicated that three of the five equivocal subjects in pedigree 161 were gene carriers (II·4, III·7, and III·11). However, in pedigree 162, six of the seven equivocal people were non-carriers (III·1, III·20, III·25, IV·4, IV·7, and IV·8), while DNA of the seventh person (III·24) was not available for genotyping. Consequently, the total number of gene carriers and non-carriers assessed in comparative clinical studies was 48 and 30 respectively (27 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 pedigree 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 difference between the two pedigrees, with a range of from 3 to 42 years (mean 11 years) in pedigree 161 and from 2 to 12 years (mean 6 years) in pedigree 162. The average number of syncopal episodes per person was lower in pedigree 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-carriers (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-carriers. 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-carriers. 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 variation existing within families may play an additional 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

Precipitating factors of sudden death in pedigrees 161, 162, 163, and 166

Subject	Sex	Age of SD (y)	Precipitating factors
Pedigree 161			
III·9	Male	16	Swimming
III·12	Female	16	Swimming
III·19	Female	17	Swimming
IV·3	Female	12	Ice skating
Pedigree 162			
II·4	Male	17	Playing tennis
III·9	Female	15	Athletics
III·10	Male	18	Running
III·22	Male	10	Swimming
Pedigree 163			
II·3	Male	6	Playing
II·10	Male	10	Playing
Pedigree 166			
II·1	Male	—	Swimming
II·2	Male	—	Swimming

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 sympathetic ganglionectomy, and five were on β blockade therapy as well as having undergone ganglionectomy. All patients receiving treatment were free of further syncopal 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 (III·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 Ala121Val mutation in KVLQT1 on chromosome 11p15.5. A common haplotype 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 identification of an Ala212Val substitution in an

unrelated family (K1807) of Italian origin and its absence in 200 unaffected controls reported in a previous study.[14] In addition, an Ala-212Glu mutation in the same KVLQT1 codon has been implicated as a cause of LQT in two families.[14] These mutations occur in the region of KVLQT1 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.[15 16] In addition to the differences associated with hearing in Jervell–Lange–Neilsen[6] and Romano–Ward forms of LQT,[4 5] 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[15] 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.[15] 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.[15]

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.[20] 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 sycopal episodes, and only one asymptomatic carrier.

The five families shared a common disease associated haplotype of 322 at D11S1318, TH, KVLQT1 (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[27] and familial hypercholesterolaemia.[28] In the latter disease, two separate "founder" mutations in the lipoprotein low density receptor shared a common haplotype.[29]

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 pedigree 162 may be associated with the underlying longer QTc interval measured in non-carriers in this family. Additionally, Weitkamp et al[17] 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[20] 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.[20] 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 ganglionectomy,[30] following a course reviewed by Schwartz et al[2] 20 years ago. This therapy is probably not directed at the underlying disease mechanisms and is not always effective.[31 32]

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.[33 34] 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.

tracing and contacting family members. Finally the members of the families participating in the study are thanked for their cooperation and interest.

1 Vincent GM, Abildskov JA, Burgess MJ. Q-T interval syndromes. *Prog Cardiovasc Dis* 1974;16:523–30.
2 Schwartz PJ, Periti M, Malliani A. The long Q-T syndrome. *Am Heart J* 1975;89:378–90.
3 Schwartz PJ, Zaza A, Locati E, Moss AJ. Stress and sudden death. The case of the long QT syndrome. *Circulation* 1991;83(suppl II):71–80.
4 Romano C, Gemme G, Pongiglione R. Aritmie cardiache rare dell'eta pediatrica. *Clin Pediatr* 1963;45:658–83.
5 Ward OC. A new familial cardiac syndrome in children. *J Irish Med Assoc* 1964;54:103–6.
6 Jervell A, Lange-Nielson F. Congenital deaf mutism. Functional heart disease with prolongation of the Q-T interval, and sudden death. *Am Heart J* 1957;54:59–68.
7 Keating M. Linkage analysis and long QT syndrome. Using genetics to study cardiovascular disease. *Circulation* 1992;85:1973–86.
8 Jiang C, Atkinson D, Towbin JA, et al. Two long QT syndrome loci map to chromosomes 3 and 7 with evidence for further heterogeneity. *Nature Genet* 1994;8:141–7.
9 Keating M, Atkinson D, Dunn C, Timothy K, Vincent GM, Leppert M. Linkage of a cardiac arrhythmia, the long QT syndrome to the Harvey ras-1 gene. *Science* 1991;252:704–6.
10 Keating M, Dunn C, Atkinson D, Timothy K, Vincent GM, Leppert M. Consistent linkage of the long-QT syndrome to the Harvey ras-1 gene locus on chromosome 11. *Am J Hum Genet* 1991;49:1335–9.
11 Schott JJ, Charpentier F, Peltier S, et al. Mapping of a gene for long QT syndrome to chromosome 4q25–27. *Am J Hum Genet* 1995;57:1114–22.
12 Wang Q, Shen J, Splawski I, et al. SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. *Cell* 1995;80:805–11.
13 Curran ME, Splawski I, Timothy KW, Vincent GM, Green ED, Keating MT. A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. *Cell* 1995;80:795–803.
14 Wang Q, Curran ME, Splawski I, et al. Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. *Nature Genet* 1996;12:17–23.
15 Curran M, Atkinson D, Timothy K, et al. Locus heterogeneity of autosomal dominant long QT syndrome. *J Clin Invest* 1993;92:799–803.
16 Dean JCS, Cross S, Jennings K. Evidence of genetic and phenotypic heterogeneity in Romano-Ward syndrome. *J Med Genet* 1993;30:947–50.
17 Weitkamp LR, Moss AJ, Lewis RA, et al. Analysis of HLA and disease susceptibility: chromosome 6 genes and sex influence long-QT phenotype. *Am J Hum Genet* 1994;55:1230–41.
18 Bazett HC. An analysis of the time-relations of electro-cardiograms. *Heart* 1920;7:353–70.

19 Corfield VA, Moolman JC, Martell R, Brink PA. Polymerase chain reaction-based detection of MN blood group-specific sequences in the human genome. *Transfusion* 1993;33:119–24.
20 Posen BM, Moolman JC, Corfield VA, Brink PA. Clinical and prognostic evaluation of familial hypertrophic cardiomyopathy in two South African families with different cardiac β myosin heavy chain gene mutations. *Br Heart J* 1995;74:40–6.
21 Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* 1975;98:503–17.
22 Pierotti MA, Radice P, Biunno I, Bornello MG, Cattadori MR, Della Porta G. Detection of two TaqI polymorphisms in the VTR region of the human HRASI oncogene. *Cytogenet Cell Genet* 1986;43:174–80.
23 Gyapay G, Morissette J, Vignal A, et al. The 1993–1994 Genethon human genetic linkage map. *Nature Genet* 1994;7:246–339.
24 Polymeropoulos MH, Xiao H, Rat DS, Merill CR. Tetranucleotide repeat polymorphism at the human tyrosine hydroxylase gene (TH). *Nucleic Acids Res* 1992;19:3753.
25 McNoe LA, Eccles MR, Reeve AE. Dinucleotide repeat polymorphism at the D11S860 locus. *Nucleic Acids Res* 1992;20:1161.
26 Lathrop GM, Lalouel JM. Easy calculations of lod scores and genetic risks on small computers. *Am J Hum Genet* 1984;36:460–5.
27 Brink PA, Ferreira A, Moolman JC, Weymer HW, Van der Merwe PL, Corfield VA. The gene for progressive familial heart block I (PFHBI), a cardiac bundle branch disorder, maps to chromosome 19q13. *Circulation* 1995;91:1633–40.
28 Brink PA, Steyn LT, Coetzee GA, Van Der Westhuysen DR. Familial hypercholestolaemia in South Africans: PvuII and StuI DNA polymorphisms in the LDL-R gene consistent with a predominating founder gene effect. *Hum Genet* 1987;77:32–5.
29 Kotze MJ, Langenhoven E, Retief AE, Seftel HC, Henderson HE, Weich HF. Haplotypes identified by 10 DNA restriction fragment length polymorphisms of the human low density lipoprotein receptor gene locus. *J Med Genet* 1989;26:255–9.
30 Schwartz PJ, Locati EH, Moss AJ, Crampton RS, Trazzi R, Ruberti U. Left cardiac sympathetic denervation in the therapy of congenital long QT syndrome. A worldwide report. *Circulation* 1991;84:503–11.
31 Bhandari AK, Scheinman MM, Morady F, Svinarich J, Mason J, Winkle R. Efficacy of left cardiac sympathectomy in the treatment of patients with the long QT syndrome. *Circulation* 1984;70:1018–23.
32 Moss AJ, Robinson J. Clinical features of the idiopathic long QT syndrome. *Circulation* 1992;85(suppl I):140–4.
33 Moss AJ, Schwartz PJ, Crampton RS, et al. The long QT syndrome. Prospective longitudinal study of 328 families. *Circulation* 1991;84:1136–44.
34 Vincent GM, Timothy KW, Leppert M, Keating M. The spectrum of symptoms and QT intervals in carriers of the gene for the long-QT syndrome. *N Engl J Med* 1992;327:846–52.