Phenotypic Variability and Unusual Clinical Severity of Congenital Long-QT Syndrome in a Founder Population

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Background—In the congenital long-QT syndrome (LQTS), there can be a marked phenotypic heterogeneity. Founder effects, by which many individuals share a mutation identical by descent, represent a powerful tool to further understand the underlying mechanisms and to predict the natural history of mutation-associated effects. We are investigating one such founder effect, originating in South Africa in approximately AD 1700 and segregating the same KCNQ1 mutation (A341V).

Methods and Results—The study population involved 320 subjects, 166 mutation carriers (MCs) and 154 noncarriers. When not taking β-blocker therapy, MCs had a wide range of QTc values (406 to 676 ms), and 12% of individuals had a normal QTc (≤440 ms). A QTc >500 ms was associated with increased risk for cardiac events (OR=4.22; 95% CI, 1.12 to 15.80; \(P=0.033\)). We also found that MCs with a heart rate <73 bpm were at significantly lower risk (OR=0.23; 95% CI, 0.06 to 0.86; \(P=0.035\)). This study also unexpectedly determined that KCNQ1-A341V is associated with greater risk than that reported for large databases of LQT1 patients: A341V MCs are more symptomatic by age 40 years (79% versus 30%) and become symptomatic earlier (7 ± 4 versus 13 ± 9 years, both \(P<0.001\)). Accordingly, functional studies of KCNQ1-A341V in CHO cells stably expressing IKr, were conducted and identified a dominant negative effect of the mutation on wild-type channels.

Conclusions—KCNQ1-A341V is a mutation associated with an unusually severe phenotype, most likely caused by the dominant negative effect of the mutation. The availability of an extended kindred with a common mutation allowed us to identify heart rate, an autonomic marker, as a novel risk factor. (Circulation. 2005;112:2602-2610.)

Key Words: arrhythmia ■ death, sudden ■ genetics ■ long-QT syndrome ■ nervous system, autonomic

The identification of genes associated with the congenital long-QT syndrome (LQTS) has had a major impact on understanding the molecular basis for ventricular arrhythmias and sudden cardiac death.1 Although great progress has been made in defining individual genes conferring the majority of genetic risk in LQTS patients and in elucidating complex genotype-phenotype correlations,2–4 there remains a substantial challenge to explain the widely observed variability in disease expression and phenotype severity. Identification of clinical and genetic variables capable of more accurately predicting the outcome of LQTS is expected to have importance for determining prognosis, selecting patients for the most appropriate therapy, and counseling asymptomatic mutation carriers (MCs).

In general, questions about disease outcome or variables predicting clinical end points are best addressed in large populations. However, the substantial genetic and allelic heterogeneity in LQTS limits the usefulness of populations composed largely of single individuals and small family units having multiple different genotypes. Populations with founder effects offer a potential advantage in this regard by providing an opportunity to observe phenotypic differences influenced by environmental or genetic factors other than the primary mutation. Identifying LQTS populations resulting from a founder effect and defining their phenotypic characteristics represent the first steps toward discovery of prognostic factors and advancing our understanding of the causes of variable genetic penetrance and expressivity.
We present data on 22 South African LQTS families all segregating the same KCNQ1 mutation (A341V) caused by a founder effect. The disease allele in all of these families descends from a common ancestor who migrated to South Africa from Western Europe more than 300 years ago. Even though these individuals share the same disease-causing mutation, there are significant phenotypic differences, including a wide distribution of the QT interval and the presence or absence of cardiac events. This large kindred therefore represents a powerful resource to study other factors, such as modifier genes or environmental variables, that modulate the severity of the clinical manifestations of the disease.

Methods

Study Population
A cohort of individuals harboring an identical LQTS-causative mutation in KCNQ1 (A341V) was investigated for the possibility of a founder effect. Starting from probands, family trees were constructed and ancestral relationships were researched through genetical studies. To exclude the possibility that the mutation arose independently on more than one occasion, which would be contrary to the founder hypothesis, haplotype data on the genomic segment encompassing KCNQ1 were also used to confirm likely lines of descent of the shared A341V mutation from the founding couple.

Clinical and genetic data concerning MCs and first-degree relatives were recorded on designed forms and included demographic information, personal and family history of disease, symptoms, and therapy. Cardiac events were defined as syncope (fainting spells with transient but complete loss of consciousness), aborted cardiac arrest (requiring resuscitation), and sudden cardiac death. MCs were classified as either symptomatic or asymptomatic. Symptomatic patients were MCs who experienced at least one episode of syncope or cardiac arrest, whereas asymptomatic MCs were those individuals without these events. Unexpected sudden death that occurred before the age of 40 years without a known cause was categorized as related to LQTS and was assumed to have occurred in MCs. Data were stored in a relational database developed jointly by authors from the University of Stellenbosch and from the University of Pavia.

All probands and family members provided written informed consent for clinical and genetic evaluations. Protocols were approved by the Ethical Review Boards of the Tygerberg Hospital of Stellenbosch University, Vanderbilt University, and the University of Pavia. Approved consent forms were provided in English or Afrikaans as appropriate.

Genotyping
Peripheral blood was collected from all index cases and family members entered into the study. Genomic DNA was extracted from lymphocytes or Epstein-Barr virus–transformed cell lines as previously described.

Polymerase chain reaction (PCR)–based detection of the A341V founder mutation, which results in the loss of a HhaI restriction enzyme site in KCNQ1 exon 7, was used to genotype members of the LQTS families. Exon 7 was PCR-amplified by use of primers X7F and X7R to generate a 190-bp amplicon for restriction digestion.

Haplotyping
Selected informative members of each LQTS-affected family were genotyped at 8 linked microsatellite loci (D11S4046, D11S1318, D11S4088, D11S4146, D11S4181, D11S1871, D11S1760, and D11S1323) that span the KCNQ1 region. Haplotypes were determined by studying family structures and by using Mendelian rules of inheritance.

ECG Analysis
A baseline ECG recorded in the absence of β-blocker therapy was available for 131 of 154 noncarriers (85%) and for 93 of 166 MCs (56%). All ECGs were coded, and the most recent one not on therapy was subsequently analyzed by one investigator (L.C.) blinded to genotype.

Baseline heart rate (HR) and duration of the QT and RR intervals in leads II and V, were measured from resting 12-lead ECGs. To allow QT values to be compared among subjects, the QT interval was corrected for HR (QTc) by use of Bazett’s formula. For comparisons of HR and QTc between symptomatic and asymptomatic patients, we used only ECGs recorded after age 15 years. This was done because the HR of healthy individuals is significantly greater before age 15 and because the absence of cardiac events before age 15 does not predict that an LQTS proband will remain asymptomatic throughout life. Thus, 7 patients with an ECG taken before age 15 were not included in this analysis, which therefore is limited to 86 patients.

Statistical Analysis
Comparisons of groups identified on the basis of the clinical characteristics and genotype were performed in univariate analysis. The Student t test was used for continuous variables, and Fisher’s exact test was used for categorical variables. QTc duration and HR in MCs were divided into tertiles, and the upper tertile of each variable, 500 ms and 73 bpm, respectively, was used as a clinical risk factor. To determine the association of the selected clinical variables with the occurrence of cardiac events, odds ratios (OR) for unadjusted data and their 95% confidence intervals (95% CI) were calculated.

Time to the first cardiac event (syncope, cardiac arrest, or sudden cardiac death) before initiation of β-blocker therapy and before age 40 years was determined by Kaplan-Meier cumulative estimates. The South African A341V population (SA-A341V) was compared with the largest published data set on 355 LQT1 patients referred to here as the LQT1 database. The age-related probability of surviving a first cardiac event was described by QTc, sex, and the specific genetic defect.

The contribution of HR, QTc duration, and sex to the risk of experiencing a cardiac event was determined by a logistic regression model in which the presence or absence of a clinical history of cardiac events was used as the dependent variable. Odds ratios and 95% CIs were computed while controlling for the covariates introduced in the model.

Data are reported as mean and SD for continuous variables; whenever the distribution was skewed, median and interquartile range were reported. Two-sided probability values <0.05 were considered statistically significant. Statistical calculations were performed by use of SPSS software (version 11.5).

Mutagenesis and Heterologous Expression of KCNQ1-A341V
Three mutations (A341V, G314S, and 543 del/ins) were constructed in a recombinant human KCNQ1 cDNA by use of PCR mutagenesis (primer sequences available on request). G314S produces a severe in vitro phenotype caused by a strong dominant negative effect, whereas 543 del/ins displays no dominant negative effect. All constructs were assembled in the bicistronic pIRE2-EGFP vector (BD Biosciences/Clontech) for mammalian cell expression experiments, then verified by restriction mapping and DNA sequencing. Expression of KCNQ1 is driven by the cytomegalovirus early promoter and enables simultaneous expression of enhanced green fluorescent protein (GFP) from the same plasmid to mark transfected cells.

Mutant KCNQ1 plasmids were expressed in a Chinese hamster ovary cell line (CHO-K1, CRL 9618, American Type Culture Collection) stably expressing wild-type KCNQ1 and its ancillary subunit KCNE1 to generate the repolarizing current I\textsubscript{K}\text{c}, as previously described. This I\textsubscript{K}\text{c} cell line was generated by stable integration of a bicistronic KCNE1-IRE2-2KCNQ1 cassette by use of targeted homologous recombination mediated by Flp recombinase. This approach enabled uniform expression of KCNQ1 and KCNE1 from a single genomic locus and resulted in a consistent level of current in
all cells. Cells were grown at 37°C in 5% CO2 in F-12 nutrient mixture medium supplemented with 10% fetal bovine serum (FBS, Atlanta Biologicals), 2 mmol/L L-glutamine, penicillin (50 U/mL)–streptomycin (50 μg/mL), and 600 μg/mL hygromycin. All tissue culture media were obtained from Life Technologies. Cells were transiently transfected by use of Fugene-6 (Roche Diagnostics Corp). After transfection (48 to 72 hours), fluorescent cells were selected by epifluorescence microscopy for use in whole-cell patch-clamp recording experiments.

Electrophysiology and Data Analysis

Whole-cell currents were measured in the whole-cell configuration of the patch-clamp technique14 by use of an Axopatch 200B amplifier (Axon Instruments Inc). The bath solution consisted of (in mmol/L): NaCl 132, KCl 4.8, MgCl2 1.2, CaCl2 2, glucose 5, HEPES (N-[2-hydroxyethyl]piperazine-N’-[2-ethanosulfonic acid]) 10, pH 7.4, ~275 mOsm/kg. The pipette solution consisted of (in mmol/L): K-aspartate 110, ATP-K2 5, MgCl2 1, EGTA (ethylene glycol-bis-[β-aminoethy] ether)] 11, HEPES 10, MgCl2 1, pH 7.3, ~265 mOsm/kg. The pipette solution was diluted 7% to 10% with distilled water to prevent activation of swelling-activated currents. Patch pipettes were pulled from thick-wall borosilicate glass (World Precision Instruments) with a multistage P-97 Flaming-Brown micropipette puller (Sutter Instruments Co) and fire-polished. Pipette resistance was 1 to 4 MΩ, and as reference electrode, a 1% to 2% agar bridge with composition similar to that of the bath solution was used. Whole-cell current traces were filtered at 5 kHz and acquired at 1 to 2 kHz. All chemicals were purchased from Sigma Chemicals.

The holding potential was —80 mV, and whole-cell currents were measured from —80 to +60 mV (in 10-mV steps) 1990 ms after the start of the voltage pulse. The access resistance and apparent membrane capacitance were estimated as described by Lindau and Neher.15 Pulse generation and data collection and analyses were performed with Clampex 8.1 (Axon Instruments, Inc) and Sigmaplot 7.0 software suites.

Figure 1. Lines of descent of the KCNQ1-A341V mutation from a common founder couple P are shown. The haplotype that segregates with the mutation is consistent with a common origin of the mutation and with minimal recombination over 10 generations. Genealogical information for pedigrees 170, in which the common haplotype segregates, and for pedigree 180, a single individual, could not be found. Haplotype construction was based on the results of the combination of alleles inherited at D11S4046, D11S1318, A341V, D11S4088, D11S4146, D11S4181, D11S1871, D11S1760, and D11S1323 in the order telomere to centromere.Common haplotypes are bordered. Index case is shown as a diamond to preserve anonymity. Circles denote females and squares males in the line of descent. Ped indicates pedigree. Year of birth is shown below individuals. The letters P, Q, and T refer to couples in the first 2 generations from which the mutation descended.

Results

Family Ascertainment, Genealogy, and Genotyping

A LQTS founder population (SA-A341V) consisting of 22 apparently unrelated kindreds was ascertained in South Africa. All index cases could be traced to a single founding couple, of mixed Dutch and French Huguenot origin, who married in approximately 1730 (Figure 1). The disease-associated haplotypes of index cases strongly support the founder hypothesis (Figure 1).

Of 345 individuals in the study population, 166 were mutation carriers, 154 were noncarriers, and 25 were not genetically tested (Figure 2).
Clinical Phenotypes

Among the 166 MCs, females (54%) and males were similarly represented; 131 (79%) had symptoms, with a median age at first cardiac event of 6 years (interquartile range, 4 to 10), and 23 (14%) suffered sudden cardiac death before age 20 years. The 26 patients here defined as asymptomatic were those older than 15 years with no events. Nine other patients without events were too young (age <15 years) to be designated as asymptomatic.16

Eighty-six MCs and 102 noncarriers, with a basal ECG recorded after age 15 (Figure 2), were analyzed for differences in QTc interval, HR, and symptoms. Baseline QTc was longer among symptomatic patients (487±45 versus 401±25 ms, P<0.001; Figure 3A). Despite sharing the same genetic defect, mutation carriers as a group exhibited a wide range of QTc values (406 to 676 ms), with 12% of individuals having a normal QTc (<440 ms). QTc was longer among symptomatic compared with asymptomatic MCs (493±48 versus 468±31 ms, P=0.026; Figure 3B). A QTc ≥500 ms was associated with an increased risk of experiencing cardiac events (OR=4.22; 95% CI, 1.12 to 15.80; P=0.033).

Because Iκs magnitude is rate-dependent, we examined the role of HR as a predictor of events. Baseline HR was not different between MCs and noncarriers (69±12 versus 70±11 bpm). The baseline HR of symptomatic MCs was also very similar to that of noncarriers (71±11 versus 70±11 bpm). By contrast, asymptomatic carriers had a significantly lower HR compared with symptomatic individuals (65±13 versus 71±11 bpm, P=0.026). MCs in the lowest 2 tertiles, defined by HR <73 bpm, were at lower risk for cardiac events compared with those with in the highest tertile, HR ≥73 bpm (OR=0.33; 95% CI, 0.19 to 0.58; P=0.005). There was no correlation between age and HR. We performed multivariate analyses to determine the clinical variables (HR, QTc, sex) best predicting risk for cardiac events in the SA-A341V population. We considered HR and QTc as categorical variables with the same cutoff used in univariate analysis. Both QTc ≥500 ms (OR=4.98; 95% CI, 1.21 to 20.55; P=0.026) and HR ≥73 bpm (OR=4.11; 95% CI, 1.03 to 16.44; P=0.046) were found to be significant risk factors for experiencing cardiac events after other covariates included in the model had been controlled for. Sex was not an independent risk factor in our analysis.

Results from both univariate and multivariate analyses identify HR and QTc as important factors in determining disease expression in this population. Figure 4A presents the distribution of symptomatic and asymptomatic MCs among 4 quadrants defined by cutoff values of HR and QTc. The smallest proportion of symptomatic subjects was found in the quadrant defined by HR <73 bpm and QTc <500 ms. In this subgroup, the risk of cardiac events was significantly lower than for all other subjects combined (OR=0.19; 95% CI, 0.06 to 0.59; P=0.005). However, there was still a significant risk of cardiac events in this subgroup, because most subjects (60%) represented in this quadrant were symptomatic. Interestingly, the impact of HR in risk stratification was stronger in the subgroup of patients with a QTc <500 ms compared with that with a QTc ≥500 ms. Indeed, there was a linearly increasing proportion of symptomatic mutation carriers from the lower to the upper tertile of HR, representing an incremental risk (OR=2.5; 95% CI, 1.11 to 5.62; P=0.026) (Figure 4B).

Clinical Severity in the South African LQTS Population

Because our ascertainment revealed relatively few symptomatic patients in the SA-A341V population and a 14% incidence of sudden death before the age of 40 years, we considered the possibility that the KCNQ1-A341V mutation segregating in these families might be associated with a
greater incidence of cardiac events compared with that reported for LQT1 subjects in general. To test this hypothesis, we compared clinical severity between the SA-A341V and LQT1 populations. In our population, the availability of information for 161 KCNQ1-A341V MCs, including 126 who were symptomatic, allowed us to analyze the cumulative event-free survival (Kaplan-Meier analysis) before the institution of β-blocker therapy and before age 40 years. Five MCs were not included in the analysis because time to first event was not available.

Compared with the LQT1 population, the SA-A341V group exhibited a more severe form of the disease. The SA-A341V carriers became symptomatic earlier than the LQT1 population (7.4 ± 4.9 versus 13 ± 9 years, \( P < 0.001 \)), with a 79% incidence of a first cardiac event by age 40 years, compared with 30% observed for LQT1 (\( P < 0.001 \)). As shown in Figure 5, the cumulative probability of suffering a first cardiac episode before β-blocker therapy was initiated and before age 40 was significantly greater (\( P < 0.0001 \)) for the SA-A341V population, in which the event-free survival is 20% by age 15, compared with 80% for the LQT1 database.

Our findings on the clinical severity of the SA-A341V phenotype could be partly explained by the significantly lower prevalence of MCs with a QTc <440 ms in this population compared with the LQT1 database (12% versus 36%, \( P < 0.001 \)). This in turn can account for the longer QTc measured in MCs in our population (487 ± 45 versus 466 ± 44 ms, \( P < 0.001 \)). Because of this concern, we performed separate Kaplan-Meier estimates of cardiac event–free survival for groups distinguished by QTc. Furthermore, to avoid the confounding role of patients with QTc <440 ms, we restricted our analysis to subjects with QTc ≥500 ms. We also constructed Kaplan-Meier curves separately by sex and observed that they were similar for males and females. Because the time course of events is much more rapid for LQT1 males, who have their events earlier in time, and because in our population, the number of males available for this analysis was small, we have chosen not to combine data for this analysis was small, we have chosen not to combine data for males and females and to present in Figure 6 only the data based on the larger and more homogeneous female group (n = 24). These analyses support the finding that the clinical severity of LQTS observed in the SA-A341V population is significantly greater than that in LQT1 in general.

**Functional Characterization of KCNQ1-A341V**

Previous studies have characterized the KCNQ1-A341V mutation in *Xenopus* oocytes or COS7 cells. These studies reported that this allele is a simple loss-of-function mutation that does not exhibit a dominant negative effect on wild-type KCNQ1, suggesting that it may cause less severe disease. However, oocytes express an endogenous *Xenopus* KCNQ1 and multiple KCNE accessory subunits. Accordingly, and
in light of our observation that South African LQTS families segregating KCNQ1-A341V have a severe clinical phenotype, we reassessed the functional consequences of this allele in a cultured mammalian cell system. In these experiments, we recognized the need to verify coexpression of wild-type and mutant KCNQ1 along with the KCNE1 accessory subunit, a potential confounding variable for interpreting previously reported COS7 cell data. Therefore, we transiently transfected KCNQ1-A341V-IRES-EGFP into CHO cells stably expressing consistent levels of wild-type KCNQ1 with KCNE1 (stable \( I_Ks \) cell line). We then performed whole-cell patch-clamp recording on cells exhibiting green fluorescence, ie, cells coexpressing both A341V and both subunits for \( I_Ks \) (Figure 7). Cells transiently transfected with the empty pIRES2-EGFP expression vector exhibited slowly activating outward current consistent with \( I_Ks \). Coexpressing KCNQ1-A341V in these cells reduced the magnitude of \( I_Ks \) by approximately 50%. By comparison, coexpression of a recessive LQTS mutant (543-del/ins) had no effect on \( I_Ks \) amplitude, whereas a strong dominant LQTS mutation (G314S) suppressed current by approximately 70% at positive voltages. These results demonstrate that KCNQ1-A341V exerts dominant suppression of \( I_Ks \) to an extent slightly less than a strong dominant mutation but behaves in a manner distinct from a pure loss-of-function allele.

**Discussion**

In the present study, we analyzed a population of LQT1 patients, established in South Africa more than 3 centuries ago and sharing a mutation identical by descent caused by a founder effect, to begin defining factors capable of explaining phenotypic variability in this disease. We identified HR, a marker of autonomic tone, as a novel factor determining risk for cardiac events in this LQT1 population. We also provided unexpected evidence for the unusual clinical severity manifested by carriers of the KCNQ1-A341V mutation in this population and further demonstrated, contrary to previous reports, that this mutation exerts dominant-negative effects on heterologously expressed \( I_Ks \). Our findings have several conceptual and clinical implications.

**Phenotypic Heterogeneity in a LQTS Founder Population**

The concept that some patients might have LQTS and also a normal QT interval (phenotypic heterogeneity) was originally proposed in 1980. Support for this hypothesis emerged with the observation that within the International LQTS Registry, approximately 5% to 6% of family members with a QTc...
<440 ms suffered cardiac events. In 1992, Vincent et al reported that in 3 LQTS families linked to chromosome 11, there was overlap in QTc duration in the range of 440 to 460 ms between MCs and noncarriers and that 6% of carriers had a QTc <440 ms. The relationship between clinical heterogeneity and the phenomenon of low penetrance was demonstrated by Priori et al in 1999 by showing that asymptomatic MCs with normal QT intervals can be identified in LQTS families.

The observation of incomplete penetrance in LQTS affects the estimation of disease prevalence, fosters the notion that a reduced "repolarization reserve" might create a substrate predisposing to drug-induced torsades de pointes, and defines a clinically important subset of LQTS patients as "silent MCs" that have a latent form of the syndrome. Explaining incomplete penetrance in LQTS is a great challenge that will require new clinical resources and insights into disease behavior. We believe that the South African LQTS founder population is such a resource and has great potential for addressing important unanswered questions about this disease.

Our present data further illustrate phenotypic heterogeneity in LQTS within a large population of related LQTS individuals carrying the same primary mutation. Given the expectation that all individuals carrying KCNQ1-A341V should have similar reductions in \( I_{Ks} \), our findings point to the existence within this South African population of additional genetic or environmental influences affecting duration of the QT interval and the probability of cardiac events. Common variants in cardiac ion channel genes similar to or including those recently described by Pfeuffer et al are excellent candidate modifiers. In this regard, we recently demonstrated that a very common KCNH2 polymorphism (K897T) can exaggerate loss of repolarizing current produced by a LQTS mutation and unmask a severe clinical phenotype. Equally plausible are modifier genes having no obvious link to myocyte electrophysiology.

**Heart Rate as a Risk Factor in LQTS**

The repolarizing current \( I_{Ks} \) activates during increased HR and is essential for QT interval adaptation during tachycardia. Without this adaptive response, the progressive reduction in the RR interval could lead to ventricular activation during the vulnerable period of the T wave, increasing the probability of ventricular fibrillation. This helps to explain why 79% of the lethal arrhythmic episodes in LQT1 patients with mutations impairing \( I_{Ks} \) occur during exercise. This is in striking contrast to the observation that most lethal episodes for LQT2 and LQT3 patients occur during startle reactions and at rest or sleep, respectively.

Adrenergic activation, as well as fast HRs (even without adrenergic activation), leads to accumulation of \( I_{Ks} \). In LQT1 patients, \( I_{Ks} \) accumulation is impaired as a result of reduced function of channels containing a mutant subunit and may contribute to reduced adaptation of action potential duration during increased HR, the well-known "failure to shorten" the QT interval on exercise characteristic of LQT1. This phenomenon also facilitates the occurrence of electrical alternans, which often manifests on the surface ECG as T-wave alternans, a phenomenon described first in an LQT1 patient under emotional stress and recognized as a marker of cardiac electrical instability often preceding onset of torsades de pointes.

The critical role of \( I_{Ks} \) in QT adaptation led us to explore whether the propensity to develop cardiac events in the SA-A341V population was related to HR. We found that, although symptomatic MCs had values almost identical to those of the noncarriers, the asymptomatic carriers had a significantly lower HR at rest. Indeed, a resting HR <73 bpm significantly lowered the probability of having arrhythmic events. Thus, a lower HR, even with the caution necessary when dealing with relatively small differences, appears to be a protective factor for patients with mutations affecting \( I_{Ks} \).

This novel finding may partially explain the high efficacy of \( \beta \)-blocker therapy for LQT1 patients. Indeed, our 2 recent studies showed that in 157 and in 187 LQT1 patients, the
long-term combined incidence of cardiac arrest and sudden death was only 1.2% and 1.1%, respectively. Thus, it appears that β-blockers are effective in LQT1 patients because they act not only on the triggers but also on the substrate by modifying HR.

Conversely, whereas patients with a resting HR ≥73 bpm were all at higher risk for cardiac events (OR=4.22, 95% CI 1.13 to 15.81; P=0.033), among the patients with a QTc <500 ms, there was a linear correlation between risk and HR level. This is practically important because it suggests that when the substrate is weaker, the arrhythmogenic role of faster rate becomes predominant, whereas it is somewhat less important in the presence of a major arrhythmogenic substrate (QTc ≥500 ms). This finding, to be accepted within the caution appropriate for extrapolations from a single ECG tracing, contributes to the novel concept, originated by this study, that HR plays a significant modulating role on the risk for cardiac events and that this arrhythmogenic role is accentuated in the presence of a moderate, but not excessive, QT prolongation.

**Clinical Severity in SA-A341V**

In studying this founder population, we made the serendipitous finding that KCNQ1-A341V is associated with a very high incidence of cardiac events. Previously, the only suggestion that different mutations could carry a different risk for cardiac events was limited to their location in the predicted topology of the gene product, and most of the interest focused on whether or not the mutations were located within or outside the pore region. Donger et al suggested that KCNQ1 C-terminal mutations are associated with a forme fruste of LQTS, and Moss et al provided evidence for a more malignant clinical course associated with KCNH2 mutations in the pore region. Subsequently, Zareba et al reported that among LQT1 patients, no differences in the risk for cardiac events were observed between those with transmembrane mutations and those with C-terminal mutations. By contrast, Shimizu et al found that LQT1 patients with transmembrane mutations were at higher risk for cardiac events.

In the present study, we found that KCNQ1-A341V is associated with an unusually severe clinical phenotype in the South African population. This conclusion is supported by multiple lines of evidence. There is a striking, and highly significant, difference in the percentage of symptomatic patients and in Kaplan-Meier survival curves between SA-A341V patients and LQT1 database patients with a diversity of mutations. Furthermore, when the analysis was limited to the more homogeneous group of females with a QTc ≥500 ms, the large difference in the probability of experiencing a cardiac event was still observed. Finally and importantly, cardiac mortality is particularly high in these patients, because 23 of 166 (14%) died suddenly.

Given the importance of this finding, we also compared our SA-A341V population to the one reported by Zareba et al, even though it included only 112 patients from 10 families (Data Supplement Figure, http://circ.ahajournals.org/cgi/content/full/CIRCULATIONAHA.105.572453/DC1). This was done because the population of the study by Zareba et al had an average QTc very similar to that of the SA-A341V patients (490±43 versus 487±45 ms). The Kaplan-Meier curves remained significantly different (P<0.01); as significant were the differences among patients with a first event by age 15 years (53% versus 80%, P<0.001) and the difference in mortality (2% versus 14%, P<0.001). This conclusively demonstrates the unusual clinical severity associated with A341V. Our data also confirm the major prognostic importance of a QTc ≥500 ms, but in a more genetically uniform population compared with previous studies.

**Dominant Suppression of I\textsubscript{Ks} by KCNQ1-A341V**

Previous studies examining the functional consequences of KCNQ1 mutations associated with LQTS have revealed a spectrum of channel dysfunction. Consistent with the autosomal dominant inheritance of Romano-Ward syndrome, many KCNQ1 mutations exert dominant negative effects on the wild-type channel in heterologous expression systems, whereas mutations associated with the Jervell and Lange-Nielsen syndrome are typically pure loss-of-function alleles. Previous characterization of KCNQ1-A341V in oocytes or COS7 cells demonstrated little or no dominant activity of this mutation, suggesting that it may be associated with a milder form of the disease. However, our survey of the phenotype associated with this allele indicates that it confers a more severe clinical picture (Figures 5 and 6). We reassessed the functional properties of this mutation by using a mammalian cell system that ensures coexpression of both wild-type and mutant channels with the KCNE1 accessory subunit. This system avoids the potential confounding influence of endogenous KCNQ1 and KCNE channel subunits that exist in Xenopus oocytes and the uncertainties associated with transient transfection of multiple separate plasmids in COS7 cells. Our findings indicate that KCNQ1-A341V exerts a dominant-negative effect on I\textsubscript{Ks}, and therefore is not a pure loss-of-function mutation. This is more consistent with an allele associated with a severe clinical phenotype.

**Clinical Implications**

The present data amplify the evidence for phenotypic heterogeneity in LQTS and indicate that even within populations sharing an identical mutation resulting from a founder effect, there is a wide spectrum of clinical manifestations. Our findings also indicate that risk stratification for LQTS patients must be more individually tailored and may have to take into account the specific mutation and probably additional relevant factors, such as HR. It is also evident that this South African LQTS population represents a useful human disease model for the identification and study of modifier genes.

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**References**


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