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Neural Control of Heart Rate Is an Arrhythmia Risk Modifier in Long QT Syndrome

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Objectives The purpose of this study was to test the hypothesis that differences in autonomic responses might modify clinical severity in long QT syndrome type 1 (LQT1) patients, those with *KCNQ1* mutations and reduced I_{Ks}, in whom the main arrhythmia trigger is sympathetic activation.

Background Some long QT syndrome (LQTS) patients experience life-threatening cardiac arrhythmias, whereas others remain asymptomatic throughout life. This clinical heterogeneity is currently unexplained.

Methods In a South African LQT1 founder population segregating *KCNQ1*-A341V, we correlated major cardiac events to resting heart rate (HR) and to baroreflex sensitivity (BRS) on and off beta-adrenergic blockers (BB).

Results In 56 mutation carriers (MCs), mean HR was lower among asymptomatic patients ($p < 0.05$). Among MCs with a QT interval corrected for heart rate ≤500 ms, those in the lower HR tertile were less likely to have suffered prior cardiac events (odds ratio [OR] 0.19, 95% confidence interval [CI] 0.04 to 0.79, $p < 0.02$). The BRS was lower among asymptomatic than symptomatic MCs (11.8 ± 3.5 ms/mm Hg vs. 20.1 ± 10.9 ms/mm Hg, $p < 0.05$). A BRS in the lower tertile was associated with a lower probability of being symptomatic (OR 0.13, 95% CI 0.02 to 0.96, $p < 0.05$). A similar trend was observed during BB. The MCs in the lower tertile for both HR and BRS were less frequently symptomatic than MCs with different patterns (20% vs. 76%, $p < 0.05$). Subjects with either ADRA2C-Del322-325 or homozygous for ADRB1-R389, 2 polymorphisms predicting enhanced adrenergic response, were more likely to have BRS values above the upper tertile (45% vs. 8%, $p < 0.05$).

Conclusions Lower resting HR and "relatively low" BRS are protective factors in *KCNQ1*-A341V carriers. A plausible underlying mechanism is that blunted autonomic responses prevent rapid HR changes, arrhythmogenic when I_{Ks} is reduced. These findings help understanding phenotypic heterogeneity in LQTS and identify a physiological risk modifier, which is probably genetically determined. (J Am Coll Cardiol 2008;51:920–9) © 2008 by the American College of Cardiology Foundation

A major question in cardiology concerns the mechanisms underlying different clinical severity among patients with the same disease. For instance, why do almost 5% of patients with an acute myocardial infarction (MI) develop ventricular fibrillation in the first few hours and often do not survive? A rational approach to this problem is to exploit studies of monogenic diseases. Long QT syndrome (LQTS), with the availability of founder populations (1) and with its well defined genotype-phenotype correlation (2), offers unique opportunities to explore factors that modify the outcome of arrhythmic disorders.

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Founder populations provide the presence of the same mutation in a relatively large number of individuals, thus allowing a comparison between mutation carriers (MCs) with and without clinical events (i.e., syncope, cardiac arrest, sudden death in the case of LQTS). Genotype-phenotype studies have revealed that, in the most common variant of LQTS—long QT syndrome type 1 (LQT1)—caused by mutations in *KCNQ1* encoding the pore-forming subunit of the delayed rectifier potassium current (slow) (I_{Ks}), most cardiac events occur under conditions associated with augmented sympathetic activity such as physical and emotional stress (2). Indeed, mutations affecting I_{Ks} create an arrhythmogenic substrate exquisitely vulnerable to adrenergic activation and to heart rate (HR) increases (3,4). The availability of a well characterized South African LQT1 founder population segregating *KCNQ1*-A341V and in which resting HR levels—assessed during a standard electrocardiogram (ECG)—seem associated with arrhythmic risk (1) offered the opportunity to test our hypothesis that the autonomic nervous system could act as an arrhythmia risk modifier in LQTS (5).

We had provided experimental (6) and clinical (7) evidence that alterations in the autonomic control of the heart, highlighted by depressed baroreflex sensitivity (BRS)—largely a marker of reduced cardiac vagal activity—are associated with increased risk for arrhythmias and sudden death after an MI. The wide distribution of BRS values in normal animals (6) suggested that these and other autonomic responses are under at least partial genetic control (8).

To define whether individual differences in autonomic responsiveness are associated with a higher or lower propensity for life-threatening arrhythmias, we assessed tonic and reflex control of HR in the South African LQT1 founder population by determining resting HR and BRS. We specifically tested the hypothesis that MCs with life-threatening arrhythmias would have had, as post-MI patients, lower BRS values compared with asymptomatic carriers. We also considered the possibility that independently transmitted functional polymorphisms of selected adrenergic receptors might contribute to BRS values, thus interacting with the *KCNQ1*-A341V mutation and increasing or decreasing arrhythmic risk.

Methods

Study population. We focused on an LQT1 founder population harboring an identical LQTS-causative mutation in *KCNQ1* (A341V) (1). Clinical and genetic data were recorded on specifically designed forms including 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) or aborted cardiac arrest (requiring resuscitation). The MCs were classified as either symptomatic or asymptomatic on the basis of their history of cardiac events. Symptomatic subjects were MCs who had experienced at any time in the past at least 1 cardiac event irrespective of therapy, whereas to be defined asymptomatic an MC had to be at least 20 years old and without prior cardiac events off therapy. Therefore, throughout the text, "risk" is used to define the association between specific parameters and probability of having suffered cardiac events at any time in the past. A QT interval corrected for heart rate (QTc) >500 ms was regarded as indicating a major arrhythmogenic substrate (1,9).

Abbreviations and Acronyms

BB = beta-adrenergic blockers
BRS = baroreflex sensitivity
CI = confidence interval
ECG = electrocardiogram
HR = heart rate
I_{Ks} = delayed rectifier potassium current (slow)
LQT1 = long QT syndrome type 1
LQTS = long QT syndrome
MC = mutation carrier
OR = odds ratio

All probands and family members provided written informed consent for clinical and genetic evaluations, as approved by the ethical review boards of the Stellenbosch, Vanderbilt, and Pavia universities.

Genotyping. Peripheral blood was collected from all index cases and family members entered in the study. From genomic deoxyribonucleic acid (DNA) the A341V mutation was detected through polymerase chain reaction and restriction digestion (1).

Adrenergic receptor gene polymorphisms were screened by different techniques. The *ADRB1*-S49G, *ADRB1*-G389R, *ADRB2*-R19C, *ADRB2*-R16G, *ADRB2*-Q27E, *ADRB2*-T164I, *ADRB3*-W64R, and *ADRA1A*-R492C were evaluated with the TaqMan 5' nucleotidase assay (ABI Prism 7900HT, Applied Biosystems, Foster City, California), and one-third of cases were randomly selected for validation by restriction fragment-length polymorphism (RFLP) methods. The *ADRA2B*-Del301-303 and *ADRA2C*-Del322-325 were evaluated by denaturing high-performance liquid chromatography analysis (Wave model 3500HT, Transgenomic, Omaha, Nebraska), and one-third of cases were randomly validated by RFLP methods.

Autonomic evaluation. All subjects were either hospitalized or stayed in a hotel close to Tygerberg Hospital for 4 days to be studied both on and off beta-adrenergic blockers (BB). Plasma concentrations of propranolol and atenolol were determined by high-performance liquid chromatography with fluorometric detection. Plasma levels below 20 ng/ml were considered "nontherapeutic."

Measurement of HR and BRS. Parameters of time- and frequency-domain HR variability (10) were calculated after environmental acclimation, while the subjects were resting comfortably. The ECG, blood pressure, and respiratory activity were simultaneously recorded for a 10-min period, digitized, and stored by a PC-based software system (Ans-Lab, Bioengineering Service Montescano, Italy).

The BRS was determined by the phenylephrine method, relating a transient increase in blood pressure (20 to 30 mm Hg) induced by bolus injections of phenylephrine (2 to 3

μg/kg, as necessary) to the resultant lengthening of the RR interval (11,12). The slope of a best-fit regression line defined BRS. Beat-to-beat RR interval and blood pressure were continuously recorded (FINAPRES, Ohmeda, Englewood, New Jersey) and then digitally converted. The BRS was calculated by 2 experienced investigators (E.V. and M.T.L.R.) blinded to each other's measurements.

Age has an important influence on BRS (12), and indeed in the 75 subjects under study whose age range was 16 to 65 years, there was a significant ($p < 0.001$) negative correlation between age and BRS ($r = -0.67$). Accordingly, we focused our analysis on the second and third age quartiles that included 38 subjects (age 26 to 47 years) and represented the middle 50% of the tested population. In this group the correlation with age was not significant (see the Results section). This approach allowed a reliable assessment of BRS while controlling for the age effect.

Statistical analysis. Continuous variables are presented as mean ± SD and compared by Student *t* test or Mann-Whitney *U* test as appropriate, without adjustment for multiple comparisons. The relationships of BRS with age and HR were analyzed by nonparametric Spearman correlations. Categorical variables were expressed as absolute and relative frequencies and were compared by the Fisher exact test or chi-square test. The change in HR and in BRS values produced by BB was evaluated by the paired-samples *t* test procedure or by the Wilcoxon signed rank test. To determine the association of HR and BRS with the occurrence of cardiac events in the population under study, these variables were divided into tertiles of their respective distribution. Unadjusted odds ratios (ORs) with 95% confidence intervals (CIs) were estimated by logistic regression. A 2-sided p value <0.05 was considered statistically significant. All analyses were performed with SPSS software (version 12, SPSS Inc., Chicago, Illinois).

Results

Figure 1 explains the relationship between the current group of study subjects (n = 83) and the previously reported population (1). Heart rate data were available for all subjects, but some did not complete the BRS study either on or off BB for various reasons.

Influence of HR on symptoms. Figure 2A illustrates that resting HR off BB was no different between the 56 MCs (41 with and 15 without symptoms) and the 27 non-MCs (65 ± 9 beats/min vs. 68 ± 9 beats/min, p = 0.08). Similarly, there was no difference when we compared only symptomatic MCs to non-MCs (66 ± 9 beats/min vs. 68 ± 9 beats/min, p = NS). By contrast, a significant difference emerged when we compared asymptomatic and symptomatic MCs: asymptomatic MCs had a significantly lower HR (61 ± 8 beats/min vs. 66 ± 9 beats/min, p = 0.038) (Fig. 2B).

The same analysis was repeated in 50 subjects (38 MCs and 12 non-MCs) during BB. Treatment reduced HR by 9 ± 6 beats/min ($p < 0.001$). When examined separately for non-MCs, symptomatic MCs, and asymptomatic MCs, the results resembled the findings observed in the absence of BB. The MCs and non-MCs had similar HR values (55 ± 9 beats/min vs. 57 ± 9 beats/min, p = NS), but the asymptomatic MCs (n = 11) had a significantly lower HR

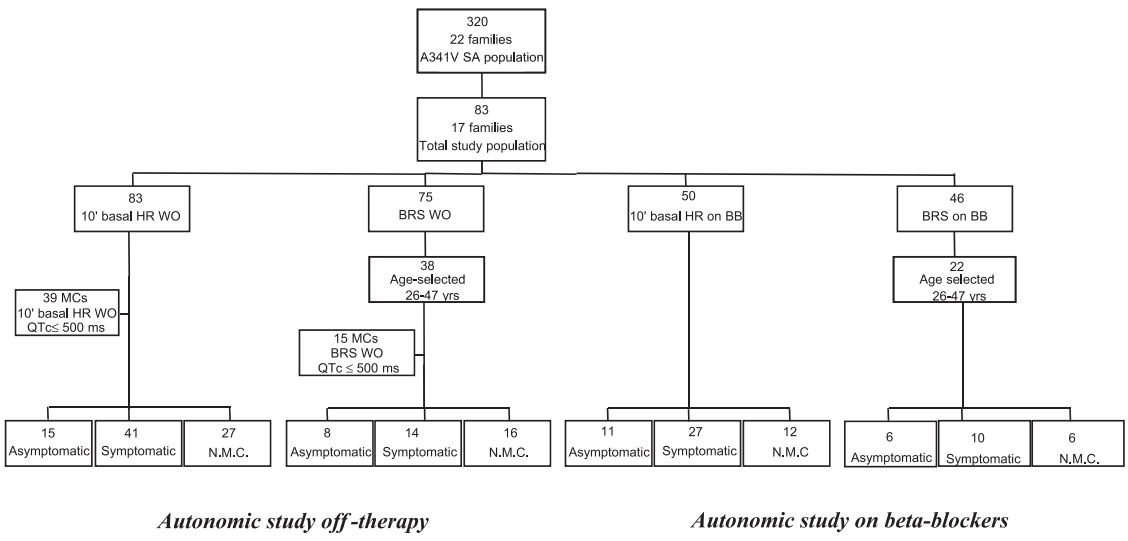

Figure 1 Study Population and Outline of the Study

Flow chart of the study population showing the number of subjects, divided in subgroups by genetic status, considered in the different analyses performed on and off beta-adrenergic blockers (BBs). For the study on BB, the number of subjects was limited to those with both measurements—on- and off-treatment—available, allowing for paired comparisons. BRS = baroreflex sensitivity; HR = heart rate; MC = mutation carriers; N.M.C. = nonmutation carriers; SA = South African; WO = Wash-Out.

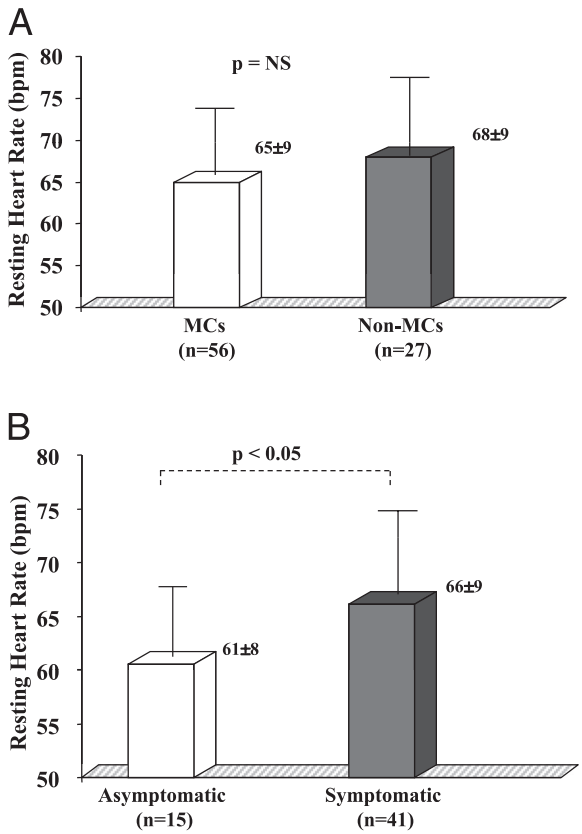

Figure 2 **Heart Rate Values**

Comparison of resting heart rate mean values off BB therapy between mutation carriers (MCs) and non-MCs **(A)** and between asymptomatic and symptomatic MCs **(B)**. bpm = beats/min; other abbreviations as in Figure 1.

compared with the 27 symptomatic MCs (51 ± 11 beats/min vs. 57 ± 8 beats/min, $p < 0.05$).

We assessed whether the level of resting HR was associated with a history of arrhythmic events in the subgroup of patients with a QTc ≤500 ms and found that MCs in the first tertile (HR ≤60 beats/min) were less frequently symptomatic compared with MCs in the other 2 tertiles (OR 0.19, 95% CI 0.04 to 0.79, $p = 0.023$) (Fig. 3). Figure 3 also provides evidence that a QTc >500 ms represents a more severe arrhythmogenic substrate in our population, on the basis of the fact that 15 of 16 (94%) MCs with a QTc >500 ms were symptomatic.

Heart rate variability, both in time- (SDNN) and frequency-domain (LF/HF), was no different between asymptomatic and symptomatic MCs (45 ± 19 ms vs. 36 ± 17 ms, and 1.5 ± 1.2 ms vs. 1.4 ± 1.4 ms, respectively).

BRS and risk for cardiac events. The analysis of BRS was limited to subjects in the 2 middle age quartiles (26 to 47 years) to avoid the influence of age (see Methods). Indeed, within this group (n = 38) there was no correlation between BRS and age ($r = -0.17$, p = NS). Furthermore, even subdividing this group according to the median value (36 years), there was no difference in BRS across the 2 subgroups.

The mean value of BRS in the entire group of 38 subjects was 16.7 ± 8.8 ms/mm Hg (Fig. 4A). As expected by a distribution of autonomic parameters independent of the LQTS mutation, the BRS values between MCs (n = 22) and non-MCs (n = 16) were no different (17.1 ± 9.7 vs. 16.3 ± 7.6, p = NS) (Fig. 4B). By contrast, a significant difference emerged when the analysis compared the asymptomatic (n = 8) with the symptomatic (n = 14) MCs (11.8 ± 3.5 vs. 20.1 ± 10.9; $p < 0.05$) (Fig. 4C).

A BRS ≤12 ms/mm Hg, which corresponds to the first tertile of the distribution among all the 38 subjects, was associated with a significantly lower probability of having suffered cardiac events compared with a BRS in the upper 2 tertiles (OR 0.13, 95% CI 0.02 to 0.96, $p < 0.05$) (Fig. 4C). Indeed, among MCs with a BRS >12 ms/mm Hg, 10 of 12 (83%) subjects were symptomatic. The BRS values in the first tertile, which represents the lower end of the spectrum of normal values among these South African families, will be referred to as "relatively low."

When we assessed whether the level of BRS was associated with a history of cardiac events in the 15 patients with a QTc ≤500 ms, we found that none of the MCs in the first tertile (BRS ≤12 ms/mm Hg) had cardiac events, whereas 80% of those with a BRS >12 ms/mm Hg were symptomatic ($p < 0.01$) (Fig. 5).

In 22 of these 38 subjects we assessed BRS both on and off BB and observed that treatment significantly increased

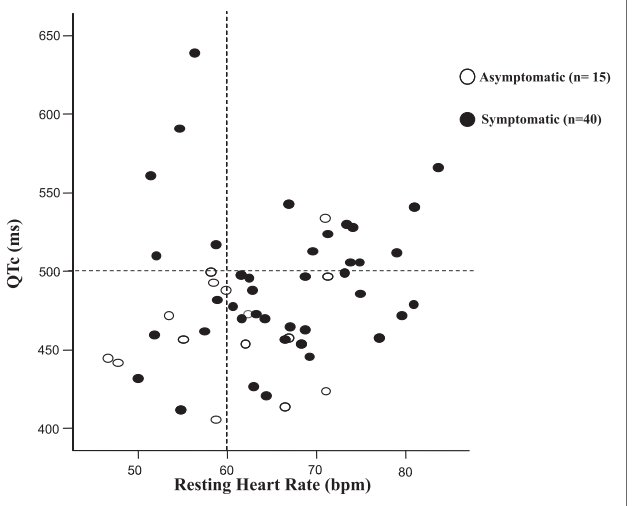

Figure 3 **Differential Risk for Arrhythmic Events According to Resting HR**

Differential risk for arrhythmic events among MCs according to resting heart rate (HR) off BB and QTc. The **dashed horizontal line** represents the predefined cut-off for QTc (≤ or >500 ms), whereas the **dashed vertical line** corresponds to the first tertile (≤60 beats/min) of the HR values distribution. Among the 56 mutation carriers included in the analysis of resting HR in the absence of BB, 1 symptomatic patient had a nonmeasurable QTc because of right bundle branch block. QTc = QT interval corrected for heart rate; other abbreviations as in Figures 1 and 2.

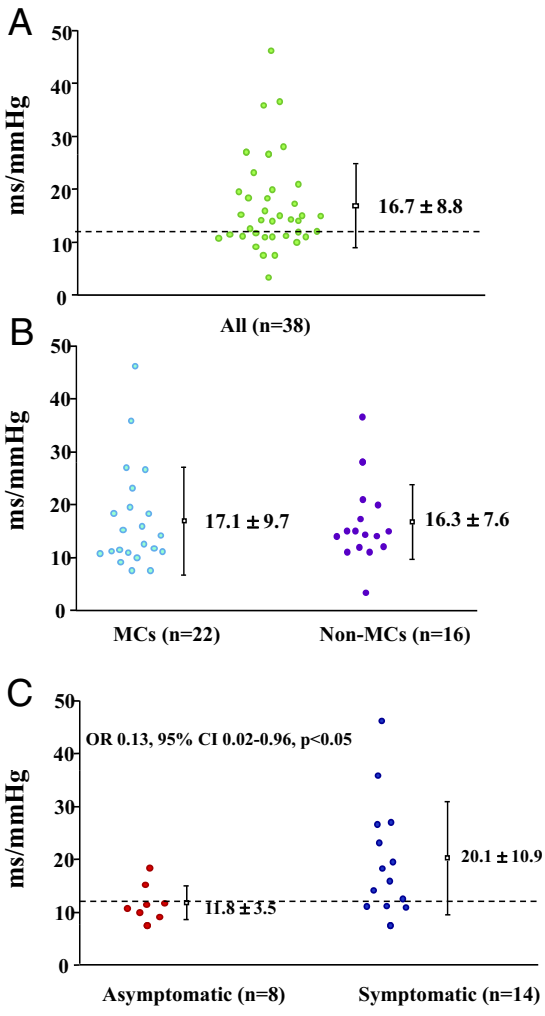

Figure 4 BRS Values

BRS values off-BB in the entire group under study ages 26 to 47 years **(A)**, in MCs and in non-MCs **(B)**, and in symptomatic and asymptomatic MCs **(C)**. Mean and SD are shown for each subgroup. The **dashed horizontal line** represents the lower tertile of BRS values. CI = confidence interval; other abbreviations as in Figures 1 and 2.

BRS by 5 ± 8 ms/mm Hg ($p < 0.05$). Interestingly, whereas a trend for greater BRS during BB therapy was evident in both symptomatic MCs and non-MCs (4.5 ± 7 and 9 ± 11 ms/mm Hg, respectively), it was practically absent in asymptomatic subjects (0.8 ± 7 ms/mm Hg). Among MCs on BB treatment and with a BRS above the lower tertile (14 ms/mm Hg), 7 of 8 (87.5%) subjects had symptoms. Therefore, both off and on BB, having a BRS above the lower tertile was significantly associated with a positive history of cardiac events.

Baroreflex sensitivity was not correlated with resting HR ($r = -0.08$), as expected (13). Given this lack of correlation, we explored the association between the presence of relatively low values of both HR and BRS and history of cardiac events. Figure 6 shows that, across the 4 possible combinations of the 2 parameters, the smallest proportion (20%) of symptomatic subjects was found among MCs in the lower tertile for both HR (≤60 beats/min) and BRS (≤12 ms/mm Hg). This frequency, increasing to 60%, 75%, and up to 87.5% when both BRS and HR were above the corresponding cut-off, was significantly ($p < 0.05$) lower than for all other subjects combined.

Influence of adrenergic receptor polymorphisms. Adrenergic receptor gene polymorphisms were screened and correlated with BRS values. The analyses were limited to 36 subjects in the 2 middle-age quartiles for whom DNA was available. We considered MCs (n = 21) and non-MCs (n = 15) together, because we expected their BRS to be independent of their mutation carrier status.

Although most of these polymorphisms had no significant correlation with BRS values, *ADRA2C*-Del322-325 and *ADRB1*-G389R revealed intriguing and significant results.

Del322-325 is a polymorphism of the alpha-2 adrenergic receptor gene. In the South African population the minor allele frequency was 6.4%, no homozygous subjects for the deletion were identified, and no deviation from Hardy-Weinberg equilibrium was observed. Subjects with *ADRA2C*-Del322-325 had higher BRS values compared with subjects without the deletion (24.9 ± 13.4 ms/mm Hg vs. 15.8 ± 7.5 ms/mm Hg, $p < 0.05$); however, all of them were also carriers of ADRB1-G389R, a well known β1 adrenergic receptor polymorphism. To test the effect of ADRB1-G389R independently from ADRA2C-Del 322-325, we determined BRS values in subjects not carrying this deletion. The BRS was 7.6 ± 0.04 ms/mm Hg in subjects homozygous for G389 (n = 2), 14.8 ± 12 ms/mm Hg in the heterozygous (n = 10), and 17.1 ± 6.9

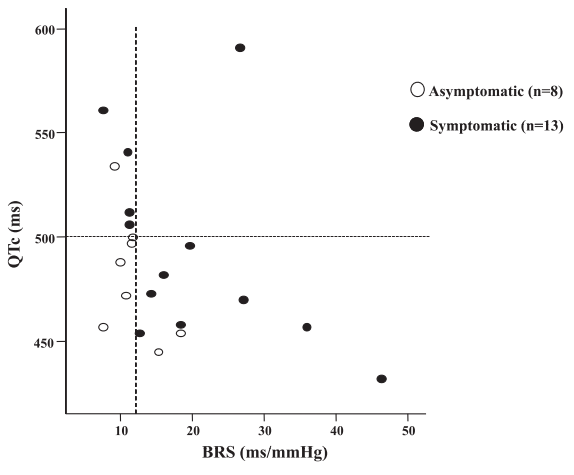

Figure 5 Differential Risk for Arrhythmic Events According to BRS in MCs With a QTc ≤500 ms

The BRS and differential risk for arrhythmic events among MCs ages 26 to 47 years with a QTc ≤500 ms. The **dashed vertical line** corresponds to the lower tertile (≤12 ms/mm Hg) of BRS values distribution. Abbreviations as in Figures 1, 2, and 3.

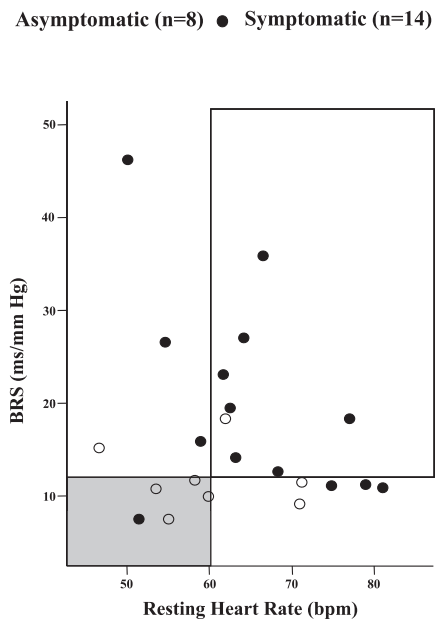

Figure 6 **Impact on Arrhythmic Risk of HR and BRS**

The BRS and resting HR off BB in the entire group ages 26 to 47 years. Differential probability of being symptomatic among MCs according to the combination of both BRS and HR values below or above the predefined cut-off. The **gray quadrant** represents the distribution of symptomatic and asymptomatic patients with relatively lower BRS (≤12 ms/mm Hg) and HR (≤60 beats/min) values in comparison with the other permutations of the 2 autonomic markers. The percentage of symptomatic MCs increases from 20% to 87.5%. Abbreviations as in Figures 1 and 2.

ms/mm Hg in the subjects homozygous for R389 (n = 19). Among these 3 genotypes, the frequency of BRS values above the upper tertile increased progressively from 0% in G389 to 10% in G389R, to 37% in R389, and jumped to 80% in subjects in whom G389R (homozygous for R and heterozygous) is associated with *ADRA2C*-Del322-325 ($p < 0.05$) (Fig. 7).

Because *ADRA2C*-Del322-325 is associated with an increased release of norepinephrine from cardiac sympathetic nerves and because *ADRB1*-R389 is associated with a greater ability to couple to adenylyl cyclase than does *ADRB1*-G389, we hypothesized that the 2 receptor polymorphisms could act synergistically to modify BRS. To test this hypothesis we combined subjects heterozygous for *ADRA2C*-Del322-325 or homozygous for *ADRB1*-R389 (Group 1, 11 families) and compared them with subjects without *ADRA2C*-Del322-325 and not homozygous for the *ADRB1*-R389 allele (Group 2, 7 families). Adrenergic responses were predicted to be enhanced in Group 1 and blunted in Group 2. Group 1 subjects had a higher frequency of BRS values above the upper tertile compared with Group 2 subjects (46% vs. 8%; $p < 0.05$) (Fig. 8). Thus, subjects with a genetic profile predicted to have augmented adrenergic responses had more brisk baroreceptor reflexes.

Discussion

The present study tested the hypothesis (5) that the different clinical severity observed among family members carrying the same LQT1 mutation might be due to variations in the autonomic control of HR, possibly of genetic origin. To reduce the possibility of chance observations, we studied members of a founder population all carrying the same mutation (*KCNQ1*-A341V); we focused on the age group in which autonomic responses were not influenced by age; and, given the potentially confounding role of BB, we performed the study both on and off treatment.

Our hypothesis was that a reduced or relatively low BRS would have been associated with a greater probability of arrhythmic events. This is the pattern that predicts increased arrhythmic and mortality risk after an MI (6,7). We actually found that, contrary to our expectations, lower BRS values were associated with a lower probability of being symptomatic.

There is, however, a plausible biological explanation for this finding. LQT1 subjects are at greatest risk for arrhythmias whenever HR changes too rapidly, at variance with patients affected by ischemic heart disease who are at risk when there is insufficient vagal activity to antagonize the arrhythmogenic sympathetic activity. When HR increases quickly, reduced I_{Ks} prevents the necessary QT adaptation (QT shortening) and a new ventricular depolarization might encroach the vulnerable phase of the T-wave, thus initiating ventricular tachycardia or fibrillation. When HR decreases quickly, the sudden RR lengthening might increase the amplitude of early after-depolarizations and initiate torsades de pointes through triggered activity. This is why for LQT1

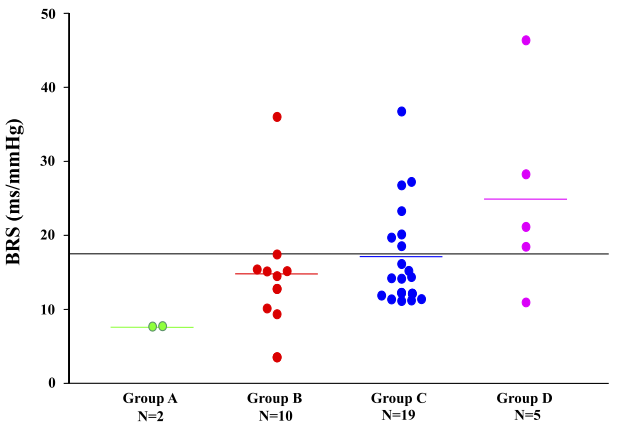

Figure 7 **BRS Values in MCs and Non-MCs According to Presence of ADRA2C-Del322-325 and ADRB1-G389**

The BRS values in MCs and non-MCs ages 26 to 47 years. Groups A, B, and C: subjects without ADRA2C-Del322-325. Group A: subjects homozygous for ADRB1-G389. Group B: subjects heterozygous for ADRB1-G389R. Group C: subjects homozygous for ADRB1-R389. Group D: subjects with ADRA2C-Del322-325 (2 also heterozygous and 3 also homozygous for ADRB1-R389). Abbreviations as in Figures 1 and 2.

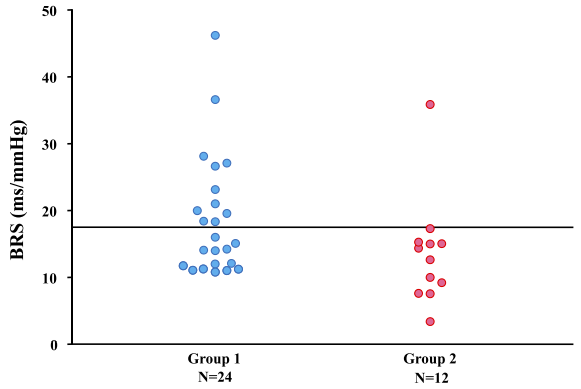

Figure 8 **BRS Values in MCs and Non-MCs According to the Presence or Absence of Adrenergic Polymorphisms**

The BRS values off-BB in subjects ages 26 to 47 years (MCs and non-MCs), according to the specific genotype. Group 1: subjects with the *ADRA2C*-Del322-325 and subjects homozygous for *ADRB1*-R389, polymorphisms resulting in increased adrenergic responses. Group 2: subjects without *ADRA2C*-Del322-325 and not homozygous for the *ADRB1*-R389 allele. The **solid horizontal line** represents the upper tertile of BRS values. Abbreviations as in Figures 1 and 2.

subjects a "blunted" autonomic response, as revealed by relatively low BRS, acts as protective factor.

HR is an arrhythmia risk modifier. In this South African founder population we recently observed that faster HRs were associated with a greater risk for cardiac events (1). That finding called attention for the first time to the possibility that resting HR might carry prognostic information in LQTS. This information, however, was limited by having been derived from a standard ECG recorded in an uncontrolled environment. In this study we assessed HR for a longer period of observation under true resting conditions. Heart rate variability did not seem to distinguish between symptomatic and asymptomatic individuals. This did not surprise us, because we had already shown in different patient populations that measures of "tonic" autonomic activity could be much less informative than measures of "reflex" autonomic activity (10,14). Furthermore, this point had been proven in experiments involving direct neural recordings of single cardiac vagal fibers destined to the sinus node and not just surrogate markers of autonomic activity such as any measure derived from HR (15).

The present results demonstrate that asymptomatic MCs had a significantly lower HR than symptomatic MCs. This was true with and without BB. Because all but 1 of the patients with a QTc >500 ms had symptoms (Fig. 3), we focused on those with a less severe arrhythmogenic substrate (i.e., QTc ≤500 ms), and it then became evident that those in the lower tertile of HR were more frequently asymptomatic. Thus, the efficacy of BB in LQT1 patients is likely to depend on 2 mechanisms of action: the direct antiadrenergic effect that antagonizes arrhythmia triggers (2) and the prevention of fast HRs.

BRS and cardiac events. We focused our study on the second- and third-age quartiles, providing ourselves with a group without influence of age on BRS, and despite a reduced sample size, the main differences observed were statistically significant.

Among healthy dogs the distribution of BRS values, which carries prognostic value in relation to the outcome during a first MI (6), has a wide range suggesting a genetic influence (8). The present data show a similar distribution in humans and especially no difference between MCs and non-MCs, which is exactly what one would expect for 2 genetic traits independently transmitted. However, when the analysis focused on the MCs it became evident that the asymptomatic carriers had significantly lower BRS values compared with MCs who suffered cardiac events. Besides the sheer numerical difference in BRS values lies a finding of meaningful prognostic significance.

Indeed, those MCs who have a BRS value in the lower tertile (≤12 ms/mm Hg) were markedly less likely to be symptomatic with an OR of 0.13. Furthermore, when the analysis was restricted to those with a less severe arrhythmogenic substrate (QTc ≤500 ms), the observed frequency of symptomatic patients was dependent on the level of BRS. In addition to the fact that none of the MCs in the lower tertile for BRS had symptoms, it is worth noting (Fig. 5) that the only 2 asymptomatic patients with a BRS >12 ms/mm Hg also had borderline QTc prolongation (445 and 454 ms). This suggests that within these South African families, when the substrate is not overwhelmingly arrhythmogenic (QTc ≤500 ms), there are 2 distinct patterns observed in asymptomatic MCs: either relatively low BRS even with a prolonged QTc (490 to 500 ms), or BRS >12 ms/mm Hg and a near-normal QTc. The same correlation between BRS values and clinical status persisted in the presence of BB.

These BRS values are not low; they are just "relatively low," because they represent the lower end of the normal distribution and might be regarded as a sign of a somewhat diminished reflex autonomic activity.

An additional observation further supports the concept of the autonomic background contributing importantly to the arrhythmic profile in these LQT1 families. The lack of correlation between BRS and HR has allowed us to meaningfully compare the MCs with lower values for both these parameters and all other subjects together. Despite the small sample size, the percentage of symptomatic patients in the lower tertiles for both HR and BRS was significantly lower (20% vs. 76%).

Altogether, these data provide evidence that the individual autonomic make-up effectively contributes to modulate the probability of being symptomatic or asymptomatic in patients with the *KCNQ1*-A341V mutation. This likely applies to other LQT1 subjects, but a specific study in LQT1 patients with different mutations will be necessary to prove this point.

Underlying mechanisms. Our objective was to test whether autonomic responses could modulate the arrhythmic history in members of a LQT1 founder population. We reasoned that, given the identical genetic arrhythmogenic substrate (1) represented by subjects carrying the same mutation, there was a unique opportunity to assess whether the arrhythmic profile is influenced by BRS values. On the basis of our previous findings in post-MI dogs and patients, we assumed that low BRS would have predicted increased risk. Our data indicated that the opposite was true.

The reason for this striking difference, besides the aforementioned effect of an impaired I_{Ks} on QT shortening during tachycardia, lies in the fact that whereas strong vagal reflexes might be life-saving in ischemic heart disease (16,17), they could actually precipitate torsades de pointes in LQT1 subjects, because sudden pauses elicit early afterdepolarizations in LQTS. Thus, those LQT1 patients who have blunted autonomic responses, indicated by relatively low values of BRS, are less frequently symptomatic. Therefore, a reduced ability to change HR suddenly, detrimental for most individuals, is favorable and protective when combined with an impaired I_{Ks}, as in LQT1 patients.

Our ability to study several of these patients on and off BB provided additional mechanistic insights. Therapy with BB drugs increases BRS, but what we did not anticipate was the striking difference between symptomatic and asymptomatic patients in their response to BB therapy. Whereas symptomatic MCs increased their BRS value by almost 5 ms/mm Hg on BB, asymptomatic carriers had practically no change in BRS. This happened despite the fact that the baseline values were higher among symptomatic MCs. This unexpected finding indicates that asymptomatic carriers have a modest resting autonomic activity (a protective factor for these patients), so that BB therapy can only slightly modify autonomic balance. It also suggests another plausible underlying mechanism: namely, that symptomatic LQT1 patients might have enhanced basal sympathetic activity. This would be reflected in slightly faster HRs and in higher BRS values, as measured by the phenylephrine method (11,12), because of the synergistic effect of the withdrawal of the enhanced basal sympathetic tone coupled with the reflex vagal activation.

Genetic risk modifiers. The search for genetic and physiological modifiers of arrhythmic risk in LQTS and in other disorders associated with sudden cardiac death is one of the most exciting challenges facing contemporary cardiology (18), but progress in this area has been limited.

We (19) and others (20) had indicated that LQTS patients who were carriers of 2 independent mutations had increased clinical severity. Thus, a second mutation could act as a modifier. However, the inherent rarity of 1 more LQTS mutation occurring in LQTS patients argued against compound mutations being a common type of genetic modifier.

In 2005 we showed that a very common polymorphism, *KCNH2*-K897T, could amplify the electrophysiological consequences of a mutation with a modest effect on the I_{Kr} current (21). That study indicated that common polymorphisms can act as genetic modifiers of clinical severity in familial arrhythmogenic disorders.

The present study carries the search for arrhythmia modifiers in a novel direction. In LQT1 patients, for whom the main arrhythmogenic trigger is sympathetic activation (2), it was reasonable to postulate a relation between autonomic responses and arrhythmic risk (5). Baroreflex sensitivity, a validated quantitative marker of autonomic responses, is partly under genetic control (22). We assumed that the wide range of BRS among individuals without structural heart disease could reflect this genetic control and that unavoidably also the carriers of LQT1 mutations would find themselves genetically bound to a propensity for having a low, intermediate, or high BRS. Our study suggests that the outcome of this association by chance between LQT1 mutations and varying levels of BRS would be a differential probability of being symptomatic. Thus, the evidence that a specific autonomic phenotype (i.e., relatively low BRS) is associated with a reduced clinical severity of an LQT1 mutation indicates that genes controlling BRS or, more broadly, autonomic responses involved in the neural control of the heart might include key modifier genes that might increase or reduce the risk for sudden cardiac death in LQTS and perhaps in other more common disorders (18,21).

These considerations provided the rationale for exploring the "adrenergic cascade," upstream and downstream, to assess the presence of polymorphisms that might modify either the amount of norepinephrine released at ventricular level or the sensitivity of the adrenergic receptors to catecholamines. We screened our LQT1 population for functional alpha- and beta-adrenoreceptor polymorphisms and found specific correlations with BRS values. Because BRS is influenced by age and not by the *KCNQ1*-A341V mutation, the analyses were performed both in MCs and non-MCs in the 2 middle-age quartiles. The most interesting results came from 2 functional polymorphisms: *ADRA2C*-Del322-325, and *ADRB1*-G389R.

The polymorphism of the alpha-2C adrenoreceptor consists of a 12-base pair (bp) in-frame deletion causing a substantial loss of agonist-mediated receptor function (23) that results in a loss of normal synaptic autoinhibitory feedback and thereby in enhanced presynaptic release of norepinephrine (24). In our South African population this deletion was associated with greater BRS values and, therefore, with increased autonomic reactivity, consistent with the increased release of norepinephrine induced by the deletion. Indeed, norepinephrine released from sympathetic efferent nerves enhances the baroreceptor reflex by sensitizing the receptor nerve endings (25).

Norepinephrine release from cardiac sympathetic nerves activates myocyte β1-adrenergic receptors that couple to the stimulatory G protein (Gs), activating adenylyl cyclase and increasing intracellular cAMP, thus augmenting cardiac

inotropy, lusitropy, and chronotropy. The G389R polymorphism of the β1-adrenergic receptors is located within a Gs-coupling domain (26), and β1R389 couples to adenylyl cyclase better than β1G389, resulting in enhanced β1-adrenergic receptor activity.

The *ADRA2C*-Del322-325 and *ADRB1*-G389R polymorphisms act synergistically, because both norepinephrine release and β1-adrenergic receptor activity are simultaneously enhanced (23). Indeed, coexistence of these 2 genetic variables is associated with an increased risk for heart failure or for its progression (23). Accordingly, we tested the hypothesis that in our study population this combination would be associated with increased reflex autonomic activity by comparing subjects with the *ADRA2C*-Del322-325 and/or homozygous for *ADRB1*-R389 with subjects with the remaining genotypes, and indeed we observed a greater baroreceptor responsiveness in the first group.

These novel genetic findings are intriguing but preliminary. They should be accepted with caution and regarded as a working hypothesis, while we pursue confirmation in different and larger founder populations.

Study limitations. The main limitation is represented by the relatively small number of individuals in whom we could assess BRS both in the absence and presence of BB. This relates to the objective difficulty of convincing patients without symptoms, all working people, to spend 4 days in or near the hospital to undergo the entire study. The limited number of asymptomatic patients depends on the already demonstrated high clinical severity of the A341V mutation, so that >80% of the MCs have symptoms (2). The severity of the A341V mutation is not related to the South African families, because we confirmed it in patients with different ethnic backgrounds (27). In contrast, the rigorous selection of study subjects (all carriers of the same mutation), the limitation of BRS analysis to the group without age influence, and the performance of the study both on and off BB therapy are all points of strength. Furthermore, it is reassuring that for the differences observed statistical significance was reached without a large study population.

Implications. The present study has identified a mechanism that helps explain why individuals carrying the same mutation producing the most common variant of LQTS, LQT1, might be more or less likely to suffer cardiac events. This finding provides a direct link between the wide range of autonomic responses present in normal individuals and arrhythmic risk and also suggests a genetic origin for this variability.

A novel concept arises from our study. Some individuals might inherit a disease-causing mutation (e.g., a LQTS mutation such as *KCNQ1*-A341V). The same individuals might inherit certain specific and relatively common gene variants (e.g., the adrenoreceptor polymorphisms investigated in our study). The genetic transmissions of the LQTS mutations and of the adrenoreceptor polymorphisms are obviously independent of each other. The coexistence of a LQTS mutation with a polymorphism creating a genetic propensity toward brisk autonomic reflexes, a combination that our data indicate increases arrhythmic risk, is simply the play of chance.

We find especially intriguing the fact that the same change in an autonomic marker, a relatively low BRS, might predict increased risk in patients with ischemic heart disease and be associated with a lower probability of being symptomatic in patients with LQT1. This highlights the predictive value of accurately determined autonomic markers but also implies a specific physiologic interaction with the underlying cardiac abnormality, an interaction that becomes easily apparent provided that the different arrhythmogenic mechanisms involved in diverse cardiac diseases are recognized.

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