Exercise Stress Test Amplifies Genotype-Phenotype Correlation in the LQT1 and LQT2 Forms of the Long-QT Syndrome

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Background—Experimental studies suggest that the interval between peak and end of T wave (Tpe) in transmural ECGs reflects transmural dispersion of repolarization (TDR), which is amplified by β-adrenergic stimulation in the LQT1 model. In 82 patients with genetically identified long-QT syndrome (LQTS) and 33 control subjects, we examined T-wave morphology and various parameters for repolarization in 12-lead ECGs including corrected QT (QTc; QT/R-R^1/2) and corrected Tpe (Tpec; Tpe/R-R^1/2) before and during exercise stress tests.

Methods and Results—Under baseline conditions, LQT1 (n = 51) showed 3 cardinal T-wave patterns (broad-based, normal-appearing, late-onset) and LQT2 (n = 31) 3 patterns (broad-based, bifid with a small or large notch). The QTc and Tpec were both larger than those in control subjects (402 ± 36 ms and 163 ± 86 ms; n = 19) in LQT2.

Conclusions—Tpe interval increases during exercise in LQT1 but not in LQT2, which may partially account for the finding that fatal cardiac events in LQT1 are more often associated with exercise. (Circulation. 2003;107:838-844.)

Key Words: electrocardiography ■ genetics ■ ion channels ■ long-QT syndrome ■ exercise

Congenital long-QT syndrome (LQTS) is a fatal disease entity caused by various mutations in at least five genes coding cardiac ion channels.1−2 Mutations in KCNQ1 and KCNH2 are most commonly identified and cause LQT1 and LQT2 forms of LQTS. Those mutations induce functional defects in either slow (I_Ks: LQT1) or rapid (I_Kr: LQT2) component of the delayed rectifier potassium current. In association with inhomogeneous functional modulation of LQTS-related ion channels, distinct phenotypic patterns of T waves have been noted in a respective genotype.3,4 Moreover, recent studies have suggested differences in the sensitivity of the genotypes to β-adrenergic stimulation.5−6 In LQT1, cardiac events (arrhythmia and sudden cardiac death) are more frequently associated with enhanced adrenergic factors (physical or emotional stress) than in other forms of LQTS.7 In this accordance, β-blockers have been reported to be most preventive against cardiac events in LQT1.7

Electrophysiological studies with single mammalian ventricular cells demonstrated that β-adrenergic stimulation enhances I_Ks and L-type Ca current (I_Ca,L) but not I_Kr.8−11 In LQT1 (reduction in basal I_Ks), β-adrenergic stimulation produces a larger prolongation of the QT interval because I_Ca,L, which is a counterpart of I_Ks, and carries inward currents, remains intact and increases. In LQT2 (reduction in basal I_Ks), phenotypic ECG change may be more prominent at slower heart rate because of its rapid activation properties.10−14 Exercise stress tests were therefore used to study β-adrenergic modulation on ECG parameters in patients with LQTS who had been identified as either LQT1 or LQT2 and compared with healthy control subjects. In both baseline and exercise conditions, we measured T-wave morphology and repolarization characteristics: QT and Tpeak-end (Tpe).

Methods

Patient Population

The study population consisted of three groups: (1) LQT1 (n = 51 from 29 unrelated families), (2) LQT2 (n = 31 from 19 unrelated families), and (3) healthy volunteers (n = 33) as a control group. We received August 5, 2002; revision received October 30, 2002; accepted October 31, 2002.

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excluded patients taking any medications that affect the repolarization, including β-blockers, during the study.

DNA Isolation and Mutation Analysis

One hundred eighty-three patients were included for genotyping under the diagnosis of LQTS (135 probands and 48 family members). Genomic DNA was isolated from leukocyte nuclei by conventional methods. The protocol for gene analysis was approved by the institutional ethics committee, and all patients gave informed consent according to the committee’s guideline. Screening for mutations of KCNQ1, KCNH2, SCN5A, KCNE1, and KCNE2 was performed with the use of polymerase chain reaction (PCR)/single-strand conformation polymorphism analyses. Briefly, PCR products were heat-denatured with formamide, applied to a 12% polyacrylamide gel, and stained with SYBR Green II (Molecular Probe). For aberrant PCR products, DNA sequencing was conducted with a DNA sequencer (ABI PRISM 320, PE Applied Biosystems). Twenty-two KCNQ1 and 19 KCNH2 mutations were identified in 29 unrelated LQT1 and 19 LQT2 probands, respectively. In those LQT1 and LQT2 subtypes, no other LQTS-associated mutations were found.

Identification of T-Wave Pattern

ST-T morphology was evaluated in all 12 ECG leads, and a representative pattern was determined in each case. When different patterns were present in different leads, the most prevalent (present in at least 4 leads including lead V5 and V6) was chosen as the representative ECG pattern.

ECG Measurements

Forty-nine patients (30 patients with LQT1 and 19 with LQT2) and 22 healthy control patients were included for the analysis with exercise stress tests.

All subjects were in sinus rhythm, and none had atrioventricular or bundle branch block. Exercise stress tests were performed according to the standard Bruce protocol. Twelve-lead ECGs were recorded at several specific heart rates from the resting state to the maximal stress state by step of ~10 beats/min. The QT was manually measured as the time interval between QRS onset (Q) and the point at which the isoelectric line intersected a tangential line drawn at the maximal downslope of the positive T wave or at the maximal upslope of the negative T wave (Tend). V5 and V6 were used for measurement because they are unipolar leads that reflect the potential from the free wall of the left ventricle. The Q-Tpeak (QTp) was defined as the time interval between QRS onset and the peak of the positive T wave or the nadir of the negative T wave. Tp was then obtained by calculating as QT minus QTp (Figure 1). When the T wave had a bifasic or bifid configuration, the peak of the T wave was defined as the former peak. The latter peak of the positive T wave was designated as a notch (Figure 2).

Measurements were performed as the mean of 3 beats in lead V5. They were corrected to heart rate according to Bazett’s method: corrected QT (QTc; QT/R-R 1/2) and corrected Tp (Tpec; Tp/R-R 1/2). During exercise tests, the QT and Tp were measured at 6 to 12 sampling points and plotted against the corresponding the R-R interval. The QT/R-R and Tp/R-R were calculated in each exercise test by fitting raw data to the simple linear regression analysis with a commercially available program (Sigma Plot 2001 ver7, SPSS Inc). Measurements were carried out by two investigators who were unaware of subject’s

Figure 1. Three typical T-wave patterns in baseline lead V5 ECGs of LQT1. A, Broad-based T-wave pattern. Both of the QTc and Tpec were prolonged. B, Normal-appearing T-wave pattern. C, Late-onset T-wave pattern. Flat ST segment was especially prolonged.

Figure 2. Two typical T-wave patterns identified in baseline lead V5 ECGs of LQT2. A, Bifid T wave with notch S, which indicates a notch lower than Tpeak. B, Bifid T wave with notch L, which is higher than Tpeak. In bifid T wave, Tpeak (Tp) was defined as the former apex and a notch as the latter apex.
genetic status. There were no significant differences in measured data between the two (data not shown).

Statistical Analysis
Data are presented as mean±SD. Two-way repeated-measures ANOVA followed by Scheffe’s test were used to compare each parameter among 3 groups and a 2-tailed Student’s t test to compare measurements at rest and during exercise. A probability value <0.05 was considered significant.

Results
Comparison of Clinical Characteristics
Thirty patients with LQT1 (59%) were symptomatic. Among them, all of the men had the first syncope attack before 16 years of age. In contrast, half of the women had the first syncope at age >16 years. The onset-age of men with LQT1 (8±3 years of age) was significantly lower than that of women (P=0.048).7 Typical triggers of cardiac events were exercise, especially swimming, and emotional stress in patients with LQT1. Seventeen patients with LQT2 (55%) were symptomatic, triggered by sleep, auditory stimuli, and bradycardia. The onset age did not significantly differ between the two sexes (Table 1).

Baseline ECGs Show Different T-Wave Patterns and Repolarization Parameters
The ECG data in the 3 study groups are summarized in Table 2. There was no significant difference in R-R intervals among the 3 groups, although patients with LQT2 showed a bradycardiac tendency. Baseline QTc and Tpec values in the 2 LQT3 groups were significantly longer than those in control patients. At variance with a previous report,17 the Tpec in LQT2 was significantly longer than that in the LQT1 group.

LQT1
Three cardinal T-wave patterns were identified: broad-based T (Figure 1A), normal-appearing T (Figure 1B), and late-onset T (Figure 1C). The broad-based T-wave pattern represented a single and smooth T wave and was seen in 43% of patients with LQT1. The normal-appearing T-wave pattern with small but significant prolongation of QT was seen in 28%. The late-onset T wave characterized by a prolonged ST segment was seen in 25%.

LQT2
Most of patients with LQT2 showed two types of bifid T-wave patterns: bifid T wave with a small notch (designated as notch S, Figure 2A) and the one with a large notch (designated as notch L, Figure 2B). The former pattern was observed in 33% and the latter seen in 25% of patients with LQT2. However, the broad-based T wave was also seen in 34% at rest.

Exercise Produces Differential Response in T-Wave Morphology and Repolarization Parameters
Forty-nine patients (30 patients with LQT1 and 19 with LQT2) and 22 healthy control patients were included for the analysis with exercise stress tests. Table 3 summarizes R-R, QTc, and Tpec values in the three groups at rest and maximal stress point. All baseline R-R, QTc and Tpec showed values similar to those evaluated in total study patients (Table 2), indicating that these subsets of patients are representative of each group. Mean ages of study patients were not significantly different between LQT1 and LQT2 subgroups (23.6±16.5 versus 25.2±13.1 years, NS).

T-Wave Morphology
In exercise, the patients with LQT1 with a broad-based T wave revealed a prominent prolongation in both QTc and Tpec without changing the T-wave morphology (Figure 3A). On the contrary, half of the late-onset T and most of the normal-appearing T patterns were changed to the broad-based pattern, resulting that 23 of 30 patients with LQT1 showed the broad-based pattern during exercise (Figure 4A). The positive predictive value (PPV) of a broad-based T wave at

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### TABLE 1. Comparison of Clinical Characteristics

<table>
<thead>
<tr>
<th></th>
<th>LQT1 29 Families</th>
<th>LQT2 19 Families</th>
<th>Control 33 Families</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Total</td>
</tr>
<tr>
<td>No.</td>
<td>15</td>
<td>36</td>
<td>51</td>
</tr>
<tr>
<td>Age, y</td>
<td>12±21</td>
<td>31±18</td>
<td>28±20</td>
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<tr>
<td>Symptomatic</td>
<td>7</td>
<td>23</td>
<td>30</td>
</tr>
<tr>
<td>Onset age, y</td>
<td>8±3*</td>
<td>25±20</td>
<td>21±19</td>
</tr>
<tr>
<td>Triggers of syncope</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercise 13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Swimming 6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypokalemia 3</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*P=0.048 between men and women with LQT1.

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### TABLE 2. Baseline Conditions: ECG Data in LQT1, LQT2, and Control

<table>
<thead>
<tr>
<th>Genotype</th>
<th>LQT1 (n=51)</th>
<th>LQT2 (n=31)</th>
<th>Control (n=33)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-R, ms</td>
<td>907±192</td>
<td>976±188</td>
<td>870±80</td>
<td>NS†</td>
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<tr>
<td>QT, ms</td>
<td>484±82</td>
<td>511±60</td>
<td>358±91</td>
<td>NS/P&lt;0.001†</td>
</tr>
<tr>
<td>QTc, ms</td>
<td>510±68</td>
<td>520±61</td>
<td>402±36</td>
<td>NS/P&lt;0.001†</td>
</tr>
<tr>
<td>Tpe, ms</td>
<td>132±52</td>
<td>191±67</td>
<td>86±20</td>
<td>P&lt;0.001†</td>
</tr>
<tr>
<td>Tpec, ms</td>
<td>143±53</td>
<td>195±69</td>
<td>99±36</td>
<td>P&lt;0.001†</td>
</tr>
</tbody>
</table>

QTc indicates QT/R-R; and Tpec, Tpe/R-R. | Between LQT1 and LQT2, †LQT1 and LQT2 compared with control, respectively.
peak exercise was 96%, and its negative predictive value (NPV) was 72%.

In the LQT2 subset, a notch on the descending T-wave limb became prominent during exercise (Figure 3B). Under the baseline condition, the bifid T-wave pattern with notch L was seen in 6 of 19 patients with LQT2, the one with notch S in 5, and the broad-based T in 6 patients (Figure 4B). In exercise, the broad-based T-wave pattern was changed into bifid T type, eventually the bifid or biphasic T-wave pattern was observed in 17 of 19 patients with LQT2 (Figure 4B). The PPV of the bifid with a notch at peak exercise was 80%, and its NPV was 90%.

**Table 3. ECG Data Before and During Exercise in LQT1, LQT2, and Control**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Peak Exercise</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>R-R, ms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LQT1</td>
<td>888±155</td>
<td>461±146</td>
<td>&lt;0.001 †</td>
</tr>
<tr>
<td>LQT2</td>
<td>1020±184</td>
<td>514±134</td>
<td>&lt;0.001 †</td>
</tr>
<tr>
<td>Control</td>
<td>816±188</td>
<td>475±64</td>
<td>&lt;0.001 †</td>
</tr>
<tr>
<td>P</td>
<td>NS*</td>
<td>NS*</td>
<td></td>
</tr>
<tr>
<td>QTc, ms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LQT1</td>
<td>511±64</td>
<td>599±54</td>
<td>&lt;0.001 †</td>
</tr>
<tr>
<td>LQT2</td>
<td>513±55</td>
<td>502±82</td>
<td>NS‡</td>
</tr>
<tr>
<td>Control</td>
<td>402±36</td>
<td>418±17</td>
<td>NS‡</td>
</tr>
<tr>
<td>P</td>
<td>NS*/P&lt;0.001 †</td>
<td>NS*/P&lt;0.001 †</td>
<td></td>
</tr>
<tr>
<td>Tpec, ms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LQT1</td>
<td>142±46</td>
<td>215±46</td>
<td>&lt;0.001 †</td>
</tr>
<tr>
<td>LQT2</td>
<td>197±70</td>
<td>163±86</td>
<td>NS‡</td>
</tr>
<tr>
<td>Control</td>
<td>127±59</td>
<td>98±21</td>
<td>NS‡</td>
</tr>
<tr>
<td>P</td>
<td>P&lt;0.001 †</td>
<td>NS*/P&lt;0.001 †</td>
<td></td>
</tr>
</tbody>
</table>

*Between LQT1 and LQT2, † LQT1 and LQT2 group compared with control, ‡ between baseline condition and peak exercise.

**Figure 3.** Representative morphologic changes in the 5 leads of ECGs during exercise in patients with LQT1 (A) and LQT2 (B) subgroups. Measured values for QTc and corrected Tpe (Tpe/R-R1/2) are shown at the bottom of each column.

**Figure 4.** Changes in T-wave pattern during exercise in LQT1 (A) and LQT2 (B) subgroups. Numbers in tables indicate absolute numbers of patients in each subgroup (30 patients with LQT1 and 19 with LQT2). Numbers in parentheses indicate numbers of patients that showed the change in T-wave pattern by exercise.
Rate-Dependent Adaptation of QT Intervals

In the LQT1 group, the QTc interval significantly prolonged in response to the shortening of the R-R by exercise. In contrast, the QTc remained unchanged by exercise in both the LQT2 and control groups (Table 3). As was shown in the previous data on QT/R-R slopes and their genotype-dependent differences, our data also suggested that the QT/R-R relation has genotype-related differences. In Figure 5A, QT values are plotted against R-R intervals in 3 representative patients. Open circles indicate data points from a LQT1 patient, closed circles those from a LQT2 patient, and closed triangles from a control patient. Three solid lines are best fit by a least-squares method. All QT/R-R values thus calculated are summarized in Figure 5B. The QT/R-R slope was significantly less steep in LQT1 than those in the other two groups.

Patients With LQT1 and LQT2 Show Different Responses in Tpe to Exercise

Exercise increased the Tpec significantly only in LQT1 (Table 3). The rate-dependent adaptation of Tpe was evaluated by plotting raw data against the R-R, as depicted in Figure 5C. In the LQT2 patient (closed circles) and the control (closed triangles), the Tpe was reduced in response to the shortening of R-R, thereby producing a positive Tpe/R-R slope. In contrast, the Tpe significantly prolonged when R-R shortened in patients with LQT1 (open circles), resulting in a negative Tpe/R-R slope.

The Tpe/R-R slopes in the 3 groups are illustrated in Figure 5D. None of the patients with LQT2 or the control patients had a negative Tpe/R-R slope. In contrast, 80% of patients with LQT1 showed a negative Tpe/R-R slope (PPV 100%, NPV 75%). Therefore, the exercise-induced QT prolongation in the LQT1 group was due mainly to the augmentation of Tpe intervals.

Discussion

The present study demonstrates that exercise amplifies the phenotypic appearance of T wave in both LQT1 and LQT2. The data also suggested that exercise produces a significant increase in both QT interval and Tpe, reflecting transmural dispersion of repolarization (TDR) only in LQT1. Since mutations in different genes are identified in LQTS, resting ECGs have been noted to differ considerably among the genotypes. Indeed, bifid T waves with a notch, which are characteristic to LQT2, were also found in about two thirds of our patients with LQT2. At variance with previous reports, the remaining one third of patients with LQT2 showed a broad-based T-wave pattern, which is thought to be typical in LQT1. Therefore, baseline T-wave morphology does not efficiently serve as a diagnostic criterion for LQTS genotyping.

On the other hand, exercise stress testing could produce distinct responses in T-wave morphology between the two groups. The broad-based T-wave pattern observed at rest in the LQT1 group remained unchanged during exercise. More interestingly, other types of T waves were changed into the broad-based pattern during exercise, which was associated
with a significant increase in the QTc and the Tpec. In contrast, the T-wave morphology was altered to the bifid pattern during exercise in most cases of LQT2.21

Arterially perfused wedge preparations6 have been used to develop pharmacological models of LQT1, LQT2, and LQT3, in which the phenotypic appearance of T wave depended on currents flowing down voltage gradients among three different cell types across the ventricular wall; epicardial, midmyocardial (M), and endocardial cells. In all 3 models, the Tpe in the transmural ECG appeared to provide an index of TDR defined usually as the time lag for repolarization between epicardial and M cells,14,16 and an amplified TDR was linked to ventricular arrhythmias such as torsade de pointes.6,14,16 In three distinct layers, epicardial and endocardial cells have intrinsically stronger net repolarizing currents (as the result of strong IKs and weak late INa ) than M cells (weak IKs and strong late INa).22,23 Therefore, a large augmentation of residual IKs by β-adrenergic stimulation would result in epicardial or endocardial cells but not in M cells, especially in the LQT1 model (in scarce IKs state).6,24,25 This may lead to an increased TDR and a broad-based T wave, which is consistent with the phenotypic appearance of ECGs during exercise in our patients with LQT1, and thereby explains the higher incidence of cardiac events with exercise in this special subset.

The cellular basis for low-amplitude T-waves with a notched configuration often seen in LQT2 has also been demonstrated by experimental studies with wedge preparations14,16: A notch on the descending limb of the T wave indicates the timing when the voltage gradient between endocardial and the M cells changes abruptly after the full repolarization of epicardial cells. A notch on the ascending limb of the T wave occurs when a gradient develops between endocardium and M region, which is capable of generating a current sufficient to change the direction of net current flow across the wall. Both types of notches were often observed in the wedge preparations perfused with IKr blockers. However, in the LQT2 model, the influence of β-adrenergic stimulation has not been yet examined on the repolarization gradient.

Study Limitations
In the present study, men with LQT1 were significantly younger than those in other groups. This may reflect the finding that the onset age of men with LQT1 was younger, as reported in previous study,7 and may affect the analysis. In regard to the study patients with exercise tolerance testing, however, this influence of a widely scattered age range could be ruled out because there was no difference in the proportion of children under 16 years of age (numbers of the children were 9 in 30 with LQT1 and 6 in 19 with LQT2). Mean ages of study patients were not significantly different. There was also no significant difference in maximum heart rate attained by exercise, excluding the age-dependent influence on ECG parameters.

At variance with a previous report,17 the Tpec in LQT2 was significantly longer than that in the LQT1 group. It may be due to the difference in definition of Tpe. Because T peak was defined as the former peak of the bifid T-wave pattern in our analyses, the Tpe interval became longer in the bifid T wave, which was the main pattern of LQT2.

In summary, the present study demonstrated that exercise-induced genotype-specific changes in the T wave and exaggerated prolongation of the QT interval in LQT15 were due principally to increase in Tpe, reflecting TDR. Exercise testing is useful to facilitate genotyping of most common variants of the LQTS, although prospective study will be needed to conclude its diagnostic value.

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References


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