GENETIC CORNER

Do LQTS Gene Single Nucleotide Polymorphisms Alter QTc Intervals at Rest and during Exercise Stress Testing?

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Background: The impact of harboring, genetic variants or single nucleotide polymorphisms (LQT-PM) on the repolarization response during exercise and recovery is unknown.

Objective: To assess the QTc interval adaptation during exercise stress testing (EST) in children with LQT polymorphisms compared to a group of age and gender matched normal controls.

Methods: One hundred forty-eight patients were age and gender matched into two groups: LQT-PM and control. Each patient underwent a uniform exercise protocol employing a cycle ergometer followed by a 9 minute recovery phase with continuous 12-lead electrocardiogram (ECG) monitoring. Intervals (RR, QT and QTc) at rest (supine), peak exercise and in recovery (1, 3, 5, 7, and 9 minutes) were measured.

Results: Forty-three patients were positive for LQT-PM and the control group consisted of 105 patients. A total of 83 SNPs were identified: *SCN5A* n = 31 (37%), *KCNE1* n = 29 (35%), *KCNH2* n = 20 (24%), *KCNQ1* n = 2 (2%) and *KCNE2* n = 1 (1%). The QTc interval measurements of the LQT-PM were longer at rest, peak exercise and all phases of recovery when compared to the control group. Neither group demonstrated abnormal QTc interval adaptation in response to exercise. Patients with homozygous SNPs had longer resting QTc intervals when compared to patients with only heterozygous SNPs (435 ± 23 ms vs. 415 ± 20 ms, respectively, P value <0.006).

Conclusions: Individuals with LQT-PM may have longer QTc intervals at rest as well as at peak exercise and all phases of the recovery period compared to normal controls. Additionally, subjects with homozygous SNPs had longer resting QTc intervals when compared to those with only heterozygous SNPs.

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Congenital long QT syndrome (LQTS) is an inherited channelopathy characterized by a prolonged QT interval, syncope, seizures, ventricular arrhythmias and sudden death.[1,2] Children and adolescents with LQTS have been shown to be at high risk of a first cardiac event.[3,4] Significant advances in the molecular understanding of LQTS have resulted in genetic testing for 13 LQTS susceptibility genes with the majority of mutations involving the LQT1 (*KCNQ1*) or LQT2 (*KCNH2*) genes. Despite progress in genomics, the diagnosis of LQTS largely depends on the Schwartz score which comprises of clinical criteria and the surface electrocardiogram (ECG) as there are several limitations to genetic testing.[2] Provocative tests such as pharmacologic stress testing and exercise stress

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testing (EST) have also been used to aid in the clinical diagnosis of LQTS.[5–8] EST is commonly used as it is noninvasive and readily performed in clinical practice with prompt interpretation of results. Patients with LQTS have abnormal repolarization reserve as manifested by QT prolongation during exercise when compared to normal controls and the presence of this phenomenon is often used to arrive at a diagnosis of LQTS.[9–11] The impact of harboring genetic variants or SNPs (LQT-PM) not generally expected to cause disease on the repolarization response during exercise and recovery is unknown. The aim of our study was to assess the QTc interval adaptation during EST in children with LQT–PM compared to a group of age and gender matched normal controls.

METHODS

Study Population

The study group consisted of patients referred to the Pediatric Arrhythmia Clinic at The Children's Hospital of Philadelphia for evaluation of suspected LQTS between January 1998 and January 2010. Inclusion criteria for this study were pediatric patients (≤21 years of age) with EST performed at our institution who had single nucleotide polymorphisms (SNPs), defined as genetic variants previously observed among healthy controls and not generally expected to cause disease as identified during genetic testing by a commercial laboratory (Class III as determined by Familion). The control group consisted of age and gender-matched controls without cardiovascular disease and with EST at our institution. Patients excluded from the study were those with EST not available for review and patients < 5 years of age due to testing compliance issues. Patients were also excluded if genetic testing revealed a disease causing LQT mutation in addition to LQT-PM.

In order to obtain an age and gender matched control group, we subdivided the study group into 4 categories: (1) 4 to 7, (2) 8 to 10, (3) 11 to 14 and (4) 15 to 21 years of age. Control patients were then age and gender-matched so that equal numbers of males/females were present in each age category in the study and control groups.

Patients/parents were asked to self-categorize their ethnicity as well into: (1) white, (2) black, (3) Hispanic, (4) American Indian, (5) Asian and (6) other.

SNP Analysis

Comprehensive genetic mutational analysis of 5 LQTS susceptibility genes (*KCNQ1, KCNH2, SCN5A, KCNE1* and *KCNE2*) was performed on all patients through a commercial laboratory (Familion, PGxHealth, New Haven, CT, USA)

Exercise Stress Test Protocol

The first EST performed at our institution was evaluated. Documented patient data included age at EST and gender as well as LQT genetic SNP subtype (*KCNQ1, KCNH2, SCN5A, KCNE1 and KCNE2*) and presence or absence of beta-blocker therapy during EST. The study protocol was approved by The Children's Hospital of Philadelphia's Institutional Review Board.

Cardiac Monitoring

A l2-lead ECG (Marquette Case-8000, Milwaukee, WI, USA) at a paper speed of 25 mm/s was recorded. Resting ECGs were obtained in the supine position after 5 minutes of rest. ECGs were then obtained 1 minute after the patient was asked to stand.

Exercise Protocol

The exercise protocol employed a cycle ergometer (SensorMedics, Yorba Linda, CA, USA). An initial phase consisted of three minutes of pedaling in an unloaded state followed by a ramp increase in work rate (Watts) to maximal exercise. The progression of cycle resistance was determined by subject weight in kilograms and designed to achieve predicted peak work rate in 10 to 12 minutes of cycling time.[12] Following the three minute warm-up phase, resistance was increased at one-minute intervals until maximal volition was achieved. Maximal volition was defined as a respiratory exchange quotient of >1.10 and was an indication that the patient had reached peak exercise capacity. After reaching peak exercise, each patient completed the EST with a 9 minute recovery period. All EST were performed at The Children's Hospital of Philadelphia.

Exercise Stress Test ECG Measurements

ECG measurements were made by two independent investigators (PA and JG) who were both blinded to the patient's LQTS status. Interobserver variability was assessed in 40 randomly selected patients that resulted in an intraclass coefficient of 0.51 and with 69% of the readings within 20ms of one another, which indicates a moderate degree of consistency. The QT (ms), RR (bpm) and QTc (ms) were measured. The QT interval was defined as the beginning of the QRS complex to the end of the T wave. In cases in which the T wave end point did not reach the isoelectric line of the ECG, the maximum downslope of the T wave and the intersection with the isoelectric line of the T-P segment was considered to be the end of the T wave.[13] U waves less than one half of the T wave amplitude were not included as a portion of the QT interval. Leads II or V_5 were used in measuring QT intervals. Intervals (QT, RR and QTc) at rest (supine), peak exercise and in recovery (1, 3, 5, 7, and 9 minutes) were measured. QTc was then calculated according to the Bazett formula ($QTc = QT/\sqrt{RR}$).[14] QT interval was measured manually.

Statistical Analysis

Mean QT, RR and QTc measurements were plotted against time in EST and differences between the polymorphism and control groups were assessed. Patients were subdivided based on age, gender and beta-blocker therapy to assess for confounding variables. Differences in EST characteristics between polymorphism subgroups were evaluated using standard statistical methods. Statistical analysis was performed using independent sample *t*-test, one-way analysis of variance (ANOVA) with Bonferroni correction post hoc and chi-square analysis (to assess gender differences). All analyses were made by a statistician using SAS version 9.1 (SAS Institute Inc, Cary, NC, USA). A *P*-value <0.05 was considered statistically significant.

RESULTS

Patient Characteristics

Between 1998 and 2010, 267 patients were referred for LQTS genetic testing. Genetic testing was feasible in 188 (70%) patients; the remaining patients were deferred due to financial or insurance constraints. A disease causing LQTS gene mutation was identified in 76 (40%) of patients and these patients were excluded from the study. Forty-three (51% male, n = 22) patients had LQT SNPs with a mean age of 13.2 ± 4.3 years and were included in the study. The control grouped consisted of 105 (58% male, n = 61) patients with a mean age of 12.0 ± 3.5 years. There was no statistical difference in age or gender in these two groups. Beta-blocker therapy was present in 49% (n = 21) of the polymorphism group and no patients in the control group received beta blocker therapy.

Table 1. Ethnicity Demographics

	LQT-PM	Control
White, n(%)	39 (91)	83 (79)
Black	Na	8 (8)
Hispanic	1 (2)	1 (1)
American Indian	1 (2)	Na
Asian	Na	Na
Other	2 (4)	13 (12)

The study and control groups were ethnically homogenous in that the vast majority classified themselves as "white," therefore statistical analysis between ethnic groups was not performed due to small sample size (Table 1).

Patient Genotype Data

Genotype data for the polymorphism patients are summarized in Table 2. A total of 83 SNPs were identified: *SCN5A* n = 31 (37%), *KCNE1* n = 29 (35%), *KCNH2* n = 20 (24%), *KCNQ1* n = 2 (2%) and *KCNE2* n = 1 (1%). The majority of SNPs occurred in three common polymorphisms (His 558 Arg, Gly 38 Ser and Lys 897 Thr) in the *SCN5A*, *KCNE1* and *KCNH2* genes, respectively. Eighteen (42%) patients had a one SNP, 11 (26%) had two SNPs, 13 (30%) had three SNPs and 1 (2%) had four SNPs (Figure 1). The majority of patients (58%, n = 25) had more than one SNP.

Patient Presentation

Family history of LQTS was the most common reason for presentation in the polymorphism group (37%, n = 16). Eleven (26%) patients presented with syncope followed by 10 (23%) patients that presented with non-LQTS symptoms. Six (14%)

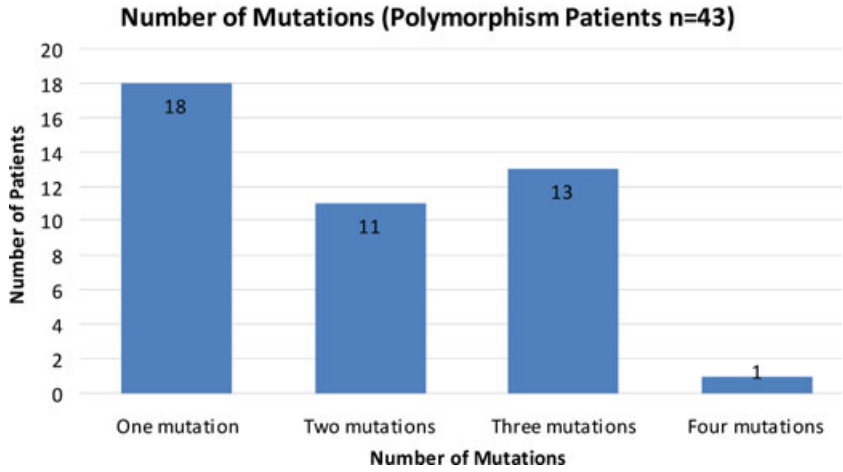

Figure 1. The number of SNP in each of the 43 patients is depicted. The majority of patients had more than 1 SNP.

Table 2. LQT-PM by Location and Amino Acid Coding

LQT Gene	Location and Coding	Exon	SNP	# of Patients
KCNQ1				
	Gly 643 Ser	Exon 16	1927 G>A	1
	IAP 54-56 dup*	Exon 1	DUP 160-8	1
KCNH2				
	Lys 897 Thr	Exon 11	2690 A>C	14
	Arg 1047 Leu	Exon 13	3140 G>T	6
SCN5A				
	His 558 Arg	Exon 12	1673 A>G	25
	Phe 2004 Leu	Exon 28	6010 T>C	1
	Pro 2006 Ala	Exon 28	6016 C>G	1
	Ser 1103 Tyr	Exon 18	3308 C>A	1
	Arg 34 Cys	Exon 2	100 C>T	2
	Arg 481 Trp	Exon 11	1441 C>T	1
KCNE1				
	Gly 38 Ser	Exon 4	112 G>A	24
	Asp 85 Asn	Exon 4	253 G>A	3
	Asp 86 Asn	Exon 4	253 G>A	2
KCNE2				
	Thr 8 Ala	Exon 2	22 A>G	1

asymptomatic patients were identified during routine ECG screening. Sixteen (32%) patients in total presented with non-LQTS symptoms at initial presentation.

QTc Interval Measurements

An example of EST tracings in LQT-PM and control patients is shown in Figure 2. The QTc interval measurements of the LQT-PM and control groups at rest, peak exercise and during 1, 3, 5, 7 and 9 minute of the recovery phase are shown in Table 3.

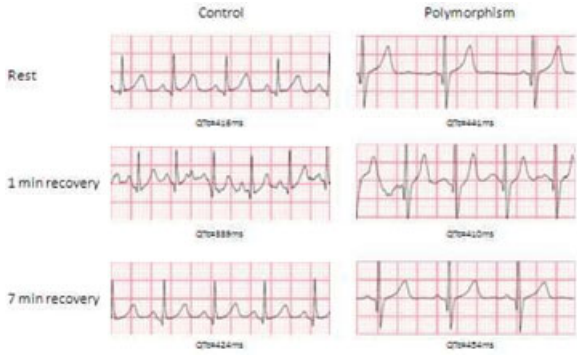

Figure 2. Comparison of ECG tracings between example control and SNP patients. At rest, the PM patient has a longer QTc but this patient has a normal adaptation response to exercise and recovery.

At peak exercise, both groups showed shortening of the QTc interval when compared to resting QTc values. Both groups had a mild increase

Table 3. QTc Interval (ms) during Exercise and Recovery in LQT-PM and Control Groups

	SNP	Control
Resting (ms), ±SD	420 ± 22	407 ± 20
Peak exercise	419 ± 31	406 ± 25
1 min recovery	414 ± 25	401 ± 22
3 min recovery	429 ± 29	408 ± 23
5 min recovery	442 ± 26	427 ± 19
7 min recovery	444 ± 27	431 ± 18
9 min recovery	442 ± 31	432 ± 18

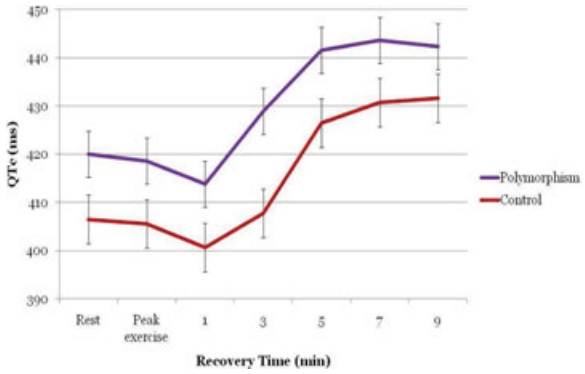

Figure 3. QTc values during exercise stress testing. The SNP patients had longer QTc intervals at all stages of the EST compared to the control group. The QTc in both groups shortens with exercise and prolongs in late recovery reflecting normal QTc adaptation.

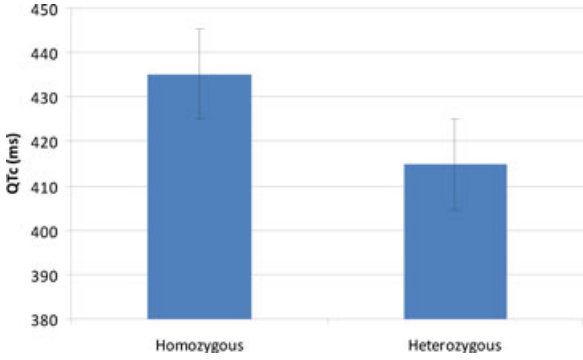

Figure 4. Resting QTc intervals in homozygous versus heterozygous SNP. Patients with homozygous SNP had longer QTc intervals at rest compared to patients with only heterozygous SNP (435 ± 23 ms vs. 415 ± 20 ms, respectively, $P < 0.006$).

in QTc intervals when comparing early recovery (1 minute) to late recovery (9 minutes). Neither group demonstrated abnormal QTc interval adaptation in response to exercise (Figure 3).

Homozygous versus Heterozygous LQT SNPs

Eleven (26%) patients had homozygous polymorphisms (His 558 Arg n = 6, Gly 38 Ser n = 5). As shown in Figure 4, patients with homozygous polymorphisms had longer resting QTc intervals when compared to patients with only heterozygous polymorphisms (435 ± 23 ms vs. 415 ± 20 ms, respectively, *P* value <0.006). There was no difference in QTc intervals in these two groups during the exercise or recovery phase.

Polymorphism Group Comparisons

Assigning value to the number of PM existing in each patient, with a heterozygous SNP counting as one PM and a homozygous SNP counting as two PM, a PM score was derived. Fourteen (33%) patients had a PM score of 1, 11 (26%) had a PM score of 2, 13 (30%) had a PM score of 3 and 5 (12%) had a PM score of 4. As depicted in Table 4, there was no correlation between increasing PM score and QTc prolongation in our PM patient population.

DISCUSSION

Repolarization dynamics during exercise and recovery may be influenced by various factors in healthy individuals as well as those affected with congenital LQTS.[8,15] Our data as well as that from other centers show that patients with disease causing SNP in the LQT1 gene appear to have diminished repolarization reserve during exercise which is manifested by a progressive or persistent pattern of QTc prolongation at higher heart rates, compared to patients with LQT2 mutations and normal controls. Patients with LQT2 mutations have QTc prolongation at intermediate heart rates such as during the late phase of recovery after an EST.[11]

Table 4. QTc Interval (ms) during Exercise and Recovery Based on Polymorphism Score

Polymorphism Score	1	2	3	4
Resting (ms) ±SD	410 ± 20	422 ± 20	421 ± 22	440 ± 25
Peak exercise	409 ± 25	431 ± 36	417 ± 32	423 ± 34
1 min recovery	412 ± 20	431 ± 27	401 ± 22	416 ± 24
3 min recovery	418 ± 30	441 ± 33	428 ± 28	430 ± 16
5 min recovery	437 ± 21	455 ± 26	433 ± 30	447 ± 23
7 min recovery	442 ± 25	447 ± 22	439 ± 33	453 ± 27
9 min recovery	436 ± 33	463 ± 26	430 ± 28	451 ± 18

The main findings of this study are: (1) patients with LQT-PM appear to have longer QTc intervals at rest as well as during peak exercise and all phases of the recovery period compared to normal controls and (2) subjects with homozygous polymorphisms had longer resting QTc intervals when compared to those with only heterozygous polymorphisms. The main clinical implication of this study is that subjects with LQT-PM may have some degree of QTc interval prolongation at rest and during all phases of the EST.

There are several limitations to this retrospective study. Foremost is the lack of a robust sample size. The interaction between certain PMs may prove to be a more relevant predictor of abnormal QTc adaptation than individual PMs. Due to limitations in sample size, we were unable to demonstrate these potential interactions. Similarly, given the heterogeneity of LQT-PM as well as co-existence of multiple SNPs in the same individual, it was not possible to assess if a particular SNP had a greater propensity to prolong the QTc interval compared to others in this study. We also excluded from the study patients with LQT-PM who also had disease causing LQT mutations and therefore the impact of having both LQT-PM and LQT disease causing mutation on the QTc intervals during EST cannot be assessed. Though our control population had no evidence of LQT channelopathy, genetic testing was not performed on this population due to economic considerations. Therefore, we can only assume that the presence of SNPs in this group is equal to that of the general population. As many clinicians are aware, there are limitations to the interpretation of EST including the inaccuracy of QTc measurement during tachycardia as well as the breakdown of Bazzett's formula at heart rates >100 bpm. Finally, the QTc response exhibited by cycle ergometry should not be generalized to other modalities of EST.

CONCLUSION

Individuals with LQT-PM may have longer QTc intervals at rest as well as at peak exercise and all phases of the recovery period compared to normal controls and therefore the exercise stress test (EST) should be interpreted with caution when the diagnosis of LQTS is being considered. However, a key distinguishing feature is that LQT-PM subjects have normal QT adaptation with exercise indicating that there was no decrease in repolarization reserve which is similar to the control population.

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