

QT Interval Is Linked to 2 Long-QT Syndrome Loci in Normal Subjects

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Background—The rate-corrected QT interval (QTc) is heritable, and the discovery of quantitative trait loci that influence the QTc would be an important step in identifying the genes responsible for life-threatening arrhythmias in the general population. We studied 66 pairs of unselected normal dizygotic (DZ) twin subjects and their parents in a sib-pair analysis. We tested for linkage of gene loci harboring genes known to cause the long-QT syndrome (LQT) to the quantitative trait QTc.

Methods and Results—We found genetic variance on QRS duration, QRS axis, T-wave axis, and QTc. Women had a longer QTc than men. Microsatellite markers were tested in the vicinity of the gene loci for the 5 known LQT genes. We found significant linkage of QTc with the loci for LQT1 on chromosome 11 and LQT4 on chromosome 4 but not to LQT2, LQT3, or LQT5. We also found linkage of the QRS axis with LQT2 and LQT3.

Conclusions—We suggest that these quantitative trait loci may represent the presence of variations in LQT genes that could be important to the risk for rhythm disturbances in the general population. (*Circulation*. 1999;99:3161-3164.)

Key Words: molecular biology ■ long-QT syndrome ■ intervals ■ genetics ■ electrocardiography

Heart failure is common in the general population and is associated with sudden cardiac death from arrhythmias.¹ The chance for a normal person to develop heart failure at some time in their life is considerable. The discovery of genes responsible for the long-QT syndrome (LQT) has enabled the understanding of molecular mechanisms involved in fatal arrhythmias.² Thus far, 4 such genes are known, and a fifth gene locus has been identified on chromosome 4. It is conceivable but not yet shown that these genes may contain lesser functionally important variants that could contribute to rhythm disturbances in the general population. The rate-corrected QT interval (QTc) is known to be influenced by genetic variance.³ The existence of highly polymorphic microsatellite markers enables testing of the hypothesis of whether or not LQT gene loci are linked to QTc in normal persons. We relied on normal monozygotic (MZ) and dizygotic (DZ) twin subjects to address this issue.

Methods

We recruited 166 pairs of twins (100 MZ and 66 DZ) by advertisement to participate in studies involving blood pressure regulation and cardiovascular phenotypes.^{4,5} The subjects were all white Germans recruited from various parts of Germany. The protocol was approved by the University's committee on the protection of human subjects, and written informed consent was obtained from all participants. Blood was obtained for determination of zygosity and other molecular genetic studies from all the twins and the parents of the DZ

twins. Each participant underwent a medical history and physical examination. None had a family history of chronic medical illness. Blood pressure was measured by a trained physician (2 measurements, 1 minute apart) with a standardized mercury sphygmomanometer, with the subject seated for 5 minutes. The mean of the 2 measurements was used. Subjects underwent echocardiography and planar ECG. A standard 12-lead ECG was performed (CARDIOVITS CS-100, Schiller AG). Duration of the QTc and RR intervals was measured in lead II. QTc was determined according to Bazett's formula.⁶ ECG parameters were scored by a computer and stored for subsequent retrieval.

For this linkage study, the DZ pairs were selected and used as ordinary sib pairs but with the advantage of perfect age matching and reduced environmental variation affecting the phenotype. The power of the twin model in elucidation of complex genetic disease has recently been emphasized by Martin et al.⁷ The MZ twins were used to estimate allele frequencies for the markers tested. Zygosity was verified with the use of 5 polymerase chain reaction–amplified microsatellite markers, as described in detail elsewhere.⁸ We examined 2 microsatellite markers at the LQT1 locus, 3 at the LQT2 locus, 3 at the LQT3 locus, 5 at the LQT4 locus, and 3 at the LQT5 locus, as shown in Table 1.

We assessed linkage for QTc as a continuous trait.⁹ Sib-pair analysis to determine linkage does not require specification of a genetic model. The underlying trait can follow either mendelian or nonmendelian modes of inheritance. Analysis was done by use of a structural equation modeling (SEM) approach,¹⁰ as implemented in the MX package.¹¹ This approach is based on variance (VAR)-covariance (COV) matrices of sibs weighted by the probability of sharing 0, 1, or 2 alleles identical by descent (IBD). Phenotypic variance was decomposed into variance due to genetic background (A), variance

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TABLE 1. Microsatellite Markers Used in Linkage Analysis

Locus	Marker
LQT1	D11S1318
	D11S4146
LQT2	D7S505
	D7S636
	D7S483
LQT3	D3S1298
	D3S1260
	D3S1100
LQT4	D4S1570
	D4S1564
	D4S402
	D4S1615
	D4S429
LQT5	D21S219
	D21S65
	D21S1252

due to the quantitative trait loci (QTL) effect (Q), and environmental variance (E):

$$\text{VAR} = A^2 + Q^2 + E^2$$

For the 3 possible IBD states (sharing 0, 1, or 2 alleles), covariance of a sib pair was then defined by

$$\text{COV}_{\text{IBD0}} = 0.5A^2 \quad \text{COV}_{\text{IBD1}} = 0.5A^2 + 0.5Q^2 \quad \text{COV}_{\text{IBD2}} = 0.5A^2 + Q^2$$

To improve estimates of total variance and genetic background, MZ twins were included in the analysis, with the covariance defined as

$$\text{COV}_{\text{MZ}} = A^2 + Q^2$$

To test for a QTL effect, the difference in model fit for models with and without a QTL effect was calculated as a χ^2 statistic. For each sib pair and each locus, the proportion of alleles IBD, based on parental genotypes and independent allele-frequency estimates, was calculated with a multipoint approach as implemented in MAPMAKER/SIBS.¹² The higher power of the variance-covariance-based analysis compared with the squared trait differences-based approach by the Elston method¹³ has been shown in a recent simulation study.¹⁴ Because we used a candidate gene approach, we accepted $P < 0.01$ to test for significant linkage, in accordance with the criteria defined by Lander and Kruglyak.¹⁵

Parameters of the quantitative genetic models were estimated by SEM with the MX program developed by Neale.¹² The variability of any given phenotype (P) within a population can be decomposed into genetic influences (A), environmental influences shared by the twins within a family (C), and effects of random environment (E):

$$P = aA + cC + eE$$

with a, c, and e as the estimated relative influence. For MZ and DZ, the covariance of their phenotype is given by

$$\text{Cov}_{\text{MZ}} = a^2 + c^2 \quad \text{and} \quad \text{Cov}_{\text{DZ}} = 0.5a^2 + c^2$$

Heritability analysis in twin studies can estimate additive components of genetic variability (estimated as a^2) as well as 2 environmental influences, shared (c^2) and nonshared (e^2) environmental influences.¹⁶ These values estimate the relative amount of the influence on interindividual differences up to a sum of 1. Genetic as well as environmental effects were estimated by the best-fit model as selected by the χ^2 value. Statistical analysis was conducted with the SPSS program. Adjustment of phenotypic values for sex and age was done by multiple linear regression with the unstandardized residuals

TABLE 2. Demographic Data and ECG Parameters in MZ and DZ Twin Subjects

Variable	MZ (mean \pm SD)	DZ (mean \pm SD)
n (pairs)	100	66
Age, y	34 \pm 15	34 \pm 12
Sex (male/female)	84/166	48/102
Height, cm	169 \pm 9	170 \pm 9
Weight, kg	67 \pm 12	70 \pm 14
Systolic blood pressure, mm Hg	125 \pm 16	123 \pm 13
Diastolic blood pressure, mm Hg	73 \pm 11	73 \pm 10
Body mass index, kg/m ²	23 \pm 4	24 \pm 4
P, ms	110 \pm 11	106 \pm 12*
PR, ms	152 \pm 20	151 \pm 22
QRS, ms	99 \pm 10	97 \pm 11
QTc, ms	414 \pm 25	416 \pm 25
P axis, $^\circ$	44 \pm 20	43 \pm 21
QRS axis, $^\circ$	55 \pm 25	49 \pm 26
T axis, $^\circ$	37 \pm 18	33 \pm 16

* $P < 0.01$.

as the corrected phenotypes. In case of significant deviations from a normal distribution, the appropriate transformations were applied before analysis.

Results

Demographic data, blood pressure values, and heredity estimates of blood pressure and ECG variables in 200 MZ and 132 DZ twins are given in Table 2. There were no significant differences between MZ and DZ twins for any of the demographic variables examined. P-wave duration was slightly longer in MZ than in DZ twins. Women had a longer QTc than men (421 \pm 25 and 405 \pm 24 ms, respectively; $P < 0.001$). Table 3 gives genetic analysis showing genetic, shared environmental, and nonshared environmental effects on ECG parameters. P-wave duration, QRS duration, QTc, QRS axis, and T-wave axis showed strong genetic effects. RR interval, P-wave duration, PR interval, and P-wave axis showed shared environmental effects, and all ECG parameters showed evidence of nonshared environmental effects. Table 4 contains linkage analysis results for the tested loci in the DZ twins. Strong inference for linkage was found at the LQT1 and LQT4 loci. No evidence for linkage was observed for the other LQT gene loci. Strong evidence for linkage to the QRS axis was found at LQT2 and LQT3, with some evidence for linkage at LQT4. Finally, some evidence for linkage to the T-wave excess was found for LQT2. We found no sex-related differences for the QTL effects. No evidence for linkage was observed for any of the other ECG parameters.

Discussion

We tested the hypothesis that LQT loci might be QTLs for the QTc in normal individuals and found that this was indeed the case for LQT1 and LQT4. We believe that these data are important because these respective genes could now be

TABLE 3. MZ-DZ Twin Analysis Showing Genetic and Environmental Effects

	Genetic Effect	Shared Environment	Nonshared Environment	χ^2/DF	<i>P</i>	<i>r</i> _{MZ}	<i>r</i> _{DZ}
RR		0.46	0.54	3.1/4	NS	0.50	0.37
P	0.46	0.12	0.42	9.6/3	0.01	0.55	0.40
PR		0.53	0.47	2.3/4	NS	0.47	0.51
QRS	0.40		0.60	7.2/4	0.01	0.41	0.08
QTc	0.52		0.48	1.3/4	0.01	0.52	0.30
P axis		0.23	0.77	2.8/4	NS	0.26	0.29
QRS axis	0.59		0.41	1.7/4	0.01	0.60	0.24
T axis	0.52		0.48	5.4/4	0.01	0.51	0.11

examined in detail for lesser allelic variants that might be functionally important. LQT syndromes are relatively rare, whereas rhythm disturbances in the general population are common. The discovery of QTLs in the normal population for QTc may elucidate causes for rhythm disturbances in the general population, allow the development of new diagnostic strategies, and enable the selection of individuals at increased risk.

Twin studies have been used previously to examine the effect of genetic variance on ECG parameters. Hanson et al³ were able to study MZ and DZ twins reared apart and showed that PR interval, QRS duration, QRS axis, QTc, and ventricular rate indicated a significant contribution of genetic effects, ranging from 30% to 60%. Although their analysis is different from the analysis we used, the heritability estimates are similar. Hanson et al³ were then able to compare data from twins reared apart and twins reared together and observed little difference in terms of ECG parameters. They provided firm evidence that genetic factors are of real importance in determining the basic physiological measures responsible for ECG components. Our MZ-DZ twin comparisons strongly support their conclusion. We were also able to confirm the finding that men have a shorter QTc interval than women of the same age.^{6,17}

DZ twins are a particularly powerful sib-pair model because of identical ages and a shared environment, at least in childhood. Interestingly, a QTL for a closely defined reading disability has been described on chromosome 6, by means of sib-pair analysis including DZ twins.¹⁸ In that study, the power of DZ twins in the sib-pair analysis was aptly demonstrated; DZ twin sib pairs exhibited a lod score twice that of nontwin affected siblings. This result would suggest that the sample size can be sharply reduced without a loss of power when DZ twin siblings are examined. The usefulness of DZ

twins in the quantitative sib-pair linkage analysis approach to genes relevant to cardiovascular disease was recently demonstrated by Austin et al,¹⁹ who found linkage between the microsomal triglyceride-transfer protein gene locus and plasma triglyceride concentrations, and also by Knoblauch et al,²⁰ who found linkage between the macrophage scavenger receptor gene locus and HDL-cholesterol concentrations. In previous studies, we found linkage between the *ACE* gene locus⁵ and the *IGF-1* gene locus²¹ and echocardiographically determined parameters of heart size in these same twin subjects.

Congenital LQT is an autosomal-dominant genetic disorder of cardiac electrical repolarization caused by mutations of ≥ 6 genes.² Four LQT genes have been identified: *KVLQT1*, *HERG*, and *Min K* encode for cardiac potassium channels, whereas *SCN5A* encodes for the cardiac sodium channel. Altered ion-channel function produces prolongation of the action potential and propensity to torsade de pointes ventricular tachycardia. A fifth gene locus has been shown on chromosome 4; however, the gene has not yet been cloned.²² The discovery of linkage to this locus in normal individuals could conceivably be useful in narrowing the region containing the responsible gene. The entire genomic structure of 3 LQT genes, including *KVLQT1*, has been described, allowing genetic screening to identify individuals at risk for this disorder.²³ Shimizu and Antzelevitch²⁴ examined the cellular basis for the ECG features of LQT1. They used a specific blocker of the *I_{Ks}* channel and prolonged the QT interval and action potential duration in an in vitro model. Our data would suggest that *I_{Ks}* channel activity is heritable, perhaps via variation in *KVLQT1*. After puberty, women with LQT are at greater risk for arrhythmias than men.²⁵ In our analysis, we found no sex-specific genetic effects on linkage with LQT loci; however, our numbers may not have been sufficient to identify a difference.

We were surprised to find strong evidence for linkage between the QRS vector and LQT2, as well as LQT3, whereas neither LQT2 or LQT3 was linked to QTc. The first locus contains *HERG*, whereas the second contains *SCN5A*. We are not aware of specific QRS-axis aberrations in LQT patients except while they experience polymorphic ventricular tachycardia.²⁶ El-Sherif et al²⁷ recently observed a localized circuit that varied its location and orientation from beat to beat, which serves to explain the transition of the QRS axis during polymorphic ventricular tachycardia in LQT. The

TABLE 4. Sib-Pair Linkage Analysis in DZ Subjects

Locus	QTc	P Wave	QRS	QRS Axis	T Axis
LQT1	<0.001	0.99	0.06	0.99	0.99
LQT2	0.76	0.99	0.99	<0.001	0.004
LQT3	0.17	0.99	0.43	0.003	0.99
LQT4	<0.001	0.99	0.25	0.05	0.42
LQT5	0.99	0.17	0.06	0.99	0.55

P values for a QTL effect are given.

presence of LQT ion channels in the conduction system might be consistent with genetic linkage with certain channel loci and the QRS axis normally. We observed a significant genetic effect on the QRS axis in the MZ-DZ twin comparison. The *HERG* locus was also linked to the T-wave axis. Phenotypic T-wave patterns are often abnormal in LQT.²⁸

The phenotype of LQT varies depending on the specific mutation involved.²⁹ Phenotypic heterogeneity is also caused by variable penetrance and expressivity. We believe that our identification of the *KVLQT1* locus as a QTL for QTc and 2 other LQT loci as QTLs for the QRS axis in normal, healthy individuals has direct clinical implications. For instance, the structure of *KVLQT1*, *HERG*, *SCN5A*, and the gene on chromosome 4, when it is cloned, will enable a strategy of multiplex sequencing in these individuals and their parents.³⁰ Allelic variants having a functional bearing on QTc or QRS axis can be identified in these healthy persons, which can then be tested in patients with congestive heart failure at risk for developing cardiac arrhythmias. For example, an allelic variant in the β_2 -adrenergic receptor gene, which strongly influences survival in heart failure patients, was recently described.³¹ Prospective strategies to influence QTc, thereby avoiding cardiac arrhythmias, could then be applied.³²

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