Common Genetic Variation in \(\textit{KCNH2}\) Is Associated With QT Interval Duration

The Framingham Heart Study

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\(\text{Background}\) —QT prolongation is associated with increased risk of sudden cardiac death in the general population and in people exposed to QT-prolonging drugs. Mutations in the \(\textit{KCNH2}\) gene encoding the HERG potassium channel cause \(30\%\) of long-QT syndrome, and binding to this channel leads to drug-induced QT prolongation. We tested common \(\textit{KCNH2}\) variants for association with continuous QT interval duration.

\(\text{Methods and Results}\) —We selected 17 single nucleotide polymorphisms and rs1805123, a previously associated missense single nucleotide polymorphism, for genotyping in 1730 unrelated men and women from the Framingham Heart Study. rs3807375 genotypes were associated with continuous QT interval duration in men and women (2-df \(P=0.002\)), with a dominant model suggested (\(P=0.0004\)). An independent sample of 871 Framingham Heart Study men and women replicated the association (1-sided dominant \(P=0.02\)). On combined analysis of 2123 subjects, individuals with AA or AG genotypes had a 0.14-SD (SE, 0.04) or 3.9-ms higher age-, sex- and RR-adjusted QT interval compared with GG individuals (\(P=0.00006\)). The previously reported association of rs1805123 (K897T) replicated under a dominant (AA/AC, 0.12 SD [SE, 0.07] or 3.1 ms higher versus CC; 1-sided \(P=0.04\)) or additive model (0.06 SD [SE, 0.03] or 1.6 ms higher per A allele; 1-sided \(P=0.01\)).

\(\text{Conclusions}\) —Two common genetic variants at the \(\textit{KCNH2}\) locus are associated with continuous QT interval duration in an unselected community-based sample. Studies to determine the influence of these variants on risk of sudden cardiac death and drug-induced arrhythmias should be considered. (\textit{Circulation}. \textit{2007};\textit{116}:1128-1136.)

\textbf{Key Words:} arrhythmia • electrocardiography • genetics • ion channels • long-QT syndrome

\(\text{S}\)udden cardiac death results in 250 000 to 350 000 deaths in the United States annually.\(^1,2\) QT interval prolongation, reflecting abnormal myocardial repolarization time, is a risk factor for sudden cardiac death and cardiovascular events in the general population in most but not all studies\(^3-10\) and for sudden infant death syndrome.\(^11\) QT interval prolongation is a complex cardiovascular trait with multiple environmental and genetic contributors, including female sex, older age, left ventricular hypertrophy, drug exposure, and genetic factors.\(^12\) Congenital long-QT syndrome (LQTS) appears to underlie a fraction of all sudden cardiac death, particularly absent structural heart disease,\(^13,14\) as well as a substantial fraction of sudden infant death syndrome.\(^15\) QT prolongation with resulting torsade de pointes is a rare but lethal side effect of many

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noncardiac medications and a major barrier to drug development.\textsuperscript{16,17} Identification of the genetic determinants of QT interval variation could improve our understanding of the pathophysiology of sudden death and suggest targets for improved pharmacotherapy and risk stratification.\textsuperscript{18}

Whereas highly penetrant mutations in ion channel genes can result in LQTS, most individual mutations are rare and do not contribute significantly to the population risk of sudden death.\textsuperscript{19} Whether common genetic variation that influences QT interval duration can reduce repolarization reserve\textsuperscript{20} and contribute incrementally to increase the risk of arrhythmias is unknown. We and others have shown that continuous QT interval variation across the range of values has a heritability of 35\%, indicating that 35\% of its variability is attributable to additive genetic factors.\textsuperscript{21} We recently identified with collaborators common variation in the nicotinic acetylcholine receptor \(\alpha 4\) subunit \(\alpha 4\beta 2\) as a major determinant of drug-induced QT prolongation as either the desired therapeutic effect of antiarrhythmic agents or an unintended side effect of noncardiac drugs. Assays for the affinity of binding to the \(\alpha 4\) subunit channel are a routine component of pharmaceutical development, but late recognition of arrhythmogenic potential has been a major barrier to the drug pipeline.\textsuperscript{24} In LQTS families with identifiable genetic mutations, 40\% occur in the \(\alpha 4\) subunit channel, resulting in LQT2.\textsuperscript{25,26} Moreover, studies have reported possible association of a missense single nucleotide polymorphism (SNP) in \(\text{KCNH2}\) (rs1805123, K897T) with QT interval duration\textsuperscript{27–30} and as a possible modifier of congenital LQTS.\textsuperscript{31}

We hypothesized that common variants in \(\text{KCNH2}\) alter QT interval duration. Therefore, we genotyped a set of common polymorphisms capturing the majority of common variation at the \(\text{KCNH2}\) locus and tested them for association with QT interval duration in participants in the Framingham Heart Study (FHS).

**Methods**

**Study Participants**

The FHS is a prospective epidemiological study established in 1948 to evaluate potential risk factors for cardiovascular disease.\textsuperscript{32-33} The offspring cohort participants studied in this report are drawn from the 5124 subjects recruited from 1971 to 1975, at which time routine resting ECGs were obtained during the course of a comprehensive examination.\textsuperscript{34,38} Original cohort participants attending examination cycle 11 from 1968 to 1971 were eligible for study in the validation sample. The FHS participants are predominantly white of European ancestry with no evidence of gross population substructure using genotype-wide SNP data.\textsuperscript{36} Participants surviving to offspring examination cycle 6 or original examination 25 (1996 to 1997) provided blood samples for DNA extraction, and DNA from a set of 1809 unrelated offspring participants was available for genetic analyses in the first stage. Subjects were excluded for known prevalent coronary heart disease (offspring cohort, 1.3\%; original cohort, 10.7\%), use of cardiac glycosides or quinidine (0.4\% and 3.6\%, respectively), atrial fibrillation (0.06\% and 1.4\%), or technically poor ECGs (0.4\% and 0.7\%), resulting in a primary study sample of 1730 men and women.

For genotyping of a missense SNP K897T (rs1805123), a slightly different DNA plate set was available (\(>80\%\) of samples in common), totalling 1746 after exclusions. For genotyping of 2 SNPs, an additional independent validation set of up to 871 FHS offspring and original cohort men and women in families was genotyped. All study participants gave written informed consent.

**Determination of ECT Intervals**

Resting 12-lead ECGs were obtained at 25 mm/s and 0.1 mV/mm on strips of lined paper (Hewlett-Packard, Palo Alto, Calif). Digital calipers were used to measure intervals on scanned ECGs as previously described.\textsuperscript{12} The QT interval was defined as the time (ms) from onset of the QRS to the return of the T wave to baseline, taking care to exclude U waves if present, in leads II, V\textsubscript{5}, and V\textsubscript{6}. If a TU complex was present, the T-wave offset was taken to be the nadir of the curve between the T and U waves. The QT interval phenotype studied was the average of the lead II, V\textsubscript{5}, and V\textsubscript{6} residuals from sex-specific models of QT interval regressed on RR interval and age (see below). We have previously shown these measures to have good reproducibility.\textsuperscript{12}

**Clinical Characteristics of the Study Sample**

The methods of risk factor assessment have been described elsewhere.\textsuperscript{37} Each examination included comprehensive assessment of cardiovascular disease, ECG, and blood testing. All covariates included in the present study were measured contemporaneously to the ECG.

**Linkage Disequilibrium Characterization and SNP Selection**

All genotyping was performed on the Sequenom (San Diego, Calif) platform that resolves allele-specific single-base extension products with mass spectrometry (MALDI-TOF) as previously reported.\textsuperscript{38,39} We constructed linkage disequilibrium (LD) maps by genotyping 53 SNPs in 93 individuals in multigenerational pedigrees from families of European ancestry recruited in Utah (Centre d’Etude du Polymorphisme Humain [CEPH] reference sample, 96 independent chromosomes). A total of 47 SNPs (89\%) met our quality control thresholds (call rate \(\geq 85\%\); Hardy-Weinberg equilibrium; \(P \geq 0.01; \leq 1\) mendelian inconsistency), and 37 were polymorphic with minor allele frequency \(\geq 5\%\), spanning a total genomic distance of 66 kilobases (kb).

As is typical of the genome, the \(\text{KCNH2}\) locus shows strong LD, allowing a parsimonious set of SNPs to be selected to capture most of the common genetic variation. Association of a trait with a genetic variant can be detected if the causal genetic variant is genotyped directly (rarely, by chance) or more commonly if it is correlated with a genotyped SNP or combination of SNP alleles (haplotype). We defined blocks of strong LD in which limited historical recombination has occurred, using the ‘solid spine of LD’ method implemented in HaploView 2.03.\textsuperscript{40} We inferred phase from unphased genotypes using an expectation-maximization algorithm\textsuperscript{41} implemented in the TagSNPs program.\textsuperscript{42} To select tag SNPs capturing most of the genetic variation at the \(\text{KCNH2}\) locus, we selected a set of SNPs within each block of strong LD that had an \(r^2\) (square of the correlation coefficient) \(\geq 0.8\) to all haplotypes with frequency \(\geq 5\%\).

We genotyped all tag SNPs and all SNPs in the gap between blocks (low LD) in 1730 FHS offspring participants. All SNPs genotyped in FHS were in Hardy-Weinberg equilibrium (1-\(df\) \(x^2\) test \(P > 0.01\)) with genotyping \(\geq 85\%\). Separately, we genotyped SNP rs1805123 (K897T) reported in the literature for association with QT...
interval duration in 1746 offspring, of whom >80% overlap with the 1730 tested above, using a modified Sequenom assay. We genotyped rs3807375 (because of nominal association in the initial sample) and rs1805123 in an additional 871 FHS subjects in families.

Statistical Analysis
Using SAS version 8.1 (SAS Institute, Cary, NC), we regressed in multivariable linear models QT interval duration on RR interval and age in sex-specific models for each of the 3 leads (II, V₃, V₅) in the entire offspring cohort with ECG data (2288 men, 2491 women). For example, the model for lead II in men was QTresidual = QTtrue − 230 − 0.133 × RRms − 0.477 × age, and in women was QTresidual = QTtrue − 223 − 0.159 × RRms − 0.316 × age. QT residuals from models for each lead and sex were standardized to a mean of 0 and SD of 1. Sex-specific averages of the standardized QT residuals from each of the 3 leads were analyzed together in sex-pooled analyses (and as a secondary sex-specific analysis). For validation genotyping in the related sample of FHS families, averaged, standardized residuals from cohort- and sex-specific models from the offspring and original cohorts were analyzed.

QT residuals were screened for association with individual SNPs using a 2-df general test of the null hypothesis that mean adjusted QT interval does not differ by genotype group (AA, Aa, aa) using linear regression. To exclude the presence of >1 association signal, we used stepwise selection to sequentially add nominally significant SNPs to a model predicting adjusted QT residuals using SAS. We tested for an interaction of sex with SNPs independently associated with QT interval duration. Association testing of 2 SNPs in the validation sample was performed with PROC MIXED to account for familial relationships. We used a 1-sided probability value for the test of the null hypothesis that the specific genetic effect observed in the derivation sample (rs3807375) or the literature (rs1805123) was not supported. Results in the 2 samples were combined through the use of inverse variance weights. Of the 17 SNPs tested for association with haplotypes ≥5% in blocks of strong LD with the haplo.stats program. The haplo.stats program uses an expectation-maximization algorithm to develop a posterior probability distribution of haplotype pairs, conditional on observed single SNP genotypes, and regresses adjusted QT interval residuals on haplotypes. A score statistic is used to determine a global probability value for the test that no haplotype is associated with a different mean QT residual (analogous to ANOVA) and haplotype-specific probability values for the test that a given haplotype is not associated with a different mean QT residual compared with all other haplotypes. We considered the global test nominal probability values the primary haplotype test.

To account for multiple tests (multiple SNPs, sex pooled and sex specific), we developed empirical gene-wide probability values for individual SNPs testing for an interaction of sex with SNPs independently associated with QT interval duration. Association testing of SNP rs1805123, reported to be associated with QT interval does not differ by genotype group (AA, Aa, aa) using linear regression. To exclude the presence of >1 association signal, we used stepwise selection to sequentially add nominally significant SNPs to a model predicting adjusted QT residuals using SAS. We tested for an interaction of sex with SNPs independently associated with QT interval duration. Association testing of 2 SNPs in the validation sample was performed with PROC MIXED to account for familial relationships. We used a 1-sided probability value for the test of the null hypothesis that the specific genetic effect observed in the derivation sample (rs3807375) or the literature (rs1805123) was not supported. Results in the 2 samples were combined through the use of inverse variance weights. Of the 17 SNPs tested for association with haplotypes ≥5% in blocks of strong LD with the haplo.stats program. The haplo.stats program uses an expectation-maximization algorithm to develop a posterior probability distribution of haplotype pairs, conditional on observed single SNP genotypes, and regresses adjusted QT interval residuals on haplotypes. A score statistic is used to determine a global probability value for the test that no haplotype is associated with a different mean QT residual (analogous to ANOVA) and haplotype-specific probability values for the test that a given haplotype is not associated with a different mean QT residual compared with all other haplotypes. We considered the global test nominal probability values the primary haplotype test.

To account for multiple tests (multiple SNPs, sex pooled and sex specific), we developed empirical gene-wide probability values for the single SNP association results. Null data sets were created by randomly sampling with replacement sets of genotypes (considered collectively to preserve their correlation) for assignment to each of the individual QT residuals. We conducted all 51 single SNP tests (3 samples and 17 SNPs) on each null data set and recorded the minimum probability value. The distribution of minimum probability values from 1000 repetitions was used to determine an empirical gene-wide probability value corresponding to the best nominal result.

Association testing of SNP rs1805123, reported to be associated with QT duration in the literature, was considered independently because it was selected to test a specific hypothesis rather than to capture common genetic variation.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Study Sample Characteristics
Our primary study sample included 859 men and 871 women (n=1730 total) with mean ages of 37.1 and 36.7 years, respectively (Table 1). The mean unadjusted QT interval in lead II was 363 ms (SD, 27 ms) in men and 361 ms (SD, 30 ms) in women. RR interval and age-adjusted QT interval duration in lead II was longer in women (394 ms) compared with men (380 ms), as has been demonstrated previously. Additional clinical characteristics are presented in Table 1.

LD in Reference Samples and Tag SNP Selection
Of 47 working SNP assays in the CEPH reference samples, 37 SNPs were polymorphic with a minor allele frequency ≥5% (average inter-SNP distance, 1870 bp; online Data Supplement Table I). Two blocks of strong LD, each containing limited haplotype diversity, were found (the Figure). Of the 66-kb genomic span of our markers (14 kb upstream, 33 kb KCNH2 locus, 19 kb downstream), 94% fell within either of the 2 blocks of strong LD. The observed haplotypes ≥5% in the 2 blocks explained 91% and 82% of the 96 chromosomes tested. The 4-kb region of lower LD between the 2 blocks was saturated with SNPs (average inter-SNP distance, 572 bp). In unrelated Framingham offspring participants, we genotyped 4 and 7 tag SNPs in blocks 1 and 2, respectively, and all 6 nonredundant SNPs in the intervening gap of low LD. Using tagger (www.broad.mit.edu/mpg/tagger/; October 2006), we found that 97% of the 37 SNPs were captured at an r² > 0.8 with a mean r² for all SNPs of 0.97. The tag SNPs genotyped in the FHS sample revealed stronger LD in the gap region than in the CEPH reference samples (online Data Supplement Figure). This difference in LD estimates likely stems from the more precise estimate of LD obtained from 3460 Framingham chromosomes compared with 96 CEPH chromosomes.

Single SNP Association Results
Of the 17 SNPs tested, we observed a minimum nominal 2-df probability value of 0.002 for intronic SNP 15 (rs3807375;
minor allele frequency, 38%) in the sex-pooled analysis (Table 2). Table 2 shows the results of association testing for the sex-pooled primary analysis with nominal \( P < 0.05 \). The results of all 51 single SNP association tests (primary sex pooled and secondary sex specific) are shown in online Data Supplement Table II. Stepwise selection confirmed that only the single independent signal from SNP 15 was present at the locus; correlation among SNPs contributed to multiple nominally significant probability values. A dominant model (AA + AG versus GG) for the minor A allele best fit the observed data (\( P = 0.0004 \)). The genotype at SNP 15 explained 0.9% of the variation in adjusted QT duration. We found similar evidence of association of SNP 15 in men and women separately (2df \( P = 0.04 \) for both; online Data Supplement Table II) and no evidence of an interaction with sex (interaction \( P = 0.79 \)).

**Adjustment for Multiple Testing**

We determined an empirical distribution of single SNP association probability values, including both the primary sex-pooled and the secondary sex-specific analyses, using null data sets in which the correlation between each individual’s phenotype and genotype had been broken. A nominal \( P = 0.002 \) as found for SNP 15 was observed in 63 of 1000

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**Table 2. First-Stage Sex-Pooled Single SNP Association Results for Adjusted QT Interval Duration With Nominal \( P < 0.05 \)**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Sample</th>
<th>LSM</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3807375 (SNP 15) ( (P = 0.002) )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>558</td>
<td>-0.08</td>
<td>0.04</td>
</tr>
<tr>
<td>AG</td>
<td>658</td>
<td>0.08</td>
<td>0.03</td>
</tr>
<tr>
<td>AA</td>
<td>227</td>
<td>0.08</td>
<td>0.06</td>
</tr>
<tr>
<td>rs758891 (SNP 20) ( (P = 0.01) )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>689</td>
<td>-0.07</td>
<td>0.03</td>
</tr>
<tr>
<td>AG</td>
<td>743</td>
<td>0.04</td>
<td>0.03</td>
</tr>
<tr>
<td>GG</td>
<td>203</td>
<td>0.10</td>
<td>0.06</td>
</tr>
<tr>
<td>rs758890 (SNP 19) ( (P = 0.01) )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>683</td>
<td>-0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>CT</td>
<td>727</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>TT</td>
<td>200</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>rs876088 (SNP 22) ( (P = 0.02) )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>1291</td>
<td>-0.04</td>
<td>0.03</td>
</tr>
<tr>
<td>AG</td>
<td>330</td>
<td>0.13</td>
<td>0.06</td>
</tr>
<tr>
<td>GG</td>
<td>24</td>
<td>-0.11</td>
<td>0.22</td>
</tr>
<tr>
<td>rs3807374 (SNP 14) ( (P = 0.03) )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>872</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>AC</td>
<td>690</td>
<td>-0.01</td>
<td>0.05</td>
</tr>
<tr>
<td>CC</td>
<td>121</td>
<td>-0.06</td>
<td>0.11</td>
</tr>
<tr>
<td>rs882156 (SNP 21) ( (P = 0.046) )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>622</td>
<td>0.09</td>
<td>0.05</td>
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<tr>
<td>AG</td>
<td>752</td>
<td>-0.02</td>
<td>0.04</td>
</tr>
<tr>
<td>AA</td>
<td>219</td>
<td>-0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>rs4725984 (SNP 13) ( (P = 0.046) )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>646</td>
<td>0.02</td>
<td>0.03</td>
</tr>
<tr>
<td>AG</td>
<td>751</td>
<td>0.05</td>
<td>0.03</td>
</tr>
<tr>
<td>AA</td>
<td>209</td>
<td>-0.11</td>
<td>0.06</td>
</tr>
</tbody>
</table>

LSM indicates least-squares mean of averaged standardized residuals across leads II, V5, and V6 (see Methods). The genetic effect on QT interval duration is shown as the LSM of the averaged standardized residuals (adjusted for age and RR interval in sex-specific models) across leads II, V5, and V6. Nominal probability values for the test that there are no differences in LSM among all 3 genotype classes are shown (2 df). The SNPs with \( P < 0.05 \) are clustered toward block 2 at the 3' end of the KCNH2 locus (SNP 15 through 37). See online Data Supplement Table 2 for nominal probability values from all single SNP association tests.
null data sets, corresponding to an empiric gene-wide corrected \( P = 0.063 \).

**Multimarker Haplotype Association Results**

We tested haplotypes with \( \geq 5\% \) frequency within 2 blocks of strong LD for association with adjusted QT interval duration. We found a nominal global \( P = 0.01 \) in block 2. The hap scores for haplotypes 2C and 2D, each containing the SNP 15 \((\text{rs3807375})\) A allele, are both positive (associated with increased QT interval), consistent with the A allelic effect seen on analysis of the single SNP (Tables 3 and 4).

**QT Association of rs3807375 in a Second Sample**

We genotyped rs3807375 in an additional 871 FHS men and women independent of the original sample. We replicated the association of the SNP with QT residual under a dominant model \((1\text{-sided} \; P = 0.02)\). On combined sex-pooled analysis for men and women together and separately are shown.\(^46\) The global \( P \) value represents a test of the null hypothesis that no difference in mean QT residual exists among carriers of any specific haplotype(s). The hap score reflects the magnitude and direction of effect of each haplotype. The hap probability value for each haplotype represents a test of the null hypothesis that no difference in mean QT residual exists among carriers of that haplotype vs noncarriers. No single haplotype probability value was lower than those of the single SNP association tests.

**Genomic Context of rs3807375**

SNP 15 \((\text{rs3807375})\) lies in intron 2 in a sequence that is not highly conserved across species. In our reference sample of 96 CEPH chromosomes, SNP 15 was perfectly correlated \((r^2 = 1.0; 95\% \; \text{CI}, 0.92 \text{ to } 1.0)\) with SNP 28 \((\text{rs1805121})\), a highly conserved synonymous coding SNP \((\text{L564L})\) 18 kb downstream in exon 7. In our CEPH reference sample genotyping, SNP 15 also was correlated with other intronic SNPs: SNP 20 \((r^2 = 0.86)\), SNP 19 \((r^2 = 0.73)\), and SNP 4 \((r^2 = 0.52)\). In 3460 independent FHS chromosomes, rs3807375 showed comparable correlation with SNP 28 \((r^2 = 0.97)\), SNP 20 \((r^2 = 0.86)\), and SNP 19 \((r^2 = 0.87)\). In HapMap samples of European ancestry \((\text{CEU})\), additional SNPs show lesser degrees of correlation: SNP 15 is partially correlated \((r^2 = 0.67)\) and rs10277237 downstream of KCNH2 \((r^2 = 0.67)\) and rs11762978 upstream of \(\text{KCNH2} \; (r^2 = 0.52)\).

**QT Association of rs1805123 (K897T)**

In addition to the LD-based approach relating common genetic variation at the \(\text{KCNH2} \) locus, we also tested

### Table 3.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Frequency</th>
<th>Hap Score</th>
<th>Hap ( P )</th>
<th>MW</th>
<th>M</th>
<th>W</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>ATGG</td>
<td>0.37</td>
<td>-1.19</td>
<td>0.24</td>
<td>0.07</td>
<td>0.22</td>
</tr>
<tr>
<td>1B</td>
<td>TTAT</td>
<td>0.29</td>
<td>-1.44</td>
<td>0.15</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>1C</td>
<td>TCAG</td>
<td>0.24</td>
<td>2.36</td>
<td>0.02</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

M indicates men; W, women. Haplotypes are identified by block with letters reflecting each of the different haplotypes \( \geq 5\% \). Nominal global and haplotype-specific probability values from haplo.stat for men and women together and separately are shown.\(^46\) The global \( P \) value represents a test of the null hypothesis that no difference in mean QT residual exists among carriers of any specific haplotype(s). The hap score reflects the magnitude and direction of effect of each haplotype. The hap probability value for each haplotype represents a test of the null hypothesis that no difference in mean QT residual exists among carriers of that haplotype vs noncarriers. No single haplotype probability value was lower than those of the single SNP association tests.

### Table 4.

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>2A</td>
<td>GCCAGG</td>
<td>0.35</td>
<td>-2.21</td>
<td>0.03</td>
<td>0.01</td>
<td>0.09</td>
</tr>
<tr>
<td>2B</td>
<td>GACAGA</td>
<td>0.23</td>
<td>-1.05</td>
<td>0.29</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>2C</td>
<td>AATGGG</td>
<td>0.23</td>
<td>1.55</td>
<td>0.12</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>2D</td>
<td>AATGGG</td>
<td>0.11</td>
<td>2.93</td>
<td>0.003</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

Definitions and commentary as in Table 3. Haplotypes 2C and 2D, each containing the minor A allele of rs3807375 \((\text{SNP} \; 15)\), both show a positive effect on QT residual consistent with the A allele effect seen on single SNP analysis.
rs1805123 (A/C, SNP 30) responsible for a lysine-threonine substitution in amino acid 897 (K897T) that has been found to be associated with QT interval in some studies. We successfully genotyped rs1805123 in 2515 FHS men and women. In 2 prior well-powered reports, modest association of rs1805123 was observed under a dominant model\(^{28}\) or an additive model of increased QTc with A allele carriage.\(^{29}\)

Under a dominant model, rs1805123 was associated with a 0.12-SD (SE, 0.07) or 3.1-ms-higher adjusted QT interval in AA or AC individuals compared with CC individuals (1-sided \(P=0.04\)). Under an additive model, rs1805123 was associated with a 0.06-SD (SE 0.03) or 1.6-ms-higher adjusted QT interval per A allele copy (1-sided \(P=0.01\)). Discrimination between a dominant or an additive model requires a much larger sample size. Our findings thus confirm a modest effect of rs1805123 (K897T) on QT interval duration.

### Discussion

**Principal Findings**

We defined a parsimonious set of tag SNPs that comprehensively capture common genetic variation at the KCNH2 locus encoding the HERG potassium channel \(\alpha\) subunit. We identified a novel association of a common variant with myocardial repolarization and replicated the previously reported association of a second independent missense variant. We found evidence of association of SNP 15 (rs3807375) with age-, sex-, and RR-adjusted QT interval duration under a dominant model in our large, community-based sample (nominal \(P=0.00006\)). We also extend previous work reported in other large community-based samples in our finding of modest supportive evidence for the association of SNP 30 (rs1805123, K897T) with QT interval duration (\(P=0.01\)).\(^{28,29}\)

**rs3807375 Association With QT Interval**

We show for the first time that rs3807375, a SNP in intron 2 with a minor allele frequency of 38%, is associated with QT interval duration (\(P=0.00006\)). Probability values in genome-wide surveys of common variant–common trait associations likely need to be \(<10^{-17}\) to have a substantial probability of not being false-positives because 10 000 000 common variants\(^{48}\) exist and a minority of them are likely to be functional. However, in a candidate gene such as KCNH2, which is among the top candidates to influence QT interval duration, we have previously estimated the posterior probability of a true-positive result to be \(\approx 65\%\) for an observed \(P<10^{-4}\).\(^{49}\)

In a 2-stage survey of common genetic variants in potassium channels in a large, community-based sample of 3966 Germans from the KORA (Cooperative Health Research in the Region of Augsburg) project, Pfeufer et al\(^{30}\) reported association of 2 variants in KCNH2 with continuous QT interval duration. They found association of rs3815459, which tags a 23% haplotype (2C in our study, h3 in the KORA article), and SNP 30 (rs1805123), which tags a 23% haplotype (2B in our study, h2 in KORA).\(^{30}\) In our study, the minor alleles of SNP 15 (rs3807375) and its perfect proxy 28 (rs1805121) are found on haplotype 2C/h3 and a related 11% haplotype 2D/h4. The association in the KORA study with SNPs on haplotype 2C but not SNP 15 or other correlated SNPs (with minor alleles on both haplotypes 2C and 2D) could stem from the limited power to detect modest effects in a screening stage of 689 subjects or a chance difference. Our observation that SNP 15 shows stronger association with QT than either haplotype has effectively fine mapped the prior association by Pfeufer et al. We are not aware of another study that has reported on the allele that we have identified and QT interval duration.

If the human genome allows identification of a functional allele without directly genotyping it through correlation with genotyped variants, but LD makes distinguishing the functional variant from all correlated and equally associated variants difficult. SNP 15 (rs3807375) is perfectly correlated with a synonymous coding SNP 28 (rs1805121) and is partially correlated with other intronic or nongenic variants. Coding SNPs in general are under significant selective constraint, as we and others have shown.\(^{50}\) Some synonymous coding SNPs have been found to interrupt exonic splicing enhancers\(^{51}\) and are generally under selective constraint.\(^{52}\) SNP 28 (rs1805121) does not fall in any of 238 exonic splicing enhancers identified by Fairbrother et al (http://genes.mit.edu/burgelab/rescue-ese/).\(^{51}\) However, deep resequencing is required to fully define the set of all candidate alleles, with much larger sample sizes needed to distinguish among the several correlated alleles at the locus. Genotyping both SNPs 15 and 28 in individuals of African ancestry could allow improved resolution of the signal of association at the locus because Yoruban HapMap samples show substantially less LD at the locus.

**rs1805123/K897T Association With QT Interval Duration**

We found modest statistical evidence in support of the association of rs1805123 (A/C), encoding a lysine-threonine change at amino acid 897 (K897T) with QT interval duration. This effect is distinct from the rs3807375 effect because the variants show minimal correlation (\(r^2=0.18\)). Several studies have examined the association of this missense SNP in exon 12 in relation to QT interval duration. Pietila et al\(^{31}\) reported a study of 226 men and 187 women from Finland and found an increased QTc to be

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**TABLE 5. Sex-Specific Mean-Adjusted QT by rs3807375 (SNP 15) Genotype in 2123 FHS Men and Women**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Men (n=1004)</th>
<th>Women (n=1119)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>376.3 (394)</td>
<td>392.3 (460)</td>
</tr>
<tr>
<td>AG</td>
<td>381.8 (462)</td>
<td>394.6 (479)</td>
</tr>
<tr>
<td>AA</td>
<td>380.2 (148)</td>
<td>396.4 (180)</td>
</tr>
</tbody>
</table>

The mean QT (ms), adjusted for RR interval of 1000 ms and age of 37 years, is shown for each sex separately. The number in each genotype class is indicated. A dominant genetic model best fits the observed data (nominal \(P=0.00006\)).
associated with C allele carriage: AA<>(AC+CC). Bezzina et al\textsuperscript{28} reported a study of 1030 men and women from Augsburg, Germany, and found a higher QTc to be associated with A allele carriage: CC<(AC+AA). Pfuefer et al\textsuperscript{29} reported a separate sample of 3966 middle-aged men and women from Augsburg and found an increasing QTc with increasing copies of the major A allele (CC<AC<AA). Most recently, Gouas et al\textsuperscript{30} reported a study of 200 men and 200 women sampled from the extremes of the QTc distribution of a community-based sample in France and found an enrichment of major allele homozygotes in the high-QTc group: (CC+AC)<AA. Although our findings are marginally statistically significant in isolation, when they are considered together with the other published findings in large samples, consistent association exists of increased QT interval duration with genotypes containing the major A allele.

An interesting report by Crotti et al\textsuperscript{31} demonstrated in cellular assays a modifier effect of the K897T polymorphism on an LQTS-causing mutation in \textit{KCNH2} found in an Italian family. The authors hypothesized that the more modest expression observed in the other LQTS-mutation carriers in the family resulted from the absence of the 897T allele, but limited numbers of individuals in each genotype class preclude definitive statements. Cellular assays of the K897T polymorphism, which resides in the carboxy terminus of the HERG channel, have suggested reduced channel expression,\textsuperscript{53} activation at more negative potentials for the minor 897T allele\textsuperscript{28,54} with hastened\textsuperscript{28} activation, and both hastened\textsuperscript{28,54} and delayed\textsuperscript{53} inactivation in different assays. Differences in assay methods could underlie apparently conflicting results. Taken in aggregate, both human genetic and cellular electrophysiological data support a functional effect of the K897T polymorphism on myocardial repolarization.

**Study Strengths and Limitations**

Strengths of our study include the large community-based sample ascertained independently of ECG or other clinical characteristics, the uniformly and reproducibly measured QT interval, and the comprehensive LD-based approach to characterizing common genetic variation at the \textit{KCNH2} locus. Limitations clearly apply. Our study sample is of European ancestry, and the relevance of the 2 variants described to populations of different continental ancestry, in whom substantial allele frequency differences are observed in HapMap, remain to be defined. Our study assayed only common variation at this locus and cannot address the role of rare variation at \textit{KCNH2} on myocardial repolarization, already well established for mendelian syndromes. Moreover, detecting more modest effects than observed here requires much larger sample sizes. Lastly, use of QT-prolonging medications in the early 1970s was sufficiently uncommon to preclude tests for interaction of \textit{KCNH2} variants with such medication use.

**Clinical Implications and Future Directions**

The identification of 2 common modest QT-modifying alleles in \textit{KCNH2} could ultimately have important implications for clinical care. HERG binding is the dominant known mechanism for drug-induced QT prolongation and resultant arrhythmias. Whether polymorphisms in \textit{KCNH2} contribute to interindividual variation in susceptibility to this disastrous complication of pharmacotherapy needs to be tested in drug-exposed patient collections. The identification of host factors that contribute to liability to cardioxic effects of common medications could improve personalized risk assessment and rational assignment to drug therapy through the development of risk prediction models incorporating both clinical and genetic factors. Ultimately, identifying the full set of variants that modulate an intermediate trait such as QT interval duration will describe a superset of variants to be tested in the general population, in high-risk subsets (eg, heart failure, after myocardial infarction, congenital LQTS), and in those exposed to QT-prolonging drugs to define their impact on clinical events.\textsuperscript{18} Although modest genetic risk factors such as those found in \textit{KCNH2} may ultimately be found to lack clinical discrimination, they can still point the way to a better understanding of myocardial repolarization in the general population.

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**Disclosures**

None.

**References**


Disordered myocardial repolarization as manifest in electrocardiographic QT interval prolongation is a consistent risk factor for sudden cardiac death. As a consequence of noncardiac medications, QT prolongation and arrhythmias, in part a result of binding to the HERG channel, are also a major impediment to bringing common drugs to market safely. We tested common genetic variants in the KCNH2 gene encoding the α subunit of the HERG channel and observed association of a novel intronic single nucleotide polymorphism and a previously reported missense single nucleotide polymorphism with continuous QT interval duration in the Framingham Heart Study. The variants contribute to modest changes in QT interval, 3.9- and 3.1-ms increases; thus, their relevance to clinical disease is currently unclear. In trials of drugs that prolong the QT interval on resting ECG, increases of 5 to 10 ms in the group mean have been associated with increased risk of arrhythmia. These group means on resting ECGs reflect some patients with little QT change and some with substantial increases who likely bear a large fraction of risk. Future tests of common variants of modest effect such as those in KCNH2 or the recently identified NOS1AP myocardial repolarization gene (≈7-ms increase) in individuals at high risk for arrhythmias (eg, heart failure, status post myocardial infarction) and those exposed to QT-prolonging drugs are needed to determine whether these small changes in group means hide substantially increased risk for certain individuals when combined with other repolarization perturbations.
Common Genetic Variation in KCNH2 Is Associated With QT Interval Duration: The Framingham Heart Study

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