Common candidate gene variants are associated with QT interval duration in the general population


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Abstract. Marjamaa A, Newton-Cheh C, Porthan K, Reunanen A, Lahermo P, Väänänen H, Jula A, Karanko H, Swan H, Toivonen L, Nieminen MS, Viitasalo M, Peltonen L, Oikarinen L, Palotie A, Kontula K, Salomaa V (Biomedicum Helsinki, University of Helsinki, Helsinki, Finland; Broad Institute of Harvard and MIT, Cambridge, MA, USA; Massachusetts General Hospital, Boston, MA, USA; University of Helsinki, Helsinki, Finland; National Public Health Institute, Helsinki, Finland; Finnish Genome Center, University of Helsinki, Helsinki; Laboratory of Biomedical Engineering, Helsinki University of Technology, Espoo; and Department of Medicine, University of Helsinki, Helsinki, Finland). Common candidate gene variants are associated with QT interval duration in the general population. J Intern Med 2008; doi: 10.1111/j.1365-2796.2008.02026.x

Objectives. QT interval prolongation is associated with increased risk of sudden cardiac death at the population level. As 30–40% of the QT-interval variability is heritable, we tested the association of common LQTS and NOS1AP gene variants with QT interval in a Finnish population-based sample.

Methods. We genotyped 12 common LQTS and NOS1AP genetic variants in Health 2000, an epidemiological sample of 5043 Finnish individuals, using Sequenom MALDI-TOF mass spectrometry. ECG parameters were measured from digital 12-lead ECGs and QT intervals were adjusted for age, gender and heart rate with a nomogram (Nc) method derived from the present study population.

Results. The KCNE1 D85N minor allele (frequency 1.4%) was associated with a 10.5 ms (SE 1.6) or 0.57 SD prolongation of the adjusted QT_Nc interval (P = 3.6 × 10^(-11)) in gender-pooled analysis. In agreement with previous studies, we replicated the association with QTNc interval with minor alleles of KCNH2 intronic SNP rs3807375 [1.6 ms (SE 0.4) or 0.08 SD, P = 4.7 × 10^(-5)], KCNH2 K897T [-2.6 ms (SE 0.5) or −0.14 SD, P = 2.1 × 10^(-7)] and NOS1AP variants including rs2880058 [4.0 ms (SE 0.4) or 0.22 SD, P = 3.2 × 10^(-24)] under additive models.

Conclusions. We demonstrate that each additional copy of the KCNE1 D85N minor allele is associated with a considerable 10.5 ms prolongation of the age-, gender- and heart rate-adjusted QT interval and could thus modulate repolarization-related arrhythmia susceptibility at the population level. In addition, we robustly confirm the previous findings that three independent KCNH2 and NOS1AP variants are associated with adjusted QT interval.

Keywords: epidemiology, genetics, KCNE1, long-QT syndrome, QT interval.

*These authors contributed equally.
Introduction

Prolonged cardiac repolarization may associate with increased morbidity and mortality in the general population [1, 2]. The distinctive feature in long QT syndrome (LQTS) is the prolongation of the QT interval on the surface ECG and high risk for ventricular tachyarrhythmias [3, 4]. The underlying mechanisms involve mutations in ten genes coding for predominantly cardiac ion channels [5]. However, these disorders are collectively rare with a recent prevalence estimate of 1/2000 for LQTS [6] and therefore cannot account for the increased population risk of ventricular arrhythmias or sudden death.

Approximately 30–40% of the variation in QT interval duration is heritable [7–9]. Several epidemiological surveys have reported common ion channel polymorphisms to be associated with QT interval duration with varying levels of statistical support [10–13]. In addition, a genome-wide association study recently identified genetic variation at NOS1AP as a modulator of repolarization [14, 15], not directly through ion channel function but perhaps by regulating intra-cardiac signalling. In the present survey, we assessed the effects of common LQTS gene variants and the recently characterized NOS1AP variants on QT interval duration utilizing a large, epidemiological cohort from the Finnish population.

Methods

Study population

The study population consisted of a two-stage stratified cluster sample of 8028 individuals drawn from the Finnish Population Information System (http://www.vaestorekisterikeskus.fi/vrk/home.nsf/www/populationinformationsystem) for the Health 2000 survey. The material was collected between September 2000 and June 2001 and it is representative of the entire Finnish population of age ≥30 years [16]. DNA samples were collected from 6334 individuals, and digital standard 12-lead ECGs were available from a total of 6295 study participants. Clinical characteristics including medications, prior heart failure, history of myocardial infarction, prevalent diabetes and smoking status were determined as described previously [16]. Subjects with complete left or right bundle branch block (n = 143), QRS duration ≥120 ms (n = 205), atrial fibrillation/flutter (n = 94), pacemaker (n = 12), use of QT interval altering medication including digoxin (n = 1064) or confirmed genetic diagnosis for one of four Finnish LQTS founder mutations [17] were excluded from the study population. A drug was considered to potentially prolong QT interval if it was listed in any of the four categories at the website http://www.qtdrugs.org (accessed November 2006). In addition, we excluded individuals taking any of the following additional agents that might affect the rate of ventricular repolarization: carbamazepine, flupentixol, levomepromazine, mefloquine, olanzapine, oxcarbazepine, periciazin, sertindole and trazodone. The final study population included 5043 individuals. The study was performed according to the declarations of Helsinki and was approved by the Ethical Committees of the Hospital District of Helsinki and Uusimaa and the National Public Health Institute. A written informed consent was obtained from the participants.

Genetic analyses

Eight nonsynonymous LQTS gene variants were selected for genotyping based on the following criteria: (i) the variant had been identified in the Finnish population based on our previous studies on LQTS [17] and (ii) the variant had evidence for a functional role in modifying cardiac repolarization in the literature (i.e. association with LQTS and/or QT interval duration or suggestive in vitro data). In addition, we assessed four recently described NOS1AP single nucleotide polymorphisms (SNPs) and an intronic KCNH2 rs3807375 SNP that have been reported to be associated with QT interval duration in other population samples [12, 15]. Genotyping was performed using Sequenom MALDI-TOF mass spectrometry (MassArray Compact Analyzer; Sequenom Inc, San Diego, CA, USA) and Applied Biosystems TaqMan SNP Genotyping Assay (Applied Biosystems, Foster City, CA, USA) according to manufacturer’s instructions (online Data Supplement Methods). All SNPs
were tested for Hardy–Weinberg equilibrium with a chi-squared test.

**ECG measurements**

A digital standard 12-lead ECG was recorded with Marquette MAC 5000 (GE Marquette Medical Systems, Milwaukee, WI, USA). The 12 leads were recorded simultaneously, and a digital median QRS-T complex was used for analyses. We used QT Guard software (GE Marquette Medical Systems, Milwaukee, WI, USA) to measure heart rate and a custom-made software for all other measurements. The QT interval measurements were based on a previously described and validated algorithm [18]. The software calculates QT interval from QRS onset to T-wave offset, and a single observer (K.P.) reviewed measurements on-screen in a blinded fashion. For final analyses, we used the mean QT interval from all 12 leads. The intraobserver coefficient of variation was 0.7% for the mean QT interval in repeated ECG measurements in our recent study [19], and the same measurement methods were used also in the present survey. QT intervals were nomogram-corrected (Nc) for heart rate [20]. The correction equations were determined separately for each 10 beats per min (bpm) heart rate range in the current study based on a previously described method [20]. In addition, the SNPs were tested for association with age-, gender- and RR interval (heart rate)-adjusted QT intervals. Left ventricular hypertrophy was considered to be present if Sokolow–Lyon voltage >35 mm [21] or gender-adjusted Cornell voltage-duration product >2440 mm ms [22] was observed.

**Statistical analyses**

Histograms of all the variables were reviewed for normality. Using a stepwise linear regression model, we adjusted the mean QT\(_{\text{Nc}}\) intervals for age and gender [23, 24] and determined the beta coefficient, 2-tailed \(P\)-value and partial \(R^2\) for each covariate in the model. In addition, we tested additional covariates i.e. left ventricular hypertrophy, acute myocardial infarction, heart failure, prevalent diabetes, smoking, diuretics usage and geographical area for a possible explanatory role in the model. As these additional covariates accounted for <1% of the variation of QT\(_{\text{Nc}}\) interval in the study population, did not reach strong statistical significance \((P > 0.001)\) and in order to use a measure comparable to other studies, the QT\(_{\text{Nc}}\) residuals were derived from the age- and gender-adjusted model. The histograms of output residuals from the adjusted model were reviewed for normality (skewness 0.39, SE 0.03, kurtosis 1.10, SE 0.07). We tested for association using both a one-degree (1df) additive model and a two-degree freedom (2df) general test. In the 1df-test, we transformed the genotype to a continuous variable that corresponded to the number of minor alleles (0, 1, 2). Appropriateness of an additive genetic model was confirmed by evaluating the genotype-specific means for the QT\(_{\text{Nc}}\) residuals. In the 2df-test, the heterozygote and minor homozygote genotypes were converted to two dichotomous variables, with the major homozygote genotype as the reference. Nominal \(P\)-values are shown throughout the text without adjustment for multiple hypotheses. The Bonferroni-corrected [25] alpha level would be \(P = 5.0 \times 10^{-3} \) (0.05/10) considering the strongly correlated NOS1AP variants that are counted as a single test. Hence, the total number of independent tests is 10. The prevalence estimates with 95% confidence intervals were derived from the weighted study population as described earlier [16]. Using four SNPs associated with QT interval duration, we constructed a QT-prolonging score composed of the sum of the predicted QT-prolonging effect of each genotype in milliseconds and tested for association of the genotype-score with QT\(_{\text{Nc}}\) on a continuous scale and in quintiles. The statistical analyses were performed in the SPSS 13.0/15.0 software (SPSS Inc, Chicago, IL, USA).

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

**Results**

The clinical characteristics of the study population are presented in Table 1. All selected SNPs were analysed in the course of the study. Genotyping call rates
ranged from 97.6 to 99.9% of the study sample, and all SNPs were in Hardy–Weinberg equilibrium ($P > 0.01$). The genotype frequencies did not differ between males and females. The gender-pooled genotype-specific mean QT$_{Nc}$ intervals are shown in Table 2. The results from the linear regression analysis of the age- and gender-adjusted mean QT$_{Nc}$ are summarized in Table 3. 

Table 1 Clinical characteristics of the Health 2000 study sample ($n = 5043$)

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
<th>Males</th>
<th>Both</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>2691 (53)</td>
<td>2352 (47)</td>
<td>5043</td>
</tr>
<tr>
<td>Age (years)</td>
<td>52.4 ± 14.9 (30–97)</td>
<td>50.2 ± 13.1 (30–97)</td>
<td>51.4 ± 14.1 (30–97)</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>64.1 ± 10.4 (37–120)</td>
<td>62.3 ± 11.1 (34–113)</td>
<td>63.3 ± 10.7 (34–120)</td>
</tr>
<tr>
<td>Mean QT interval (ms)</td>
<td>391.9 ± 29.6 (288–532)</td>
<td>384.7 ± 29.7 (279–536)</td>
<td>388.5 ± 30 (279–536)</td>
</tr>
<tr>
<td>Mean QT$_{Nc}$ interval (ms)</td>
<td>398.9 ± 18.7 (338–512)</td>
<td>387.6 ± 19.3 (323–514)</td>
<td>393.6 ± 19.8 (323–514)</td>
</tr>
<tr>
<td>Mean QTc interval (ms)</td>
<td>401.9 ± 20.4 (333–504)</td>
<td>388.6 ± 22.1 (323–514)</td>
<td>393.6 ± 22.2 (323–514)</td>
</tr>
<tr>
<td>Left ventricular hypertrophy</td>
<td>418 (15.5)</td>
<td>459 (19.5)</td>
<td>877 (17.4)</td>
</tr>
<tr>
<td>Prevalent heart failure</td>
<td>31 (1.2)</td>
<td>8 (0.3)</td>
<td>39 (0.8)</td>
</tr>
<tr>
<td>History of myocardial infarction</td>
<td>35 (1.3)</td>
<td>77 (3.3)</td>
<td>112 (2.2)</td>
</tr>
<tr>
<td>Prevalent diabetes</td>
<td>82 (3.0)</td>
<td>105 (4.5)</td>
<td>187 (3.7)</td>
</tr>
<tr>
<td>Current smoking</td>
<td>457 (17.0)</td>
<td>670 (28.5)</td>
<td>1127 (22.3)</td>
</tr>
<tr>
<td>Diuretic usage</td>
<td>72 (2.7)</td>
<td>28 (1.2)</td>
<td>100 (2.0)</td>
</tr>
</tbody>
</table>

Nc, nomogram–corrected for heart rate; QTc, QT interval corrected for heart rate according to the Bazett’s formula [59]. Values are presented as mean ± SD (range) for continuous variables, and as number of subjects (%) for categorical variables. Left ventricular hypertrophy assessed with Sokolow-Lyon voltage [21] and/or gender-adjusted Cornell voltage-duration product [22] criteria.

Table 2 Mean QT$_{Nc}$ intervals by genotype for 12 candidate gene single nucleotide polymorphisms

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Amino acid change</th>
<th>Major homozygotes</th>
<th>Heterozygotes</th>
<th>Minor homozygotes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>%</td>
<td>QT$_{Nc}$</td>
<td>%</td>
</tr>
<tr>
<td>KCNH2</td>
<td>rs3807375</td>
<td></td>
<td>32.4</td>
<td>392.2</td>
<td>49.4</td>
</tr>
<tr>
<td>KCNH2</td>
<td>rs1805123</td>
<td>K897T</td>
<td>67.9</td>
<td>394.3</td>
<td>28.8</td>
</tr>
<tr>
<td>KCNH2</td>
<td>rs3621042</td>
<td>R1047L</td>
<td>86.8</td>
<td>393.6</td>
<td>12.6</td>
</tr>
<tr>
<td>SCN5A</td>
<td>R190G</td>
<td></td>
<td>99.1</td>
<td>391.9</td>
<td>0.9</td>
</tr>
<tr>
<td>SCN5A</td>
<td>rs1805124</td>
<td>H558R</td>
<td>64.0</td>
<td>392.9</td>
<td>32.0</td>
</tr>
<tr>
<td>SCN5A</td>
<td>A572D</td>
<td></td>
<td>93.8</td>
<td>393.5</td>
<td>6.1</td>
</tr>
<tr>
<td>KCNE1</td>
<td>rs1805128</td>
<td>D85N</td>
<td>97.3</td>
<td>393.2</td>
<td>2.6</td>
</tr>
<tr>
<td>KCNE1</td>
<td>rs1805127</td>
<td>G38S</td>
<td>34.4</td>
<td>393.1</td>
<td>49.0</td>
</tr>
<tr>
<td>KCNE2</td>
<td>rs2234916</td>
<td>T8A</td>
<td>99.0</td>
<td>393.5</td>
<td>1.0</td>
</tr>
<tr>
<td>NOS1AP</td>
<td>rs2880058</td>
<td></td>
<td>42.3</td>
<td>390.5</td>
<td>45.5</td>
</tr>
<tr>
<td>NOS1AP</td>
<td>rs4657139</td>
<td></td>
<td>41.3</td>
<td>390.4</td>
<td>46.1</td>
</tr>
<tr>
<td>NOS1AP</td>
<td>rs10918594</td>
<td></td>
<td>43.8</td>
<td>390.7</td>
<td>44.0</td>
</tr>
<tr>
<td>NOS1AP</td>
<td>rs10494366</td>
<td></td>
<td>41.6</td>
<td>390.7</td>
<td>45.7</td>
</tr>
</tbody>
</table>

Percentages refer to prevalence estimates. SNP = single nucleotide polymorphism.

KCNE1 D85N (rs 1805128) minor allele A was identifiable in 127 study participants in heterozygous form resulting in a prevalence estimate of 2.6% (95% CI
Table 3 Effect of SNPs on age-, gender- and heart rate (Nc)-adjusted QT interval in Health 2000

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Genotypic model</th>
<th>Allelic model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Heterozygote</td>
<td>Minor homozygote</td>
</tr>
<tr>
<td>KCNH2</td>
<td>rs3807375</td>
<td>2.1 (0.12)</td>
<td>2.9 (0.16)</td>
</tr>
<tr>
<td></td>
<td>K897T</td>
<td>-2.6 (-0.14)</td>
<td>-4.9 (-0.27)</td>
</tr>
<tr>
<td>KCNH2</td>
<td>R1047L</td>
<td>-0.5 (-0.03)</td>
<td>-10.8 (-0.58)</td>
</tr>
<tr>
<td>SCN5A</td>
<td>R190G</td>
<td>-0.5 (-0.03)</td>
<td></td>
</tr>
<tr>
<td>SCN5A</td>
<td>H558R</td>
<td>1.4 (0.08)</td>
<td>3.1 (0.17)</td>
</tr>
<tr>
<td>SCN5A</td>
<td>A572D</td>
<td>0.3 (0.02)</td>
<td>7.1 (0.39)</td>
</tr>
<tr>
<td>KCNE1</td>
<td>D85N</td>
<td>10.5 (0.57)</td>
<td>20.6 (1.12)</td>
</tr>
<tr>
<td>KCNE1</td>
<td>G38S</td>
<td>0.6 (0.03)</td>
<td>0.7 (0.04)</td>
</tr>
<tr>
<td>KCNE2</td>
<td>T8A</td>
<td>0.1 (0.01)</td>
<td>-</td>
</tr>
<tr>
<td>NOSIAP</td>
<td>rs2880058</td>
<td>4.5 (0.24)</td>
<td>7.7 (0.41)</td>
</tr>
<tr>
<td>NOSIAP</td>
<td>rs4657139</td>
<td>4.5 (0.24)</td>
<td>7.5 (0.41)</td>
</tr>
<tr>
<td>NOSIAP</td>
<td>rs10918594</td>
<td>3.9 (0.21)</td>
<td>6.5 (0.35)</td>
</tr>
<tr>
<td>NOSIAP</td>
<td>rs10494366</td>
<td>4.0 (0.22)</td>
<td>6.6 (0.36)</td>
</tr>
</tbody>
</table>

Values are differences from major homozygotes in milliseconds. Beta coefficients standardized to the SD of the age-, gender- and nomogram-adjusted residuals are shown in parentheses. Genotypic and allelic models refer to the genetic models described in detail in the Methods. R² = Proportion of variance explained. The standard deviation of age-, gender- and nomogram-adjusted QT residuals is 18.39.

2.2–3.2) in the population, and three subjects were homozygous carriers of the allele. Each additional copy of the D85N minor allele was associated with a 10.5 ms higher adjusted QT interval (SE 1.6, \( P = 3.6 \times 10^{-11} \)). Accordingly, whilst the mean QT interval in subjects without this allele was 393 ± 20 ms (\( n = 4684 \)), the corresponding value in D85N homozygotes was 404 ± 20 ms (\( n = 127 \)) and 415 ± 23 ms in homozygotes (\( n = 3 \)).

A 2.6 ms (SE 0.5, \( P = 2.1 \times 10^{-7} \)) shortening of the adjusted QT interval was observed per KCNH2 K897T minor C allele. The KCNH2 intronic SNP rs3807375 was associated with a 1.6 ms (SE 0.4) increase of adjusted QT interval per minor allele QTc (\( P = 4.7 \times 10^{-10} \)). The SNP rs3807375 has previously been tested under a dominant model [12], and therefore we analysed this intronic variant accordingly (effect size 2.3 ms, SE 0.6, \( P = 5.4 \times 10^{-5} \)). The four NOSIAP variants were in strong linkage disequilibrium (online Data Supplement Table 2) and resulted in statistically significant association with age- and gender-adjusted QTc under an additive model of inheritance. The strongest association was observed for SNP rs2880058 with a 4.0 ms (SE 0.4) increase of adjusted QT interval per minor G allele copy (\( P = 3.2 \times 10^{-24} \)). The effect sizes and significance of each SNP on age-, gender- and RR interval-adjusted mean QT interval were similar to the age- and gender-adjusted mean QTc (data not shown). Secondary analyses excluding all individuals older than 60 years, with QTc ≥ 500 ms, history of myocardial infarction and prevalent heart failure did not alter the results (data not shown). A secondary analysis excluding individuals with a history of myocardial infarction yielded equivalent results (data not shown).

Using the genotype effect size for each of the four SNPs KCNH2 rs3807375 and K897T, KCNE1 D85N, and NOSIAP rs2880058, we constructed a score ranging from 0 to 35.0 points based on the effect in milliseconds of each QT-prolonging genotype relative to the reference genotype. A score of 0 results for an individual who carries none of the genotypes associated with longer QT interval. A 1 point increase in the QT-prolonging score was associated with a 0.89 ms increase in the age- and gender-adjusted QTc interval (\( P = 4.6 \times 10^{-38} \)). For each quintile increase in the QT-prolonging score, the adjusted QTc interval increased by 2.4 ms (\( P = 1.6 \times 10^{-12} \)).
Accordingly, the mean QT<sub>Nc</sub> in the first quintile was 388 ± 19 ms compared with a mean QT<sub>Nc</sub> of 398 ± 20 ms in individuals in the fifth quintile \( (P = 8.3 \times 10^{-23}) \). In addition, the odds ratio for a prolonged QT<sub>Nc</sub> interval (QT<sub>Nc</sub> > 419 ms, the upper 90th percentile) was 1.11 (95% CI 1.08–1.13) per quintile increase in the QT-prolonging score \( (P = 1.5 \times 10^{-16}) \).

Discussion

Principal findings

Using a large, epidemiological study cohort of more than 5000 individuals of Finnish origin with well-characterized ECG phenotypes, we report association of three common LQTS gene variants in KCNE1 and KCNH2 and several correlated NOS1AP variants with QT interval duration.

**KCNE1 D85N association**

The most notable finding was the strong association of the KCNE1 D85N variant [26] present in 2.6% of the population and resulting in a 10.5 ms age-, gender- and nomogram-corrected QT-interval prolongation in heterozygous individuals. In a prior report by Gouas et al., this polymorphism in minK, the regulatory subunit of I<sub>Ks</sub> channel, was examined in 200 subjects with the shortest and 200 with the longest QT interval from amongst 2008 participants in a French community-based study [10]. Minor allele carriers had an increased odds of being in the longer QT group \( (P = 0.03) \).

In studies based on clinical samples, the D85N has been identified in solitary patients with torsade de pointes in the absence of recognizable disease-causing mutations in other LQTS genes [27, 28]. Wei et al. studied 96 acquired LQTS patients and 46 subjects with normal QT intervals following anti-arrhythmic drug therapy [29]. KCNE1 D85N appeared more prevalent in acquired LQTS subjects (7.3%) than in drug-tolerant patients (2.2%) [29]. Salisbury et al. identified 11 carriers (11%) of D85N in a cohort of 98 LQTS-gene negative patients, whereas 364 healthy controls had a significantly lower minor allele frequency with only nine heterozygous carriers (2.5%) [30]. A study by Westenskow et al. reported KCNE1 D85N in altogether thirteen carriers of LQTS-causing KCNQ1 or KCNH2 mutations who had evidence of a longer QTc compared to the noncarriers of the D85N minor allele [31]. In vitro electrophysiological studies have suggested a functional role of the D85N in reducing the I<sub>Ks</sub> current under voltage clamp conditions [31, 32].

Thus, in aggregate, substantial evidence supports the role of the KCNE1 D85N as a modifier of long QT syndrome and drug-induced torsade de pointes. Our finding of a striking effect of this SNP on baseline QT interval duration in a large unselected population supports the relevance of this variant to the general population. Ultimately, these findings may contribute to identification of patients with genetically determined reduced repolarization reserve [33, 34] and increased risk of arrhythmias in general (LQTS) or upon exposure to QT-prolonging drugs.

**KCNH2 (HERG) K897T and rs3807375 associations**

We were the first group to report the common K897T substitution in the KCNH2 gene and proposed a plausible mild phenotypic effect of this variant amongst female LQTS1 patients [35]. Upon studying 1030 Caucasian men and women, Bezzina et al. described longer QTc interval duration amongst carriers of the K897T major allele A consistent with our earlier findings [36]. Gouas et al. showed a higher frequency of the K897T minor alleles amongst 200 individuals with the shortest QTc intervals from 2008 healthy French subjects [10]. Pfeufer et al. reported a nearly 2 ms shortening of the QT interval per K897T C allele copy in a population-based study of 3966 individuals from southern Germany [13] similar to the most recent findings by Newton-Cheh et al. [12]. Conflicting results exist in a study of randomly selected healthy Finnish individuals, which observed QT prolongation amongst female carriers of the minor allele [37]. Discrepant results have also been reported amongst the numerous in vitro electrophysiological experiments concerning the functional consequences.
of the KCNH2 K897T polymorphisms [36, 38–40]. In fact, the consequences of the K897T variant may differ in patients with varying degree of genetically modified I\(K_r\) current [40]. Nevertheless, our present results confirm the majority of in vivo findings that the K897T minor allele is associated with moderate shortening of the QT interval under resting conditions.

In addition, we replicate the finding that an intronic KCNH2 SNP (rs3807375) is associated with QT interval under an additive or dominant genetic model as previously reported by Newton-Cheh et al. [12]. This variant marks a pair of haplotypes at the locus, one of which was found to be associated with QT interval duration in the same direction in a study by Pfeuffer et al. [13]. The missense K897T and intronic rs3807375 show weak correlation (\(r^2 = 0.15\)) and thus likely represent independent signals.

**NOS1AP variant association**

In addition to the LQTS gene polymorphisms, we robustly confirmed the association of rs10494366 and three other NOS1AP SNPs in linkage disequilibrium, all of which showed association of minor alleles with longer QT interval (\(P = 8.3 \times 10^{-19}\) to \(3.2 \times 10^{-24}\)). The stronger statistical support up to five orders of magnitude suggests that SNPs other than rs10494366 are more strongly correlated to the as yet unrecognized causal variant. A genome-wide association study by Arking et al. first reported the association of NOS1AP rs10494366 with modest but consistent QT prolongation [14] and subsequently these results have been replicated in a total of five published independent population-based samples until now [14, 15, 41]. The pathophysiological mechanisms mediating the NOS1AP effect on myocardial repolarization remain to be elucidated, but it is now evident that these SNPs, explaining up to 2% of the variation of QT interval in our model, do indeed modify myocardial repolarization.

**SCN5A H558R variant possible association**

SCN5A H558R [42] has been under intense investigation for a contribution to various arrhythmias including atrial fibrillation [43] and Brugada syndrome [44], but its role in myocardial repolarization has remained uncertain amongst community-based samples. Gouas et al. reported modest association of the H558R minor allele with an increased odds of being in the longer QTc group in the D.E.S.I.R. study (\(P = 0.01\)) [10]. Similarly, a study by Aydin et al. demonstrated an association of H558R minor allele on QT interval prolongation in a twin cohort (\(P = 0.025\)) [45]. We find modest replication of the association of the H558R minor allele with a 1.5 ms increase in age-, gender- and nomogram-corrected QT for each minor allele (\(P = 2.0 \times 10^{-3}\)), but consider the existing evidence of association of this variant with QT interval duration to be inconclusive.

**Other LQTS gene variants**

KCNH2 R1047L [46] has been described to be associated with dofetilide-induced torsade de pointes [28, 47] and leads to slower activation and inactivation kinetics in vitro [47]. According to our previous molecular genetic studies in LQTS patients, SCN5A A572D [48] and SCN5A R190G [17] appear to be slightly enriched amongst LQTS patients (6% vs. 4% controls for A572D and 2% vs. 0.6% controls for R190G) [17]. KCNE1 G38S [49] was reported to be associated with QT prolongation in men in a family-based study from Israel [50], but a study by Akyol et al. in a German sample neither found a modifying effect of the G38S minor allele on QT interval duration [51], nor did the smaller study by Gouas et al. [11]. The polymorphism KCNE2 T8A has previously been reported to be associated with drug-induced torsade de pointes [27, 52, 53], but recently a study by Pfeuffer et al. found no effect of this SNP on QT at the population level [13]. Based on our analyses in over 5000 genotyped and phenotyped individuals, the KCNH2 R1047L, SCN5A A572D, SCN5A R190G, KCNE1 G38S and KCNE2 T8A polymorphisms seem unlikely to modify the resting QT interval to a large extent in the Finnish population. It is unsettled, however, whether these variants account for alterations in QT interval in interaction with other genetic factors or with extrinsic stresses such as exercise, hypokalemia, ischemic or structural heart disease or drug exposure [54].
Potential clinical impact

Despite the marked significance levels in the current study, the allelic effect sizes remain relatively modest with the exception of KCNE1 D85N. The $R^2$ values are all under 2.5% despite the noticeable prevalence of these common variants and reflect the relatively modest effects of these variants. As QT interval prolongation is associated with increased mortality in patients with chronic [55] and acute [2] coronary artery disease and in men with cardiovascular disease [56] as well as in the general population [1, 57], even subtle additive changes may reduce repolarization reserve and contribute to increased risk of arrhythmias. Furthermore, even small changes caused by common variants may become important at the population level. In the present survey, we constructed a genotype score of four validated QT-altering SNPs. The 10 ms difference in mean QTc intervals amongst individuals with QT prolonging scores below the 20th and greater than the 80th percentiles of the QT genotype score is comparable to the QT prolonging effects of drugs withdrawn from the market [58] and thus potentially of clinical importance. Nevertheless, as about one third of the variation in QT interval duration is heritable [8], and the SNPs in the present study explain only a fraction of that, it is evident that other as yet unidentified heritable factors, independently or in interaction, must contribute to variability in cardiac repolarization.

Study strengths and limitations

The strength of the current study builds on the large, stratified cluster sample of over 5000 individuals with surface ECGs and DNA that was designed to reflect the whole Finnish population of ≥30 years old. The collection of clinical data without regard to phenotype enabled assessment of potential confounding factors. Systematic evaluation of the ECG parameters by a single observer yielded precise ECG phenotypes. The correction for heart rate was based on the nomogram-method from the present study population thus allowing accurate adjustments. Furthermore, the availability of alternative methods for adjusting QT interval for heart rate (nomogram, RR-interval in a linear model) and the equivalence of results from them confirms the general relevance of the findings in the current study sample and in other populations that may use different QT adjusting methods. To detect more subtle effects, an even larger population sample would be required. This limitation applies particularly to the four SNPs that failed to demonstrate statistically significant effects on the evaluated QT parameters. Our study involved participants of Finnish origin only. As the association studies on myocardial repolarization have thus far been restricted to the European ancestry, we cannot draw any definitive conclusions regarding the effects of these variants in populations of other ancestries.

Conclusions

In conclusion, we have demonstrated that the KCNE1 D85N missense SNP is associated with a 10.5 ms prolongation of the age-, gender- and nomogram-adjusted QT interval. Despite the relative infrequency of the polymorphism, this variant stands as a plausible independent risk factor for repolarization-related arrhythmogenesis. In addition, we have convincingly replicated the association of the KCNH2 intronic rs3807375 and missense K897T SNPs and several correlated NOS1AP variants in modulating the QT interval duration at the population level.

Conflict of interest statement

No conflicts of interest to declare.

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Supporting Information
Additional supporting information may be found in the online version of this article.

**Table S1** PCR primer and extension primer sequences used in genotyping.
Table S2 The pairwise linkage disequilibrium structure and $r^2$ values of NOS1AP variants.

Methods S1 Extended methods.

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