GENETIC EPIDEMIOLOGY

Gender and effects of a common genetic variant in the NOS1 regulator NOS1AP on cardiac repolarization in 3761 individuals from two independent populations

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Background
A longer heart-rate corrected QT interval (QTc) is associated with increased risk of ventricular arrhythmias. Women have longer resting QTc and are more likely than men to develop drug-induced QT prolongation. Recent studies have shown association between resting QTc and a common variant (rs10494366) of the NOS1 regulator, NOS1AP. We investigated the association between rs10494366 in NOS1AP and QTc, and assessed gender-specific NOS1AP associations with QTc during rest and after exercise.

Methods
We investigated the SNP associations with resting QTc in 919 women and 918 men from 504 representative families in the UK GRAPHIC study, and with QTc at rest and at 3 min recovery after exercise in 699 women and 1225 men referred for exercise testing in the Finnish FINCAVAS study.

Results
In the GRAPHIC study the minor allele (G) of the NOS1AP SNP rs10494366 prolonged QTc by 4.59 ms (95% CI 2.77–6.40; P = 7.63/10^7) in women, but only by 1.62 ms (95% CI –0.15 to 3.38; P = 0.073) in men (gender-SNP interaction term P = 0.025). In the FINCAVAS study the G allele significantly prolonged QTc in both women (P = 0.0063) and men (P = 0.0043) at 3 min recovery after exercise, but at rest an association was only seen in women (P = 0.020 excluding outliers).

Conclusions
A common NOS1AP variant prolongs QTc with a difference between genders. Further studies should aim to confirm this finding and to

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fatal cardiac events are often triggered by exercise.17 As QT exercise.

Studies of Mendelian disorders of the QT interval have implicated ion channel genes.1,22,23 As QT exercise.

A common intron 1 variant of the NOS1AP gene (rs10494366) has recently been associated with resting QTc in a genome-wide association scan28 and replicated in independent populations.28,31,32 NOS1AP spans over 299 kb of DNA on Chromosome 1 (1q23.3), including 10 exons and 1.5 kbs of coding sequence. The gene is responsible for the production of the nitric oxide synthase 1 (neuronal) adaptor, a cytosolic protein which regulates NO synthesis by facilitating interactions between the post-synaptic N-methyl-D-aspartate receptor (NMDAR) and neuronal nitric oxide synthase (nNOS) at neuronal synapses. NOS1AP is ubiquitously expressed.33,34

Methods

Keywords Cardiac repolarization, QTc, NOS1AP, gender, genetic association

Introduction

The QT interval represents the duration of ventricular depolarization and subsequent repolarization. A delay in cardiac repolarization manifests as a long QT interval and favours the development of cardiac arrhythmias, notably torsade de pointes (TdP). Mendelian disorders of the QT interval predispose to sudden cardiac death (SCD).1,2 Even lengthening of the heart-rate corrected QT interval (QTc) within the normal range is associated with raised coronary heart disease (CHD) incidence, CHD mortality and all-cause mortality.3–5 Furthermore, many otherwise beneficial drugs fail to be approved, need to be withdrawn or are relegated to second-line status because they prolong cardiac repolarization and increase risk of TdP.6–9 In fact, QT prolongation is the commonest cause for withdrawal or restriction of drugs that have already been marketed.10

The QT interval shortens after puberty in males but not females11 resulting in a longer QT in women than in men. Women are two to three times more likely to develop drug induced TdP than are men6,12–14 and greater caution is required when prescribing drugs known to prolong cardiac repolarization in women.7 Women with long QT syndrome are at higher risk than men of cardiac arrest and sudden cardiac death.15,16 In the long QT syndrome subtype LQTS1, fatal cardiac events are often triggered by exercise.17 Indeed, QT interval changes after exercise may unmask latent long QT syndrome.18,19 Furthermore, women show greater adaptation of the QT interval to exercise than men.20,21 Thus, the heart may be dissimilarly modulated by genetic factors in rest and in exercise.

Studies of Mendelian disorders of the QT interval have implicated ion channel genes.1,22,23 As QT interval is an accurately measurable, highly heritable quantitative trait (heritability estimates 35–41%),24–26 recent studies have focused on genetic determinants of cardiac repolarization in the general population.27–30 A common intron 1 variant of the NOS1AP gene (rs10494366) has recently been associated with resting QTc in a genome-wide association scan28 and replicated in independent populations.28,31,32 NOS1AP spans over 299 kb of DNA on Chromosome 1 (1q23.3), including 10 exons and 1.5 kbs of coding sequence. The gene is responsible for the production of the nitric oxide synthase 1 (neuronal) adaptor, a cytosolic protein which regulates NO synthesis by facilitating interactions between the post-synaptic N-methyl-D-aspartate receptor (NMDAR) and neuronal nitric oxide synthase (nNOS) at neuronal synapses. NOS1AP is ubiquitously expressed.33,34

Given differences in QTc biology between men and women, the gender specific effects of NOS1AP are of obvious interest. Any difference in the NOS1AP gene effects on QTc between men and women may provide further valuable insight into the biology of cardiac repolarization and the increased susceptibility of women to drug induced TdP. The reports of studies to date28,31,32 are inconclusive in this respect. Similarly, whether the genotype at the NOS1AP SNP affects QTc interval after exercise is unknown. The objectives of our study were therefore: (i) to investigate whether the association between resting QTc and the NOS1AP SNP, rs10494366, could be replicated in a population-based sample of nuclear families; (ii) to evaluate the gender-specific effects of the NOS1AP SNP on resting QTc in the same population; and (iii) to investigate overall and gender-specific association between NOS1AP and QTc during rest and after exercise in a cohort of individuals referred for exercise stress tests.
syndrome, missing rs10494366 genotype or with unexplained Mendelian inconsistencies in the genotype data. Leicestershire Research Ethics Committee approved the study and all subjects provided written informed consent.

The Finnish Cardiovascular Study (FINCAVAS) is an ongoing study focusing on the genetic background of exercise stress test responses. The participant pool of the present study consists of patients who underwent exercise stress testing at the Tampere University Hospital between October 2001 and December 2004. All patients willing to participate in the study were recruited (n = 2210). Referrals were for the following reasons: diagnosing CHD (n = 873); diagnosing arrhythmias (n = 414); evaluations of drug therapy (n = 295); evaluation of working capacity (n = 346); evaluation of status prior to an invasive operation (n = 262); and investigation after acute myocardial infarction (n = 149). Some patients had more than one indication. The exercise test was performed by electrical bicycle ergometer, measuring and digitally recording 12-lead ECG continuously at 500 Hz with the CardioSoft exercise ECG system (Version 4.14, GE Healthcare, Freiburg, Germany). QT interval was measured at rest and after 3 min of recovery using the GE algorithm implemented in Case Workstation software (GE Healthcare, Freiburg, Germany). The research ethics committee approved the study and all subjects provided written informed consent prior to the study.

Genotyping

In the GRAPHIC study, the SNP was detected by allelic discrimination using 15 ng of DNA, 36 mM of each primer pair, 8 mM of both allele specific fluorescent probes and TaqMan® Universal PCR Master Mix, No AmpEraser® UNG containing: AmpliTaq Gold DNA Polymerase, dNTPs and ROX passive reference [Applied biosystems (ABI), Foster City, Ca, USA]. PCR was performed on a DNA Engine Tetrad thermal cycler (MJ Research) in 96 well plates, using a cycling protocol of 95°C for 10 min followed by 45 cycles of 92°C for 15 s and 60°C for 1 min. Fluorescence was detected post PCR using the ABI Prism 7900HT Sequence Detector System and genotypes called using ABI Prism SDS software Version 2.1 (ABI, Foster City, Ca, USA). A similar TaqMan-based protocol was adopted for the FINCAVAS study, using 384 well plates.

Statistical analysis

Estimates of the magnitudes of the SNP effects and the SNP-gender interactions were obtained from the GRAPHIC study family data by fitting generalized linear mixed models (GLMMs) using Gibbs sampling in WinBUGS. This is a variance components based approach that takes full and appropriate account of the correlation of phenotypes within families. Narrow sense heritability was defined as the proportion of the QTc variance attributable to additive polygenic effects. Gender-specific analyses were carried out using generalized estimating equations (GEE) with an exchangeable correlation structure and robust standard errors to account for the correlation induced by mother-daughter and father-son relationships, as well as single-sex sibships. For analyses of Hodges-corrected QTc-intervals in the FINCAVAS data, linear regression models were employed. Age and SNP (rs10494366) were included as covariates in all analyses and an additive genetic model was assumed. We utilized the statistical packages STATA (release 9.1, STATACorp LP, College Station, TX, USA) and SPSS (release 14.0, SPSS Inc., Chicago, IL, USA) for the GEE and linear regression analyses, respectively. In both datasets, we tested the sensitivity of NOSIAP–QTc association tests to the exclusion of certain groups of participants.

In silico analysis

To identify SNPs that may potentially produce similar association results as a result of strong linkage disequilibrium (LD) with rs10494366 we analyzed the LD relationships between the SNP of interest and all other SNPs located up to 2.5 Mb from rs10494366 in CEPH HapMap Phase II data (Release 21a), illustrating the pairwise r² values using the ssSNPer web interface. We also assessed potential functional significance of these SNPs in silico using SNP Function Portal and investigated potential gene–gene interactions using the SNPs3D database.

Supplementary methods

Additional details of statistical analyses (including power calculations) and in silico analysis methods are provided in a supplementary file.

Results

Phenotypic characteristics

Table 1 shows key characteristics of the GRAPHIC and FINCAVAS participants. The narrow sense heritability of QTc in the GRAPHIC study families was 40.5% (95% CI 30.2–50.4%). Resting QTc was longer in women than in men, particularly in the GRAPHIC participants (P < 0.001). In the FINCAVAS participants, resting QTc was longer than in GRAPHIC participants (P < 0.001), and also more variable (Figure 1). These differences are to be expected. In contrast to the essentially healthy GRAPHIC study population, all FINCAVAS participants—both men and women—had been referred for exercise stress tests. Furthermore, their mean age was substantially higher (57 vs 39 years) and more participants...
Table 1 Characteristics of the GRAPHIC and FINCAVAS study participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>GRAPHIC Study</th>
<th>FINCAVAS Study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males (n = 919)</td>
<td>Females (n = 918)</td>
</tr>
<tr>
<td>Age in years: mean (SD)(^a)</td>
<td>39.25 (15.09)</td>
<td>39.18 (13.84)</td>
</tr>
<tr>
<td>Height (m): mean (SD)</td>
<td>1.78 (0.064)</td>
<td>1.64 (0.064)</td>
</tr>
<tr>
<td>Weight (kg): mean (SD)</td>
<td>83.23 (14.07)</td>
<td>69.35 (13.50)</td>
</tr>
<tr>
<td>Body mass index (kg/m(^2)): mean (SD)</td>
<td>26.32 (4.27)</td>
<td>25.80 (4.88)</td>
</tr>
<tr>
<td>Current smoker: (n) (%)</td>
<td>200 (21.8%)</td>
<td>162 (17.7%)</td>
</tr>
<tr>
<td>History of CHD: (n) (%)</td>
<td>6 (0.7%)</td>
<td>1 (0.1%)</td>
</tr>
<tr>
<td>History of hypertension: (n) (%)</td>
<td>134 (14.6%)</td>
<td>153 (16.7%)</td>
</tr>
<tr>
<td>Hodges corrected QTc at rest (ms): mean (SD)</td>
<td>398.70 (19.46)</td>
<td>409.93 (20.05)</td>
</tr>
<tr>
<td>Hodges corrected QTc at 3 min recovery after exercise (ms): mean (SD)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Taking drugs which may prolong QTc (excluding inhalers): (n) (%)</td>
<td>13 (1.4%)</td>
<td>35 (3.8%)</td>
</tr>
<tr>
<td>Taking drugs which may prolong QTc (including inhalers): (n) (%)</td>
<td>33 (3.6%)</td>
<td>60 (6.5%)</td>
</tr>
<tr>
<td>Taking (\beta)-blockers: (n) (%)</td>
<td>22 (2.4%)</td>
<td>22 (2.4%)</td>
</tr>
</tbody>
</table>

\(^a\) Age ranges were: 18–60 years in both men and women in the GRAPHIC study; 17–84 years in men and 15–84 years in women from the FINCAVAS study.

Figure 1 Distribution of QTc values in the GRAPHIC and FINCAVAS studies
(64 vs 50%) were male. Mean QTc at 3 min recovery from exercise stress testing was lower than resting QTc, particularly in women. The correlation coefficients between resting QTc and 3 min recovery QTc in FINCAVAS were modest at 0.50 for men and 0.53 for women.

Genetic associations

**GRAPHIC**

In the GRAPHIC study parental generation, rs10494366 had a minor allele (G) frequency of 39.5%, comparable to that reported previously and did not deviate from Hardy–Weinberg equilibrium \((P = 0.89)\). The rs10494366 was strongly associated with resting QTc \((G \text{ allele effect } 3.08 \text{ ms}; \ P = 1.3 \times 10^5)\) in the GRAPHIC population, accounting for 4.7% of the variance attributable to additive genetic effects \((1.3\% \text{ of total QTc variance})\). Analysis by gender revealed a striking difference. The genotype effect was highly significant in women \((P = 7.63 \times 10^7)\) whereas in men the association did not reach statistical significance at the 5% level (Table 2). A significant gender–SNP interaction was observed: each copy of minor allele \((G)\) of rs10494366 prolonging QTc by an estimated 2.82 ms more in women than in men \((P = 0.025)\). The estimated effect of rs10494366 was similar in mothers and in daughters (Table 2). We did not find evidence that the associations we observed in women were modified by estrogens, as the genotype effect remained highly significant after adjusting for menopausal status or combined oral contraceptive use \((P = 1.03 \times 10^6 \text{ and } P = 7.97 \times 10^7, \text{ respectively})\).

In sensitivity analyses, we obtained a similar pattern of findings in women and in men: (i) after excluding individuals with QTc values outside the first and 99th centiles (Table 3); (ii) after excluding individuals with cardiovascular diseases or individuals taking drugs which may prolong QTc \((n = 143)\); (iii) using Bazett or Fridericia-corrected QTc or (iv) after adjustment for current smoking status, body mass index, waist-hip ratio or alcohol intake as covariates (data not shown).

**FINCAVAS**

In the FINCAVAS population, rs10494366 had a lower minor allele (G) frequency (34.2%), and did not deviate from Hardy–Weinberg equilibrium \((P = 0.58)\). We observed a similar pattern of findings between FINCAVAS and GRAPHIC in that the estimated effect of rs10494366 on QTc was consistently larger in women \((P = 0.0063)\) and \((P = 0.0043)\) with or without exclusion of outliers (Tables 2 and 3). In contrast to the findings at rest, rs10494366 showed statistically significant association with QTc at 3 min recovery from exercise testing in both women \((P = 0.0063)\) and men \((P = 0.0043)\) with or without exclusion of outliers (Tables 2 and 3). We also obtained similar findings when we altered the thresholds for excluding outliers.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Associations between rs10494366 in NOS1AP and resting and 3 min recovery QTc (all subjects)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants</td>
<td>Phenotype</td>
</tr>
<tr>
<td>---------</td>
<td>----------</td>
</tr>
<tr>
<td>Women</td>
<td>Resting QTc</td>
</tr>
<tr>
<td></td>
<td>QTc at 3 min recovery after exercise</td>
</tr>
<tr>
<td>Men</td>
<td>Resting QTc</td>
</tr>
<tr>
<td></td>
<td>QTc at 3 min recovery after exercise</td>
</tr>
</tbody>
</table>

*Effect size estimates were similar in mothers \([\text{coefficient } 4.22 \text{ (95\% CI } 1.51 \text{ to } 6.92); \ P = 0.0022]\) and in daughters \([\text{coefficient } 4.38 \text{ (95\% CI } 2.04 \text{ to } 6.73); \ P = 0.00025]\). Mean ages: mothers 51.8 years, daughters 26.0 years.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Associations between rs10494366 in NOS1AP and resting and 3 min recovery QTc, excluding outliers (&lt;1st and &gt;99th centile)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants</td>
<td>Phenotype</td>
</tr>
<tr>
<td>---------</td>
<td>----------</td>
</tr>
<tr>
<td>Women</td>
<td>Resting QTc</td>
</tr>
<tr>
<td></td>
<td>QTc at 3 min recovery after exercise</td>
</tr>
<tr>
<td>Men</td>
<td>Resting QTc</td>
</tr>
<tr>
<td></td>
<td>QTc at 3 min recovery after exercise</td>
</tr>
</tbody>
</table>
outliers, after exclusion individuals with prior diagnoses of CHD, conduction disturbances or taking beta-blockers (data not shown).

In silico analysis
Of the 8304 SNPs located in the 5 Mb region surrounding rs10494366, only 39 SNPs showed high LD ($r^2 > 0.5$) and all were located within NOS1AP. None of these SNPs showed functional significance in silico. In addition to the known interaction with NOS1, seven other potential gene–gene interactions of NOS1AP were identified involving SYN2, SYN1, REG1A, KCNA5, GRIN1, GRIN2B and DLG4 (Table 4).

Discussion
Our findings provide unequivocal replication of the association of NOS1AP variant rs10494366 with QTc in two independent populations, and are also consistent with gender differences in the associations of this common variant in the NOS1AP gene with QTc.

We found NOS1AP gene–QTc association in two populations with different characteristics. GRAPHIC participants are broadly representative of the general population, whilst FINCAVAS participants, referred for exercise tests, represent a group of patients encountered in secondary care. Utilizing the FINCAVAS population, we were able to study the genetic association with QTc after exercise, as well as during rest. The FINCAVAS participants exhibited more variable QTc intervals than the mostly healthy GRAPHIC study population. Although not apparent in all women at rest, the association was revealed in FINCAVAS women by excluding outlying observations. Furthermore, the estimated SNP effect on QTc was larger after exercise in both FINCAVAS men and women, indicating that exercise may unmask the effects of NOS1AP, at least in diseased individuals. We focused on QTc at 3 min recovery in the FINCAVAS study rather than peak exercise QTc, as the latter is more difficult to measure accurately and showed a substantially larger variance (SD 30.9 in men and 27.5 in women using the GE algorithm).

The evidence of association between NOS1AP and QTc, based on our study and published evidence to date, is compelling. Furthermore, our findings suggest that there are differences between men and women in the effect of the NOS1AP gene on QT interval. The findings are likely to reflect real biological differences rather than artifacts for the following reasons. First, there is a priori evidence of substantial differences in QT biology between women and men, including very different risks of drug induced QT prolongation and TdP in women.

Second, we had adequate power to detect a clinically important effect in males or females alone. For example, in the GRAPHIC study, the power to detect, in either sex, an effect as large as that actually observed in females was >99% at $P < 0.01$. Third, we found a consistent pattern of findings between two independent populations. Consistent findings across independent populations, despite differences in genetic background and sampling characteristics, enhances confidence that the gender differences in the associations we report reflect biological processes rather than biases or over-elaborate data exploration. Fourth, our findings are unlikely to be confounded by population substructure, because the allele frequency of rs10494366 has not been shown to vary across UK regions and we showed the NOS1AP–QTc association in the family-based GRAPHIC study.

Table 4: Potential gene–gene interactions involving NOS1AP identified from the SNPs3D database

<table>
<thead>
<tr>
<th>Interacting gene</th>
<th>Gene symbol</th>
<th>Biological process related to the interaction</th>
<th>Clinical relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synapsin 2</td>
<td>SYN2</td>
<td>Neurotransmitter secretion, synaptic transmission</td>
<td>Schizophrenia</td>
</tr>
<tr>
<td>Synapsin 1</td>
<td>SYN1</td>
<td>Neurotransmitter secretion, synaptic transmission</td>
<td>Epilepsy</td>
</tr>
<tr>
<td>Regenerating islet-derived 1 alpha (pancreatic stone protein, pancreatic thread protein)</td>
<td>REG1A</td>
<td>Cell proliferation</td>
<td></td>
</tr>
<tr>
<td>Nitric oxide synthase 1 (neuronal)</td>
<td>NOS1</td>
<td>Nitric oxide synthesis, synaptic transmission</td>
<td>Depression</td>
</tr>
<tr>
<td>Potassium voltage-gated channel 5</td>
<td>KCNA5</td>
<td>Delayed rectifier potassium channel activity, potassium ion transport</td>
<td></td>
</tr>
<tr>
<td>Glutamate receptor, ionotropic, N-methyl D-aspartate 1</td>
<td>GRIN1</td>
<td>Potassium ion transport, synaptic transmission</td>
<td>Learning/memory</td>
</tr>
<tr>
<td>Glutamate receptor, ionotropic, N-methyl D-aspartate 2B</td>
<td>GRIN2B</td>
<td>Potassium ion transport, synaptic transmission</td>
<td>Learning/memory</td>
</tr>
<tr>
<td>Discs, large homolog 4</td>
<td>DLG4</td>
<td>Synaptic transmission</td>
<td>Learning/memory</td>
</tr>
</tbody>
</table>
(analysed in a manner that took full account of the implications of that design). The findings are also unlikely to be confounded by phenotypic or lifestyle characteristics which commonly confound traditional epidemiology studies, since genotypes are randomly allocated at conception.\textsuperscript{18,45} For example, as age has been shown to be associated with QTc,\textsuperscript{46} age might have confounded the NOS1AP–QTc association if there had been any association between NOS1AP and age. However, we found no association between the G allele of rs10494366 and age in our study (\(P=0.78\) and \(P=0.40\) in GRAPHIC and FINCAVAS, respectively).

In contrast to many contemporary genetic epidemiology studies, ours was focused on a single genetic variant. We were able to adopt this efficient and targeted approach because of the previous fine mapping by Arking et al.\textsuperscript{28} We also identified the same genetic association signal and, although we cannot be certain about the precise ‘causal variant’, all SNPs in strong LD (\(r^2>0.5\)) with rs10494366 were within NOS1AP. Until the report of Arking et al.,\textsuperscript{28} the involvement of NOS1AP in QT regulation was unknown. We found gender differences in the NOS1AP–QTc association at rest. Studies reported previously have been inconclusive in this respect. Arking et al. described stronger NOS1AP–QTc association in women than in men in American adults of European ancestry, but not in a Southern German population.\textsuperscript{29} Post et al.\textsuperscript{32} found no significant gene-by-gender interaction in a study of 763 individuals from 377 old order Amish families. Aarnoudse et al.\textsuperscript{31} reported no difference in the effect of the SNP between men and women in a population of 5374 older individuals (mean ages 68.2 and 70.4 years in women and men, respectively). Only the Arking paper presented the gender-specific estimates.\textsuperscript{28} A more recent study has also reported NOS1AP–QTc association in a study population enriched for type 2 diabetes, but without gender-specific associations.\textsuperscript{51}

Given that the assessment of the interaction between NOS1AP and gender on QTc was not a primary focus of these studies, and that these studies individually had limited power to detect such an interaction, this published evidence neither confirms nor refutes our findings. Further studies and, ideally further reporting of previous studies, will be required to investigate whether the gender differences in NOS1AP–QTc associations we noted are evident in other populations with different age distributions and with comorbidities.

The gender differences we found were attenuated after exercise, suggesting that nNOS signalling may be especially important in regulating resting QTc in women but that on exercise nNOS signalling is of central importance in both genders. Gender differences in QT prolongation at puberty\textsuperscript{11} together with greater susceptibility of women than men to drug-induced QT prolongation\textsuperscript{12} suggest that sex hormones influence cardiac repolarization. In the GRAPHIC study, the genotype effect on QTc was similar after adjusting for menopausal status or for combined oral contraceptive use. Although this suggests that neither endogenous estrogens nor exogenous estrogens alter the association between NOS1AP and QTc, we cannot rule out such an effect on the basis of our data alone. Testosterone levels have been implicated in QT behaviour in humans\textsuperscript{52} and in animal models testosterone shortens cardiac repolarization and administration of NG-nitro-L-arginine methyl ester (L-NAME), which suppresses the activities of all isoforms of NOS,\textsuperscript{53} reverses testosterone effects.\textsuperscript{52} nNOS appears to have a role in intracardiac vagal stimulation,\textsuperscript{54} but has other effects on cardiac physiology which are incompletely understood,\textsuperscript{55} and alternative pathways could explain the associations we observed in females and with QTc after exercise. Although an effect via its interaction with NOS1 could explain the effect of NOS1AP on QTc, we identified seven other genes that interact with NOS1AP. These genes encompass a range of different functions, including cardiac ion transport and it is possible that the effects of NOS1AP on QTc are mediated via its interactions with one or more of these proteins.

The G allele of the NOS1AP SNP rs10494366 is common (frequency 34–40%).\textsuperscript{28} Each additional copy of the G allele was associated with a prolongation of QTc by over 4.5 ms in GRAPHIC study women at rest and by around 3 ms in FINCAVAS women and men at 3 min recovery after exercise (differences between homozygote groups of around 9 and 6 ms, respectively). Clinical exposures causing QTc prolongation of a similar magnitude raise serious concerns about the risk of TdP and SCD. In fact, terfenadine was withdrawn from the market after reports of a 5 ms prolongation of QTc in a drug trial in healthy volunteers warrants an expanded ECG safety evaluation in later stages of drug development.\textsuperscript{7} This suggests that our results are of potential clinical relevance. Our gender and exercise related findings suggest that the NOS1AP variant interacts with other influences on QTc interval and raises the possibility that the pathway through which NOS1AP affects QTc may also be modifiable by drugs. If NOS1AP gene variants are found to influence the risk of drug-induced QTc prolongation, this could have profound implications for drug development. In combination with other predictors, such knowledge may allow the survival of some promising drug molecules that would otherwise be abandoned—this may potentially provide an excellent example of pharmacogenomics in action. If a consistent pattern of findings is observed in other populations, further studies should aim to establish whether NOS1AP genotype, alone or in combination with other genetic variants, can help predict individual QT response to certain drugs.
As neither rs10494366 nor its statistically similar SNPs show an obvious potential to affect transcription or translation in silico, future functional in vitro studies will be warranted to dissect the molecular background of the detected association with QTc. As in all observational studies, we cannot completely rule out the possibility that our reported associations may be due to chance or bias. But these explanations seem unlikely given the rigour with which we avoided biases in phenotype and genotype measurement, the limitation of our genotyping to a single SNP, the statistical power of our analysis, the a priori evidence of gender differences in QT biology and, crucially, consistent patterns of findings in two independent populations.

Conclusions
In summary, we have shown a strong association of the NOS1AP SNP, rs10494366, on QTc interval in two populations and gender differences in the association. This common SNP is responsible for QTc prolongation to an extent comparable with some QT-prolonging drugs that cause concern to clinicians, regulatory bodies and the pharmaceutical industry due to the risk of TdP and sudden death. Our findings, and consistent findings in other populations, will provide a basis for functional studies to further elucidate the biochemical pathways underlying cardiac repolarization. Further studies will be required to establish whether the NOS1AP genotype could help predict response to certain drugs and whether the predictors may differ between women and men. If this proves to be the case, then the genetic association we have shown could have widespread clinical relevance, as knowledge of NOS1AP genotype could not only help predict individuals at risk from QT prolonging drugs but it may also provide a pharmacogenomic approach to saving some potentially useful drug molecules from extinction.

Supplementary material
Supplementary data are available at IJE online.

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Conflict of interest: None declared.

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