The common non-synonymous variant G38S of the KCNE1-(minK)-gene is not associated to QT interval in Central European Caucasians: results from the KORA study

Mahmut Akyol, Shapour Jalilzadeh, Moritz F. Sinner, Siegfried Perz, Britt M. Beckmann, Christian Gieger, Thomas Illig, H.-Erich Wichmann, Thomas Meitinger, Stefan Kääb, and Arne Pfeufer

1 Institute of Human Genetics (IHG), GSF National Research Centre of Environment and Health, Ingolstädter Landstr. 1, D-85764 Neuherberg, Germany; 2 Institute of Human Genetics (IHG), Technical University of Munich, Klinikum rechts der Isar, Trogerstr. 32, D-81675 Munich, Germany; 3 Department of Medicine I, Ludwigs-Maximilians University Munich, Klinikum Grosshadern, Marchioninistr 15, D-81377 Munich, Germany; 4 Institute of Medical Informatics (IMEI), GSF National Research Centre of Environment and Health, Ingolstädter Landstr. 1, D-85764 Neuherberg, Germany; and 5 Institute of Epidemiology (EPI), GSF National Research Centre of Environment and Health, Ingolstädter Landstr. 1, D-85764 Neuherberg, Germany

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Aims The QT interval in the general population is a complex trait with 30–50% heritability. QT prolongation is associated with an increased risk of sudden death. A recent family-based study found an association between QT interval and the common non-synonymous Glycin 38 Serine variant (G38S, rs1805127) of the KCNE1 gene coding for the minK-potassium channel subunit. We intended to replicate this finding in a large population sample of central European Caucasian ancestry as part of our ongoing search for genetic variants predisposing to arrhythmias.

Methods and results We studied 3966 unrelated individuals from the KORA S4 population-based study without atrial fibrillation, pacemaker implant, or pregnancy. Individuals were genotyped by MALDI-TOF mass spectrometry. We did not detect any significant association between the genotypes of the G38S variant and the QT interval in the entire population or in any gender.

Conclusion Unlike the common Lysine 897 Threonine variant of KCNH2 (K897T, rs1805123) the G38S variant of KCNE1 does not appear to have a strong modifying effect on QT interval. However, we cannot rule out an effect of G38S on QT in other ethnic groups, under exercise or medications or on the risk for arrhythmias and sudden death.

KEYWORDS
Cardiac repolarization; Genetic association study; Single nucleotide polymorphism (SNP); Genetic epidemiology

Introduction

The analysis of genetic variants modifying cardiac electrophysiological traits has met increased attention in recent years. Beyond cellular electrophysiology of ion channels and other candidate proteins and molecular cloning of disease genes in families affected by mendelian arrhythmogenic diseases, it is believed to provide a third and independent route to the identification of genes and gene products involved in cardiac electrophysiology. One of the prerequisites of this approach is the use of population samples that are large, free of underlying population stratification, have been carefully phenotyped, and have biosamples available.

In addition, these studies are motivated to enable tests that can identify individuals predisposed to arrhythmias useful in primary and secondary prevention.

One trait finding particular attention of researchers is the QT interval. This is mostly because of its relatively high heritability, accurate measurability, and its associated predisposition to sudden cardiac death. Several previous association studies have analysed whether non-synonymous SNPs in cardiac ion channel candidate genes modify the QT interval. One of these SNPs is the Lysine 897 Threonine variant of the KCNH2 gene (K897T, rs1805123). The rarer T897 allele was reproducibly found to be associated with a shortened QT interval following an additive model of allelic effects. A recent study of 441 men and women sampled within families reported association of another common
non-synonymous SNP in another cardiac ion channel gene, the Glycine 38 Serine variant of the KCNE1 gene (G38S, rs1805127). The KCNE1 gene encodes the MinK protein, which forms the beta-subunit of the cardiac IKs channel. The allele frequency of the minor S38 allele has been reported to be between 16.4 and 48.5% in different ethnic groups. In the study, however, G38S had an exceptionally low minor allele frequency of 3.3%. It was found to be associated to QT interval only in men, in whom it accounted for 2.2% of QT variance in a multivariate linear regression model (P < 10e – 4). Male probands with heterozygous G38S genotype had on average 21.7 ms longer QT intervals compared with GG38 homozygotes, equaling to a difference of almost one standard deviation (SD) (σ = 23.7 ms). In our study, we intended to replicate the previous result in 3966 probands from the KORA S4 survey, a large population-based sample of Central European Caucasian origin.

Methods

Individuals

Between 1999 and 2001, we conducted an epidemiological survey of the general population living in or near the city of Augsburg, Southern Germany (KORA S4). This was the fourth in a series of population-based surveys originating from our participation in the WHO MONICA project. The study population consisted of unrelated residents of German nationality born between 1 July 1925 and 30 June 1975 identified through the registration office. A sample of 6640 subjects was drawn with 10 strata of equal size according to gender and age. Following a pilot study of 100 individuals, 4261 individuals (66.8%) agreed to participate in the survey, who were ethnic Germans with very few exceptions (≥99.5%). From 4115 probands, a positive consent, a DNA sample as well as an electrocardiogram (ECG) recording were available. After the application of exclusion criteria, atrial fibrillation, pacemaker or defibrillator implant, or ongoing pregnancy, 3966 individuals were used for association analysis. A detailed description of probands and phenotypic criteria, including QT interval as well as the corrected QT interval according to Bazett’s formula, has been suggested from Framingham Heart Study data with the correction parameters derived from a multivariate linear regression model including the covariates heart rate (RR interval), sex, and age within KORA S4 as previously described. Correction factors were determined separately for each sex. The QT interval corrected for age-, sex-, and rate was determined for men:

\[ QT_{\text{RAS}} = QT - [0.152 \times (\text{RR} - 1000 \text{ ms})] - (0.318 \text{ ms/year} \times (\text{age} - 60 \text{ years})) \]

and for women:

\[ QT_{\text{RAS}} = QT - [0.154 \times (\text{RR} - 1000 \text{ ms})] - (0.207 \text{ ms/year} \times (\text{age} - 60 \text{ years})) - 4.58 \text{ ms} \]

where RR denotes RR interval in milliseconds.

DNA extraction and genotyping

DNA was extracted from EDTA anticoagulated blood using a salting out procedure. The G38S variant of the KCNE1 gene was determined using PCR, primer extension, and MALDI-TOF mass spectrometry in a 384-well format (Sequenom, San Diego, USA) as previously described. Hardy-Weinberg equilibrium (HWE) P-values were calculated using the STATA statistical software package.

Genotype phenotype association analysis

SNPs were tested for association to QT, QTc-Bazett, and QTc-RAS as the dependent variables by applying two-tailed one-degree-of-freedom linear regression test (1df) and two-tailed two-degree-of-freedom ANOVA analysis (2df). The 1df test has a relatively higher power to detect weak effects, whereas the 2df test accounts for dominance and recessivity by allowing the trait increase of each genotypic change to take an individual value.
To determine gender specific differences of SNP-phenotype associations, we performed sex-specific regression analysis in the total sample. Sample sizes of males (n = 1959) and females (n = 2007) were similar and therefore comparable for effect size. Although this study was intended to replicate a previous significant finding, in light of the grossly different allele frequency we did not use one-tailed but two-tailed statistics. All reported significance levels have not been adjusted for multiple testing.

We designed our study using n = 3966 individuals to be able to detect the effect of the G38 heterozygous genotype described to prolong QT interval by about 0.2 standard deviations at the given allele frequencies (Table 2).

Table 2  Power calculation

<table>
<thead>
<tr>
<th></th>
<th>Effect size estimator (d)</th>
<th>Significance level (α)</th>
<th>GG38</th>
<th>GS38</th>
<th>SS38</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entire sample (n)</td>
<td></td>
<td></td>
<td>1591</td>
<td>1770</td>
<td>555</td>
</tr>
<tr>
<td>Power to discriminate</td>
<td>0.2 SD</td>
<td>0.001</td>
<td>0.994</td>
<td>0.936</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>&gt;0.999</td>
<td>0.998</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0 SD</td>
<td>0.001</td>
<td>&gt;0.999</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>&gt;0.999</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men only (n)</td>
<td></td>
<td></td>
<td>772</td>
<td>872</td>
<td>293</td>
</tr>
<tr>
<td>Power to discriminate</td>
<td>0.2 SD</td>
<td>0.001</td>
<td>0.776</td>
<td>0.545</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>0.982</td>
<td>0.926</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0 SD</td>
<td>0.001</td>
<td>&gt;0.999</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>&gt;0.999</td>
<td></td>
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</tr>
</tbody>
</table>

Power calculation to detect an effect of the G38S variant in the study population. Power was calculated separately for each allelic step (GG to GS and GS to SS) in two-tailed T-tests (1df) with the detected genotype frequencies from the study population. An effect size (d) in the published magnitude of 21.7 ms QTc equals about 1 SD, an effect size of 0.2 SD corresponds to a change of 3.4 ms in QTc.

Table 3  Association results

<table>
<thead>
<tr>
<th></th>
<th>GG38</th>
<th>GS38</th>
<th>SS38</th>
<th>P (1df)</th>
<th>P (2df)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (n)</td>
<td>1591</td>
<td>1770</td>
<td>555</td>
<td></td>
<td></td>
</tr>
<tr>
<td>QT</td>
<td>408.1±28.0</td>
<td>407.2±28.2</td>
<td>409.7±27.5</td>
<td>0.5797</td>
<td>0.1655</td>
</tr>
<tr>
<td>QTc-Bazett</td>
<td>423.1±22.0</td>
<td>422.6±21.2</td>
<td>423.5±21.4</td>
<td>0.9263</td>
<td>0.6493</td>
</tr>
<tr>
<td>QTc-RAS</td>
<td>417.7±17.5</td>
<td>417.2±17.0</td>
<td>418.7±16.8</td>
<td>0.5498</td>
<td>0.1650</td>
</tr>
<tr>
<td>Men (n)</td>
<td>772</td>
<td>872</td>
<td>293</td>
<td></td>
<td></td>
</tr>
<tr>
<td>QT</td>
<td>407.7±30.3</td>
<td>406.8±29.4</td>
<td>407.7±28.3</td>
<td>0.8393</td>
<td>0.8187</td>
</tr>
<tr>
<td>QTc-Bazett</td>
<td>419.9±22.8</td>
<td>419.2±21.9</td>
<td>421.7±23.0</td>
<td>0.5006</td>
<td>0.2460</td>
</tr>
<tr>
<td>QTc-RAS</td>
<td>418.1±18.6</td>
<td>417.3±17.7</td>
<td>419.2±17.5</td>
<td>0.6904</td>
<td>0.2995</td>
</tr>
<tr>
<td>Women (n)</td>
<td>819</td>
<td>898</td>
<td>262</td>
<td></td>
<td></td>
</tr>
<tr>
<td>QT</td>
<td>408.5±25.6</td>
<td>407.5±27.0</td>
<td>412.0±26.4</td>
<td>0.2636</td>
<td>0.0548</td>
</tr>
<tr>
<td>QTc-Bazett</td>
<td>426.0±20.7</td>
<td>426.0±20.0</td>
<td>425.6±19.3</td>
<td>0.7747</td>
<td>0.9444</td>
</tr>
<tr>
<td>QTc-RAS</td>
<td>417.3±16.4</td>
<td>417.0±16.4</td>
<td>418.2±15.9</td>
<td>0.6842</td>
<td>0.5589</td>
</tr>
</tbody>
</table>

Association of uncorrected and corrected QT interval measurements to the G38S genotypes in the entire sample and stratified by gender. Significance levels are given from two-tailed tests. A total of 3916 of 3966 individuals were successfully genotyped corresponding to a call rate of 98.7%. The genotype frequencies were in HWE (P = 0.080).

Discussion

We could not confirm the previously published strong effect of the non-synonymous G38S variant of the KCNE1 gene in men or any other effect of this variant on QT interval.
Our non-replication is in line with a previous investigation that also could not find any evidence of association between G385 and QT interval length, but was less powered to do so. Power simulations showed that we would have been adequately powered to detect an effect if it existed in the range of 1 SD as in the initial publication as well as down to the range of 0.2 SD, which equals the magnitude of the effect of K897T in KCNH2.

It appears unlikely that the non-replication can be explained by a lack of precision of the QT interval measurement or another unsuitability of the samples as we have successfully replicated the association of the KCNH2 K897T SNP to QT interval in the same sample in a previous publication. In addition, a novel QT-L for QT interval in the promoter of the NOS1AP (CAPON) gene could be identified in this sample and reproducibly confirmed in others. The population-representative recruitment of individuals from one geographic area with limited recent immigration increases the homogeneity of the sample and thus increases the power to detect true positives and likewise reduces the probability of false positives due to population stratification.

Non-synonymous variants are generally considered to be likely causal variants themselves and not just markers associated by linkage disequilibrium to causal variants in their vicinity. Therefore, also in individuals from other ethnic groups we would expect no association between this variant and the QT interval. However, we cannot rule out the possibility that G385 may be a causal variant only on certain genetic backgrounds or that it may be in linkage disequilibrium to neighbouring causal variants only in some ethnic groups. Independent replication studies of similar size in individuals of such groups will be the only way to resolve the issue whether this negative association result is dependent on ethnicity or is universally valid. Similarly, the non-replication does not rule out the possibility that the two alleles of G385 may still exert subtle differences on the repolarization process. It makes it likely that such an effect does not exist on repolarization at rest, but an effect limited to exercise, intake of medications, or other conditions may well exist. We likewise cannot rule out a modification of the risk of arrhythmias or sudden cardiac death by G385 via more complex repolarization- or non-repolarization-driven effects.

Effects of other variants within the KCNE1 gene may also be present. Two other QT-modifying variants have been previously described, the Intron 2 variant IVS2–128 G>A and the promoter variant rs727957. Both of them await independent replication.

This finding necessitates the use of large sample sizes for future studies of QT interval and sudden cardiac death in order to obtain statistically significant and reproducible associations as—despite the relatively high heritability of the QT interval—the contribution of individual variants is recognized to be rather low.

The QT interval is a valuable endophenotype to investigate the predisposition to complex arrhythmias but can nevertheless replace disease phenotypes such as VT/VF or sudden cardiac death in future investigations. We are now starting to anticipate the substantial size of study samples carrying these phenotypes that will have to be recruited in future to obtain reliable and reproducible genetic associations for complex arrhythmias.

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Conflict of interest: none declared.

References

Clinical vignette

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Failed Amplatzer Septal Occluder device implantation due to an embryonic septal remnant

Gianluca Rigatelli1*, Gabriele Braggion1, Paolo Cardaioli1, and Giuseppe Faggian2

1Interventional Cardiology Unit, Division of Cardiology, Rovigo General Hospital, Italy and 2Department of Cardiac Surgery, Verona University Medical School, Verona, Italy

* Corresponding author: EndoCardioVascular Therapy Research, Via WA Mozart 9, 37040 Legnago, Verona, Italy. Tel: +39 03471912016; fax: +39 044220164.
E-mail address: jackyheart@hotmail.com

Embryonic remnants of incomplete septation may complicate occlusion device implantation in secundum atrial septal defects (sASD) even if stiff devices such as the Amplatzer Occluder are used.

A 35-year-old woman was referred to our center for evaluation of a sASD. Transesophageal echocardiography revealed a haemodynamically relevant sASD with a mean diameter of 22 mm, a virtually absent anterosuperior rim (aortic rim) and remaining floppy rims, and an apparently soft membrane at the inferior aspect of the interatrial septum which was concluded to be a remnant of incomplete septation (EM) (Panel A). Because the patient initially refused the surgical option, a percutaneous closure was attempted.

By intracardiac echocardiography (UltraICE, Boston Scientific Corp.), the diameters of the sASD at the aortic valve and four-chamber planes were 29 and 32 mm, respectively. A soft membrane (EM) was visible at the aortic valve plane but was felt to be too soft to influence device opening (Panel B; AS, anterosuperior rim; EM, embryonic membrane; PI, posteroinferior rim; RA, right atrium). To test the stiffness of the rims, a sizing balloon was passed across the ASD and inflated till an indentation was evident thus obtaining a sizing balloon ASD with a diameter of 28 mm. After calculating a mean diameter from the sizing balloon and the ICE measurement, the operators opted for a 32 mm Amplatzer ASD Occluder. The device was successfully implanted (Panel C; RA, right atrium; ASO, Amplatzer Septal Occluder). It was postulated that once the embryonal septal remnant had been caught by the device, it became stiffer and anchored to the true septum primum rim. It was therefore decided to remove the device surgically. Direct surgical inspection confirmed the suspicion (Panel E; EM, embryonic membrane). The partially endothelialized device (Panel F) was removed and the defect repaired with a patch. Embryonic remnants of interatrial septum formation, such as the one presented above, are quite rare but should be carefully evaluated for potential technical constraints to percutaneous closure. Once this structure has been caught up by the two retention disks of the device, it may become quite stiff and can deviate the disks from the true rim.

See online supplementary material for a colour version of the figure available at European Heart Journal online.