A common polymorphism in KCNH2 (HERG) hastens cardiac repolarization

Connie R. Bezzina a,b,* , Arie O. Verkerk a, Andreas Busjahn c, Andreas Jeron d, Jeanette Erdmann d, Tamara T. Koopmann a, Zahurul A. Bhuiyan a,b, Ronald Wilders e, Marcel M.A.M. Mannens b, Hanno L. Tan a, Friedrich C. Luft c, Heribert Schunkert d, Arthur A.M. Wilde a

aExperimental and Molecular Cardiology Group, Room M0-052, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands
bDepartment of Clinical Genetics, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
cFranz Volhard Clinic and Max Delbrück Center for Molecular Medicine, Berlin, Germany
dInternal Medicine, University of Regensburg, Regensburg, Germany
eDepartment of Physiology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

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Abstract

Objective: Genetic variants of cardiac ion channels may influence cardiac repolarization. Thereby such variants may modulate the penetrance of primary electrical disorders, contribute to differences in susceptibility to drug-induced QT-prolongation between individuals, or contribute to rhythm disturbances in the context of structural heart disease. Since the current encoded by KCNH2 (HERG; I Ks ) is a primary determinant of repolarization, we conducted association studies between the respective alleles of the common amino acid-changing polymorphism at codon 897 (2690A>C; K897T) within HERG and rate-corrected QT interval (QTc).

Methods and Results: Association analysis in Caucasian subjects (n=1030) revealed a significant association of this polymorphism with QTc (P=0.0025) with CC homozygotes having a significantly shorter QTc (388.5±2.9 ms) compared to AA homozygotes (398.5±0.9) and heterozygotes (AC, 397.2±1.2). The latter two genotypes were associated with comparable mean QTc's, suggesting that the 2690C-allele is recessive. After stratification by sex, the polymorphism was more predictive of QTc in females (P=0.0021), a finding that was replicated in a second population sample (n=352) from the same ethnic background (P=0.044). To assess whether this polymorphism could represent a 'functional' polymorphism, we compared the biophysical properties of K897- and T897-HERG channels by whole-cell voltage clamp. Compared to the K897 channel, the T897 channel displayed a shift of −7 mV in voltage dependence of activation and increased rates of current activation and deactivation.

Conclusion: As confirmed in modeling studies, these changes are expected to shorten action potential duration by an increase in I Ks . This recapitulates the shorter QTc in females homozygous for the 2690C-allele.

Keywords: K-channel; Ion channels; ECG; Epidemiology; Arrhythmias

1. Introduction

The electrocardiographic (ECG) QT interval is a measure of cardiac ventricular repolarization. A prolonged baseline QT interval may predispose to sudden death in patients with coronary artery disease [1] or myocardial infarction [2,3]. Large population studies have shown that a prolonged QT interval may also predict cardiovascular death in normal, apparently healthy individuals [4,5]. Moreover, in dofetilide-treated patients with moderate to...
severe heart failure and reduced left ventricular systolic function, a short baseline QT interval was associated with reduced mortality [6].

A prolonged QT interval may reflect inter-individual variability but may also occur as an adverse reaction to some commonly used antiarrhythmic agents as well as various medications including antibiotics, antihistamines and antipsychotics, which block \( I_{Kr} \) (described below) [7]. Some of these have also been implicated in the development of life-threatening torsade de pointes ventricular tachycardia, particularly in females [8] and in individuals with a prolonged QT interval at baseline [9].

The QT interval reflects ventricular repolarization during which a net outward current restores the membrane potential of the depolarized myocyte back to the resting membrane potential. The onset of this repolarization phase is primarily mediated by the delayed rectifier \( K^+ \) current, which is comprised of the rapidly and slowly activating components \( I_{Kr} \) and \( I_{Ks} \), respectively. Mutations in genes encoding for ion channel subunits of these currents (\( KCNH2 \), \( KCNE2 \) for \( I_{Kr} \); \( KCNQ1 \), \( KCNE1 \) for \( I_{Ks} \)) are associated with specific subtypes (2, 6, 1, and 5, respectively) of the long QT syndrome (LQTS) [10], wherein a decrease in outward \( K^+ \) current [11] results in prolongation of the action potential (AP). This prolongation, which is translated into a prolonged QT interval on the surface ECG, renders the heart vulnerable to torsade de pointes and sudden cardiac death.

ECG parameters, including QT interval, are biometrical traits known to be influenced by genetic variance [12] and loci for cardiac ion channel genes have been demonstrated to constitute quantitative trait loci for these parameters in normal, healthy individuals [13]. These observations imply that, aside from the monogenic disorders of the LQTS, cardiac ion channel genes contain allelic variants that have a functional bearing on ECG parameters including QTc in the general population. Thereby, such variants may underlie (at least in part) inter-individual variation in ECG parameters, modulate the penetrance or expressivity of primary electrical disorders such as LQTS [14] and Brugada syndrome [15,16], modify the propensity to drug-induced LQTS [7], or contribute to rhythm disturbances in the context of structural heart disease, a hypothesis that has gathered considerable support in recent years [17,18].

Since \( I_{Kr} \) is a primary determinant of repolarization, we assessed the impact of the common 2690A>C nucleotide change corresponding to the K(lysine)897T(threonine) amino acid polymorphism within the \( KCNH2 \) gene (also called HERG for human ether-a-go-go-related gene) that encodes the pore-forming subunit of the \( I_{Kr} \) channel, on the ECG by association studies between the respective alleles of this polymorphism and QTc in two independent samples of the general population. The occurrence of such an amino acid-changing polymorphism raises the possibility that the two channel variants (K897 and T897) could differ in their electrophysiological properties and thereby impact on ECG parameters in the general population. Thus we also studied the biophysical consequences of this polymorphism by whole-cell voltage clamp and computer simulations.

2. Methods

2.1. Study population

2.1.1. Sample I

We identified 1030 healthy Caucasians through a population-based survey, namely the Augsburg MONICA (Monitoring of Trends and Determinants in Cardiovascular Disease, study 3) study, with normal ECG without signs of acute myocardial infarction or unstable angina pectoris at the time of examination and recording of the ECG [19]. Each participant underwent a physical examination and a medical history was obtained before blood was taken for genetic studies. The investigation conforms with the principles outlined in the Declaration of Helsinki and written informed consent was obtained from all participants.

A standard 12-lead resting ECG was recorded and stored in a digitized fashion using a Hewlett Packard Pagewriter XLi (Hewlett Packard, Palo Alto, CA, USA). QT interval in individual leads was computed by the appropriate software (Interpretive Cardiograph A.0.1.00; Hewlett Packard) and double-checked by an investigator blinded to the clinical data. Mean corrected QT interval (QTc) was calculated according to Bazett’s formula. Individuals with an ECG showing a bundle branch block pattern with a QRS duration greater than 120 ms were excluded from further analysis.

2.1.2. Sample II

We recruited 176 pairs of twins (monozygotic, 116; dizygotic, 60; Table 1) by advertisement to participate in studies involving blood pressure regulation and cardiovascular phenotypes. The subjects were all German Caucasians from various parts of Germany. The protocol was approved by the University’s (Berlin) committee on the protection of human subjects and written informed consent was obtained from all participants. Blood was obtained for the determination of zygosity and other molecular genetic studies. Each participant underwent a medical history and physical examination. None had a family history of chronic medical illness. Subjects underwent echocardiography and planar ECG. A standard 12-lead electrocardiogram was performed (CARDIOVITS CS-100, Schiller, Baar, Switzerland). The QT- and RR-intervals were measured in lead II. QTc was determined according to Bazett’s formula. The ECG parameters were scored by a computer and stored for subsequent retrieval.
2.2. Genotype analysis

Genotyping for the KCNH2 K897T polymorphism was done by restriction analysis (sample I: \( n=730 \); sample II: all) or by DNA sequencing (sample I: \( n=300 \)). For restriction analysis, a fragment encompassing the polymorphism was amplified using primers: 5'GGGAGCTTGGGGCCTGACC3' (forward; P1) and 5'TCCCTCTCCCCGCTACAC3' (reverse; P2). P2 differed from the KCNH2 sequence such that in combination with the 2690A>C change a restriction site for BsoAI was created (Fig. 1B). The nucleotide that differed is underlined.

For sequencing, a fragment containing the polymorphism was amplified using primers: 5'AAGGGCCCTGATACTGATTTT3' (forward; P3) and 5'TTCCAGCTCCAGCCCTCAC3' (reverse, P4). The reverse primer was used for sequencing, which was carried out using the ABI BigDye Terminator Sequencing kit (Applied Biosystems; Fig. 1A).

Mass spectrometric analysis of primer extension products, a technique amenable to high-throughput analysis, was also set up (data not shown). Primer extension was carried out on shrimp alkaline phosphatase-treated PCR products of primers P3 and P4, using the following primer: 5'TCCGCAGGCGACGGAC3' in a 10-\( \mu l \) reaction consisting of dCTP, ddATP, ddGTP (100 \( \mu \)mol/l each), 20 pmol extension primer and 1 U Thermo Sequenase DNA polymerase (Amersham) in Thermo Sequenase buffer. Thermal cycling conditions were 94 °C for 3 min followed by 30 cycles of 94 °C for 10 s, 57 °C for 1 min, 72 °C for 30 s. Before mass spectrometric analysis on a MALDI-TOF (Bruker Biflex) system, products of primer extension were purified using the Genopure oligo DNA purification system (Bruker).

2.3. Site-directed mutagenesis and cell culture

The HERG/GFPires construct containing K897-HERG [20] cloned in the GFPires plasmid [21] for bicistronic expression of the HERG channel protein and GFP reporter (i.e. translation of the protein of interest and marker from the same mRNA due to the presence of an internal ribosomal entry site (IRES) between the two cloned genes), was kindly provided by David C. Johns (Johns Hopkins University, Baltimore, MD, USA). T897-HERG
was created by PCR overlap-extension mutagenesis. The final PCR product was digested with SacI and XhoI and cloned into HERG/GFPires, creating the construct T897-HERG/GFPires. The mutated insert and ligation regions were sequenced fully to ensure that the clone was free of polymerase errors.

Human embryonic kidney (HEK-293) cells were transiently transfected with the HERG/GFPires or T897-HERG/GFPires construct using lipofectamine and cultured at 37 °C. Cells exhibiting green fluorescence were selected 36–48 h after transfection for electrophysiological experiments.

### 2.4. Electrophysiological experiments

Cells were superfused with solution (36±1 °C) containing (mmol/l): NaCl 140, KCl 5.4, CaCl₂ 1.8, MgCl₂ 1.0, glucose 5.5, HEPES 5.0, and pH 7.4 (NaOH). Membrane currents were recorded in the ruptured-patch whole-cell configuration of the patch-clamp technique. Patch pipettes (3–5 MΩ) were pulled from borosilicate glass, heat-polished, and filled with solution containing (mmol/l): K-gluconate 125, KCl 20, MgCl₂ 1.0, EGTA 5, MgATP 5, HEPES 10, and pH 7.2 (KOH). Voltage control, data acquisition, and analysis were accomplished using custom software. Potentials were corrected for the liquid junction potential. Membrane currents and potentials were low-pass filtered on-line (cut-off frequency 2 kHz) and digitized at 5–10 kHz. Cell size for cells transfected with HERG/GFPires (8.3±0.7 pF) did not differ significantly from those with T897-HERG/GFPires (8.9±1.4 pF).

The activation, deactivation, and inactivation kinetics of the HERG current were determined by the voltage clamp protocols as diagrammed in Fig. 3 and described previously [22–24]. For all protocols, holding potential was −80 mV and the pacing intervals 15 s. Tail current amplitude, normalized to maximum tail current, was used to construct steady-state (in)activation curves (Fig. 3C and E). The (in)activation curves were fitted using the Boltzmann equation $I/I_{\text{max}} = A/[1.0 + \exp ((V - V_i)/k)]$ to determine the membrane potential for half-maximal (in)activation $V_i$ and the slope factor $k$. The time course of (in)activation (Fig. 3C, inset, and Fig. 3F) was fitted by the monoequivalent equation $I/I_{\text{max}} = A_t \times 1/[1.0 - \exp (-t/\tau_t)] + A_s \times [1.0 - \exp (-t/\tau_s)]$, where $A_t$ and $A_s$ are fractions of fast and slow inactivation components, and $\tau_t$ and $\tau_s$ are the time constants of fast and slow inactivating components, respectively [22,24,25]. The time course of recovery from inactivation (Fig. 3F) was measured as the fast component of a biexponential equation [22,25]. Data are expressed as mean±S.E.M. and compared using a Student’s $t$-test with a significance level $P<0.05$.

### 2.5. Computer simulations

Functional differences between the K897- and T897-HERG channels were tested by computer simulations using the Priebé and Beuckelmann [26] human ventricular cell model. The experimentally observed shift in the steady-state activation curve of T897 versus K897 was implemented by a −7 mV shift in the $I_{kr}$ rate constants $\alpha_{kr}$ and $\beta_{kr}$. The approximately twofold increase in the rate of $I_{kr}$ activation and deactivation of T897 versus K897 was implemented by doubling $\alpha_{kr}$ and $\beta_{kr}$, respectively.

### 2.6. Statistical analysis

The QTc values of monozygotic twins (sample II) were converted into singleton values by calculating the average. Genotype-dependent differences in QTc were determined with univariate analysis of variance (sample I) or mixed model analysis (sample II). In the latter model the individuals were treated as repeated measures within the random factor family. The within-family correlation was 0.45. In both analyses, genotype, gender and genotype–gender and age–gender interactions were used as independent between group factors and age as covariate. Furthermore, the analyses were repeated for both genders separately where appropriate. Difference in allele frequency was assessed using the normal approximation of the binomial distribution. All numbers are presented as mean±S.E.M. Differences were considered significant for $P<0.05$. All statistics were calculated using SPSS software (version 11, SPSS).

### 3. Results

#### 3.1. Association studies

A total of 1382 Caucasian (German) subjects, recruited as two separate samples at two different centers (Regensburg and Berlin) were evaluated in this study (Table 1). Sample I was initially evaluated. Findings were then replicated in a second smaller sample (II). All subjects were genotyped for the K897T polymorphism, with the exception of the monozygotic twins, in whom only one of the pair was genotyped. As expected of two samples of the same ethnicity, minor allele (2690C) frequency was similar between the two; 23.5% and 24.6% for samples I and II, respectively ($P=0.725$). The allelic distribution was in Hardy–Weinberg equilibrium. We evaluated the influence of this polymorphism on QTc, in multifactor analysis that included age, sex, and genotype–sex and age–sex interactions, as factors and covariates.

Mean QTc values per genotype for the samples as a whole and after separation by gender are given in Table 2. In sample I, the K897T polymorphism was significantly associated ($P=0.0025$) with QTc (Table 3). CC homo-
zygotes had the shortest QTc (388.5 ms) compared to AA homozygotes (398.5 ms) and heterozygotes (AC, 397.2 ms). Sex and age were both strongly associated ($P < 0.001$) with QTc (Table 3). After stratification of the population by sex, the K897T genotype was more predictive of QTc in females than in males ($P = 0.002$, females; $P = 0.34$, males; Table 3). Female CC homozygotes had the shortest mean QTc (388.5 ms) compared to AA homozygotes (403.0 ms) and AC heterozygotes (402.1 ms) (Table 2, Fig. 2). In males, although the association with genotype was not significant, QTc tended to be shorter in CC homozygotes compared to AA homozygotes (Table 2, Fig. 2).

Table 3
Influence of covariates, including K897T genotype, gender and age, in a multi-variate/factor model

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Whole population</th>
<th>Male $P$</th>
<th>Female $P$</th>
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<tbody>
<tr>
<td><strong>Sample I</strong></td>
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<tr>
<td>$KCNH2$ K897T genotype</td>
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<td>0.34</td>
<td>0.002</td>
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<td>0.001</td>
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<tr>
<td>Sex</td>
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<td></td>
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<tr>
<td>Sex×K897T genotype</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Sex×age</td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sample II</strong></td>
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</tr>
<tr>
<td>$KCNH2$ K897T genotype</td>
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<td>0.088</td>
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<td>0.001</td>
<td>0.220</td>
</tr>
<tr>
<td>Sex</td>
<td>&lt;0.001</td>
<td></td>
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</tr>
<tr>
<td>Sex×K897T genotype</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Sex×age</td>
<td>0.05</td>
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</tr>
</tbody>
</table>

We next sought to replicate the association of this polymorphism with QTc in females in a second independent sample from the same population recruited at a different center (sample II). Again, the association between the K897T genotype and QTc was found to be statistically significant in females ($P = 0.044$, Table 3).

Finally, since the mean QTc values of the AA and CC homozygotes differed much more than those of the AA homozygotes and AC heterozygotes, a recessive model was tested (sample I: CC $n = 58$, versus AA/AC $n = 972$). CC homozygotes had a significantly shorter QTc (388.5±2.9 ms) compared to those with AA/AC genotypes (398.0±0.7 ms) ($P < 0.001$). Similarly, female CC homozygotes had a significantly shorter QTc (389.0±3.9 ms; $n = 27$) compared to females with AA/AC genotypes (402.6±0.9 ms; $n = 494$) ($P < 0.01$).
Fig. 3. Characteristics of K897 and T897 HERG currents. (A) Representative examples of K897 (left) and T897 (right) currents elicited by a two-step voltage clamp protocol (inset). P1 activated steady-state HERG current. The current magnitude progressively increased and then decreased with voltage according to voltage-dependent inactivation. P2 elicited HERG tail currents; their peak is due to fast recovery from inactivation secondary to repolarization. The subsequent current decline is due to deactivation. (B) The average amplitudes of steady-state (circles) and peak tail (squares) currents did not differ significantly between K897 and T897. (C) The voltage for half-maximal activation shifted from $-23.7 \pm 1.8$ in K897 to $-30.8 \pm 1.6$ in T897 ($P = 0.005$); the slope factor $k$ (mV) was 7.3 $\pm$ 0.6 and 7.2 $\pm$ 0.5, respectively ($P = 0.86$). The activation time course (fitted to the rising phase of the current during P1, panel A) for T897 was faster than for K897 (inset, asterisks, $P$, 0.05). (D) Deactivation characteristics determined using a two-step protocol (left inset). P1 activated steady-state HERG current. P2 served to elicit HERG tail currents. The deactivation current–voltage (I–V) relationship was determined from the peak of the deactivating tail current during P2. This I–V relationship did not differ significantly between K897 and T897. The deactivation time course (analyzed by fitting the decaying phase of the current during P2 to a biexponential function) for T897 was faster than for K897, as shown by the decrease in both time constants (right inset, asterisks, $P < 0.05$). (E) Voltage dependence of inactivation of K897 and T897 determined by a three-step protocol (inset). During P1, HERG current activation occurred and was followed by inactivation. Repolarization during P2 allowed recovery from inactivation. The voltage dependence of inactivation was determined from the peak of the tail current measured during P3. This I–V relationship did not differ significantly between K897 and T897. Voltage for half-maximal inactivation: $-39.9 \pm 3.7$ (K897), $-40.5 \pm 3.5$ (T897) ($P = 0.90$); slope factor $k$ (mV): 20.0 $\pm$ 1.7 (K897), and 19 $\pm$ 1.2 (T897) ($P = 0.62$). (F) Time constants of HERG current inactivation (circles) and recovery from inactivation (squares). The time constant of inactivation was determined using a three-pulse protocol (right inset). The first two steps served to (in)activate and recover from inactivation, respectively (see E). The time constant of inactivation, derived from fitting the declining phase of the current during P3, did not differ significantly between K897 and T897. The time constant of recovery from inactivation was determined using a two-pulse protocol (left inset). P1 served to activate and inactivate HERG channels. Repolarization during P2 elicited a tail current due to fast recovery from inactivation. This recovery process was measured as the fast time constant of a biexponential fit to the tail current (the slower time constant of this fit reflects the concurrent deactivation process). The fast time constant did not differ significantly between K897 and T897.
3.2. Electrophysiological data

Fig. 3 shows typical K897- and T897-HERG currents expressed in HEK-293 cells (panel A), and their activation (panels B–C), deactivation (panel D), and inactivation (panels E and F) characteristics. The average amplitudes of steady-state and peak tail currents did not differ significantly between K897 and T897 (Fig. 3B). The voltage dependence of activation of T897 displayed a 7 mV shift into the negative direction ($P=0.005$) compared to K897 (Fig. 3C). Moreover, the activation time course for T897 was faster than for K897 ($P<0.05$), as shown by the approximately twofold decrease in the activation time constant (Fig. 3C, inset). The deactivation current–voltage (I–V) relationship did not differ significantly between K897 and T897 (Fig. 3D). However, the deactivation time course for T897 was ~1.5 times faster ($P<0.05$) than for K897, as shown by the decrease in both time constants (Fig. 3D, right inset). The voltage dependence of inactivation (Fig. 3E) and the time constants of inactivation (Fig. 3F left, and left inset) and recovery from inactivation (Fig. 3F left, and left inset) did not differ significantly between K897 and T897.

3.3. Physiological implications

Our electrophysiological experiments demonstrate that the T897-HERG channel differs from the K897 channel by alterations in both activation (~7 mV shift of steady-state activation curve and approximately twofold increase in rate of activation) and deactivation parameters (approximately twofold increase in rate of deactivation). To assess the physiological implications of these differences, we carried out computer simulations using the comprehensive mathematical model of a human ventricular cell by Priebe and Beuckelmann [26]. Fig. 4A shows the effect of altered activation parameters. The altered activation of the T897 channel (solid lines) compared to the K897 channel (dashed lines) results in a larger $I_{Kr}$ amplitude (bottom). As a consequence, repolarization of the AP is accelerated (top), resulting in an 11.5% decrease in AP duration at 90% repolarization (APD$_{90}$). Fig. 4B shows the effect of altered deactivation parameters. The more rapid deactivation of $I_{Kr}$ (bottom) results in a 0.7% increase in APD$_{90}$. The net effect of the alterations in both activation and deactivation parameters is a decrease in APD$_{90}$ by 10.7% (Fig. 4C).

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**Fig. 4.** Functional effects of the K897T HERG polymorphism. Dashed lines show the control action potential (top) and the HERG encoded membrane current $I_{Kr}$ (bottom) of the Priebe–Beuckelmann human ventricular cell model [26] at steady 1-Hz stimulation. Solid lines show the effects of incorporating the experimentally observed changes in $I_{Kr}$ kinetics of the K897T polymorphism. (A) Effect of altered activation parameters (~7 mV shift in steady-state activation curve and twofold increase in rate of activation). (B) Effect of altered deactivation parameters (twofold increase in rate of deactivation). (C) Combined effect.
4. Discussion

Association studies between the respective alleles of the KCNH2 K897T polymorphism and QTc in a large sample of Caucasian subjects recruited in Germany demonstrated that this polymorphism is significantly associated with QTc. CC homozygotes displayed a significantly shorter QTc compared to AA homozygotes and AC heterozygotes. The latter two genotypes were associated with comparable mean QTc’s, suggesting that the 2690C-allele is recessive. After stratification by gender, the effect of the K897T genotype on QTc was found to be most predictive in females, in whom mean QTc was reduced by ~14 ms in CC homozygotes compared to AA homozygotes and AC heterozygotes. This (female) gender-specific effect of the K897T genotype on QTc was replicated in a second (smaller) independent sample of the (German) Caucasian population recruited at a different center.

We next investigated whether the K897T change itself could be responsible for these effects, i.e. whether it represents a ‘functional polymorphism’. Electrophysiologically characterized of the T897-HERG channel (reflecting the situation in CC homozygotes) revealed that this channel exhibited biophysical properties similar to those of the K897-HERG channel (which reflects the situation in AA homozygotes) with the exception of voltage dependence of steady-state activation (shifted by ~7 mV), and time courses of $I_{Ks}$ activation and deactivation (accelerated approximately twofold). As confirmed by computer simulation, these changes increase $I_{Ks}$ thereby augmenting net outward current during the repolarization phase of the cardiac AP. This hastens repolarization, which would translate into a shorter QTc on the surface ECG.

The K897 residue in KCNH2 is evolutionary highly conserved from mouse and rat to human [27–29], suggesting that replacing this basic residue with threonine (T) could affect the function of the channel. Together with the fact that the biophysical properties of the T897 channel explain the observed phenotype for this allele, this implies that it is highly probable that it is the K897T change itself, and not another polymorphism in tight linkage disequilibrium with it, that is functionally responsible for the QTc phenotype. In further support of this, sequence analysis of the entire coding region of KCNH2 in six CC homozygotes and 26 AC heterozygotes (all Caucasian) identified no further amino acid-changing polymorphisms in these individuals. Nevertheless, yet-unknown polymorphisms within the non-coding regions that could be in tight linkage disequilibrium with the polymorphism described herein and that could impact on gene function cannot be completely ruled out.

Although the mechanism whereby the K897T polymorphism is preferentially associated with a shorter baseline QTc in females remains to be elucidated, this finding is important for several reasons. Compared with men, women exhibit a longer QTc both at baseline (this study and Ref. [30]) as well as in the setting of the congenital LQTS [31]. This disparity in QTc between males and females stems from postpubertal QTc shortening in men [32], the mechanism for which is hitherto unknown. The longer QTc in females renders them more susceptible to the development of torsade de pointes in various settings of QT-prolongation, including the congenital LQTS wherein among known mutation carriers, females are more likely to be symptomatic [33]. A similar female preponderance is found in the setting of drug-induced QT-prolongation [8,34]. The polymorphism described herein could attenuate QTc-prolongation in these settings in female CC homozygotes, and could underlie, at least in part, some degree of inter-individual susceptibility to the development of QTc-prolongation and life-threatening cardiac arrhythmias subsequent to $I_{Kr}$-blocking drugs in females. In support of this proposition is the fact that female CC homozygotes had a mean QTc (389.0±3.9 ms) comparable to the mean (all genotypes) QTc in males (392.9±1.0 ms; Table 2).

In addition, the K897T polymorphism could contribute to the variable penetrance in the congenital LQTS. In support of such a role, Laitinen et al. [35] noted that among 28 families in which the KCNQ1-Fin mutation—a founder mutation for LQTS in Finns—segregated, female CC homozygotes compared to AA homozygotes and AC heterozygotes. This underscores the importance of confirming associations in different populations. The disparity between the two studies could reflect population differences such as population-specific differences in the occurrence of additional (functional) polymorphisms (possibly in the promoter region) that influence the effect. Besides, our study analysed a much larger number of individuals (521 females versus 187) and thus we were able to compare all three genotypes separately (AA versus AC versus CC, as opposed to AA versus AC and CC combined). In particular, we tested association using 27 CC homozygotes whereas the number of homozygotes in the other study was five. Notwithstanding the disparity, both studies point to a role for the KCNH2 gene in inter-individual variability of QTc interval.

The kinetic differences between the T897 and K897 channels are modest compared with those observed for mutations found in patients with manifest LQTS [37], consistent with the proposition that polymorphisms act to modify the phenotype of stresses such as pathogenic mutations or pharmacological provocation [38]. Furthermore, this finding is consistent with the increasingly recognized view that inter-individual susceptibility to QT-
prolongation lies in the co-inheritance of multiple subsets of polymorphisms that act in concert in determining one’s risk [18]. In such cases, determination of their separate contribution requires association studies in large population samples and their effect also being substantiated through functional studies.

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