Long-QT mutation p.K557E-Kv7.1: dominant-negative suppression of $I_{Ks}$, but preserved cAMP-dependent up-regulation

Roel L.H.M.G. Spätjens¹, Markéta Bébarová², Sandrine R.M. Seyen¹, Viola Lentink¹, Rosalie J. Jongbloed³, Yvonne H.J.M. Arens³, Jordi Heijman¹,⁴, and Paul G.A. Volders¹*

¹Department of Cardiology, Cardiovascular Research Institute Maastricht, Maastricht University Medical Centre, PO Box 5800, Maastricht 6202 AZ, The Netherlands; ²Department of Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic; ³Department of Clinical Genetics, Maastricht University Medical Centre, Maastricht, The Netherlands; and ⁴Institute of Pharmacology, Faculty of Medicine, University Duisburg-Essen, Essen, Germany

Received 14 January 2014; revised 26 July 2014; accepted 29 July 2014; online publish-ahead-of-print 18 August 2014

Time for primary review: 32 days

Aims
Mutations in KCNQ1, encoding for Kv7.1, the α-subunit of the $I_{Ks}$ channel, cause long-QT syndrome type 1, potentially predisposing patients to ventricular tachyarrhythmias and sudden cardiac death, in particular, during elevated sympathetic tone. Here, we aim at characterizing the p.Lys557Glu (K557E) Kv7.1 mutation, identified in a Dutch kindred, at baseline and during (mimicked) increased adrenergic tone.

Methods and results
K557E carriers had moderate QTc prolongation that augmented significantly during exercise. $I_{Ks}$ characteristics were determined after co-expressing Kv7.1-wild-type (WT) and/or K557E with minK and Yotiao in Chinese hamster ovary cells. K557E caused $I_{Ks}$ loss of function with slowing of the activation kinetics, acceleration of deactivation kinetics, and a rightward shift of voltage-dependent activation. Together, these contributed to a dominant-negative reduction in $I_{Ks}$ density. Confocal microscopy and western blot indicated that trafficking of K557E channels was not impaired. Stimulation of WT $I_{Ks}$ by 3’-5’-cyclic adenosine monophosphate (cAMP) generated strong current up-regulation that was preserved for K557E in both hetero- and homozygosis. Accumulation of $I_{Ks}$ at fast rates occurred both in WT and in K557E, but was blunted in the latter. In a computational model, K557E showed a loss of action potential shortening during β-adrenergic stimulation, in accordance with the lack of QT shortening during exercise in patients.

Conclusion
K557E causes $I_{Ks}$ loss of function with reduced fast rate-dependent current accumulation. cAMP-dependent stimulation of mutant $I_{Ks}$ is preserved, but incapable of fully compensating for the baseline current reduction, explaining the long QT intervals at baseline and the abnormal QT accommodation during exercise in affected patients.

Keywords
Long-QT syndrome type 1  •  $I_{Ks}$  •  Potassium channel  •  Adrenergic regulation  •  KCNQ1

1. Introduction
The congenital long-QT syndrome (LQTS) is a potentially life-threatening cardiac disorder characterized by an increased QT interval on the electrocardiogram (ECG), reflecting prolonged ventricular repolarization. Patients may have variable symptoms, ranging from mild palpitations to syncope and sudden death due to torsades de pointes (Tdp)/ventricular fibrillation. Mutations leading to LQTS have been identified in at least 16 different genes encoding for cardiac ion channels or their interacting proteins.

LQTS type 1 (LQTS1) accounts for ~35% of all cases of LQTS and is caused by mutations in the KCNQ1 gene, encoding Kv7.1, the α-subunit of the slowly activating delayed rectifier K⁺ ($I_{Ks}$) channel. To date, >250 different KCNQ1 mutations associated with LQTS1 have been reported.

While expression of the homotetrameric Kv7.1 channel only elicits a rapidly activating K⁺ current, co-assembly with the modulatory β-subunit minK, encoded by the KCNE1 gene, causes marked slowing of the activation and a strong increase in current amplitude, typical of $I_{Ks}$. Under basal unstimulated isolated myocyte conditions, $I_{Ks}$ plays a minor role in ventricular repolarization. However, its contribution to the so-called repolarization reserve becomes significant when action potential duration (APD) is prolonged due to a decrease in other repolarizing currents and can be modulated through various factors.
regulatory processes, notably β-adrenergic receptor (βAR) stimulation and fast heart rate. These can cause significant up-regulation of \( k_s \), allowing it to play an important role in the shortening of APD. βAR regulation of \( k_s \) requires 3'-5'-cyclic adenosine monophosphate (cAMP)-dependent activation of protein kinase A (PKA) anchored to the channel by the A-kinase anchoring protein (AKAP) Yotiao. PKA subsequently phosphorylates Serine 27 at the Kv7.1 N-terminus, resulting in a strong current up-regulation. Furthermore, Yotiao plays an important role in the translation of this phosphorylation to enhanced \( k_s \).

The complexity of the \( k_s \) macromolecular complex and the important role of neuronal modulation of \( k_s \) in cardiac repolarization make a multilevel approach imperative when determining the cellular and clinical effects of a given KCNQ1 mutation. Here, we report the results of a translational study on the LQTS1 mutation p.K557E, obtained by combining analyses of the ECGs of carriers at rest and during exercise, with a cellular electrophysiological characterization of mutant \( k_s \) and its cAMP-dependent regulation, confocal imaging, and mathematical modelling.

2. Methods

An extended overview of the Methods is provided in the Supplementary material online. This study conforms to the Declaration of Helsinki and was performed according to a protocol approved by the local ethics committee of Maasstricht University Medical Centre, The Netherlands. Patients gave written informed consent for DNA diagnostics and ECG research.

2.1 Clinical and genetic diagnosis

LQTS was diagnosed if the heart rate-corrected QT interval (Bazett’s equation) exceeded 440 ms in the standard 12-lead ECG at baseline and/or during exercise testing. The latter was performed using a standard Bruce protocol. Pace and inclination were increased every 3 min until exhaustion or for other clinical reasons to halt, followed by a 5-min recovery period. The ECG was monitored constantly.

Patients were genotyped using standard molecular techniques, as previously described.

2.2 Site-directed mutagenesis and cell transfection

K557E was generated by site-directed mutagenesis based on wild-type (WT) human KCNQ1 using the QuikChange® II XL kit. Chinese hamster ovary (CHO) cells were cultured at 37°C/5% CO₂ in Ham’s F-12 medium supplemented with 10% fetal calf serum and 1% penicillin/streptomycin. For patch-clamp recordings in WT or homozygous mutant (K557Ehom) experiments, KCNQ1 (0.5 μg; WT or mutant) and KCNE1 (1 μg) cDNA were transiently co-transfected in CHO cells using Fugene® 6 (Roche Diagnostics Nederland B.V., Almere, The Netherlands/Promega C/5% CO₂ in 80% humidified air). KCNQ1-GFP (0.5 μg) was combined with untagged K557E-GFP cDNA and 2 μg of untagged KCNE1 cDNA were used. For K557Ehet, either WT KCNQ1-GFP (0.5 μg) combined with untagged K557E (0.5 μg) and KCNE1 (2 μg), or mutant K557E-GFP (0.5 μg) combined with untagged WT KCNQ1 (0.5 μg) and KCNE1 (2 μg) were transfected. Forty-eight hours after transfection, confocal images were obtained on a Leica TCS-SPE confocal microscope and further analysed using LAS AF Lite software v2.2.1.

2.5 Computational modelling

Using a detailed computational model of the canine epicardial myocyte (with βAR stimulation incorporated), the effects of K557E on the action potential (AP) configuration were studied. Parameters of βAR-stimulated and -unstimulated WT or K557Einh \( k_s \) were based on the experimental data obtained in patch-clamp studies in CHO cells in the absence or presence of cAMP/OA, after correction for temperature. The model subsequently determined the total current in response to βAR stimulation with isoproterenol based on the \( k_s \) phosphorylation status, as previously described.

2.6 Statistical analysis

Results are expressed as mean ± SEM from n patients/cells. An unpaired Student’s t-test, a one-way analysis of variance, or a two-way analysis of variance with Tukey’s post hoc test was used to determine statistical differences when appropriate. A P value < 0.05 was considered statistically significant.

3. Results

3.1 Genetic and clinical characterization

An A to G point mutation at nucleotide position c.1669 of the KCNQ1 gene (NCBI reference sequence NM_000218.2), causing a change from lysine to glutamic acid at protein position 557 (p.K557E) in the coiled-coil domain of the channel, was identified in the proband and various members of a Dutch family (Figure 1). Non-paternity was excluded for the proband. The mutation had occurred de novo. Baseline QTc averaged 476 ± 13 ms in K557E carriers (n = 8; age: 22 ± 7 years) vs. 400 ± 9 ms in unaffected siblings (P < 0.05; n = 5; age: 32 ± 15 years). During treadmill exercise testing, the QTc interval of mutation carriers (age: 37 ± 10 years) prolonged by 91 ± 11 ms (~19%) at peak exercise (from 474 ± 11 ms at rest to 564 ± 12 ms, n = 5, P < 0.005). After 1 and 2 min of recovery, QTc prolonged further to 604 ± 21 and 598 ± 15 ms, respectively, all P < 0.005 vs. their corresponding QTc at rest, unlike the findings in an unaffected family
member (subject III:3, age: 27 years) in whom QTc was 389 ± 6, 382 ± 1, 418 ± 8, and 425 ± 8 ms for rest, peak exercise, 1-min, and 2-min recovery, respectively (Figure 2A). These control values are consistent with reports in the literature.19 Figure 2B and C show representative ECGs and QTc and RR findings in a K557E mutation carrier, in whom QTc prolonged by 34 ± 2% after 1-min recovery from exercise (subject IV:1). Symptomatic carriers (n = 3 of eight family members) experienced palpitations and/or spells, but none had syncope or sudden cardiac death.

3.2 Subcellular localization of WT and mutant channel complexes

To determine the effect of K557E on the membrane expression of the mutant \(I_{Ks}\) channel, confocal microscopy of CHO cells expressing C-terminally GFP-tagged Kv7.1-WT or Kv7.1-K557E subunits in combination with untagged minK subunits was performed. As depicted in Figure 3A, Kv7.1-WT-GFP/minK and Kv7.1-K557E-GFP/minK channels, as well as their co-expression in heterozygous conditions (K557Ehet), showed clear membrane localization. Intensity plots confirmed membrane expression for all groups (Figure 3B). Moreover, western blot experiments showed no differences in Kv7.1 expression in lysates or membrane fractions, and no difference in Kv7.1 lysate-to-membrane ratio of CHO cells from WT, K557het, or K557E hom groups (Figure 3C). Together, these data indicate that K557E did not disturb processing and trafficking of the mutant channel protein.

3.3 Biophysical properties of WT and K557E-mutant \(I_{Ks}\) channel complexes

A slowly activating \(K^{+}\) current was elicited during a 5-s depolarizing pulse from −80 to +60 mV by the WT \(I_{Ks}\) channel complex expressed in CHO cells (Figure 4A, ‘+cAMP/OA’). The mutant current was significantly down-regulated for both K557Ehet and K557E hom. Key differences between WT, K557Ehet, and K557E hom are summarized in Table 1.

To determine the voltage dependence of activation, \(I_{Ks}\) tail densities at −40 mV were plotted as a function of the prepulse potential during 5-s depolarizing pulses between −50 and +90 mV for WT, K557Ehet, and K557E hom (Figure 4C). In the WT \(I_{Ks}\) channel complex, the activation threshold was approximately −20 mV. K557Ehet, and particularly K557E hom, showed a rightward shift of the activation curve (see Supplementary material online, Figure S1). The slope factors were comparable for WT and K557E hom but significantly increased for K557Ehet (Table 1). Quantitative analysis of a conceptual model of the \(I_{Ks}\) channel population suggested that the increase in slope factor for K557Ehet results from a variable combination of WT and mutant subunits, with some channels opening at more negative potentials than others (see Supplementary material online, Figure S2).

Where comparison with WT was possible, the activation kinetics (\(\tau_{act}\)) of K557E hom were slower, whereas deactivation kinetics (\(\tau_{deact}\)) were significantly accelerated for all membrane voltages (\(V_m\)) (Figure 4D). For K557E hom, \(\tau_{deact}\) was intermediate to that of WT and K557E heterozygous and was significantly accelerated vs. WT (\(P < 0.05\) for \(V_m\) more negative than −60 mV). In contrast, \(\tau_{act}\) was significantly slower for all \(V_m\) where comparison with WT was possible (\(P < 0.05\)).

3.4 cAMP-mediated stimulation of WT and mutant channel complexes

Figure 4A (+cAMP/OA) shows also the averaged current traces during a 5-s depolarizing pulse from −80 to +60 mV after stimulation with cAMP/OA, for WT, K557Ehet, and K557E hom \(I_{Ks}\) channels. The relative change in \(I_{Ks}\) tail amplitude for the different variants (normalized to the value after 1 min of wash-in of OA and cAMP) is shown in
In all, a significant increase in tail current was observed upon cAMP/OA-dependent stimulation. WT I_{Ks} did not increase when the wash-in protocol was performed without cAMP/OA in the pipette. As an additional negative control, Kv7.1 mutant G589D was tested since this mutation has previously been shown to prevent binding of Yotiao and subsequent cAMP-dependent up-regulation when expressed heterozygously.15 Indeed, G589Dhet mutant I_{Ks} did not increase when stimulated with cAMP/OA (Figure 4B). The steady-state effect of cAMP-dependent stimulation on the current–voltage relationship of the different channels was determined after a 5-min wash-in period (Figure 4C). cAMP-dependent stimulation resulted in increased WT, K557E_{het} and K557E_{hom} I_{Ks}. However, the stimulatory effect of cAMP/OA could not compensate for the reduction in the basal current density caused by the K557E mutation in either heterozygous or homozygous conditions.

Furthermore, cAMP-dependent stimulation of the I_{Ks} channel has been reported to result in several gating alterations, including a significant leftward shift of the voltage dependence of activation.20,21 This effect was indeed observed for WT and K557E_{hom}. For K557E_{het}, V_{1/2} was unchanged (39 ± 2 mV). Slope factors for WT, K557E_{het} and
During cAMP/OA treatment were 14 ± 0.33 ± 2, and 15 ± 1 (Table 1 and see Supplementary material online, Figure S1). Also during cAMP/OA, \( \tau_{\text{act}} \) remained significantly slower for both K557E\textsubscript{het} and K557E\textsubscript{hom} compared with WT for overlapping \( V_m \) (Figure 4D). All \( \tau_{\text{deact}} \) were significantly accelerated for K557E\textsubscript{hom} compared with WT. K557E\textsubscript{het} remained intermediate (\( P < 0.05 \) for \( V_m \) more negative than −60 mV).

3.5 Rate-dependent accumulation of K557E-mutant \( I_{Ks} \)

In addition to cAMP-dependent up-regulation, \( I_{Ks} \) can also be increased through accumulation at rapid stimulation rates.\(^7\) The latter mechanism is determined by the \( I_{Ks} \) kinetics.\(^7\,^8\) To test the ability of K557E-mutant \( I_{Ks} \) to accumulate, we used AP clamp pulses with the interpulse interval set to 20 ms to maximally promote \( I_{Ks} \) accumulation. Accumulation of WT \( I_{Ks} \) was significantly larger than for K557E\textsubscript{het} (+841% vs. +208%, isoproterenol AP morphology). In addition, accumulation was significantly more pronounced with the isoproterenol AP waveform compared with the baseline AP morphology (Table 1; Figure 5A and B). When cAMP/OA was added to the pipette solution, these differences were even more pronounced (+2231% vs. +491% compared with Beat 1 for WT and K557E\textsubscript{het}-isoproterenol AP morphology). Despite functional accumulation in K557E\textsubscript{het}, the final \( I_{Ks} \) density after 100 pulses did not reach the level of WT \( I_{Ks} \). The dependence of \( I_{Ks} \) accumulation on interpulse interval was further investigated using square voltage-clamp pulses to +20 mV (see Supplementary material online, Figure S3). Consistent with the results with AP clamp pulses, there was a significant accumulation of WT \( I_{Ks} \) at short interpulse intervals. K557E\textsubscript{het} also showed accumulation, but to a much lesser extent. Of note, for both WT and
Figure 4  Baseline electrophysiological characteristics of WT, K557Ehet, and K557Ehom $I_{Ks}$ channels. (A) Averaged current density traces for WT, K557Ehet, and K557Ehom in the absence ($-$; $n = 13, 16$, and $9$ cells, respectively) or presence ($+$; $n = 15, 11$, and $9$ cells, respectively) of intrapipette cAMP/OA during a 5-s depolarizing pulse to $+60$ mV (holding potential was $-80$ mV in all experiments). Inset: zoom on the tail current densities at baseline. (B) Relative tail current change for WT ($n = 15$ cells), K557Ehet ($n = 9$ cells), K557Ehom ($n = 9$ cells), and G589Dhet ($n = 6$ cells) upon repetitive pulses from $-70$ to $+60$ mV (in the case of K557Ehom, pulses to $+90$ mV were used) with cAMP/OA in the pipette solution. Values were normalized to the current density obtained after 1-min wash-in of cAMP/OA. After 5 min of wash-in, WT, K557Ehet, and K557Ehom conditions showed a significant increase in tail current density (at $t = 5$ min; $P < 0.05$ for all three conditions). G589Dhet showed no increase on cAMP/OA stimulation. As an additional negative control, WT $I_{Ks}$ without cAMP/OA in the pipette (WT neg.; $n = 6$) was used. (C) Tail current–voltage relationship for WT, K557Ehet, and K557Ehom after 5-s depolarizing pulses from $-50$ to $+90$ mV in absence (left; $n = 13, 16$, and $7$, respectively) and presence (middle; $n = 15, 11$, and $9$, respectively) of cAMP/OA. Voltage protocol is shown at the right side. * indicates significant differences of K557Ehet and K557Ehom vs. WT current. (D) Activation and deactivation kinetics of WT (circles), K557Ehet (squares), and K557Ehom (triangles) channels in absence (left; $n_{act} = 13, 5$, and $4$ cells; $n_{deact} = 7, 7$, and $8$ cells, respectively) and presence (middle; $n_{act} = 11, 6$, and $4$ cells; $n_{deact} = 8, 7$, and $5$ cells, respectively) of cAMP/OA. Time constants derived from monoexponential fits of the raw current traces were plotted as a function of the membrane potential. For the K557Ehet channel, slowing of the activation and an acceleration of the deactivation kinetics were observed. K557Ehom currents were too small in this voltage range to fit the activation kinetics. Voltage protocol is shown at the right side.
augmentation generally occurred up to interpulse intervals of 100 ms (see Supplementary material online, Figure S3).

### 3.6 Effect of K557E on APD during βAR stimulation

The effect of K557E on ventricular repolarization was additionally investigated using a computational model of βAR stimulation in the canine ventricular myocyte. The non-phosphorylated and phosphorylated populations of $I_{Ks}$ channels in the model reproduced characteristics of WT or K557Ehet $I_{Ks}$ measured in the absence or presence of cAMP/OA, respectively (see Supplementary material online, Figure S4). K557Ehet caused a dominant-negative reduction in $I_{Ks}$ amplitude during the AP (Figure 6A). Under basal conditions, $I_{Ks}$ plays a minor role in ventricular repolarization, and, consistently, loss of $I_{Ks}$ by K557E resulted only in a minimal APD prolongation in the model.

### Table 1 Comparison of WT, K557Ehet, and K557Ehom $I_{Ks}$ properties in the absence or presence of cAMP/OA

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>K557Ehet</th>
<th>K557Ehom</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peak current density at +80 mV (pA/pF)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>300 ± 39 (13)</td>
<td>59 ± 7 (16)*</td>
<td>4 ± 1 (7)*</td>
</tr>
<tr>
<td>cAMP/OA</td>
<td>465 ± 70 (15)**</td>
<td>121 ± 16 (10)**</td>
<td>22 ± 4 (9)**</td>
</tr>
<tr>
<td><strong>Tail current density at +80 mV (pA/pF)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>52 ± 7 (13)</td>
<td>12 ± 2 (16)*</td>
<td>0 ± 0 (7)*</td>
</tr>
<tr>
<td>cAMP/OA</td>
<td>84 ± 14 (15)**</td>
<td>21 ± 4 (10)**</td>
<td>7 ± 1 (9)**</td>
</tr>
<tr>
<td>$V_{1/2}$ (mV)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>12 ± 0 (13)</td>
<td>42 ± 3 (11)*</td>
<td>122 ± 2 (7)*</td>
</tr>
<tr>
<td>cAMP/OA</td>
<td>-1 ± 0 (15)**</td>
<td>39 ± 2 (14)</td>
<td>106 ± 1 (13)**</td>
</tr>
<tr>
<td><strong>Slope factor</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>16 ± 0 (13)</td>
<td>34 ± 3 (11)*</td>
<td>18 ± 2 (7)</td>
</tr>
<tr>
<td>cAMP/OA</td>
<td>14 ± 0 (15)**</td>
<td>33 ± 2 (14)</td>
<td>15 ± 1 (13)**</td>
</tr>
<tr>
<td><strong>Accumulation of control AP (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>341 ± 93 (7)***</td>
<td>188 ± 50 (6)***</td>
<td>N.D.</td>
</tr>
<tr>
<td>cAMP/OA</td>
<td>1923 ± 503 (8)***</td>
<td>297 ± 42 (6)***</td>
<td>N.D.</td>
</tr>
<tr>
<td><strong>Accumulation of isoproterenol AP (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>841 ± 229 (7)***</td>
<td>208 ± 52 (6)***</td>
<td>N.D.</td>
</tr>
<tr>
<td>cAMP/OA</td>
<td>2231 ± 504 (8)***</td>
<td>490 ± 83 (6)***</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate numbers of cells investigated.

* $P < 0.05$ vs. WT; ** $P < 0.05$ vs. baseline for a given group; *** $P < 0.05$ current at pulse 100 vs. pulse 1; N.D. not determined.

**Figure 5** Rate-dependent accumulation of K557E-mutant $I_{Ks}$. (A) Representative examples of $I_{Ks}$ densities, obtained with 100 consecutive AP clamps with an interpulse interval of 20 ms. On top, AP-clamp waveforms are displayed with the baseline AP on the left (APD = 255 ms) and isoproterenol (iso) AP on the right (APD = 180 ms). Below, for each condition, 100 superimposed $I_{Ks}$ traces are shown to document the accumulation. Note different y-scale (black vertical lines; 100 pA/pF) for WT + cAMP/OA. Time course of accumulation (time constant determined by fitting peak current—pulse number relationship with a mono-exponential curve) was similar for both AP morphologies, but significantly faster in WT than in K557Ehet (5102 ± 340 vs. 8107 ± 767 ms; $P < 0.01$). (B) Bar chart showing 1st and 100th beat for each condition and waveform (*$P < 0.05$ Beat 1 vs. 100). Although accumulation did happen for K557Ehet, the absolute current amplitudes remained lower than in WT, even when compared with WT without cAMP/OA stimulation. For WT, WT + cAMP/OA, K557E, and K557E + cAMP/OA, n values are 7, 8, 6, and 6 cells, respectively, for both clamp types.
The p.K557E mutation is located in the distal C-terminus of Kv7.1, a region that has been implicated in channel assembly, trafficking, and regulation of the macromolecular channel complex.\(^{11,22,23}\) Despite being located in this region, p.K557E does not impair trafficking of \(I_{Ks}\), channels to the membrane (Figure 3). Accumulation of K557E-mutant current at fast rates was partly preserved and cAMP-dependent up-regulation was intact (Figures 4 and 5), but these processes could not overcome the severe gating alterations caused by K557E, including a positive shift of the voltage dependence of activation, deceleration of the activation, and acceleration of the deactivation (Figure 4).

The pronounced shift of the voltage dependence of activation (\(>100 \text{ mV}\)) by K557E\(_{\text{homo}}\) (Table 1, see also Supplementary material online, Figure S1) is particularly striking since this mutation is located distant from the S4-voltage sensor of Kv7.1. This may suggest that the conformation of the Kv7.1 C-terminus after the change of charge at amino-acid position 557 (from positive to negative) can modulate the voltage sensor or the activation gate. Although homology models of the structure of the Kv7.1 transmembrane region have been described, no structural model is currently available for the Kv7.1 C-terminus. Even in heterozygous conditions mimicking clinical K557E carriernesship, an 82% reduction in current density at \(+60 \text{ mV}\) was found compared with WT \(I_{Ks}\), indicating that K557E subunits suppress WT Kv7.1-subunit function in a dominant-negative manner. The exact mechanism of this remains unclear. Previous work has suggested that the Kv7.1 distal C-terminus may be involved in channel regulation by phosphatidylinositol 4,5-bisphosphate (PIP2).\(^{24}\) A similar phenotype, involving a rightward shift in voltage dependence of activation, strongly reduced \(I_{Ks}\), and preserved cAMP-dependent stimulation, was seen in Xenopus oocytes homogeneously expressing the p.R555C Kv7.1 mutant. This Kv7.1-R555C was shown to have weaker apparent affinity for PIP2.\(^{24,25}\) Thus, impaired PIP2 regulation could also contribute to the loss of function of Kv7.1-K557E.

4.2 Role of βAR stimulation in K557E carriers

A leucine zipper motif in the C-terminus of Kv7.1 has been shown to be involved in the binding of the AKAP Yotiao and subsequent Yotiao-modulated PKA-dependent regulation of the channel.\(^{11}\) To investigate the effect of the K557E mutation on this regulation, we performed patch-clamp experiments with cAMP and OA in the pipette solution.\(^{11,12}\) Significant \(I_{Ks}\) up-regulation was observed for WT, K557E\(_{\text{hetero}}\), and K557E\(_{\text{homo}}\), indicating that the K557E mutation did not disturb cAMP-dependent regulation of the \(I_{Ks}\) channel. Despite of this and despite preserved rate-dependent accumulation of \(I_{Ks}\), the severely altered baseline current characteristics dominated the loss of function caused by the K557E mutation. Therefore, mutant \(I_{Ks}\) is small in the AP-voltage range, both under baseline and βAR-stimulated conditions, resulting in a significant AP prolongation in the latter case, as supported by our mathematical simulations (Figure 6). These findings are in agreement with a decrease in the available repolarization reserve in affected patients. Thus, although cAMP-dependent regulation is not impaired in these carriers, they may still have an increased risk for the development of ventricular tachyarrhythmias during increased sympathetic tone.

4.3 Implications of the present findings

Several studies have identified genetic and biophysical characteristics of KCNQ1 mutations that influence their observed clinical severity. For example, decelerated channel activation has been associated with a higher risk for cardiac events.\(^{26}\) Although also manifest in K557E, clinical severity is mild with this mutation. With regard to location, mutations in the transmembrane, cytoplasmic-loop and pore regions of Kv7.1 predispose to more frequent and more severe cardiac events than those in the N- or C-terminus.\(^{27-29}\) Mutations causing a dominant-negative
reduction of $I_{Ks}$ in heterologous expression systems have been reported to have a more severe phenotype than haploinsufficient Kv7.1 mutations. Balancing these characteristics in the context of our findings on K557E makes it difficult to classify phenotypic severity for this mutation and indicates the importance of advanced cellular electrophysiological examination, as we did. Currently, <10% of all reported LQTS1 mutations have been investigated electrophysiologically, and an even smaller number has been evaluated under conditions of (mimicked) $beta$AR stimulation. Given the importance of sympathetic stimulation in arrhythmogenesis in LQTS1 patients, baseline biophysical characteristics alone are likely insufficient to determine the complete functional effects of a mutation. The clinical phenotype of patients carrying the p.K557E-Kv7.1 mutation, showing QTc prolongation in response to exercise, is similar to that recently reported by Wu et al. for the p.G269S-Kv7.1 mutation. However, in that study, excessive prolongation of QT intervals on exercise was suggested to be due to blunted cAMP/PKA-dependent $I_{Ks}$ up-regulation. Since cAMP-dependent $I_{Ks}$ up-regulation is preserved in p.K557E carriers, this suggests that various molecular impairments of $I_{Ks}$ can prevent the recruitment of sufficient net repolarizing current during sympathetic stimulation, thereby resulting in inappropriate QT prolongation and an increased risk for ventricular tachyarrhythmias.

Additionally, the use of in silico models is one promising approach to support the prediction of the clinical outcome for mutation carriers, but it requires more robust validation over time. The gap between LQTS1 clinical phenotype and experimental characterization could also be bridged using patient-specific induced pluripotent stem cells that are differentiated into cardiac myocytes. Although promising to provide a better link between genotype and phenotype for individual patients, it may be challenging to precisely characterize the mutant behaviour in such derived cardiomyocytes due to the intrinsic heterozygous condition and the influence of other ion channels and genetic modifiers. In this study, we performed a detailed characterization of the biophysical properties of K557E based on experimental recordings in CHO cells and computational modelling, and we show that this approach provides crucial information for a better understanding of the clinical findings.

4.4 Limitations
We performed a detailed electrophysiological characterization of p.K557E in Kv7.1 identified in a single Dutch family. We showed differences in baseline QTc between mutation carriers and non-affected family members. Moreover, carriers had abnormal QTc responses during exercise and recovery. Only one non-carrier underwent a full exercise testing protocol, which rendered a detailed statistical comparison between QTc development in carriers and this non-carrier inappropriate. It cannot be excluded that (genetic) factors other than p.K557E-Kv7.1 contribute to the observed LQTS phenotype in this family. The response of WT- and K557E-Kv7.1 to $beta$AR stimulation was evaluated using intracellular cAMP/OA due to the absence of representative endogenous $beta$AR signalling complexes in CHO cells. Differences between $I_{Ks}$ stimulation through $beta$AR and via intracellular cAMP may exist. We employed a computational model of the canine left-ventricular epicardial AP18 to investigate the effects of p.K557E-Kv7.1 on APD. The model shows impaired APD shortening in response to simulated $beta$AR stimulation (based on $I_{Ks}$ measurements in the presence of cAMP/OA), suggesting an important role of normal $I_{Ks}$ for repolarization accommodation during exercise. However, a quantitative comparison with the exercise tests of patients with p.K557E-Kv7.1, who showed QTc prolongation with exercise, is not possible due to species differences (human vs. canine), differences between cellular and in vivo conditions, differences between simulated isoproterenol and in vivo sympathetic stimulation with heterogeneous autonomic innervation, and potentially other genetic and acquired factors that modulate cardiac repolarization. These are beyond the scope of the current modelling efforts.

4.5 Conclusions
The K557E mutation causes a dominant-negative loss of function of the $I_{Ks}$ channel, which relates to severe gating disturbances. Trafficking is not affected. The resulting significant reduction of $I_{Ks}$ leads to a reduced repolarization reserve, associated with QTc prolongation in patients, which is most apparent during exercise testing. Intact $beta$AR regulation and partly preserved rate-dependent current accumulation may underlie, at least partly, the relatively mild phenotype of mutation carriers.

Supplementary material
Supplementary material is available at Cardiovascular Research online.

Acknowledgements
The authors thank Miren David, Helma Kuipers, Jos Broers, and Inge Boulet, Maastricht University, for helpful discussions and technical assistance. The authors also wish to thank Assoc. Prof. Jiri Simurda, CSC (Masaryk University, Brno, Czech Republic), for expert suggestions during the preparation of this manuscript. The human Yotiao construct was kindly provided by Prof. Robert S. Kass, Columbia University, NY, USA. Human KCNQ1 and KCNE1 constructs were a kind gift from Prof. Dirk J. Snyders, University of Antwerp, Belgium.

Conflict of interest: none declared.

Funding
P.G.A.V. is supported by The Netherlands Heart Foundation (NHS2010B216) and a Vidi grant from the Netherlands Organization for Scientific Research (ZonMw 91710365).

References


