Functional characterization of the common amino acid 897 polymorphism of the cardiac potassium channel KCNH2 (HERG)

Kristian J. Paavonen\textsuperscript{a,b}, Hugh Chapman\textsuperscript{a}, Päivi J. Laitinen\textsuperscript{b}, Heidi Fodstad\textsuperscript{b}, Kirsi Piippo\textsuperscript{b}, Heikki Swan\textsuperscript{c}, Lauri Toivonen\textsuperscript{c}, Matti Viitasalo\textsuperscript{c}, Kimmo Kontula\textsuperscript{b}, Michael Pasternack\textsuperscript{a,*}\textsuperscript{1}

\textsuperscript{a}Institute of Biotechnology, Viikinkaari 9, PO Box 56, 00014, University of Helsinki, Helsinki, Finland
\textsuperscript{b}Department of Medicine, University of Helsinki, Helsinki, Finland
\textsuperscript{c}Department of Cardiology, University of Helsinki, Helsinki, Finland

Received 17 January 2003; accepted 14 May 2003

Abstract

Objective: To determine whether the amino acid 897 threonine (T) to lysine (K) polymorphism of the KCNH2 (HERG) potassium channel influences channel performance or patient phenotype. Methods: The phenotypic effects of this polymorphism were investigated in vitro by electrophysiological experiments in HEK-293 cells and in vivo by exercise electrocardiography in a group of LQTS patients carrying the same genetically proven KCNQ1 mutation. Results: When expressed in HEK-293 cells, the 897T isoform of the KCNH2 channel exhibited changes in inactivation and deactivation properties, and a smaller current density than the more common 897K isoform. Western blot experiments indicated that the decreased current density associated with 897T was caused by reduced channel expression. During a maximal exercise test in 39 LQT1 patients carrying an identical KCNQ1 mutation (G589D) and showing a prolonged QT interval (\(\text{>440} \text{ ms}\)), QT intervals were longer in patients carrying the 897T allele than in those homozygous for the 897K allele. Conclusions: The K897T variation has an effect on channel function and clinical phenotype. Our data warrant further investigations into the significance of this polymorphism in drug-induced and inherited LQTS.

© 2003 European Society of Cardiology. Published by Elsevier B.V. All rights reserved.

Keywords: Arrhythmia; ECG; K-channel; Long QT syndrome; Membrane currents

1. Introduction

The long QT-syndrome (LQTS) is an acquired or congenital channelopathy that predisposes to potentially fatal arrhythmias, and is usually manifested by a prolonged QT-interval on the electrocardiogram [1–4]. Acquired LQTS is a disorder caused by a variety of drugs and metabolic abnormalities [5], while the inherited forms of LQTS are caused by mutations in the genes encoding the potassium channel \(a\)-subunits KCNQ1 (previously KvLQT1) [6] or KCNH2 (previously HERG) [7], the sodium channel SCN5A [8], the potassium channel \(b\)-subunits minK [9] or MiRP1 [10], ankyrin-B [11] or the inward rectifier potassium channel Kir2.1 [12].

Drug-induced changes in the QT interval show high interindividual dispersion. It is an intriguing possibility that common polymorphisms of ion channels or their regulatory proteins could alter channel function or drug sensitivity and hence contribute to the variance in observed QT intervals. Indeed, a number of polymorphisms have been found in the genes encoding ion channels implicated in the pathogenesis of LQTS [13–16]. As an example, a polymorphism in the KCNH2 \(b\)-subunit, MiRP1, has been demonstrated to increase sensitivity to sulfamethoxazole [16].

KCNH2, in particular, is affected by a wide variety of drugs, some of which bind preferentially to certain states
or conformations of the channel [17]. KCNH2 would therefore be a likely candidate for a determinant of polymorpha-linked drug sensitivity. In a recent paper we reported a polymorphism in the KCNH2 gene, located in the C-terminal domain of the channel at nucleotide 2690 (A>C), resulting in substitution of a basic lysine by a neutral threonine at amino acid 897 (K897T). This polymorphism is common in the Finnish population: a survey of 81 healthy blood donors indicated [13] that approximately 64% of Finns are K897K homozygotes, 32% are K897T heterozygotes and 4% are T897T homozygotes. Screening of a group of LQTS patients of type LQT1 carrying the KCNQ1-Fin founder mutation [18] showed KCNH2 genotype-related differences in QTc interval, with K897K subjects displaying a longer QTc interval than those with the K897T or T897T genotypes [13]. Also, in a population of middle-aged Finnish women, the K897T polymorphism was found to alter the duration and transmural dispersion of ventricular repolarization [19].

The present study was conducted to investigate in detail the pathophysiological consequences of the KCNH2 K897T polymorphism. First, we used the whole cell patch clamp method to compare the biophysical properties of the 897K and 897T variants of KCNH2 subunits in a heterologous expression system. Second, exercise electrocardiograms as well as clinical data of LQTS patients were analysed to determine whether this polymorphism alters the repolarization of the heart, or predisposes to arrhythmia.

2. Materials and methods

2.1. In vitro studies

The wild type KCNH2 cDNA (KCNH2-897K; U04270) in the plasmid pcDNA3.1 (Invitrogen, Carlsbad, CA, USA) was kindly provided by Dr. Gail Robertson (University of Wisconsin, Madison, WI, USA). The KCNH2-897T cDNA construct containing the nucleotide substitution A2690C, predicted to result in substitution of threonine for lysine at amino acid 897, was made using the QuikChange site-directed mutagenesis kit (Stratagene, CA, USA). The DNA construct was sequenced to rule out the introduction of undesired mutations. Transient transfections into a human embryonic kidney cell line (HEK-293) and an African green monkey cell line (COS-7) were performed using the calcium phosphate precipitation method. In addition, some transfections of COS-7 cells were made using Effectene Transfection Reagent (Qiagen, Valencia, CA, USA). Enhanced green fluorescent protein (EGFP) cDNA was co-transfected with the experimental constructs to identify the transfected cells for electrophysiological recordings.

Whole cell membrane currents were measured at room temperature (22–24 °C) unless otherwise stated using an EPC-9 amplifier and Pulse/Pulsefit software (HEKA, Lambrecht, Germany). The extracellular solution used in the recordings contained 150 mmol/l NaCl, 5.4 mmol/l KCl, 1.8 mmol/l CaCl2, 1 mmol/l MgCl2 and 5 mmol/l HEPES (pH 7.4 with NaOH). The recording pipettes were filled with a solution containing 150 mmol/l KCl, 2 mmol/l MgCl2, 5 mmol/l Bapta and 10 mmol/l HEPES (pH 7.2). Recordings were performed approximately 48 h after transfection. Input resistances of all cells were over 100 MΩ and patch pipette resistances were 2–3 MΩ. The series resistance was compensated in all experiments to ≥70%. All electrophysiological recordings were performed on transiently transfected HEK-293 cells unless otherwise stated.

The voltage clamp protocols are detailed in the figure legends. The half-maximal voltages ($V_{1/2}$) and slope factors ($k$) of activation and inactivation were estimated by fitting a Boltzmann equation to the data. The time constants of inactivation and recovery from inactivation were determined by fitting a single exponential, while the deactivation time course was obtained by fitting a double exponential.

Forskolin was dissolved in DMSO, and perfused into the cell bath in the extracellular solution at a concentration of 10 mmol/l. The final DMSO concentration was under 0.1% in all experiments and the control solution contained a similar concentration of DMSO with no effect on channel performance.

Preparation of membrane protein fractions and Western blotting experiments were performed as described previously [20,21]. Membrane proteins from transfected HEK-293 and COS-7 cells were subjected to SDS–polyacrylamide gel electrophoresis and incubated with 1:10 000 diluted KCNH2-antiserum (Alomone labs, Jerusalem, Israel) at room temperature overnight. The epitope of the antibody used comprised amino acids 1106–1159 at the C-terminus of the KCNH2 protein. The antibody was detected with an ECL detection kit (Amerham Pharmacia Biotech, UK).

2.2. Patients and their clinical examinations

DNA samples and clinical data were available from 261 LQTS patients (120 males and 141 females, aged 3–89 years) who were established carriers of the founder mutation KCNQ1-Fin (G589D) [18]. Determination of the KCNH2 polymorphism was carried out as described previously [13].

Exercise electrocardiograms (ECGs) were measured from 53 carriers of the KCNQ1-Fin mutation (26 men, 27 women, aged 14–54 years). The exercise test was performed with a bicycle ergometer during continuous heart rate monitoring, the initial load being 30 W followed by stepwise increments of 15 W each minute until exhaustion. Resting heart rates and resting QT-intervals were measured from lead II, and the QT-interval was adjusted to heart rate as described by Bazett [22]. QT intervals during exercise
tests and recovery at heart rates from 100 to 150 beats per min (BPM) were determined at intervals of 10 beats per min. Heart rates were determined from RR intervals and QT-intervals from three successive beats from lead V3. The QT intervals were measured manually from the onset of the QRS complex to the end of the T-wave defined as the intersection of the isoelectric line and the tangent of the maximal downward slope of the T-wave. The observers were blinded to the patients’ genotypes at all times. None of the patients had hypertension or coronary heart disease, nor were any taking medication altering cardiac repolarization including beta-blockers. The K897T and T897T carriers were pooled together for statistical analysis. Written informed consent had been obtained from all study subjects and the study protocol had been approved by the Ethical Review Committee of the Helsinki University Central Hospital. The investigation conforms with the principles outlined in the declaration of Helsinki [23].

2.3. Statistical methods

Group data are expressed as mean±S.E.M. The chi-square test was used to study dichotomous variables while statistical comparisons between groups were performed by analysis of variance (ANOVA). If significant effects were indicated, a Student’s t-test was used to evaluate the significance of differences between means. Generalized linear models were constructed to study repeated measures. A repeated measures ANOVA was used to compare differences between the groups. A P<0.05 was deemed to indicate statistical significance. SPSS 9.0 (SPSS Inc, Chicago, IL, USA) was used for data analysis.

3. Results

3.1. Experiments in vitro

The 897K and 897T isoforms were studied electrophysiologically in vitro. Depolarizing voltage steps followed by repolarization to −60 mV resulted in the 897T isoform of KCNH2 displaying the characteristic inward rectification and subsequent large tail currents similar to those of the more common isoform 897K. The variant 897T form of the KCNH2 channel exhibited a similar half-maximal activation voltage (V1/2) as the 897K form (−5.9±7.4 mV vs. −7.6±7.0 mV, n=34 and n=27, respectively; Fig. 1C). The slope factor for the 897T channel tended to be somewhat larger than the corre-

Fig. 1. Typical whole cell current recordings in HEK-293 cells: (A) a cell transfected with KCNH2-897K; and (B) with KCNH2-897T. From a holding potential of −80 mV, the cells were depolarized to −60, −40, −20, 0, +20 and +40 mV for 2 s and then stepped to −60 mV at 10-s sweep intervals. (C) Plot of voltage dependence of activation in 897K and 897T cells. The peak tail current after repolarization to −60 mV was measured and normalized for each cell to the current at +60 mV and then averaged.
sponding value for 897K (7.4±1.2 mV vs. 7.0±1.0 mV, n=34 and n=27, respectively, P=0.1).

As wild type KCNH2 channels are modulated by cAMP both directly and indirectly via PKA [24], we speculated that the substitution of lysine for threonine at codon 897 could either provide an additional target for PKA phosphorylation or disrupt two putative protein phosphorylation sites at nearby amino acids 890 and 895 [25]. Consequently, a series of experiments were conducted using forskolin (10 mmol/l) which activates adenylate cyclase leading to an increase in cAMP and PKA stimulation. The principal effect of forskolin, apart from a decrease in current, is a depolarizing shift of the activation curve [25]. However, in the presence of forskolin, there was no significant difference in the $V_{1/2}$ of 897K and 897T channels ($V_{1/2}=3.1±4.7$ mV; n=5 and 5.0±5.1 mV; n=6, respectively). The differences in deactivation and inactivation parameters between the two isoforms were comparable to those at baseline. This suggests no difference between the two isoforms in PKA modulation.

The time course of current activation was then investigated at $+20$ mV. Using an envelope of tail currents protocol, the rate of activation was found to be similar for the two isoforms (Fig. 2A, B). In contrast, there was a difference in the rate of deactivation between the KCNH2 isoforms, though this became insignificant with increasingly hyperpolarized test voltages. In the latter experiment, the test pulses of various voltages were applied after a 2 s depolarizing pulse to $+20$ mV and the following decay currents were analyzed by fitting a double exponential function. Both the fast and the slow deactivation time constants were significantly slower for the 897T variant channel at $-40$ mV (Fig. 2C, D).

The rate of inactivation was studied using a three step voltage protocol [26]. The channels were activated and inactivated by a step to $+50$ mV, whereafter the inactivation was removed with a transient hyperpolarization step and the subsequent step to test voltages enabled current inactivation to be observed (Fig. 3A). At all the test voltages used from $+40$ mV to $-20$ mV the rate of inactivation was slower for the variant 897T channel than for 897K (all $P<0.05$) (Fig. 3B). Another three step protocol was used to investigate the voltage dependence of the distribution of channels between the open and inactivated states (steady-state inactivation) (Fig. 3C) [26,27]. As shown in Fig. 3D, there was a shift in the voltage

![Fig. 2](https://academic.oup.com/cardiovascres/article-abstract/59/3/603/346876/10.1111/j.1528-1247.2002.01756_1){#fig2}
Fig. 3. The effect of the K897T polymorphism on channel inactivation properties. (A) The time course of inactivation was examined using the voltage protocol shown: a 2-s step to +50 mV from the holding potential of −80 mV was applied followed by a brief (7 ms) hyperpolarizing step to −120 mV and then a step to various test potentials. Representative inactivating currents from the two isoforms recorded at the start of the third step (at +20 and −20 mV) of the protocol are shown. The currents are scaled to peak current observed for the 897K. A single exponential function was fitted to these currents to give the inactivation time constants. (B) The voltage dependence of the rate of inactivation ($n = 10$ and 12, for 897K and 897T, respectively). The rate of inactivation was significantly slower for the 897T isoform at all test potentials ($P < 0.05$). (C) The voltage dependence of channel availability (steady state inactivation). KCNH2 channels were activated and inactivated by a 3-s step to +90 mV. The subsequent 12.5 millisecond step to the test potentials enables a fraction of the channels to recover from inactivation. The relative number of channels available to become activated is measured as the instantaneous current at +20 mV. Recordings obtained at −10 to −130 mV from a KCNH2-897K cell are shown. A correction for deactivation during the step to the test potentials was done as described before [26,27]. D. A plot of the mean values for steady state inactivation for the KCNH2 isoforms 897K and 897T from 3 and 4 cells, respectively, with fits using the Boltzmann function ($V_{1/2} = −92.0 ± 3.2$ mV and $−103.5 ± 1.8$ mV; $k = 32.8 ± 7.1$ mV and $26.4 ± 2.0$ mV, for 897K and 897T respectively).

dependence with the variant 897T exhibiting a more negative $V_{1/2}$ than the more common isoform, suggesting that there are fewer channels available for 897T at any given membrane potential. From the protocol used to measure the deactivation time course, the rate of recovery from inactivation was also obtained as a monoexponential fit to the initial increase in tail current amplitude. This rate was similar for the two KCNH2 isoforms (data not shown).

We also ran a series of experiments at physiological temperature (36°C) to find out whether the changes in
channel properties observed at room temperature would appropriately reflect those recorded at physiological temperature. The elevated temperature did not bring about isoform-specific alterations different from those observed at room temperature.

The maximum available KCNH2 current was much larger in the cells expressing the 897K channel subunits than in those expressing the variant 897T, although the cell capacitances corresponding to total cell membrane area in the two groups did not differ. The respective current densities in the two groups were 64±10 pA/pF and 34±4 pA/pF (n = 25 and n = 33, respectively, P = 0.004; Fig. 4A). The increased steady state inactivation is expected to result in a decrease in the available current, but this alone is not sufficient to explain the differences in current density observed between the isoforms [27]. The remaining difference could be due to a decreased expression of the KCNH2-897T subunit in comparison to the 897K subunit, and/or to a change in the single channel properties. We determined the cell membrane expression of 897K and 897T using the Western blotting technique. In agreement with a previous report, the Western blots displayed two KCNH2 bands with the higher molecular weight (HMW) band corresponding to the complexly glycosylated membrane-associated (functional) KCNH2 channel and the lower to an intracellular core-glycosylated precursor form of the channel (Fig. 4B) [20,21]. This was verified by subjecting the intact transfected cells to extracellular proteinase K treatment, which selectively abolished the HMW band (data not shown). In HEK-293 cells, the overall expression of the 897T isoform was much reduced when compared to that of the 897K isoform. A similar difference in expression was seen in COS-7 cells when transfected using either the calcium phosphate method or the lipid based Effectene Reagent (data not shown). Visually, no difference in EGFP expression was noted between the two isoforms. The ratio of the membrane-associated form to the precursor form was also reduced for the 897T variant (Fig. 4B), suggesting that the surface targeting of the 897T channel subunits is impaired.

Collectively, these data indicate that the surface expression of KCNH2-897T, and thus, the number of functional channels, is reduced in comparison to KCNH2-897K in HEK-293 and COS-7 cells, which is in good agreement with the corresponding electrophysiological data indicated above. Although a difference in single channel properties between 897K and 897T cannot be excluded, the reduced expression of the 897T isoform together with the observed increase in inactivation is sufficient to explain the reduction in macroscopic currents reported above.

3.2. In vivo observations

We assumed that due to the large reserve of \( I_{Kr} \) and \( I_k \), repolarizing currents in healthy individuals [28], the penetrance of the KCNH2-897T variant allele would be weak in individuals without any challenge on this reserve, such as administration of \( I_{Kr} \), or \( I_k \) blocking drugs, or occurrence of mutations in KCNQ1, KCNH2 or other LQTS-related channels. Accordingly, we studied the phenotypic effects of the 897T allele in a group of patients who were carriers of the KCNQ1-Fin mutation, a founder gene accounting for approximately 30% of all LQTS cases in Finland. In this group of 261 LQTS patients, 47/153

![Fig. 4](https://academic.oup.com/cardiovascres/article-abstract/59/3/603/346876)
(31%), 22/89 (25%) and 3/19 (16%) of the patients with the genotypes K897K, K897T and T897T, respectively, were symptomatic having a history of syncope or documented cardiac arrhythmia, indicating no significant genotype-related risk of cardiac events in this specific group (P=0.3).

Because of the reduced membrane expression of the isoform KCNH2-897T and its location near two putative phosphorylation sites, we speculated that it would influence the repolarization of the heart during exercise. Patients with the K897K genotype and those with the genotypes K897T and T897T showed very similar QT intervals during exercise and recovery (Fig. 5A, B). Splitting the KCNQ1-Fin group into groups of males and females did not bring about a difference in QT intervals (data not shown). In contrast, when the patients with prolonged QT intervals (QTc > 440 ms at rest, n = 39) were considered separately, a significant KCNH2-genotype-dependent difference in the QT interval dynamics was noted. Resting QTc intervals were similar in these two groups, being 469±5 in the K897K group (n = 22) and 477±5 ms in the K897T + T897T group (n = 17). In this group, 6/22 (27%), 6/15 (40%) and 2/2 (100%) of the patients with the genotypes K897K, K897T and T897T, respectively, were symptomatic (P=NS). During exercise, patients with the K897T + T897T genotypes had longer QT intervals than those with the K897K genotype, while no KCNH2-genotype related difference was observed during the recovery phase at any heart rate (Fig. 5C, D).

4. Discussion

Sudden, unexpected death is the most severe repercussion of LQTS. Numerous factors are known to predispose LQTS patients to potentially fatal arrhythmias [29–31]. However, additional knowledge of factors predisposing some LQTS patients to arrhythmia are urgently needed. Polymorphisms of cardiac ion channel genes, or genes modifying their function, could be underlying factors in the phenotypic dispersion observed in LQTS patients. We previously identified a common 897T variant of KCNH2 with an allele frequency of about 16% in the Finnish population; it occurs in hetero- or homozygous form in 36% of the population. We show here that KCNH2-897T channels have biophysical properties in vitro which differ in respect to channel inactivation and deactivation from those of KCNH2-897K channel.

KCNH2-897T channels inactivate more slowly but show a higher degree of inactivation, an alteration that is expected to decrease channel function. It is also possible that changes in KCNH2-897T inactivation properties can alter drug sensitivity. This is because several commonly used drugs inhibiting KCNH2 function bind preferentially to the inactivated state [32,33]. The decreased rate of inactivation accompanied with unaltered recovery from inactivation would predict that steady state inactivation should decrease, rather than increase as found here. Similar findings have, however, been reported for KCNH2 mutations located in the S4 region [34,35] and the outer mouth

![Fig. 5. QT interval dynamics during an exercise test in a group of LQT1 patients carrying the same KCNQ1 mutation (G589D). (A) all patients during exercise (n = 53). (B) all patients during recovery. (C) patients with a resting QTc≥440 milliseconds (n = 39) during exercise. (D) patients with a resting QTc≥440 milliseconds during recovery.](https://academic.oup.com/cardiovascres/article-abstract/59/3/603/346876)
of the pore region [34] as well as the C-terminus [36]. For these particular mutations no changes in rates to and from the inactivated state were observed, but shifts in the steady state inactivation were seen. These data indicate the complex nature of the inactivation process, and the rate constants determined in this study may therefore not fully describe the inactivation process [37].

A significant slowing of the deactivation rate was also seen with the KCNH2-897T channels at more depolarized potentials, an effect that would increase the outward current during cardiac repolarization shortening the action potential. The rate of deactivation of KCNH2 channels is a major determinant of action potential duration [21] and its alteration appears to negate the opposing effect expected from the shift in steady state inactivation. This dominant effect of deactivation on outward current has been seen with mutations in the PAS domain [38] and may explain the shorter QTc associated with the T897T genotype in LQT1 patients carrying the same KCNQ1 mutation [13].

In addition to the change in inactivation and deactivation properties observed in transiently transfected HEK-293 cells, we also observed a decreased functional expression of the KCNH2-897T isoform. The types of alterations we observed in both the channel gating and expression are in accordance with the role of the C-terminus suggested by splice variant and deletion constructs [39,40]. Moreover, the primary channel dysfunction brought about by many C-terminal mutations is in changes in protein trafficking to the plasma membrane so that the precursor form of KCNH2 is retained within the endoplasmic reticulum [20,36,41].

The Western blots from both HEK-293 and COS-7 cells supported a decreased surface expression of the 897T isoform. In whole cell recordings the current density was reduced by approximately 50%, whereas the Western blots indicated a substantially larger decrease in surface expression. Although we did not quantify this in detail, a likely explanation for the above is that a relatively larger subset of 897T cells was discarded from analysis since they did not express KCNH2 current despite of expressing EGFP. We cannot, however, exclude the possibility of a compensatory increase in the single channel current of the 897T isoform.

The reduced expression of KCNH2-897T variant observed in HEK-293 cells may reflect a reduction of the number of functional channels in the heart, and hence result in an increased sensitivity to factors influencing the maximum available repolarizing current, such as specific drugs or mutations of other LQTS-causing genes and other genes modulating the cardiac action potential. This is supported by our findings that in spite of similar QTc values at rest, LQT1 patients with the K897T and T897T genotypes exhibit longer QT-intervals during exercise than patients with the K897K genotype. In a recent study, the K897T polymorphism was reported not to alter channel performance or expression [42]. However, this study used a different expression platform (Xenopus oocytes) and the electrophysiological parameters were examined in less detail than in our present report; for example, in the measurement of current deactivation rates at only a single voltage was used by Scherer et al. [42]. We observed appreciable differences in current deactivation rates only at less negative voltages (Fig. 2C, D).

Clearly, further studies would be needed to clarify whether the KCNH2-K897T polymorphism is a risk factor in a number of offending situations, such as strenuous physical exercise, myocardial ischemia and concurrent administration of drugs affecting cardiac repolarization. Our present data suggests that the KCNH2-K897T polymorphism may exert a significant effect on individuals challenged with other factors influencing the QT interval, such as a carrier status for another QTc prolonging gene. On the contrary, a recent study failed to reveal any change in the frequency of the 897K and 897T alleles in drug-associated torsade de pointes associated with acquired LQTS [43]. However, it should be emphasized that even a small change of risk associated with the K897T polymorphism would be accentuated by the high prevalence of this polymorphism, at least in the Finnish population.

5. Limitations of the study

Heterotetramers of the KCNH2 channel subunits were not investigated in the present study. It is possible that the biophysical qualities and expression of the heteromeric channels would be different from those of the homomeric ones.

Acknowledgements

This work was supported in part by the Finnish Academy, the Finnish Foundation for Cardiovascular Research, the Sigrid Juselius Foundation, the Johnson & Johnson Pharmaceutical Research & Development and the Helsinki University Science Fund. We also thank Mrs. Mari Palviainen for her expert assistance with the experiments.

References


