

D242N, a K_V7.1 LQTS mutation uncovers a key residue for I_{Ks} voltage dependence

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Abstract

K_v7.1 and KCNE1 co-assemble to give rise to the I_{Ks} current, one of the most important repolarizing currents of the cardiac action potential. Its relevance is underscored by the identification of more than 500 mutations in K_v7.1 and, at least, 36 in KCNE1, that cause Long QT Syndrome (LQTS). The aim of this study was to characterize the biophysical and cellular consequences of the D242N K_v7.1 mutation associated with the LQTS. The mutation is located in the S4 transmembrane segment, within the voltage sensor of the K_v7.1 channel, disrupting the conserved charge balance of this region. Perforated patch-clamp experiments show that, unexpectedly, the mutation did not disrupt the voltage-dependent activation but it removed the inactivation and slowed the activation kinetics of D242N K_v7.1 channels. Biotinylation of cell-surface protein and co-immunoprecipitation experiments revealed that neither plasma membrane targeting nor co-assembly between K_v7.1 and KCNE1 was altered by the mutation. However, the association of D242N K_v7.1 with KCNE1 strongly shifted the voltage dependence of activation to more depolarized potentials (+50mV), hindering I_{Ks} current at physiologically relevant membrane potentials. Both functional and computational analysis suggest that the clinical phenotype of the LQTS patients carrying the D242N mutation is due to impaired action potential adaptation to exercise and, in particular, to increase in heart rate. Moreover, our data identify D242 aminoacidic position as a potential residue involved in the KCNE1-mediated regulation of the voltage dependence of activation of the K_v7.1 channel.

Keywords: K_v7.1 KCNE1 Long QT syndrome Electrophysiology

1. Introduction

Cardiac channelopathies are responsible for about a quarter of non-ischemic sudden cardiac deaths, the most prevalent and well-known disorder within this group being the congenital Long QT syndrome (LQTS), which is more predominant in women than in men, since women exhibit a longer QT interval [1, 2]. The prolonged QT_c interval (usually >480 ms) in the electrocardiogram (ECG) in these patients is caused by a delayed repolarization of the cardiac action potential (AP). Such LQT intervals can cause a polymorphic ventricular tachycardia called *torsades de pointes* and, in some cases, sudden cardiac death. Fifteen genes are associated with LQTS [1]. Among all of them, the *KCNQ1* gene accounts for more than 30% of the identified pathological genetic variants and mutations in LQTS causing the LQTS type 1 (LQTS1). *KCNQ1* gene codifies for K_v7.1 channels that, after assemble with KCNE1 subunits, generate the I_{Ks} current that greatly contributes to the repolarization of the cardiac AP [3-5]. Assembly with KCNE1 profoundly modifies the biophysical characteristics of the current: it slows the activation and deactivation kinetics, shifts the voltage dependence of the activation to more positive potentials, removes the inactivation, increases unitary conductance, increases PIP₂ sensitivity and confers sensitivity to the β adrenergic-PKA mediated activation, reconstituting the characteristics of the native cardiac I_{Ks} current [6-10]. This current becomes predominant at fast cardiac rate due to its extraordinary slow activation and deactivation kinetics that lead to an accumulation of K_v7.1-KCNE1 channels in the activated-closed states, facilitating channel opening when it is required [11]. Accordingly, exercise and emotional stress trigger arrhythmias in more than 80% of the LQTS1 patients making evident the key role of this current during elevated heart rates [12].

In this study, we analyzed the functional consequences of the *KCNQ1* mutation D242N (chr11:2572053G>A, c.724G>A) localized in the exon 5, found in two patients (twins) with LQTS. This genetic variant has been previously identified in other genetic studies [13-18]. D242 is located in the C-terminal half of the S4 domain facing the surface boundary of the S4-S5 linker. The S4 segment is the major contributor of the voltage sensing domain, thanks to a string of charged amino acids. Here we show that D242N mutation disturbs the charge balance of the K_v7.1 sensor domain leading to a significant right shift of the voltage dependence of activation determined by KCNE1 interaction. Our data show that D242N K_v7.1-KCNE1 channels generate negligible currents at physiological membrane potentials supporting its major role in the LQTS phenotype of the patients.

2. Materials and Methods

The institutional review board at The Heart Hospital, Institute of Cardiovascular Science (London) approved the study, and the patients provided written informed consent. The study conforms to the Declarations of Helsinki. There is an online extended Materials and Methods section.

2.1. Mutagenesis and Cell transfection

The D242N mutation was introduced in the pEYFP-N1-*KCNQ1* by PCR using QuickChange II XL Site-Directed Mutagenesis Kit (Agilent Technologies) following manufacturer's instructions. COS7 and HEK293 cells were transiently transfected with K_v7.1-pEYFP (WT and/or D242N) and KCNE1-pECFP by using Fugene6 (COS7) or MetafecteTM Pro (HEK293), following the manufacturer's instructions.

2.2. Protein purification and Western blot analysis

Twenty-four hours after transfection, HEK293 cells were washed twice with ice-cold PBS and the appropriate procedure was performed, either for biotinylation or immunoprecipitation experiments. Cell surface proteins biotinylation was carried out with Pierce Cell Surface Protein Isolation Kit (Pierce) following manufacturer's instructions. For the immunoprecipitation assay we used Protein A-Sepharose beads (GE Healthcare) that were coated with anti-KCNE1 or anti-K_v7.1 antibodies (Alomone Labs).

Protein samples, containing equal amounts of protein were incubated at 65°C for 10min in 250mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol and 2% β-mercaptoethanol and size-separated in 8% SDS-PAGE gel. Gels were blotted onto an Immobilon-P nitrocellulose membrane (Millipore) and processed as recommended by the antibodies supplier.

2.3. Confocal microscopy

HeLa cells seeded on a glass coverslip were fixed 24h after transfection of K_v7.1-pECFP-N1 or D242N K_v7.1-pECFP-N1, KCNE1-pEYFP-N1 and Akt-PH-pDsRed or pDsRed- ER. Cell preparations were imaged under Zeiss LSM 880 confocal microscope (Zeiss) equipped with an argon multiline laser (458 for pECFP, 488 and 514nm for pEYFP) and a red light laser (DPSS 561 for pDsRed).

2.4. Electrophysiological recordings

Currents were recorded from transiently transfected COS7 cells using the perforated amphotericin B-patch-clamp technique with an Axopatch 200B amplifier (Axon Instruments) as described [19, 20].

2.5. Computational analysis

To estimate the effects of the mutation in heterozygous conditions (D242N/WT K_v7.1-

KCNE1) on the APD, the O'Hara-Rudy (ORd) model was used [21] (see supplemental methods). The effects of β -adrenergic stimulation were simulated by acting on I_{Ks} , I_{CaL} and I_{up} according to previous computational studies [22]. The pseudo-ECG signal was also computed on a one-dimensional fiber taking into account the transmural heterogeneity, as previously described [23].

2.6. Statistics

Data are presented as mean values \pm SEM. Two-way ANOVA repetitive measurements test followed by Bonferroni test were used to assess statistical significance when appropriate. A value of $P < 0.05$ was considered significant (see online Material and Methods section).

3. Results

3.1. Patient characteristics: clinical analysis and genetics

Two 20 years-old dizygotic twins (female and male) were evaluated after presenting with episodes of syncope and one nocturnal seizure pre-diagnosis. Both twins were genetically examined and it was found that they carried the *KCNQ1* mutation D242N (chr11:2572053G>A, c.724G>A) localized in the exon 5. Cardiac examination and echocardiogram were unremarkable. In both patients, the ajmaline challenge for Brugada Syndrome was normal. The sister had a clinical history of two syncopes while standing and one nocturnal seizure in childhood but an unremarkable ECG apart from a J point elevation of 0.5mm inferior laterally and mildly prolonged QT interval at rest (QTc 473ms). The QT interval was prolonged with exercise, reaching a maximum QTc of 527ms (Figure 1A, Table S1). The twin brother had a one syncopal episode following playing a computer game, normal ECG with J point elevation of 0.5mm in inferior leads and a QTc at rest of 410ms. With exercise he initially developed a bifid T wave which was normalized at higher heart rates and the QTc increased to 522ms. A marked prolongation of QTc was seen at peak exercise, with rapid normalization during recovery (Figure 1B, Table S2). An ECG from their father, aged 54 years, was available for evaluation. J point elevation of 1.5mm in inferior, lateral leads with mild ST elevation consistent with early repolarization and mild notching in V1 were the only notable findings. QTc interval at rest was normal (421ms). There was no family history of sudden death. An ECG from the mother was not available for evaluation.

3.2. Functional characterization of D242N K_v7.1 channels

First, we analyzed the subcellular localization of the mutant and we observed a similar pattern of trafficking between WT and D242N K_v7.1-CFP channels characterized by a high ER retention (Figure 1A and B). Currents were elicited by applying 5.5s pulses to potentials between -80 and +100mV from a holding potential of -80mV in 20mV steps. The activation kinetics of mutant channels was slower than that of WT channels (Figure 2C and 2D, Figure S2). The current-voltage (I-V) relationship of D242N K_v7.1 showed a mild loss-of-function at membrane potentials positive to +40mV compared to WT K_v7.1 channels (Figure 2E), without changes in the activation curve (Figure 2F).

As it is shown in Figure 2C, tail currents of D242N K_v7.1 currents did not exhibit the typical hook of WT K_v7.1 channels [3, 4]. To analyze the voltage dependent of inactivation of WT and D242N K_v7.1 channels using the tail current analysis previously reported [24]. As shown in Figure 2G, inactivation of WT K_v7.1 channels was evident in the hook of the tail currents recorded at -120mV after 5.5s depolarizing pulses to different potentials, between -60 and +100mV. This hook is attributed to a rapid recovery from inactivation. Two components of tail currents can be measured in the tails: the instantaneous current (I_{inst}) and the extrapolated one ($I_{extrapol}$) obtained from a monoexponential fit of the tail current after the initial hook. The ratio $I_{inst}/I_{extrapol}$ was used to analyze the channel availability (Figure 2H). The half-inactivation voltage was -19.1 ± 2.1 mV (n=5) in WT K_v7.1 channels. As observed, D242N K_v7.1 channels lost the inactivation.

3.3. Loss of function of D242N K_V7.1-KCNE1 channels

To investigate the effects of the D242N mutation on K_V7.1 currents in the physiologically relevant channel complex (K_V7.1-KCNE1), WT or D242N K_V7.1 channels were co-transfected with KCNE1 subunits in HEK293 and COS7 cells. First, we analyzed the trafficking of these complexes to the plasma membrane, which, in some mutations, explains the decrease of the current [23, 25, 26]. Subcellular distribution of D242N K_V7.1-KCNE1 resembled that of WT K_V7.1-KCNE1 channels (Figure 3A and 3B). Also, the WT and the mutant K_V7.1 channels colocalized and coimmunoprecipitated with KCNE1 to the same extent (Figure S1A and S1B). Despite the high intracellular retention observed, the targeting of both, WT and D242N K_V7.1, to the plasma membrane was further confirmed by a cell surface biotinylation assay with no major differences between them (Figure S1A and S2B).

Secondly, we studied the electrophysiological characteristics of the D242N K_V7.1 channels assembled with KCNE1 subunits (Figure 3C and 3D). Coexpression of the mutant channel with KCNE1 caused a dramatic current inhibition compared to WT K_V7.1-KCNE1. Thus, the current generated by D242N K_V7.1-KCNE1 channels after depolarizing from a holding potential of -80mV to +60mV for 5.5s was significantly smaller than that elicited by WT K_V7.1-KCNE1 channels (438.6±80.8pA *vs.* 2571.0±568.4pA in D242N and WT K_V7.1-KCNE1 channels, respectively, n=10/15, P<0.01) (Figure 3C-3E). Figure 3F shows the activation curves obtained for WT and mutant complexes. Thus, the loss-of-function produced by the mutation seems to be due to a positive-shift of the voltage dependence of activation of K_V7.1-KCNE1 complex. Finally, the deactivation kinetics was faster in D242N than in WT K_V7.1-KCNE1 channels (59.6±7.7ms *vs.* 435.8±71.7ms, n=16/10, P<0.01, Figure 3C and 3D), which represents loss of function during AP repolarization.

The activity of I_{Ks} is crucial for cardiac AP repolarization. More precisely, it contributes to the adaptation to heart rate changes [22, 27, 28]. Slow activation of I_{Ks} ensures that the current activated in a single cardiac AP does not reach the maximal value and, due to its incomplete deactivation during short diastole periods, can be further increased upon repeated depolarizations, leading to a cardiac AP shortening in response to fast heart rate [29]. In order to elucidate if the rate-dependency of the I_{Ks} generated by the mutant channel was modified, trains of 70 pulses to +50mV, from -80mV, were applied at 1, 2 and 2.5Hz, mimicking low (resting) and fast (exercise) heart rates (Figure 4A). As previously described, for WT K_V7.1-KCNE1 complexes [23], a significant increase in the current was observed at fast rates (≥ 2 Hz). Figure 4B shows the current amplitude of each pulse plotted as a function of the pulse number. Thus, cells expressing D242N K_V7.1-KCNE1 channels, exhibited a smaller increase in the current magnitude at all frequencies tested.

3.4. Heterozygous condition

We mimicked the heterozygous condition of the patients reported by co-expressing WT, D242N K_V7.1 channels with KCNE1 (Figure 5A). Heteromeric D242N/WT K_V7.1-KCNE1 channels exhibited intermediate electrophysiological characteristics between WT and mutant homomeric channels. Figure 5B, shows the I-V relationship of the heterozygote exhibiting

similar current magnitude than WT K_v7.1-KCNE1 channels. Figure 5C shows the activation curves for WT/D242N K_v7.1-KCNE1 heteromeric channels, which exhibited a mild shift of the V_h to positive potentials vs. WT K_v7.1-KCNE1 channels but smaller than homomeric D242N K_v7.1-KCNE1. These results indicate that the mutation has not a dominant negative effect against WT channels. Also, the deactivation kinetics was faster than in WT K_v7.1-KCNE1 channels (313.8 ± 89.2 ms vs. 513.6 ± 37.0 ms, $n=5/15$, $P<0.05$), but slower than in D242N/WT K_v7.1-KCNE1 channels (313.8 ± 89.2 ms vs. 59.6 ± 7.7 ms, $n=5/16$, $P<0.05$).

3.5. D242N mutation impairs cardiac AP rate adaptation

A human ventricular cell computer model (O'hara Rudy model) was used to examine the effects of mutant channels (D242N/WT K_v7.1-KCNE1, mimicking the heterozygous condition) on AP morphology in endocardial, epicardial and M cells (Figure 6). At basal rate (60bpm), the mutation had negligible effects for all cell types, whereas at high rate (180bpm) and concomitant β -adrenergic stimulation (mimicking exercise conditions) the mutant AP failed to adapt its duration to the same extent than the WT one (e.g. 281 vs. 267ms in M cells, Figure 6A right panels), due to a smaller amount of I_{Ks} current (Figure 6A, middle panels). The impaired rate adaptation exhibited by the mutant was also observed by computing the rate dependence curve (APD₉₀ vs. pacing cycle length) for the three cellular types (Figure 6C).

In order to relate the modeled changes in APD₉₀ to QTc (i.e., to test if QTc increases at high heart rates as those observed during exercise in patients could be reproduced in simulations), pseudo-ECGs for WT/D242N K_v7.1-KCNE1 heterozygous conditions were computed. In Figure S5 the results from simulations run exactly at the same heart rates experimentally recorded in twin #1 at baseline and under exercise (84bpm vs. 163bpm plus β -adrenergic stimulation) are shown. Only slight rate adaptation can be observed, and the simulated QTc was significantly prolonged from 387ms to 493ms, similarly to experimental recordings (Figure S5 and Table S1).

4. Discussion

In this study we have characterized the LQTS-associated mutation D242N in the K_v7.1 channel. We observed that: 1) the affected patients carrying this mutation lost the adaptation to fast heart rate, being this effect fairly well reproduced by our computing model. 2) The voltage-dependence of D242N K_v7.1 channels was similar than in the WT channels, although the activation kinetics was slower and a large decrease in the inactivation was observed. 3) Neither cell surface targeting nor assembly with KCNE1 was affected. However, 4) upon co-assembly with KCNE1, the activation curve was shifted to positive potentials by 50mV. This shift prevents D242N K_v7.1-KCNE1 channel opening at physiological membrane potentials. All these findings uncover the molecular mechanism of the LQTS phenotype presented by patients carrying D242N K_v7.1 mutation and also a K_v7.1 residue involved in the KCNE1-mediated voltage dependent activation of K_v7.1 channel.

Cell surface targeting of D242N K_v7.1 in the absence of KCNE1 was not affected when compared to WT K_v7.1 channels, thus suggesting that the electrophysiological changes

observed are due to modifications in the gating of the channel and not to problems in the trafficking of the channel, as expected for a mutation in the S4 segment (main part of the voltage sensor domain of the channel). The alignment of the S4 segment sequences of different voltage-gated channels reveals that Kv7.1 is unique among them. It displays an unusual paucity of net positive charges (+3) compared to other members of the voltage-gated ion channels superfamily, such as Kv1.1 (+7), even within Kv7 family (+5) (Figure 7A). Interestingly, residue D242 is not present in other potassium channels families, which might be indicative of a particular role in Kv7 channels. The loss of a negative charge caused by the mutation under study, D242N, unbalances the charge equilibrium of the distal S4. But surprisingly, the voltage-dependence activation of this mutant channel was similar to that observed in WT Kv7.1 channels. This behavior is comparable to the effect of D242W substitution, which does not shift the voltage-dependence activation of the channel [30]. On the contrary, the D242A mutation shifts the voltage dependence of activation to negative membrane potentials and converts this channel into a constitutively open channel [31]. These data suggest that residue volume might be more important than the charge at this position. On the other hand, D242N Kv7.1 current exhibited slower activation kinetics and a decreased inactivation, confirming the functional role of S4 in the channel gating [31, 32].

The charge paucity of the C-terminal half of the S4 domain has been proposed to underlie the susceptibility of Kv7.1 to exhibit different behaviors upon KCNE interaction [31]. Co-assembly of D242N Kv7.1 and KCNE1 subunits markedly shifted the voltage dependence of activation to positive potentials by 50mV and thus, at physiological membrane potentials (from -20 to +20 mV), the currents elicited by the mutant complexes were negligible producing the delayed repolarization observed in LQTS patients carrying the D242N mutation. It has been described that D242W Kv7.1-KCNE1 complexes also expressed currents of small magnitude compared to WT Kv7.1-KCNE1, which was attributed to a low membrane expression of the complex [30]. However, here we show that D242N Kv7.1-KCNE1 complexes are localized in the cell membrane and their assembly with this beta-subunit is not modified *vs.* WT Kv7.1-KCNE1. The D242A mutation did not produce significant effects on the voltage dependence when the channel was assembled with KCNE1 subunits [31]. Based on these data, it is unlikely that the effect of D242N mutation can be attributed solely to the negative charge lost. It is worthwhile to consider residues M238 and L239, in the S4 segment, that according to Smith's model [33] are in the proximity of D242 (Figure 7B). Mutations of these residues to the more voluminous tryptophan generated very small currents only in the presence of KCNE1, which suggested that these residues might pack tightly when the channel assemble with KCNE1 [30]. Moreover, mutation of two phenylalanines that have been predicted to be involved in the modulation of Kv7.1 channels by KCNE1 (F232 in S4 and F279 in S5) to different amino acids, produced a clear side-chain volume-dependent shift in the midpoint of the activation curve when the channels were expressed together with KCNE1 [23, 34]. Thus, it might be hypothesized that the slight increase in volume from aspartic to asparagine [35] and/or the presence of a polar uncharged residue might modify the packing environment between D242N Kv7.1 and KCNE1. The rearrangement of the WT Kv7.1 channel in the presence of KCNE1 induces a positive shift in the activation curve of the channel [4]. D242N is boosting this feature, either by stabilizing

the resting state of the channel or by destabilizing the activated state. In fact, deactivation kinetics is much faster in D242N K_v7.1-KCNE1 than in WT K_v7.1-KCNE1 channels by about 10-fold. These data highlights the importance of D242 in the fine-tuning modulation of K_v7.1 activity by the KCNE1 subunit.

The potassium current generated by the heteromeric D242N/WT K_v7.1-KCNE1 channels exhibited a similar magnitude and activation kinetics than WT K_v7.1-KCNE1 channels, likely due to the mild shift in the voltage-dependence of activation induced by this mutation. However, the deactivation kinetics of the current generated by these complexes remained faster than WT K_v7.1-KCNE1, which may affect the fast heart rate adaptation. These results suggest that, in cardiomyocytes, this mutation would induce a delay in the repolarization phase of the cardiac AP. This effect ultimately triggers the observed prolongation of the QT interval detected in D242N-LQTS1 patients and an almost complete loss of the adaptation to high heart rates. This result may be attributed to the acceleration of the deactivation kinetics observed in the D242N/WT K_v7.1-KCNE1 channels; since the contribution of I_{Ks} to the AP repolarization is rate dependent [28] and the channels accumulate in the pre-open states, which leads to a rate-dependent increase of current, causing AP shortening [22, 36]. Our results suggest that this mutation, also in heterozygous, favors the closed state of the channel, which indicates that they cannot properly respond to heart rate changes like WT K_v7.1-KCNE1 channels. The two symptomatic twins with a confirmed diagnosis of LQTS1, by clinical and genetic studies, were recommended with beta-blockers and they did not exhibit recurrences of syncope during a 10 years follow-up, thus confirming that this mutation in heterozygous is mostly relevant under β -adrenergic stimulation (i.e., during exercise).

Our computational analysis fully confirmed this interpretation and provided a plausible explanation for the clinical manifestations of the mutation with a slight decrease in the QT interval during exercise, but a great increase in the QTc. While the mutation has negligible effects on the action potential at baseline, a failure to adapt to exercise conditions was identified (see Figure 6A and Figure S5). At the cardiomyocyte level, the I_{Ks} current amplitude increases with heart rate and β -adrenergic stimulation. In our simulation model, we were able to separately quantify the effects of these two main factors participating to exercise adaptation. Our simulation results suggest that both factors equally contribute since if rate is increased with no β -adrenergic stimulation, a small decrease in APD adaptation is observed (e.g. WT: from 356 to 298 ms, D242N/WT: from 359 to 305 ms, at 180 bpm) and a similar effect is produced by simulation of β -adrenergic stimulation alone with no change in rate (WT: from 298 to 267 ms, D242N/WT: from 305 to 281 ms).

5. Conclusion

We provide evidence that the D242N K_v7.1 mutation exhibits a loss-of-function when associated with KCNE1 regulatory subunits and that the decrease in the current is caused by a positive shift of 50mV in the activation curve rather than trafficking defects. The patients carrying this mutation lost the adaptation to fast heart rate, being this effect fairly well reproduced by our computing model.

Disclosures

None.

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Figure legends

Figure 1

ECGs of the patients. ECG detail (leads I, II, III) of affected twins sister (**A**) and brother (**B**) taken at baseline and peak exercise showing a significant prolongation of QT interval. Frequency QT corrected for the frequency using Bazzet (QTc) and Rautaharju methods (QTp).

Figure 2

Trafficking and characterization of D242N K_v7.1 channels. HeLa cells were transfected with either K_v7.1-CFP (WT) or D242N K_v7.1-CFP (D242N), and Akt-PH- pDsRed as plasma membrane (PM) marker (A) and DsRed-ER as endoplasmic reticulum (ER) marker (B). Aa-Ac WT PM surface expression. Ad-Af D242N PM surface expression. Ba-Bb WT ER retention. Bd-Be D242N ER retention. Aa, Ad, Ba and Bd show K_v7.1 channels, either WT or D242N, in grey scale. Ab, Ae, Bb and Be show merge images of K_v7.1 channels (WT or D242N) in green, and marker (PM or ER) in blue. Colocalization is highlighted in cyan. Ac, Af, Bc and Bf are histograms of the pixel-by-pixel analysis of the arrow section in merge panels. Green profile corresponds to channels (WT or D242N) and blue profiles to subcellular marker (PM or ER). Scale bars, 10 μm. **C** and **D** display representative currents of WT and D242N K_v7.1 channels after applying the pulse protocol shown at the top of the Figure. Inset: Enlarged tail currents recorded at -50mV. **E**, I-V relationships of current measured at the end of the 5.5s depolarizing pulses for WT (black circles) and D242N K_v7.1 channels (white circles). **E**, Activation curves obtained by plotting the normalized tail currents vs. membrane potential with midpoint activation (V_h) and slope (s) values of $V_h = -19.9 \pm 3.2$ mV, $s = 11.3 \pm 2.2$ mV vs. $V_h = -13.8 \pm 4.1$ mV and $s = 14.7 \pm 1.7$ mV, for WT and D242N channels, respectively ($P > 0.05$, $n = 9/6$). **G** shows current traces of WT and D242N K_v7.1 channels obtained after applying a 3-pulse protocol shown in the top. Cells were pulsed at +40, +60, +80 and +100mV for 2s before applying a 20ms interpulse at -130mV to allow channels to recover from inactivation. The membrane potential was returned to the test potential after 20ms to -130mV interpulse to assess the extent of recovery. **H**, plot of the degree of inactivation measured at each test potential. E, F and H, values are mean ± SEM of $n = 10/5$ experiments, * $P < 0.05$ and ** $P < 0.01$.

Figure 3

KCNE1 produces a positive shift of D242N K_v7.1-KCNE1 voltage dependence of activation. HeLa cells were transfected with either K_v7.1-CFP (WT) or D242N K_v7.1-CFP (D242N), KCNE1YFP, and Akt-PH- pDsRed as PM marker and DsRed-ER as ER marker. Aa-Ad WT-KCNE1 PM surface expression. Ae-Ah D242N-KCNE1 PM surface expression. Ba-Bd WT-KCNE1 ER retention. Be-Bh D242N-KCNE1 ER retention. Aa, Ae, Ba and Be show K_v7.1 channels, either WT or D242N, in greys scale. Ad, Ag, Bc and Bg show merge images of K_v7.1 channels (WT or D242N) in green, KCNE1 in red, and subcellular marker (PM or ER) in blue. Triple colocalization in highlighted in white. Ad, Ah, Bd and Bh are histograms of pixel-by-pixel analysis of the arrow section in merge panels. Green profile

corresponds to channels (WT or D242N), red profile to KCNE1 and blue profile to subcellular marker (PM or ER). Scale bars, 10 μm . Representative current traces elicited by WT Kv7.1-KCNE1 (**A**) and D242N Kv7.1-KCNE1 channels (**B**). The pulse protocol is shown at the top. **C**, I-V relationships after plotting the current magnitude at 5.5s vs. membrane potential. **D**, The voltage dependence of activation obtained by plotting the tail current vs. membrane potential exhibited V_h and s values of $V_h=+32.9\pm 3.6\text{mV}$, $s=16.6\pm 0.7\text{mV}$ vs. $V_h=+82.6\pm 3.9\text{mV}$ ($P<0.01$, $n=10/13$) and $s=13.4\pm 1.4\text{mV}$, for WT and D242N channels, respectively ($P>0.05$, $n=10/13$). Black circles: WT Kv7.1; white circles: D242N Kv7.1. Values are mean \pm SEM of $n=10/13$ experiments. * $P<0.05$ and ** $P<0.01$

Figure 4

Rate dependency of D242N Kv7.1-KCNE1 currents. **A**, Rate-dependent current increase of WT (filled symbols) and D242N (empty symbols) Kv7.1-KCNE1 channels at different frequencies: 1Hz (squares), 2Hz (circles) and 2.5Hz (triangles). The pulse protocol consisted in 70 pulses of 350ms in duration at different frequencies (1, 2 and 2.5Hz) is represented at the top. **B**, Graph showing the instantaneous current amplitude of the pulses of each train plotted as a function of pulse number. Values are mean \pm SEM of $n=5$ experiments.

Figure 5

Characteristics of the heterozygous D242N/WT Kv7.1-KCNE1. **A**, Representative traces obtained after applying the pulse protocol shown at the top. **B**, I-V relationship of the heterozygous condition obtained by plotting the current density at 5.5s vs. membrane potential. The figure also shows the I-V relationships of WT Kv7.1-KCNE1 (solid line) and that of D242N Kv7.1-KCNE1 homozygous (shaded line). **C**, Activation curves obtained after plotting the tail current values vs. membrane potential and fit the data with a Boltzmann equation. This panel also shows the activation curves of WT Kv7.1-KCNE1 and that of D242N Kv7.1-KCNE1 homozygous. Values are mean \pm SEM of $n=5$ experiments.

Figure 6

Computational analysis of D242N/WT Kv7.1-KCNE1 on the ventricular AP. Comparison of ventricular APs (**A**), and I_{Ks} current (**B**) in WT Kv7.1-KCNE1 and D242N/WT Kv7.1-KCNE1 conditions for endocardial (left), epicardial (middle), and M (right) cells. Simulations were performed mimicking baseline (60bpm) and exercise (180bpm and β -adrenergic stimulation) conditions, using the ORd model. (**C**) Rate dependence curve for WT Kv7.1-KCNE1 (black) and D242N/WT Kv7.1-KCNE1 (red) simulating the effect of exercise at the cellular level (no β -adrenergic stimulation at CL=1000ms, 25% of β -adrenergic stimulation at CL=700ms, 50% of β -adrenergic stimulation at CL=500ms, 75% of β -adrenergic stimulation at CL=400ms and 100% of β -adrenergic stimulation at CL=333ms).

Figure 7

The C-terminal of S4 segment of Kv7.1 is unique among voltage-gated ion channels. **A**, Amino acid sequence alignment of Kv7.1 transmembrane S4 domain with Kv7.4, another member of the Kv7 family and other representative members of the voltage-gated potassium

channel subfamilies (Kv1.1, Kv2.1, Kv3.1, Kv4.1 and Kv11.1) and voltage-gated ion channel superfamily (Cav1.1 and Nav1.1). The positions of S4 charged residues in Kv7.1 are indicated at the top. Positively and negatively charged residues are colored in green and in red, respectively. The COOH-half region of every voltage-gated ion channel is highlighted in yellow. **B**, Cartoon representation of the closed (top) and opened (bottom) states of Kv7.1 channel based on the Smith et al model [33]. Side view with the extracellular solution above and the intracellular media below. Only two monomers are colored in blue and green for better visualization. The D242 residue is indicated by spheres surrounded by residues in stick representation. Only polar hydrogens are shown. The PyMOL Molecular Graphics System, Version 1.7 Schrödinger, LLC was used.

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