

In Silico Investigation of Short QT Syndrome-Linked Potassium Channel Mutations on Electro-Mechanical Function of Human Atrial Cells

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Abstract

Short QT syndrome (SQTs) patients are prone to atrial arrhythmias. However, the link between SQTs gene mutations and atrial pro-arrhythmia is not well understood. This study investigated the functional impact of two SQTs-related gain-of-function potassium channel mutations on the electro-mechanical activities of human atrial cells.

*A contemporary human atrial action potential (AP) model was coupled to the Rice et al. mechanics model. Markov formulations of the rapid and slow delayed rectifier currents, I_{Kr} and I_{Ks} , the α subunits of which are encoded by the *hERG* and *KCNQ1* genes, respectively, were implemented in wild type (WT), N588K-*hERG* (SQT1), and V307L-*KCNQ1* (SQT2) conditions. The Markov models were validated against experimental data through simulated voltage and AP clamp experiments.*

*The N588K-*hERG* and V307L-*KCNQ1* mutations were found to accelerate atrial repolarisation by increasing outward potassium currents during phase 3 of the AP, which reduced action potential duration at 90% repolarisation (APD_{90}). Secondary effects of the mutations resulted in impaired contractile force, an effect which was lessened when stretch-activated channels (SACs) were incorporated.*

1. Introduction

The short QT syndrome (SQTs) is a recently discovered genetic disorder that is characterised by a short QT interval on the ECG, and is associated with increased incidence of cardiac arrhythmias and sudden death [1]. Symptomatic atrial fibrillation (AF) has been reported as a common clinical presentation of the SQTs [2]. Whereas several studies have investigated ventricular arrhythmia substrates in the SQTs, the underlying mechanisms behind which SQTs increases susceptibility to atrial arrhythmias such as AF remain unclear.

Variants 1 and 2 of the SQTs, referred to hereinafter as SQT1 and SQT2 respectively, are caused by gain-of-function mutations to repolarising potassium channels.

SQT1 is caused by mutations to the *hERG* gene which encodes the α subunit of channels mediating the rapid delayed rectifier current, I_{Kr} , and SQT2 is caused by mutations to the *KCNQ1* gene which encodes the α subunit of channels carrying slow delayed rectifier current, I_{Ks} [1].

McPate *et al.* and other studies demonstrated that the SQT1-related N588K-*hERG* mutation results in severely impaired inactivation, causing a gain-of-function to I_{Kr} [3,4]. The SQT2-related V307L-*KCNQ1* mutation has been shown to cause a gain-of-function to I_{Ks} through a significant negative shift in channel activation [5,6]. Here, an electro-mechanical human atrial cell model was developed to investigate how these mutations with distinct gain-of-function mechanisms create substrates favourable to the development of atrial arrhythmias.

2. Methods

2.1. Model development

Markov formulations of the rapid and slow delayed rectifier currents, I_{Kr} and I_{Ks} , taken from [7] were incorporated into the Colman *et al.* human atrial cell model [8]. The Nelder-Mead simplex algorithm was used to obtain the optimal fit between simulated I_{Kr} and I_{Ks} currents to end pulse (shown) and peak tail currents from whole-cell patch clamp recordings of WT- and SQTs-mutant *hERG* and *KCNQ1* channels expressed in CHO cells, performed at 37°C [3,5] (Figure 1). Conductances were chosen based on the relative contributions of I_{Kr} and I_{Ks} during repolarisation as determined under normal and experimental I_{Kr} blocker (E-4031) conditions [9].

AP clamp experiments were simulated in WT- and SQTs-mutant conditions using mathematical human atrial cell model AP waveforms as dynamic inputs to generate current profiles (Figure 2). WT-/N588K-*hERG* I_{Kr} currents were compared with traces from a previous AP clamp study [10], and WT-/V307L-*KCNQ1* currents were validated against current traces from El Harchi *et al.* [5].

The electrophysiology model was coupled with the Rice *et al.* cardiac myofilament model [11] to construct

an electro-mechanical model. As in a previous study [12], minor modifications were made to transition rates in the mechanics model to fit the Ca^{2+} -tension relationship observed experimentally in human atrial myocytes at 20°C [13], with Q_{10} correction accounting for force development at physiological temperature.

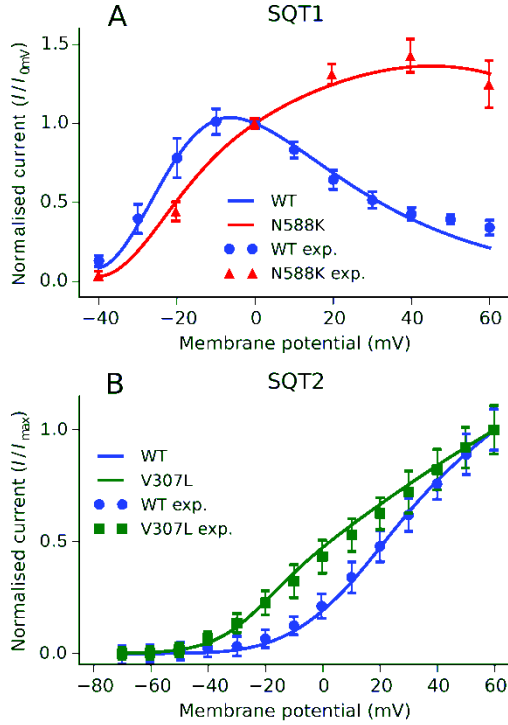


Figure 1: Simulated and experimental end pulse currents from SQT1-related WT- and N588K-hERG I_{Kr} channels normalised to the current at 0 mV (A), and SQT2-related WT- and V307L-KCNQ1 I_{Ks} channels normalised to the maximal current (B).

A stretch-activated current, I_{SAC} , was incorporated into the mechanics model as a source of mechano-electric feedback. The formulation for the current, taken from [14], is given by

$$I_{SAC} = g_{SAC} \cdot P_m \cdot (V_m - E_{SAC}), \quad (1)$$

where g_{SAC} is the maximum conductance of the channel, P_m is the stretch-dependent open probability of the channel, and E_{SAC} is the reversal potential, set to 0 mV [15]. I_{SAC} was assumed to be permeable to Na^+ , K^+ , and Ca^{2+} ions with a permeability ratio of $P_{Na}:P_K:P_{Ca} = 1:1:1$, where P_X is the permeability of I_{SAC} to ions of type X.

2.2. Single cell investigations

The effect of the SQTS-related mutations on the AP morphology, which exhibited a spike and dome shape,

was determined both with and without inclusion of I_{SAC} . To quantify SQTS-induced changes to electro-mechanical function, the APD_{90} and active force were computed. Active forces were normalised to the WT no I_{SAC} case.

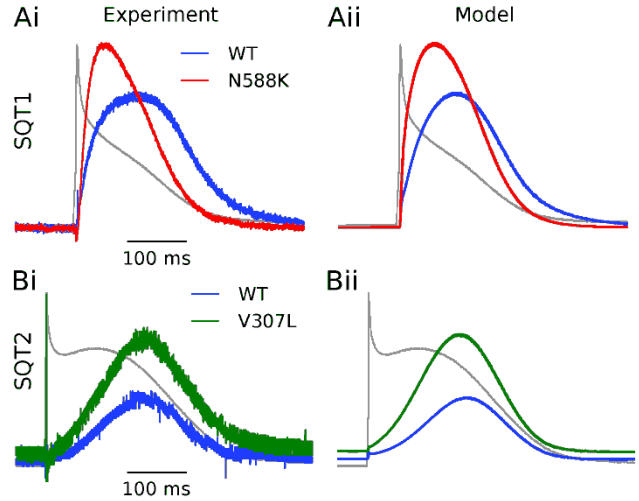


Figure 2: Comparison of experimental and simulated WT- and N588K-hERG I_{Kr} current profiles from an AP clamp using the Nygren *et al.* human atrial cell model [16] (Ai-Aii), and WT- and V307L-KCNQ1 I_{Ks} current profiles from an AP clamp using the CRN human atrial cell model [17] (Bi-Bii). Amplitudes of simulated current profiles are scaled to match those of the experimental traces for ease of comparison.

3. Results

3.1. Model validation

The Markov formulations of I_{Kr} and I_{Ks} were found to accurately reproduce experimental kinetics data (Figure 1). Markov chain formulations are based on specific kinetic states, enabling more physiologically accurate simulations of kinetic changes in SQTS mutations; namely (i) profoundly impaired inactivation in N588K-hERG mutant channels and (ii) the significant negative shift in channel activation associated with the V307L-KCNQ1 mutation.

Simulated AP clamp experiments revealed that kinetic changes underlying the SQTS gene mutations alone resulted in a gain-of-function to I_{Kr} (SQT1) and I_{Ks} (SQT2). Furthermore, Markov formulations of I_{Kr} and I_{Ks} reproduced morphological changes to current profiles seen experimentally [10,5].

3.2. SQTS effects on electrical activity

The increased current density of I_{Kr} in the SQT1-related N588K-hERG mutation resulted in accelerated

atrial repolarisation during phase 3 of the AP, in agreement with a previous study [18]. The SQT2-mediated increase in I_{Ks} due to the V307L-KCNQ1 mutation abbreviated atrial repolarisation to a lesser extent than the SQT1 mutation (Figure 3A). I_{SAC} was found to modulate the AP in a subtle way. Given that E_{SAC} was set to 0 mV, at positive membrane potentials SACs produced an outward current, whereas at negative membrane potentials an inward current was generated

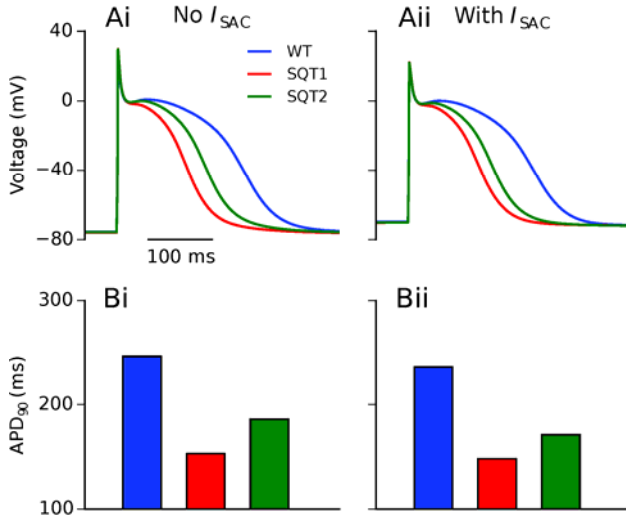


Figure 3: Effects of the SQT1-related N588K-hERG and SQT2-related V307L-KCNQ1 mutations on the AP morphology with and without I_{SAC} (Ai-Aii). Summary of SQTs-induced changes to APD₉₀ with and without I_{SAC} (Bi-Bii). BCL=1000 ms.

The APD₉₀ was shortened more significantly by the SQT1-related N588K-hERG mutation than the SQT2-related V307L-KCNQ1 mutation at a BCL of 1000 ms (Figure 3B). Upon inclusion of I_{SAC} , the difference in APD₉₀ reduction between SQT1 and SQT2 was reduced slightly. This suggests that N588K-hERG I_{Kr} currents are more sensitive to stretch-induced changes in AP morphology than I_{Ks} currents in V307L-KCNQ1 mutant channels. SACs shortened the APD₉₀, which is consistent with several other studies – see [15] for a review.

3.3. SQTs effects on mechanical activity

In addition to changes to the AP morphology, SQTs mutations were shown to modulate mechanical activity. The active force generated during an AP was decreased in both SQTs mutations, more so for the SQT1-related N588K-hERG mutation than the SQT2-related V307L-KCNQ1 mutation at a BCL of 1000 ms (Figure 4A), consistent with the greater reduction in APD. Including I_{SAC} had the effect of slightly offsetting the SQTs-induced negative inotropic effect, causing a decrease in

the proportional force reduction between WT and mutation conditions. This effect was more prominent in the SQT1 mutation, reflecting the aforementioned different responses of the SQTs mutant forms to stretch-induced changes in AP morphology. The impact of SQTs mutations on peak normalised active force both with and without I_{SAC} is summarised in Figure 4B.

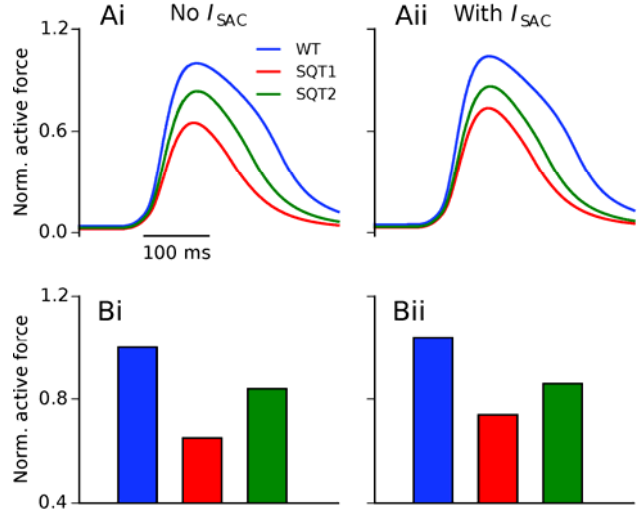


Figure 4: Effects of the SQT1-related N588K-hERG and SQT2-related V307L-KCNQ1 mutations on force generation during an AP with and without I_{SAC} (Ai-Aii). Summary of SQTs-induced changes to normalised active force with and without I_{SAC} (Bi-Bii). BCL=1000 ms.

4. Discussion

Markov formulations of repolarising currents I_{Kr} and I_{Ks} have been incorporated into a human atrial cell model and coupled with an established cardiac mechanics model. The resulting electro-mechanical model has been validated using simulated voltage and AP clamps and used to investigate consequences of SQTs gene mutations on human atrial electro-mechanical function.

Our simulation results showed that two potassium channel mutations in the SQTs with distinct gain-of-function mechanisms both caused accelerated atrial repolarisation due to increased outward K⁺ currents during phase 3 repolarisation. This is likely to reduce the effective refractory period in tissue, stabilising re-entrant circuits. The changes to AP morphology caused by the SQTs mutations led to reduced active force. This is mediated by a decrease in calcium transient amplitude.

Whereas there is currently no reported evidence of reduced atrial contractility in the SQTs *per se*, a recent study identified impaired ventricular systolic function in SQTs patients [19]. It is possible that atrial systolic function is impaired by similar mechanisms, which could potentially facilitate the development of atrial

arrhythmias.

The stretch-activated current, I_{SAC} , was shown to counter the SQTs-induced reduction in active force. This is consistent with a previous study [14], which suggested that SACs may serve as compensatory factors which offset impaired contractility in the SQTs. Increased inotropy in the presence of stretch is due to increased intracellular calcium concentration, $[Ca^{2+}]_i$, which is the trigger for mechanical contraction. Our results indicate that SACs may be a necessary consideration in future studies on cardiac electro-mechanical function.

The more profound reduction in APD_{90} and active force in the SQT1-related N588K-hERG mutant form than the SQT2-related V307L-KCNQ1 mutation is likely a reflection of the relative contributions of I_{Kr} and I_{Ks} to atrial repolarisation. However, pacing rate and autonomic modulation are also likely to be important factors determining the degree to which SQTs mutations alter electro-mechanical function.

In conclusion, an investigation into the effects of SQTs-linked potassium channel mutations on electro-mechanical function of human atrial cells has revealed reduced APD and impaired contractile function as potential mechanisms by which pro-arrhythmia in the human atria is increased.

Acknowledgements

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