Linking a Novel Mutation to its Short QT Phenotype through Multiscale Computational Modelling

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Abstract

The aim of this work was to assess the link between a newly identified KCNQ1 mutation and the short QT syndrome clinically observed in the patients. We applied two human Action Potential models, the ten Tusscher - Panfilov (TTP) and the O'Hara Rudy (ORd). We also simulated the effects of adrenergic stimulation on action potential, since the basal adrenergic tone should likely affect the I_{Ks} influence on QTc in vivo. Finally, we simulated the pseudo ECG taking into account the heterogeneity of the cardiac wall.

Simulations predict a shortening of the action potential consistent with the patient phenotype: using the TTP model the shortening was largely more pronounced (e.g. from 397 to 297 ms in M cells) than with the ORd (e.g. from 332 to 318 ms in M cells). When simulating the β-adrenergic stimulation, the APD shortening was enhanced in ORd model.

Pseudo ECG results confirm the reduction of the QT interval caused by mutation.

1. Introduction

Sudden cardiac death in a young person is a shocking event that has puzzled physicians for decades. In recent years, many of the underlying cardiac pathologies have been discovered. These include structural abnormalities such as hypertrophic cardiomyopathy and nonstructural disorders associated with unstable rhythms that lead to sudden cardiac death.

The best known of these "channelopathies" are the long QT syndromes, which result from abnormal potassium and sodium currents in cardiomyocytes. More recently, interest has been growing in a disorder that may carry a similarly prognosis but that has an opposite finding on electrocardiography (ECG), the Short QT syndrome (SQT). SQT is a heterogeneous genetic channelopathy that causes both atrial and ventricular arrhythmias and that has been documented to cause sudden cardiac death (SCD) in the setting of an abnormally short QT interval, ranging from 248 to 300 ms (Bazett corrected QT, (QTc)), and structurally normal heart. Six genes encoding ion channel subunits have been implicated in the pathogenesis of SQT syndrome, and further genetic culprits are suspected.

SQT2 is caused by gain of function mutations in the *KCNQ1* gene encoding the alpha subunit of the $K_v7.1$ channel (I_{Ks}) [3]. The index case of the family was a 37 years old man who died unexpectedly. His son, an athletic healthy 23 years old, was referred recently for cardiac evaluation. ECG showed sinus bradycardia (55bpm), normal PR, QRS, and slightly short QTc interval of 356ms. Exercise test was normal of note; QTc shortened with the exercise, measuring 350ms at maximum workload.

In this study, we investigate and simulate the biophysical and cellular phenotype of a new characterized *KCNQ1* mutation, F279I, found in a patient with SQT, on the action potential clamp and on the pseudo ECG using two different human cardiac action potential (AP) models, the ten Tusscher-Panfilov (TTP) and O'Hara-Rudy (ORd).

2. Methods

2.1. Electrophysiology technique

A blood sample from the patient and his sister was sent for genetic study of most prevalent genes related to channelopathies. Genetic studies on the index case son's DNA excluded mutations in *KCNH2, SCN5A, KCNE1, KCNE2, KCNJ2* and *RyR2*. Analysis of the *KCNQ1* gene revealed a novel heterozygous mutation (change from phenylalanine to isoleucine at 279 in $K_v7.1$ (p.F279I) within the S5 transmembrane segment), which was not

reported before. Among the K_v7 family $K_v7.1$ is the unique channel carrying a phenylalanine at the equivalent position; suggesting that this residue might be important for the unique properties of the cardiac I_{Ks}. No other potential SQT mutations were identified and neither in his daughter. To examine the effects of the F279I in this physiologically relevant channel complex, either wild type (WT) $K_v7.1$ or the mutant F279I subunit were co-transfected with KCNE1.

Membrane currents were measured using the perforated-patch configuration of the patch-clamp technique with the amphotericin. Currents were recorded using an Axopatch 200B amplifier (Axon Instruments), were filtered at 1 kHz (4-pole Bessel filter) and sampled at 2 kHz. Micropipettes were pulled from borosilicate glass capillary tubes (Narishige, GD-1) on a programmable horizontal puller (Sutter Instruments Co.) and heat-polished with a microforge (Narishige). Micropipette resistance was 1-3 MΩ. Capacitance and series resistance compensation were optimized. pClamp version 9 software (Axon Instruments) was used for data acquisition and analysis. Currents were recorded at room temperature (21-23ºC) at a stimulation frequency of 0.03 Hz. The voltage dependence of activation curves were fitted with a Boltzmann equation: $y = 1/[1 + \exp(-(V-V_h)/s)]$, in which s represents the slope factor, V the membrane potential and V_h the voltage at which 50% of the channels are open.

2.2. Computational analysis

To evaluate the electrophysiological consequences of the F279I mutation at the level of the cardiac AP, we introduced the I_{Ks} model obtained with the mutation, modifying the ten Tusscher-Panfilov (TTP) human ventricular myocyte model [1]. We changed the activation kinetics in agreement with voltage-clamp recordings of $K_v7.1$ current in the presence of KCNE1 to simulate the mutant condition (Table 1).

To test the model-dependency of the simulation results, we carried out the simulation also using the O'Hara-Rudy (ORd) human ventricular myocyte model [2]. We repeated all the simulations, with both models, by introducing the effects of β-adrenergic stimulation by increasing I_{Ks}, (scaled by a factor of 2.66), I_{CaL} (scaled by a factor of 1.5) and I_{up} (scaled by a factor of 1.5) according to a previous computational study [4].

Single cell simulations. Model differential equations were implemented in Matlab (Mathworks Inc., Natick, MA, USA) and solved with a variable order solver (ode15s). Pacing at 1 Hz was maintained until a steady state AP was reached and APD was measured as the interval between AP upstroke and the 90% repolarization level (APD_{90}).

Multicellular simulations. One dimensional fiber (1.64 cm length) composed by 25 endo-, 25 M-, 50 mid- and 65 epi-cardial cells has been considered. Model equations have been translated into cellML language using COR environment and monodomain equations have been solved with Chaste Software [5]. Pseudo-ECG signal has been computed as described by Glukhov et al. [6].

3. Results

3.1. Electrophysiological results

The mutant channel displayed ~1.8 fold acceleration of the fast component of the activation kinetics in comparison to the WT (Table 1). Furthermore, the initial sigmoidal activation, characteristic of WT was absent (Figure 1, middle panel).

The voltage dependence of channel opening for the mutant was shifted towards more negative potentials, without changes in the slope (Figure 1, bottom panel; Table 1). Altogether, the shift in the activation curve and the faster activation kinetics indicate that F279I channels open faster and at more negative potentials than WT channels. These biophysical properties would predict a gain of function.

Table 1. Activation kinetics and voltage dependence of WT and F279I channels. V_h is the voltage at which 50% of the channels are open, s represents the slope, τ_f and τ_s are the time course of activation, fast and slow, respectively.

	WT	F279I
V_h (mV)	32.7 ± 3.2	7.0 ± 4.1*
s (mV)	17.1 ± 0.8	17.9 ± 1.2
τ_f (ms)	794.1 ± 35.9	431.8 ± 27.4**
τ_s (ms)	3564.3 ± 481.2	2781.5 ± 209.6

mean±SEM *: P<0.001; **: P<0.01.

Table 2. Action potential duration at 90% repolarization (APD_{90}) of ventricular (endocardial, epicardial and midmyocardial) cells with WT and F279I I_{Ks}, without and with β-adrenergic stimulation (β-as), simulated with TTP and ORd models.

		APD_{90} (ms)					
		Endo		Epi		M	
		TTP	ORd	TTP	ORd	TTP	ORd
Basal	WT	300	261	301	228	397	332
	F279I	194	251	196	220	297	318
β-as	WT	242	246	242	211	340	297
	F279I	149	226	151	195	238	274

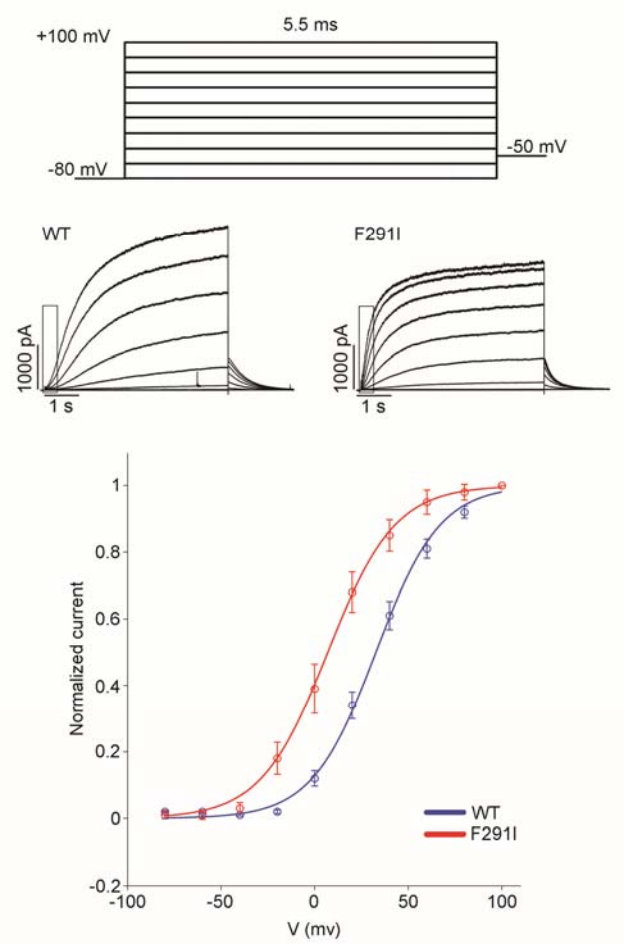

Figure 1. Top: voltage clamp protocol; pulses were applied from a holding potential of -80 mV to +100 mV in 20 mV-steps. Tail currents were recorded at -50 mV. Middle: WT (left) and F291I (right) currents records. Bottom: activation curves were obtained by plotting the normalized tail current versus the membrane potential. WT blue curve, F291I red curve.

3.2. Computational results

The TTP ventricular cell computer model was used to study the effects of mutant channels on AP morphology in epicardial and M cells (Figure 2). In particular, concerning the steady state activation we introduced a negative shift of 25 mV between wild type and mutant. In addition, we scaled the activation time constant, τ_f, and τ_s, by a factor equal to the ratio between measured F279I, and WT values (Table 1).

Simulated I_{Ks} in the F279I homozygous condition shortened the AP with respect to the WT one, from 397 to 297 ms in M cells (Figure 1A, right panel). The F279I-induced AP shortening is due to the presence of a significant increase in the outward K^+ current during phases 3 of the AP (Figure 2A, lower panels). The F279I channel did not induce significant additional changes in AP morphology with respect to WT. When simulating the β-adrenergic stimulation, the APD behavior was similar, as shown in Table 2 and figure 2B.

In the ORd model the mutation also induced APD shortening, but much less pronounced (Figure 3A). APD reduction was almost negligible in basal conditions while it was enhanced by adrenergic stimulation (up to 23 ms shortening in M cells, Figure 3B, right panel).

Pseudo-ECG was simulated in order to assess the QT interval shortening using both models as described in the methods. The results are shown in figure 4: where we can appreciate a significant reduction of the interval using the TT model and less with the ORd.

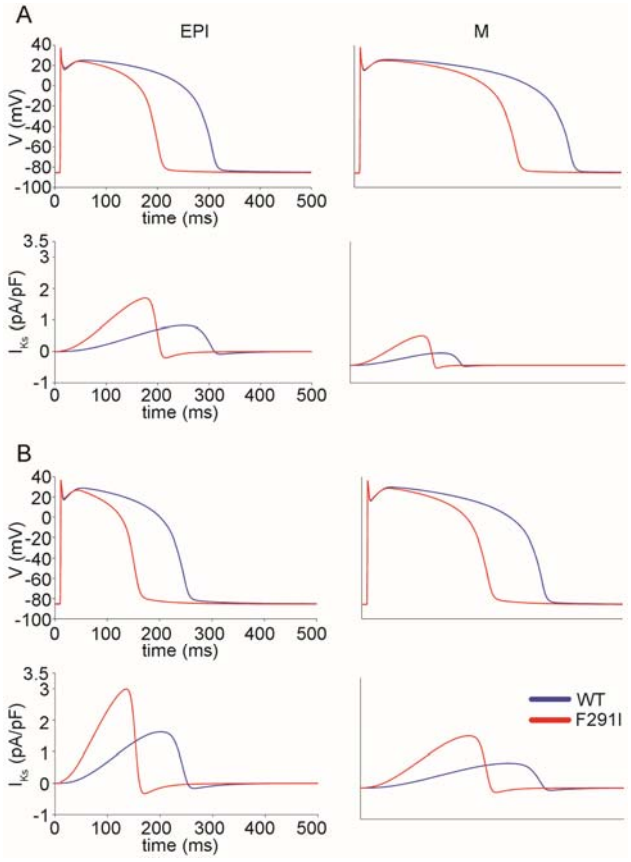

Figure 2. (A) Comparison of ventricular AP (top) and I_{Ks} current (bottom) in wild-type (WT, blue curve) and F279I (red curve) conditions for epicardial (left) and M (right) cells (basic cycle length: 1000 ms), simulated using the TTP model. (B) Simulation of β-adrenergic stimulation in the same conditions as in (A).

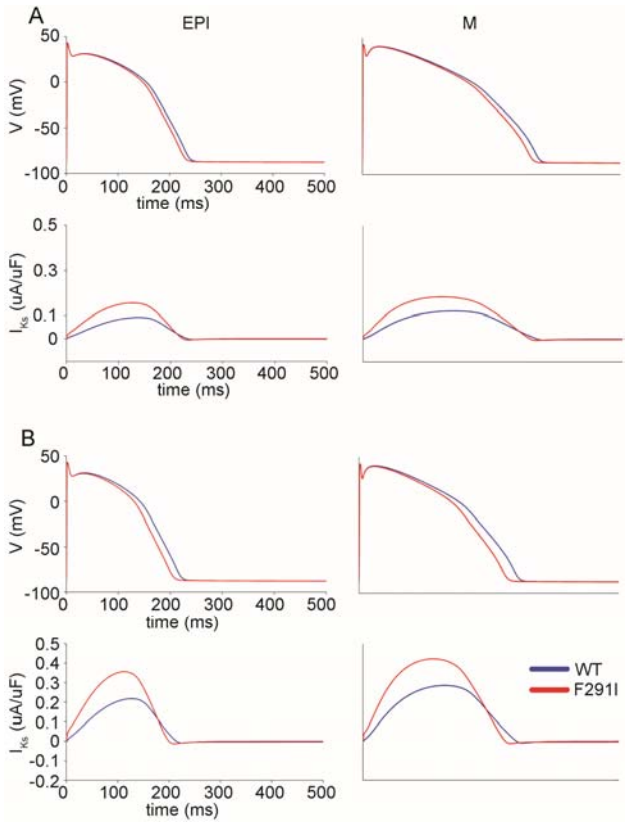

Figure 3. (A) Comparison of ventricular action potentials (top) and I_{Ks} current (bottom) in wild-type (WT, blue curve) and F279I (red curve) conditions for epicardial (left) and M (right) cells (basic cycle length: 1000 ms), simulated using the ORd model. (B) Simulation of β-adrenergic stimulation in the same conditions as in (A).

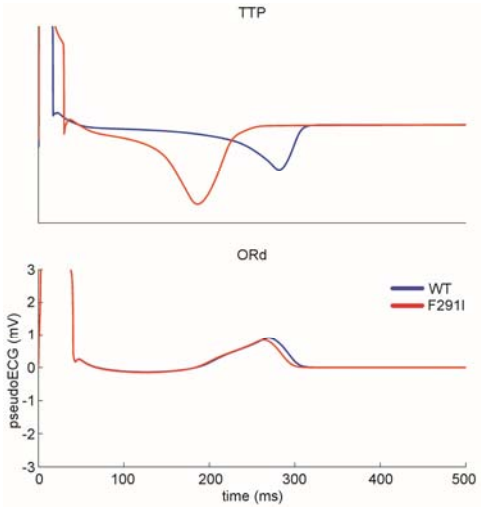

Figure 4. Pseudo ECG performed with TTP (top) and ORd (bottom) model.

4. Discussion and conclusion

$K_v7.1$ is the alpha subunit of the ion channel complex underlying I_{Ks} that, together with I_{Kr}, are the major repolarizing current in ventricle. Gain of function mutations on $K_v7.1$ cause SQT2. This mutation modifies the gating of the WT channel.

The electrophysiological characteristics associated with the mutation are expected to lead enhance repolarization with shortening of the QT interval (leftward shift of the activation curve, faster activation kinetics).

The use of mathematical models to simulate mutant conditions proved useful in order to demonstrate its effects on the AP. The significant differences between the results with the two models is due to the different formulation of I_{Ks}. The weight of this current in human APD is actually still a matter of debate and deserves a detailed analysis. Also the compute of the pseudo ECG confirmed the shortening effects of the QT interval.

References

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