A Human-Based Computational Investigation Into Sarcomeric and Ionic Remodelling in Hypertrophic Cardiomyopathy

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Abstract

Hypertrophic cardiomyopathy (HCM), an inherited cardiac disease, is one of the leading causes of sudden cardiac death in the young. There is no pharmacological therapy in use that specifically targets HCM pathophysiology. HCM caused by mutations in the gene coding for the sarcomeric protein troponin T is particularly arrhythmogenic; the mechanisms remain unknown. Guided by human experimental data, we investigate sarcomeric remodelling caused by the R92Q troponin T mutation and HCM ionic remodelling using a human-based computational model of an adult ventricular cardiomyocyte. Arrhythmogenic triggers in the form of early afterdepolarisations (EADs) were absent when considering R92Q sarcomeric remodelling alone, but sarcomeric remodelling increased the frequency of EADs associated with HCM ionic remodelling. An arrhythmogenic ionic mechanism was identified: the sarcomeric mutation increased calcium myofilament sensitivity which led to prolonged calcium transient decay, resulting in prolonged inward I_{NCX} which generated EADs. This pathway was targeted in the simulations with a sarco/endoplasmic reticulum Ca^{2+}-ATPase activator, which reduced EAD frequency. This highlights the potential of computational precision medicine to investigate mutation-specific pathomechanisms and identify therapeutic targets in inherited cardiomyopathies.

1. Introduction

Hypertrophic cardiomyopathy (HCM) is an inherited cardiac disease, with a frequency of one in 500, characterised by thickening of the interventricular septum and left ventricle. It is caused by mutations in genes encoding proteins in the sarcomere. Carriers of the *TNNT2* missense mutation R92Q, affecting the troponin T (TnT) regulatory protein located on the thin-filament of the sarcomere, have a higher incidence of sudden cardiac death compared to other *TNNT2* mutations, other sarcomeric mutations and patients with no identifiable mutation [1]. It is currently not known why the R92Q TnT sarcomeric mutation is particularly arrhythmogenic.

The cellular electrophysiological properties of cardiomyocytes influences arrhythmogenicity. Cellular ionic remodelling, that is, up- and down-regulation of various ion currents in the cell, is known to occur in HCM and has been characterised in isolated human cardiomyocytes from HCM myectomy patients [2].

Sarcomeric mutation-induced remodelling is thought to lead to this downstream HCM ionic remodelling. With the R92Q TnT mutation, this is thought to be due to an increase in Ca^{2+} myofilament sensitivity, as shown in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) and transgenic mice [3, 4].

Previously, a cellular computational electromechanical model of R92Q sarcomeric remodelling was studied [5]. Separately, there exists a cellular computational electrophysiological-only model of HCM ionic remodelling [6]. Here, we investigate the relative contributions of the sarcomeric and ionic aspects of remodelling to the HCM cellular pro-arrhythmic phenotype using a combined computational electromechanical model of both R92Q sarcomeric and HCM ionic remodelling. The model is exploited to investigate mutation-specific pathomechanisms in HCM, and uncover tailored therapies for their correction in the high-risk group of patients with thin-filament mutations.

2. Methods

ToR-ORd-Land was used as the baseline electromechanical model of a human ventricular cardiomyocyte [7]. Upon simulated pacing, this model outputs action potentials (APs), intracellular calcium transients (CaTs) and active tension (T_a) curves (Figure 1). A population of models (PoM) approach was used to account for cell-to-cell and patient-to-patient variability; 1000 initial models were generated by varying the conductances using latin hypercube sampling between 50–150% of the original value for major ionic currents as in [6].

Calibration of AP, CaT and T_a curves to human non-diseased myocyte experimental data as described in [5–8], reduced the population down to 440 models to form the

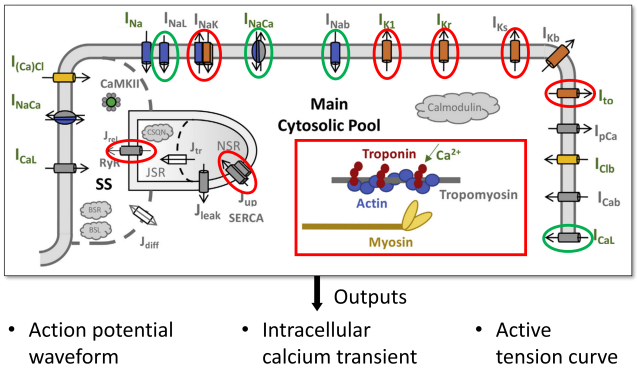

Figure 1. ToR-ORd-Land electromechanical model of a human ventricular cardiomyocyte [7]. Green and red circles: ionic current up- and down-regulation, respectively, as per HCM ionic remodelling. Red square: HCM sarcomeric remodelling.

non-diseased control population. From this, three diseased populations were generated by varying parameters in the models. The R92Q sarcomeric remodelling population was generated by increasing Ca^{2+} myofilament sensitivity (Ca_{50}; -30%) and preferring the non-blocking tropomyosin state (K_{TmBlock}; -20%) for all models as in [5], and as per experimental data [3, 4]. A HCM ionic remodelling population was generated by varying 17 parameters, mainly ion channel conductances, as in [6] (except +10% for I_{CaL} due to new data), as informed by experimental data [2]. Finally, a combined R92Q sarcomeric and HCM ionic remodelling population was generated using both sets of parameters simultaneously. The simulated diseased populations recapitulated the HCM phenotype as per experimental data [2, 3, 5], including hypercontractility and prolongation of action potential duration (APD), relaxation and CaT decay (data not shown).

Simulations were performed in MATLAB (The MathWorks Inc., Natick, MA, USA). Each model was paced at 1 Hz until steady-state, only the last beat was taken into consideration. A stimulus current of $-53\,\mu\text{A}/\mu\text{F}$ of 1 ms duration was used. Early afterdepolarisations were detected with the threshold: $\mathrm{d}V/\mathrm{d}t > 0.005$ mV/ms at any point 150 ms after V_{peak}, where V is the membrane potential.

3. Results

For an arrhythmia to occur, a trigger is required which is timed within a vulnerable window and which occurs within a suitable sustaining substrate of the heart. One type of trigger of arrhythmias is early afterdepolarisations (EADs) — these are abnormal depolarisations in phase 2 or 3 of the AP. The fraction of models which displayed EADs in each of the populations is shown in Figure 2a. No EADs were observed in the control and R92Q sarcomeric remodelling populations. Combined R92Q and ionic remodelling (R92Q + Ionic) was more arrhythmogenic than HCM ionic remodelling (HCM Ionic) alone (7.27% vs 5.45%).

Phenotypically, the main observed difference between both populations was a prolonged CaT decay in the R92Q + Ionic population (Figure 2b; median 556 ms vs 483 ms). Analysis of individual baseline models (without PoM conductance variations) revealed no significant differences between R92Q + Ionic and HCM Ionic in ion channel currents over time, except for the Na^+/Ca^{2+} exchanger current (I_{NCX}). The inward mode of I_{NCX} under R92Q + Ionic remodelling was found to be protracted relative to the HCM Ionic model (Figure 2c). This prolonged inward depolarising I_{NCX} at late times coincides with phase 2 and 3 of the AP, and thus was hypothesised to be a source of EADs.

Late Na^+, L-type Ca^{2+} and NCX current blocks were applied to each population of models from 0 to 60% block. The EAD frequency was measured at each percentage block. NCX current block was more effective than other currents blocks at decreasing EAD frequency in the R92Q + Ionic population as compared to the HCM Ionic population (Figure 2d; $\Delta_{\text{EAD Freq.}} = 0\%$ for 20% NCX block but 1.4% for 20% NaL block). Excluding the increase in calcium sensitivity in the R92Q + Ionic model restored the calcium transient decay time and frequency of EADs to the HCM Ionic model level (data not shown) — demonstrating that the calcium myofilament sensitivity aspect of the R92Q model was responsible for the calcium transient decay time prolongation, rather than the tropomyosin positioning.

It was hypothesised that by decreasing the calcium transient decay time, one may be able to decrease EAD frequency. The sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) mediates the reuptake of calcium, J_{up}, into the network sarcoplasmic reticulum (NSR). We modelled stimulation of SERCA by increasing J_{up} by +60% in all models in the R92Q + Ionic population. This resulted in a decrease in calcium transient decay time (Figure 2b). Correspondingly, the EAD frequency decreased more than two-fold upon SERCA stimulation (Figure 2a) (7.27% to 2.95%). The reduction in calcium transient decay time (Figure 2e) shifts the I_{NCX} curve back to the left (Figure 2f), that is, reducing the level of depolarising current at phase 2 and 3 of the AP, putatively explaining the reduction in EAD frequency.

SERCA stimulation was varied (J_{up} multiplier in the range [0.8, 2.0], on top of the initial 0.75 ionic remodelling multiplier; data not shown) at fast and slow pacing rates for the R92Q + Ionic model. As J_{up} was increased, T_a and CaT amplitude decreased under 4000 ms basic cycle length (BCL) stimulation but the amplitudes increased under 1000 ms BCL stimulation, suggesting that heart rate could alter the effects of SERCA stimulation in HCM.

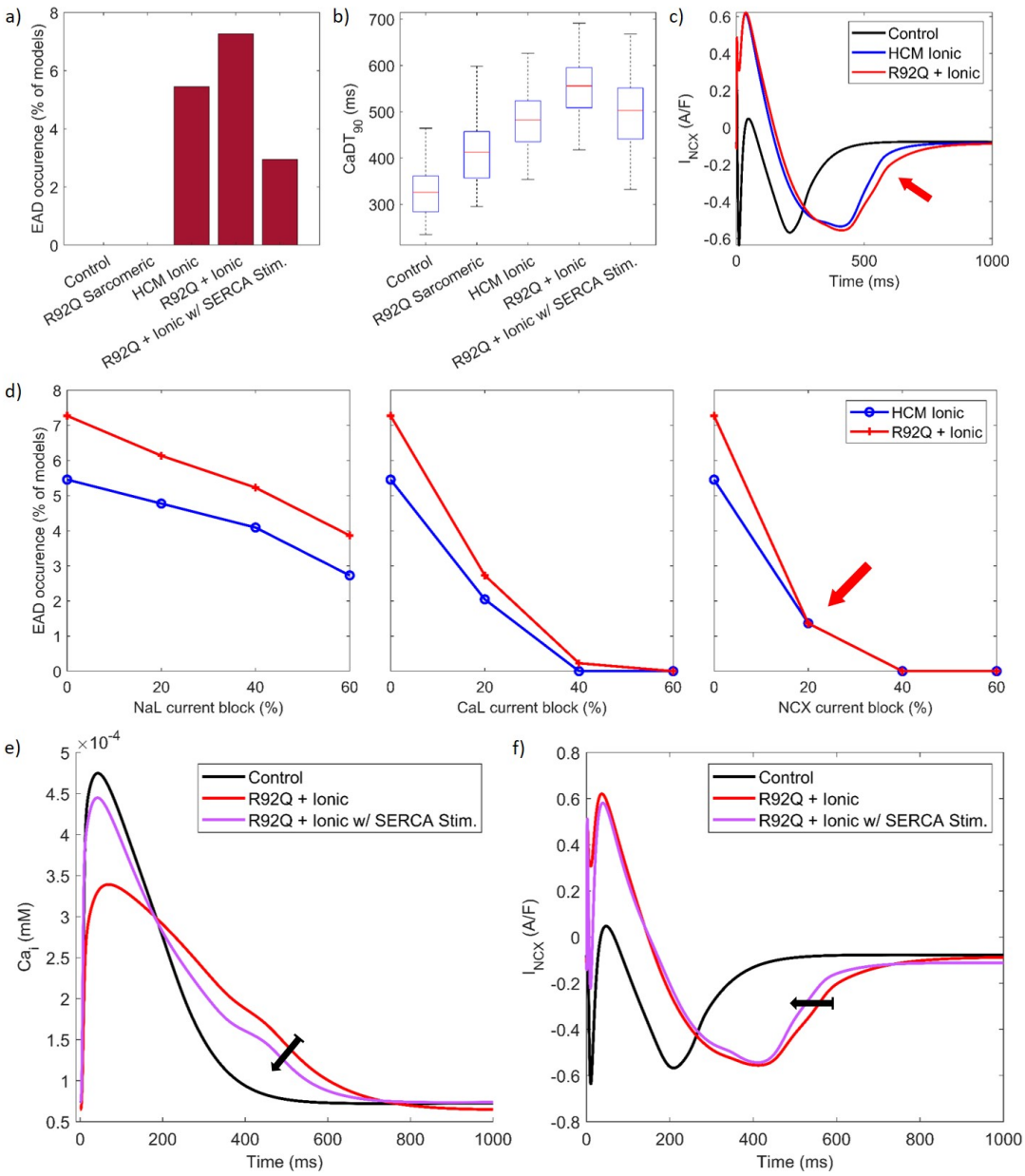

Figure 2. a) EAD frequency per population; b) calcium transient time from peak to 90% decay per population; c) NCX current for ionic only and combined remodelling; d) EAD frequency for ionic and combined remodelling as a function of current blocks; e) intracellular calcium transient under SERCA stimulation; f) NCX current under SERCA stimulation.

While excessive SERCA stimulation caused SR Ca^{2+} overload, moderate stimulation raised the low $[Ca^{2+}]_{NSR}$ in HCM ionic remodelling back up to control levels.

4. Discussion

This computational study has explored the relative contributions of the sarcomeric and ionic aspects of remodelling to HCM arrhythmogenesis. Sarcomeric remodelling in isolation did not cause EADs, only downstream ionic remodelling resulted in EADs. The combination of sarcomeric and ionic remodelling generated more EADs than ionic remodelling in isolation, due to the sarcomeric increase in Ca^{2+} sensitivity.

The canonical mechanism for EAD generation is reactivation of L-type Ca^{2+} channels due to a prolonged ventricular AP. These simulations suggest the following arrhythmogenic mechanism in R92Q TnT HCM simulated

human cardiomyocytes: the sarcomeric mutation raises the calcium myofilament sensitivity, this prolongs the calcium transient decay time, which in turn leads to prolonged depolarising late I_{NCX} resulting in EADs.

This study showed that stimulating SERCA decreased EAD frequency in the presence of combined sarcomeric and ionic remodelling. Experimentally stimulating SERCA, by phospholamban ablation, in R92Q HCM transgenic mice prevented development of the HCM phenotype [9]. In addition, early adenoviral SERCA2a overexpression in transgenic HCM mice improved cardiac function [10]. Our simulations provide the first investigation in a human-based system, overcoming the limitation of significant electrophysiological differences between mice and humans, and support a study in human cells.

No drug currently exists that specifically activates SERCA. Thapsigargin acts on SERCA but inhibits it, rather than increases its activity; the investigational drug istaroxime non-specifically activates SERCA but causes $[Ca^{2+}]_i$ overload through inhibition of Na^+/K^+-ATPase. Current HCM therapeutic research approaches involve sarcomere myosin de-activators such as mavacamten, however this may not be effective in patients with non-thick filament mutations. A Ca^{2+} flux altering SERCA activator may be particularly useful for patients with thin-filament mutations — providing a precision medicine approach arising from pharmacogenomics. The human-based simulations presented here provide mechanistic support to the concept that a specific SERCA activator could provide a novel anti-arrhythmic therapeutic for HCM patients.

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