Populations of Models Show Increased Arrhythmogenicity of the Hypertrophic Cardiomyopathy MYBPC3-c.772G>A Mutation

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Abstract

Several mutations can lead to hypertrophic cardiomyopathy (HCM), making it the most common genetic heart disease. Given the large heterogeneity of phenotypes shown by mutation carriers, robust patient stratification is still lacking, limiting treatment efficacy. In this work, we adopt a population of models approach to investigate the arrhythmic potential of the recently discovered MYBPC3-c.772G>A mutation, to provide an additional risk assessment tool. According to this objective, we built control and mutated populations of 10000 electro-mechanical models of single adult ventricular myocytes, randomizing 19 parameters (maximal conductances, kinetics and contractile element parameters) and calibrating them according to experimental data ranges. The mutation was modeled by applying ionic and contractile remodeling measured on patients myectomy samples. Linear and logistic regressions were performed to assess the parameters and mutation influence on action potential biomarkers and early-after depolarizations (EADs) occurrence. The results highlight a higher propensity of mutated cells in showing an arrhythmic behavior, with Ca^{2+}-related and K^+ currents parameters driving EADs susceptibility. In conclusion, this work provides detailed insights on HCM arrhythmia mechanisms, potentially aiding risk stratification of patients.

1. Introduction

Recent works [1, 2] have discovered a founder-effect mutation in the myosin-binding protein C3, involved in sarcomere organization and thus in cardiac contraction modulation. Experiments on both hiPSCs and adult myocytes obtained from patients myectomies have shown that the mutation determines faster cross-bridge cycling between myosin and actin filaments. However, this does not translate in an increased force generation due to the compensatory effect of electrophysiological remodeling. In particular, lower expression of repolarizing potassium channels, and slower Ca^{2+}-handling kinetics, balance the direct effect of the mutation. However, this 1) comes at the cost of possible increased arrhythmic risk and 2) does not reduce the increased energy consumption given by faster contraction kinetics. Eventually, indeed, the mutation leads to a hypertrophic cardiomyopathy (HCM) phenotype in a population of patients from northeastern Tuscany, Italy. Previous works on sarcomeric mutations have highlighted how ion channel remodeling (mainly K^+ currents down-regulation as well as late sodium and L-type calcium currents up-regulation [3]) leads to increased susceptibility of single cells and tissues to arrhythmia [4, 5]. Here, we build on this to assess the electrophysiological effects of the MYBPC3-c.772G>A mutation. Using mathematical models and adopting a population approach, we evaluate possible increased arrhythmic risk markers given by this specific mutation.

2. Methods

2.1. Single cell model

For the simulations, we employed the "BPS-Land" adult ventricular myocyte model developed in our group [6]. The electrophysiological part is a development of the TorOrd model [7], able to reproduce the inversely proportional relationship between action potential duration and extracellular calcium concentration [8]. The contractile part is represented by the Land et al. model [9], allowing for fully coupled electro-mechanical feedback.

2.2. Population of models

Following the consolidated approaches adopted in our and other laboratories [4, 10], we generated a population of 10000 single cells using Latin hypercube sampling in the range $[-50\%, +200\%]$. We randomized 19 parameters: 11 maximal conductances and fluxes (g_{Na}, g_{NaL}, g_{K1}, g_{Kr}, g_{Ks}, g_{to}, g_{NaCa}, g_{NaK}, $J_{rel,max}$, $J_{up,max}$, P_{CaL}), 3 kinetics parameters (τ_f, τ_s, τ_{Ca}; respectively the voltage-dependent fast and slow I_{CaL} time constants and the Ca^{2+}-dependent one) as well as 5 parameters of the contractile element (n_{perm}, $Ca_{50,TRPN}$, $Ktm_{unblock}$, mu, nu). Simulations lasted 200 s, and the last action

potential was analyzed. Action potential (AP), calcium transient (CaT) and active tension (AT) biomarkers were computed as in previous works [4, 6, 8]. Calibration was performed by accepting only the cells whose biomarkers fell within the experimental ranges (min, max). Since for active tension the latter were too restrictive to obtain a sufficient number of cells in the population, we used a cost function as in [11] to soften this requirement while preserving reliable biomarkers values.

2.3. Modeling the mutation

Model parameters were changed according to mutation-specific experimental reports [1] and unspecific HCM remodeling from previous computational studies [5]. Semi-automatic parameter optimization (based on a gradient-descent approach) was adopted to better constrict the changes based on weaker data sources (e.g. mRNA expression instead of patch-clamp recordings). Changes in APD_{90}, $CaTD_{90}$, CaT_{amp}, AT_{peak} and AT_{RT50} (mut vs. ctrl) from [1] were used to guide the optimization process. The final parameter set defining the c772G>A mutation is reported in Table 1.

Parameter	Scaling Factor	Parameter	Scaling Factor
G_{NaL}	2.4	J_{rel}	0.47
P_{Nab}	2.65	τ_{Caf}	1.35
G_{to}	0.3	τ_{Cas}	1.2
G_{Kr}	0.81	r^2_{cell}	1.9
G_{Ks}	0.55	P_{CaL}	1.02
G_{K1}	0.7	k_{off}	0.8
G_{NCX}	2.15	mu	1.52
P_{NaK}	0.7	J_{up}	0.98

Table 1. Optimized BPS model parameter changes for the MYBPC3.c772G>A HCM mutation.

The mutation was applied to the calibrated control population; checks were made on intracellular ionic concentrations ($5 < Na_i < 15\,\mathrm{mM}$, $0 < Ca_i < 10\,\mathrm{mM}$), repolarization failures and early after-depolarizations in order to discard cells showing abnormal dynamics.

2.4. Sensitivity analysis

To assess the importance of model parameters in modulating biomarkers and pro-arrhythmicity, a sensitivity analysis was performed. On one hand, a least-square linear regression approach as in [12] was adopted; on the other, logistic regression was performed as in [13] to investigate the influence of the 19 parameters on EADs inducibility. For the latter, the protocols adopted were the same as in [6]: fast pacing for 1500 beats at a basic cycle length (CL) of $275\,\mathrm{ms}$ followed by a $10\,\mathrm{s}$ pause to induce Ca^{2+} overload for DADs and either $20\,\mu\mathrm{M}$ quinidine or $0.1\,\mu\mathrm{M}$ dofetilide adiministration on top of slow pacing ($CL = 4000$ and $5000\,\mathrm{ms}$, respectively) to elicit EADs. Quinidine administration was simulated by blocking I_{Na}, I_{Kr}, I_{CaL}, I_{Ks} and I_{to} maximal conductances by 59.5%, 98.3%, 86.4%, 87.8% and 90.6%, respectively, according to their IC50 values. Dofetilide was instead simulated by blocking I_{Kr} maximal conductance by 93.8% with altered ionic concentrations ($[K^+]_o$ = 5 mM, $[Ca2^+]_o$ = 2 mM and $[Na^+]_o$ = 137 mM).

3. Results

Following the population approach described above, we obtained 119 calibrated cells on which we applied the MYBPC3-c.772G>A mutation. Following the parameter changes, 11 cells had to be discarded according to the criteria stated in the Methods, remaining with a mutant population of 108 cells. Figure 1 reports AP, CaT and AT traces for the calibrated control, mutated and discarded cell populations.

The analysis of the biomarkers (Figure 2) shows an APD prolongation ($+63.4\%$) and an increase in triangulation (APD_{90}-APD_{40}: $+63.3\%$) in the mutant population, while the calcium transient is prolonged to a less extent ($+13.8\%$ in $CaTD_{90}$) and relaxation times are shorter (-15.8% in RT_{95}). Peak tension is also similar in the two groups ($20.4\pm9\,\mathrm{kPa}$ of the mutation vs. $18.9\pm7\,\mathrm{kPa}$ in control), as found experimentally despite the hypertrophic phenotype. The linear regression showed similar results between control and mutant population in terms of biomarkers dependence on model parameters, with $Ca_{50,TRPN}$ having a major role in modulation CaT duration and amplitude and, as a consequence, AT peak.

Interestingly, the number of induced after-depolarizations grows substantially with the dofetilide protocol: 29 cells show EADs in the mutant population vs 3 in CTRL (2 vs 1 with quinidine). Logistic regression results (Figure 3) show that G_{NaCa}, $Ca_{50,TRPN}$ and P_{Ca} are the 3 parameters whose increase drives EAD occurrence most, while G_{NaK}, G_{Kr} and $J_{up,max}$ are the 3 most protective ones.

4. Discussion and Conclusion

Pioner et al. [1] reported that the MYBPC3-c.772G>A mutation – which causes an acceleration of the cross-bridge dynamics enhancing tension generation – leads to a compensatory electrophysiological remodeling determining action potential and calcium transient prolongations. As a consequence, the peak in the active tension developed by adult ventricular myocytes is similar to healthy control cells due to the slower calcium-handling dynamics. This is reflected in our results with the mutant population (Figures 1 and 2), for which the biomarkers show sub-

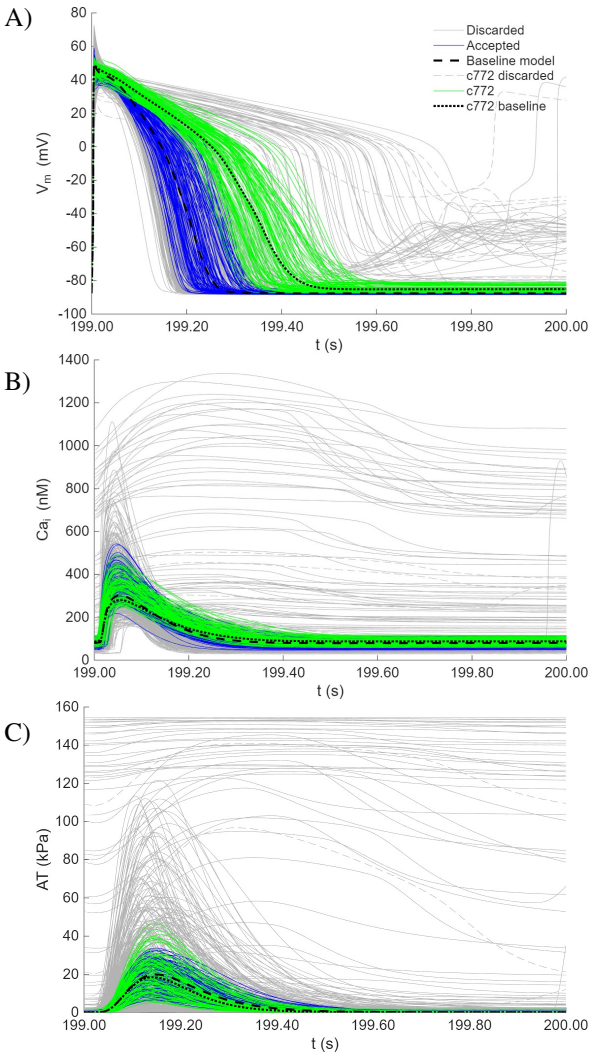

Figure 1. Action potential (A), calcium transient (B) and active tension (C) traces of the MYBPC3-c.772G>A population (green) vs. control (blue).

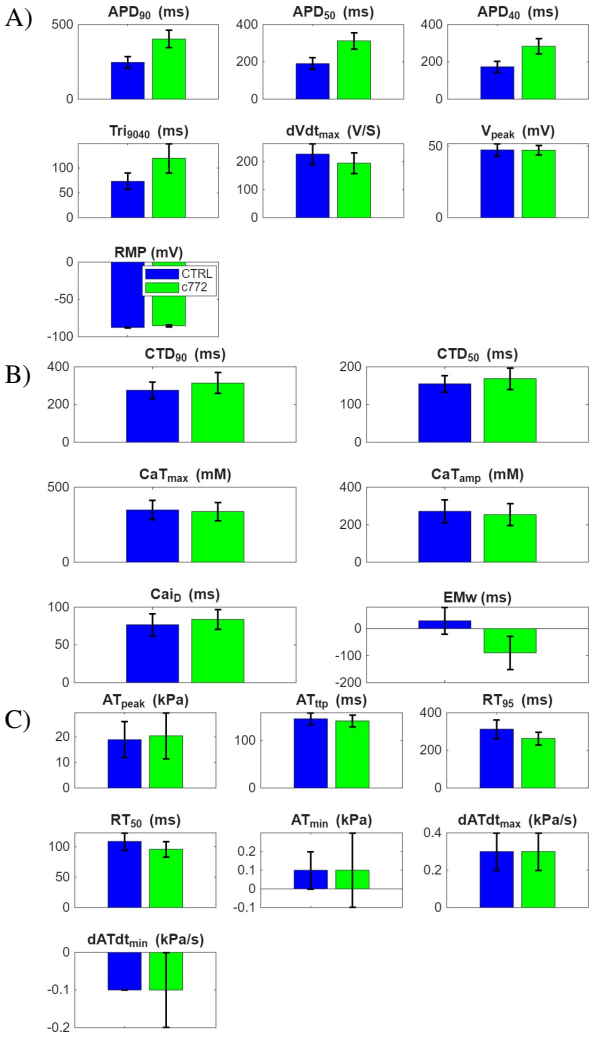

Figure 2. Action potential (A), calcium transient (B) and active tension (C) biomarkers (bottom) of the MYBPC3-c.772G>A population (green) vs. control (blue).

stantial AP prolongation and a consequent mild calcium transient duration increase, leading to limited increases in active tension ($+7.9\%$). Despite the absence of statistically significant difference, the experiments showed a tendency towards a reduction in the AT peak (-12.6%), possibly suggesting that the model shows less sensitivity to the compensatory mechanism than in vitro myocytes.

Our following analysis suggests that this electrophysiological compensation (as one would expect from the substantial APD prolongation) comes at the cost of an increased risk of arrhythmia, quantified as the occurrence of early after-depolarizations. Indeed, the reduction of the repolarization reserve due to remodeling, determines a higher propensity of mutated cells to show EADs upon further block of I_{Kr} due to dofetilide administration. In this context, logistic regression identified Ca^{2+}-related parameters (such as the maximal conductance of the sodium-calcium exchanger) as the main factors determining increased pro-arrhythmicity. To check if overfitting affected the logistic regression results, analysis with parameter subsets (4-6) were performed. In these conditions the coefficient results were similar to the results of Figure 3, where all parameters ($n = 19$) were considered. The limitations of the present work include the small number of cells of the healthy population that pass the calibration phase. This is due, on one hand, to the restrictive ranges for active tension parameters found experimentally and, on the other, to the sensitivity of the Ca^{2+}-tension curve of the BPS model. Despite this, the number of calibrated cells we obtained ($n = 119/10000$) is still large enough to allow compari-

son between healthy and mutant populations.

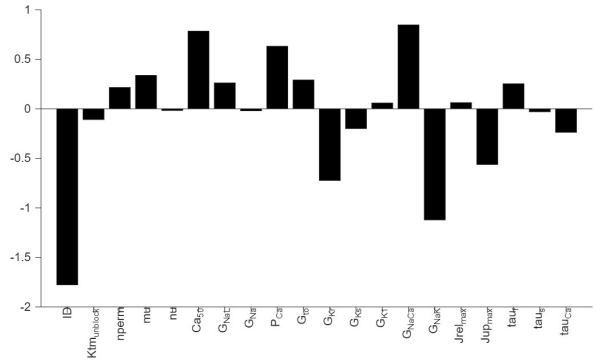

Figure 3. Coefficients of the logistic regression for the dofetilide protocol.

In conclusion, our analysis could guide pharmacological interventions to reduce the increased arrhythmic risk of the MYBPC3-c.772G>A mutation, pointing to a reduction in the sodium-calcium exchanger or L-type calcium currents, as well as a sensitization of troponin to Ca2+ (lowering $Ca_{50,TRPN}$). On the opposite, based on this results one could act on enhancing the sodium-potassium pump, the rapid delayed rectifier currents or increasing of the SERCA uptake rate. In general, the population of model approach adopted in the present study can provide useful information in the effort towards mutation-specific stratification of different HCM variants.

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