The HMC Related MYBPC3:c.772G>A Mutation: A Model Dependency Study

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Abstract

Hypertrophic cardiomyopathy (HCM) is responsible for the thickening of left ventricle walls due to genetic mutations that affect the sarcomere, with prevalence of 1 out 500 in the general population. Even if HCM may be a silent disease, diastolic dysfunction, heart failure and sudden cardiac death are reported as adverse events. Recent studies described the HCM-related c.772G>A variant, that affects the myosin binding protein C (MYBPC3), both from the clinical and the cellular perspective, thanks to the enrollment of HCM patients who underwent left ventricle septum myectomy.

Our aim is to simulate the effects of the HCM c.772G>A mutation using the electrophysiology ventricular models by O'Hara-Rudy (ORd), Tomek-Rodriguez (ToRORd) and Bartolucci (BPS) integrated with the Land contractile model. The results are analyzed from a model dependency point of view. First, we identified the most influential parameters thanks to sensitivity analysis (SA) under control conditions. Then we compared three different HCM remodelings: the first using values from previous works, the second through model specific calibration; the third one used averaged values from the previous calibration. The comparison between HCM remodelings and experimental data highlighted the importance of calibrating the parameters depending on the model in use.

1. Introduction

Hypertrophic cardiomyopathy (HCM) is a common genetic disease with a prevalence of 1/500 in the general population and often results in adverse events, such as sudden cardiac death (SCD), diastolic dysfunction, heart failure (HF) and atrial fibrillation (AF).

HCM mutations mainly affect genes that encode proteins that form the sarcomere, responsible for contraction and the development of force. These mutations may alter the functionality and expression levels of genes that encode for $\beta-$myosin heavy chain (MYH7), troponin T (TNNT2), troponin I (TNNI3) and the myosin binding protein C (MYBPC3). A recent study [1] characterized the *MYBPC3*:c.772G>A variant, showing that it reduces the expression of MYBPC3, resulting in faster cross-bridge cycling. The prolongation of the action potentials (AP) and calcium transients (CaT), on the other hand, reveal a counterbalancing effect that preserves the twitch duration.

Computational models are a powerful tool for revealing the mechanisms that underlie pathophysiological conditions [2, 3] and provide information to identify potential treatment targets. Recently, several human-specific cardiac models that combine electrophysiological and contractile descriptions have been developed [4–6]. However, results may differ on the basis of the adopted model.

Our work aims to evaluate and quantify the effects of the *MYBPC3*:c.772G>A mutation on AP, CaT and active tension (AT) on three different adult ventricular single cell models: the O'Hara-Rudy (ORd) [7], the Tomek-Rodriguez (ToROrd) [8] and the Bartolucci (BPS) [6] models describing the electrophysiological behavior have been integrated with the mechanical description provided by Land. To do so, we performed sensitivity analysis, implemented the HCM remodeling available in the literature, and calibrated the models to better reproduce experimental data. Finally, we performed a second sensitivity analysis on each calibrated HCM model and highlighted similarities and differences under physiological and pathological conditions.

2. Methods

Sensitivity analysis (SA) allows one to identify which parameters affect biomarkers the most. We scaled the parameters responsible for HCM remodeling and generated a population of N=1000 cells. The scaling factors were randomly extracted from a log-normal distribution [9], with σ=0.1873, which roughly corresponds to the range $[-50\% + 50\%]$ with respect to the nominal value. The matrix that collects the correlation weights was computed through a Non linear Iterative Partial Least Square (Nipals) algorithm.

The model-dependent set of parameters to describe HCM remodeling was identified through an automatic calibration framework. Starting from values according to [2], the optimal set was reached using the MatLab built in method $fminsearch$. The sum of absolute differences

between experimental and simulated action potential duration at 90% of repolarization ($APD90$), the calcium decay at 90% with respect to the calcium peak ($CaTD_{50}$), the calcium amplitude ($CaTamp$), the peak of active tension (AT_{peak}) and the relaxation time at 50% (AT_{rt50}) was used as a cost function.

We ran all simulations and plotted the results using MatLab (Mathworks, Natick,MA). The equations were integrated with the ode15s solver, at a pacing frequency of 1 Hz, for 200 beats. The external ionic concentrations were set to $[K^+]_o$=5.4 mM, $[Ca^{2+}]_o$=1.8 mM, $[Na^+]_o$=144 mM.

3. Results

3.1. Sensitivity Analysis

Calcium permeability ($P_{Ca,L}$), the rapid potassium conductance (G_{Kr}), the activity of the $Na^+ - Ca^{2+}$ exchanger (G_{NCX}), the calcium uptake (J_{up}) and release (J_{rel}) of the sarcoplasmic reticulum (SR) and the transition rate from weak to strong binding in the contractile subunit (k_{ws}) are the parameters with higher correlation with APD_{90}, $Ca_{TD,50}$, Ca_{amp}, AT_{peak} and $AT_{rt,50}$.

In control conditions, G_{Kr} showed a high negative correlation with APD_{90} (-0.90, -0.78 and -0.82, respectively, for ORd, ToRORd and BPS). ToRORd shows a mild positive correlation with late sodium conductance ($G_{Na,L}$) and G_{NCX}. J_{up} has strong negative correlation with $Ca_{TD,50}$. ORd and ToRORd share moderate positive correlations between Ca_{amp}, $P_{Ca,L}$ and J_{up} and moderate negative correlation with J_{leak}, (see Figure 1 panel B and C). In BPS, Ca_{amp} is positively correlated with (J_{rel}) (+0.52) and negatively with J_{leak} (-0.57) (Figure 1 panel C). AT_{peak} showed negative correlation with G_{NCX} (ORd=-0.45, ToRORd=-0.38, BPS=-0.50), while with $P_{Ca,L}$, ORd and ToRORd reported higher positive correlations than BPS (+0.61, +0.66 vs +0.40). $AT_{rt,50}$ showed negative correlations with J_{up} and k_{ws}, to different degrees. Furthermore, ToRORd reported a positive correlation with $P_{Ca,L}$ (+0.54) and a negative (-0.36) with G_{Kr}.

SA has been performed for the second time after HCM remodeling calibration: APD_{90} preserves strong negative correlation with G_{Kr}; ToRORd shows a slight increase in positive correlation with $G_{Na,L}$, while the correlation with G_{NCX} becomes lower than 0.30. On the other hand, $Ca_{TD,50}$ does not show significant changes. Ca_{amp} correlations are unchanged in ToRORd, while HCM remodeling leads to a lower sensitivity for G_{NCX} and J_{leak} in ORd. The contribution of $P_{Ca,L}$ becomes stronger (+0.36) in BPS. AT_{peak} shows consistent positive correlation with $P_{Ca,L}$ (+0.50,+0.66, +0.40, respectively in ORd, ToRORd and BPS). AT_{peak} reports a positive correlation with (J_{up}) in ORd and ToRORd and a negative one (-0.43) in BPS. Finally, $AT_{rt,50}$ has consistent negative correlations with (J_{up}); in BPS, k_{ws} negatively correlates with $AT_{rt,50}$, (-0.43) weaker than in control conditions (-0.77).

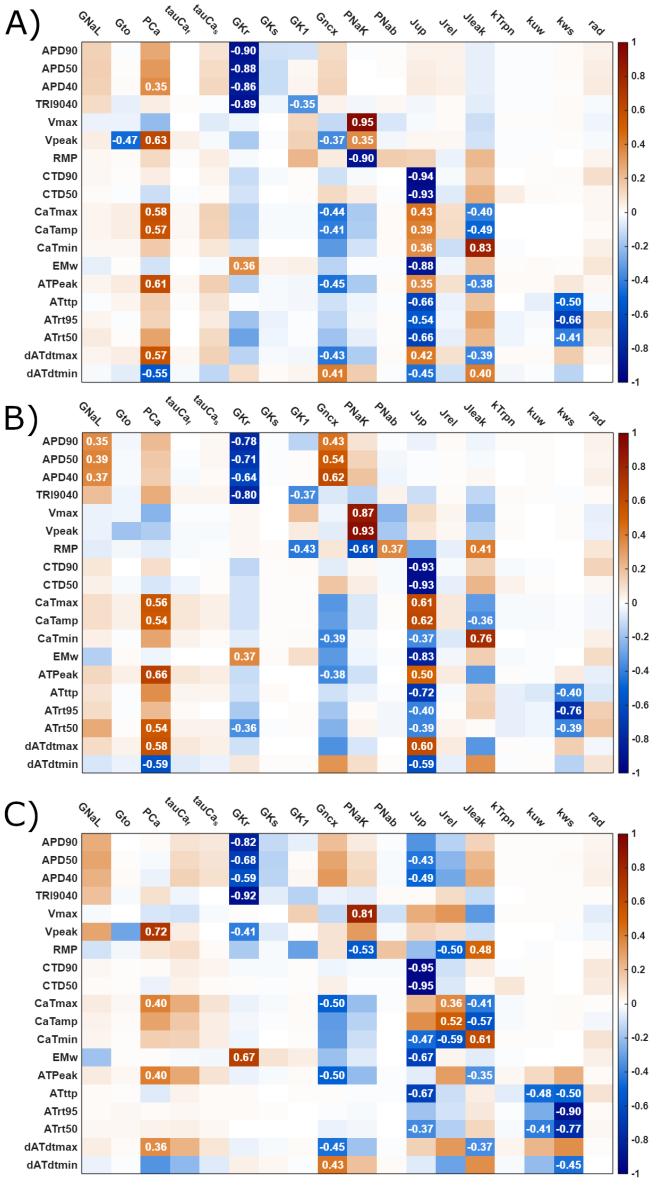

Figure 1. Sensitivity analysis of A) ORd Land B) ToRORd and C) BPS in CTRL conditions. Red, blue and white squares report respectively positive, negative and negligible correlations between parameters and biomarkers

3.2. HCM Remodeling and Parameter Calibration

The HCM remodeling was first implemented using the values provided by Passini using the ORd electrophysio-

logical model [2]. Timecourses of AP, CaT and AT are depicted in Figure 2, red traces. APD_{90} prolongation was larger than in experimental data (+76.7%,+97.6%,+89.1%, respectively for ORd, ToR and BPS, versus 70.2% exp). CaT resulted in slower decay and increased amplitude. The simulations showed a consistent behavior with the experimental data, even if Ca_{amp} changes *in silico* are greater. A large discrepancy between models and experiments is found in changes of AT_{peak}. The models, indeed, show a significant increase in active tension, while the experiments report a reduction (-12.6%).

For such a reason, we performed a model-specific calibration. The new values of the remodeling parameters allow the models to closely reproduce the experimental data: APD_{90} and AT_{peak} are in good agreement.However, the models show a reduction of Ca_{amp}, which is not consistent with *in vitro* data. Table 1 summarizes the changes in the calibrated parameters compared to the initial values. $P_{Ca,L}$ decreases and G_{Kr} increases to accelerate repolarization.

Finally, the calibrated parameters are averaged in order to test a more general set of values for HCM remodeling. This new set leads to inconsistent results, especially for changes in AT_{peak}. On the one hand, the reduction of AT_{peak} is too marked in ORd and ToRORd (respectively, -46.0%, -21.9%). On the other hand, BPS showed an increase in tension (+26.0%)

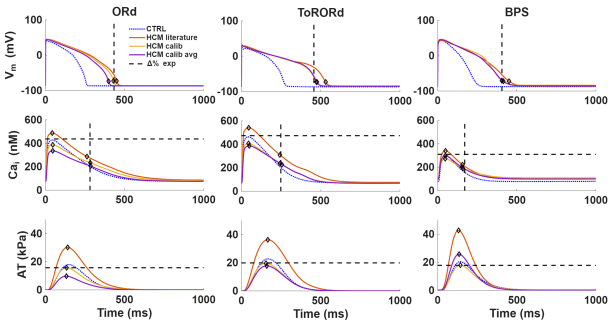

Figure 2. Timecourses of AP (first row), CaT (second row) and AT (third row) relative to ORd Land, ToRORd Land and BPS Land models. Each model was simulated in CTRL (blue traces), HCM remodelling according literature (red), HCM remodelling after optimization (yellow) and averaged optimal HCM remodelling (purple). Black dashed traces report experimental data. Black diamonds represent simulated biomarkers

4. Discussion and Conclusion

With this study, our goal is to assess how the choice of the computational model affects the results in the simulation of the HCM-related MYBPC3:c.772G>A mutation. To do that, we performed SA under control conditions and downstream calibration of the parameters involved in HCM remodeling. Under physiological conditions, beyond a common strong negative correlation with G_{Kr}, only ToRORd presents a mild $G_{Na,L}$ positive correlation with APD_{90}, In BPS, Ca_{amp} is mainly controlled by the contributions of J_{rel} and J_{leak}, while in ORd and ToRORd, $P_{Ca,L}$ provides moderate positive correlation. The models show similar mechanisms for the control of AT_{peak}, with higher sensitivity for $P_{Ca,L}$ in ORd and ToRORd, compared to BPS. The implementation of HCM remodeling slightly changes the working point of the

Table 1. Parameters scaling factors used for replicate HCM remodelling before and after model dependent optimization and relative variations with respect to initial guess from literature

Model	Parameter Scaling	*HCMLit*	*Opt*	Δ%
ORd	$P_{Ca,L}$	1.40	1.17	-16.6
	GK_r	0.55	0.58	5.1
	G_{NCX}	1.30	1.47	13.0
	J_{up}	0.85	0.87	2.5
	J_{rel}	0.80	0.82	2.7
	k_{ws}	2.00	1.89	- 5.3
ToRORd	$P_{Ca,L}$	1.40	1.00	-28.6
	GK_r	0.55	0.62	12.7
	G_{NCX}	1.30	1.47	13.0
	J_{up}	0.85	0.94	10.6
	J_{rel}	0.80	0.77	-3.8
	k_{ws}	2.00	1.94	-3.0
BPS	$P_{Ca,L}$	1.40	1.02	-27.7
	GK_r	0.55	0.81	47.3
	G_{NCX}	1.30	2.15	65.6
	J_{up}	0.85	0.98	15.5
	J_{rel}	0.80	0.47	-41.1
	k_{ws}	2.00	1.52	-24.0
OptAvg	$P_{Ca,L}$	1.40	1.06	-24.4
	GK_r	0.55	0.67	21.7
	G_{NCX}	1.30	1.70	30.6
	J_{up}	0.85	0.93	9.5
	J_{rel}	0.80	0.69	-14.1
	k_{ws}	2.00	1.78	-10.8

Table 2. % changes of APD_{90},$CaTD_{50}$ CaT_{amp},AT_{peak} and AT_{rt50} with with respect to control conditions in the three remodelling scenarios

	Δ *biom%*	*ORd*	*ToRORd*	*BPS*	*EXP*
HCM lit	Δ *APD90*	76.7	97.6	89.1	70.2
	Δ *CaTD50*	15.6	21.1	13.6	23.9
	Δ *CaTamp*	14.2	18.8	6.2	2.6
	Δ *ATpeak*	67.5	59.7	109.3	-12.6
	Δ *ATrt50*	5.4	21.9	-13.7	0.3
HCM Opt	Δ *APD90*	69.2	76.3	69.7	70.2
	Δ *CaTD50*	24.8	21.9	16.2	23.9
	Δ *CaTamp*	-13.3	-14.1	-28.0	2.6
	Δ *ATpeak*	-12.8	-13.6	-12.2	-12.6
	Δ *ATrt50*	0.4	9.6	-2.5	0.3
HCM Opt Avg	Δ *APD90*	56.6	77.4	73.7	70.2
	Δ *CaTD50*	25.2	27.4	11.3	23.9
	Δ *CaTamp*	-26.1	-18.7	-12.2	2.6
	Δ *ATpeak*	-46.0	-21.9	26.0	-12.6
	Δ *ATrt50*	-2.2	11.4	-12.7	0.3

models, but the fundamental control mechanisms are preserved. Under HCM conditions, ORd and ToRORd show lower correlations between AT_{peak}, G_{NCX} and J_{leak} than under control. ToRORd preserves control of Ca_{amp} and AT_{peak}, while AT relaxation, under HCM, is completely controlled by J_{up}, losing the contributions from the membrane due to $P_{Ca,L}$ and G_{NCX}.

The analysis of the timecourses of AP, CaT and AT shows the difference between the HCM remodeling approaches tested. The higher agreement with the experimental data of the calibrated models is not surprising and highlights the need for model-dependent HCM remodeling. Remarkable is the discrepancy in the behavior of AT while using the averaged calibrated set (see Figure 2, last row,purple traces) in BPS, not consistent with the other models and *in vitro* data; even if the Ca^{2+} peak is comparable with control conditions, AT increases.

Comparison of the effects of drug administration with selective current blocks on HCM-remodeled models may represent a future step to further assess the impact of the adopted model on predictions.

Acknowledgments

F.M., A.F., E.R. and S.S. were funded by the European Union - Project 101137115 — SMASH-HCM. C.B. and S.S. have received funding from the European Union - NextGenerationEU through the Italian Ministry of University and Research under PNRR - M4C2-I1.3 Project PR_00000019 “HEAL ITALIA” to S.S. CUP J33C22002920006.

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