

# The HCM related MYBPC: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 of 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 c772G>A variant, that affects the myosin binding protein C (MYBPC), 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 c772G>A mutation using the electrophysiology ventricular models from O'Hara-Rudy (ORd), Tomek-Rodriguez (ToRORd) and Bartolucci (BPS) integrated with the contractile model from Land. 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. Finally we compared SA under HCM to control conditions. SA pointed out that  $I_{Ca,L}$ ,  $I_{Kr}$ ,  $I_{NCX}$ ,  $J_{up}$  and  $J_{rel}$  show high correlations with AP duration (APD90), Calcium amplitude (CaTamp) and Active Tension (AT). The comparison between the HCM remodelings 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:C772G>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 on the identification of 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:C772G>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. To do so, 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  $\sigma=0.1873$ , 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 ( $APD_{90}$ ), the calcium decay at 90% with respect to the calcium peak ( $CaTD_{50}$ ), the calcium amplitude ( $CaT_{amp}$ ), the peak of active tension ( $AT_{peak}$ ) and the relaxation time at 50% ( $AT_{rt,50}$ ) 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  $APD_{90}$ ,  $CaTD_{50}$ ,  $CaT_{amp}$ ,  $AT_{peak}$  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  $CaTD_{50}$ . ORD and ToRORD share moderate positive correlations between  $CaT_{amp}$ ,  $P_{Ca,L}$  and  $J_{up}$  and moderate negative correlation with  $J_{leak}$ , (see Figure 1 panel B and C). In BPS,  $CaT_{amp}$  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,  $CaTD_{50}$  does not show significant changes.  $CaT_{amp}$  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).

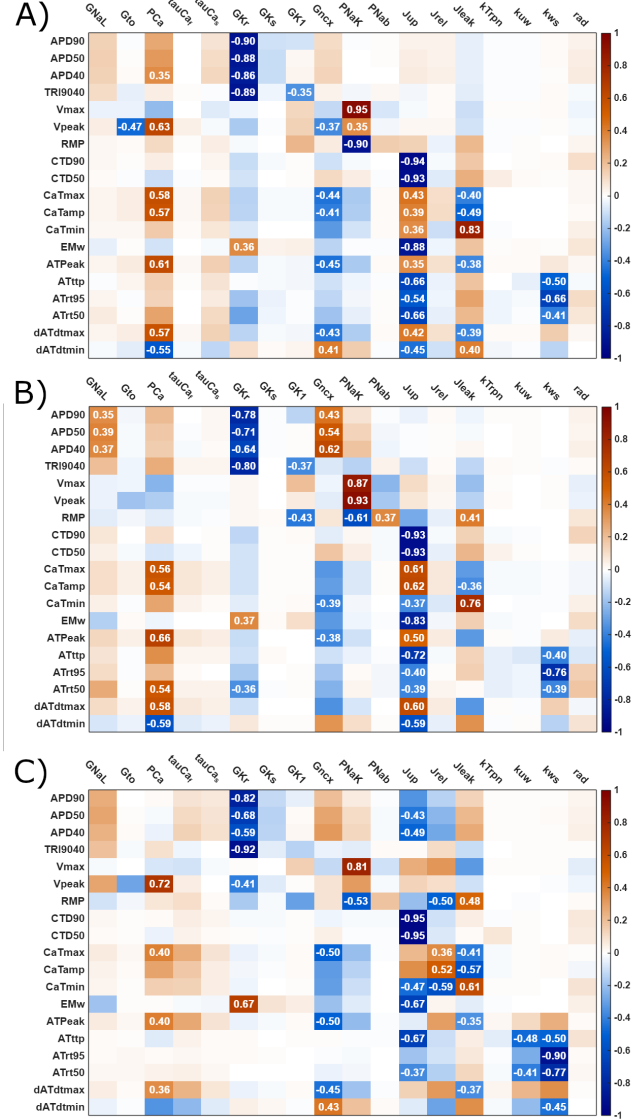


Figure 1. Sensitivity analysis of A) ORD and B) ToRORD and C) BPS in CTRL conditions. Red, blue and white squares report respectively positive, negative and negligible correlations between parameters and biomarkers

### 3.2. HCM remodeling and parameter calibration

The HCM remodeling was first implemented using the values provided by Passini using the ORd electrophysiological 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, shows 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 allows the models to closely reproduce the experimental data:  $APD_{90}$  and  $AT_{peak}$  are in good agreement. However, the models shows a reduction of  $Ca_{amp}$ , 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%)

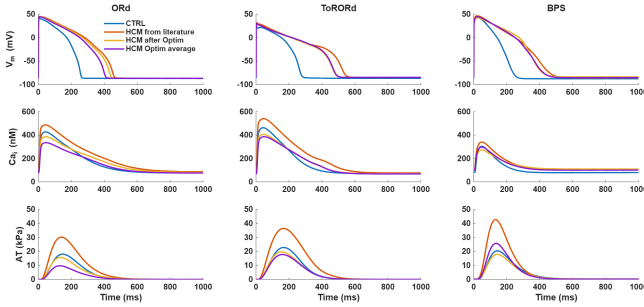


Figure 2. Timecourses of AP (first row), CaT (second row) and AT (third row) relative to ORd Land, ToRORd Land and BPS Land models. Each model was simulated in CTRL (blue traces), HCM remodelling according literature (red), HCM remodelling after optimization (yellow) and averaged optimal HCM remodelling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

### 4. Discussion and Conclusion

With this study, our goal is to assess how the choice of the computational model affects the results in the simu-

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	HCM Lit	Opt	$\Delta\%$
ORd	$P_{Ca,L}$		1.40	1.17	-16.6
	$G_{Kr}$		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
ToRORd	$k_{ws}$		2.00	1.89	-5.3
	$P_{Ca,L}$		1.40	1.00	-28.6
	$G_{Kr}$		0.55	0.62	12.7
	$G_{NCX}$		1.30	1.47	13.0
	$J_{up}$		0.85	0.94	10.6
BPS	$J_{rel}$		0.80	0.77	-3.8
	$k_{ws}$		2.00	1.94	-3.0
	$P_{Ca,L}$		1.40	1.02	-27.7
	$G_{Kr}$		0.55	0.81	47.3
	$G_{NCX}$		1.30	2.15	65.6
OptAvg	$J_{up}$		0.85	0.98	15.5
	$J_{rel}$		0.80	0.47	-41.1
	$k_{ws}$		2.00	1.52	-24.0
	$P_{Ca,L}$		1.40	1.06	-24.4
	$G_{Kr}$		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 respect to control conditions in the three remodelling scenarios

	$\Delta biom\%$	ORd	ToRORd	BPS	EXP
HCM lit	$\Delta APD_{90}$	76.7	97.6	89.1	70.2
	$\Delta CaTD_{50}$	15.6	21.1	13.6	23.9
	$\Delta CaT_{amp}$	14.2	18.8	6.2	2.6
	$\Delta AT_{peak}$	67.5	59.7	109.3	-12.6
	$\Delta AT_{rt50}$	5.4	21.9	-13.7	0.3
HCM Opt	$\Delta APD_{90}$	69.2	76.3	69.7	70.2
	$\Delta CaTD_{50}$	24.8	21.9	16.2	23.9
	$\Delta CaT_{amp}$	-13.3	-14.1	-28.0	2.6
	$\Delta AT_{peak}$	-12.8	-13.6	-12.2	-12.6
	$\Delta AT_{rt50}$	0.4	9.6	-2.5	0.3
HCM Avg	$\Delta APD_{90}$	56.6	77.4	73.7	70.2
	$\Delta CaTD_{50}$	25.2	27.4	11.3	23.9
	$\Delta CaT_{amp}$	-26.1	-18.7	-12.2	2.6
	$\Delta AT_{peak}$	-46.0	-21.9	26.0	-12.6
	$\Delta AT_{rt50}$	-2.2	11.4	-12.7	0.3

lation of the HCM-realted MYBPC:c772G>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 a 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 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.

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