In Silico Investigation of the CACNA1C N2091S Mutation in Timothy Syndrome

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Abstract

Experimental studies demonstrated that CACNA1C-N2091S led to a gain-of-function in the L-type calcium current (I_{CaL}) linked to heritable Timothy Syndrome, but mechanisms by which the N2091S mutation promotes and perpetuates ventricular fibrillation remain unclear. This study sought to investigate the proarrhythmic effects of N2091S-induced I_{CaL}. Using a dynamic ventricular myocyte model, we simulated I_{CaL}, APs, Ca^{2+} transients ($[Ca^{2+}]_i$) and sarcoplasmic reticulum (SR) calcium profiles ($[Ca^{2+}]_{SR}$) in three cell types. Effects of the N2091S mutation on cell electrophysiology were quantified by changes in I_{CaL} density, $[Ca^{2+}]_i$ amplitude ($[Ca^{2+}]_{i(max)}$), SR calcium content ($[Ca^{2+}]_{SR(max)}$), action potential duration (APD) and AP shape. It was shown that the N2091S mutation increased $I_{CaL(max)}$, $[Ca^{2+}]_{i(max)}$, $[Ca^{2+}]_{SR(max)}$ and APD in three cell types. Compared with ENDO and EPI cells, MCELL cells with excessive prolongation of APD due to the N2091S mutation facilitated inducibility of early afterdepolarization (EAD)-mediated triggered activity. And the different EAD inducibility among the three cell types can amplify the electrical difference and thereby dispersion of repolarization, increasing susceptibility to ventricular arrhythmias. Thus, the N2091S mutation confers not only a trigger, but also a substrate for lethal ventricular arrhythmias.

1. Introduction

Timothy syndrome (TS) is a rare disorder that affects many parts of the body including the heart, digits (fingers and toes), and the nervous system[1]. TS is caused by gain-of-function mutations of the *CACNA1C* gene, which encodes the L-type Ca^{2+} channel, $Ca_V1.2$, and is characterized by QT prolongation, congenital heart disease, cognitive and behavioral problems[2].

$Ca_V1.2$ plays an important role in regulating excitation-contraction coupling and modulating cellular excitability [3]. Abnormalities in $Ca_V1.2$ due to gain-of-function mutations of the *CACNA1C* gene have been suggested as factors contributing to ventricular arrhythmogenesis[4-6]. Recently, Sutphin et al. identified the variant p.N2091S (c.6272A> G) and functional analysis revealed the N2091S variant led to a 105% gain-of-function in the L-type calcium current (I_{CaL}) and minor kinetic alterations including a -3.4 mV shift in the half-maximal voltage of activation ($V_{a,0.5}$)[7]. However, the ionic mechanisms by which the *CACNA1C*-N2091S mutation promotes and perpetuates ventricular fibrillation remain unclear.

In this study, changes in I_{CaL} channel kinetics arising from the *CACNA1C* N2091S mutation were incorporated into the dynamic ventricular myocyte models for representing endocardial (ENDO), middle (MCELL) and epicardial (EPI) cells. Using the validated cell models, we investigated its effects on action potential (AP) and calcium transient ($[Ca^{2+}]_i$). Changes in I_{CaL}, AP profile, AP duration (APD), $[Ca^{2+}]_i$, sarcoplasmic reticulum calcium content ($[Ca^{2+}]_{SR}$) and activation fraction of Ca^{2+}/calmodulin-dependent protein kinase II ($CaMKII_{active}$) were quantified to analyze pro-arrhythmic effects of the *CACNA1C* N2091S mutation.

2. Methods

The well-known TP06 model for human ventricular myocytes was modified to incorporate CaMKII regulation in our previous study. Some modifications to the modified TP06 model were included to reproduce I_{CaL} channel kinetics arising from the *CACNA1C* N2091S mutation. In the cellular model, an ordinary differential equation was used to describe the transmembrane potential *V*:

$$\frac{dV}{dt} = -\frac{I_{ion}}{C_m} \quad (1)$$

where *t* is the time, C_m is the capacitance across the cell membrane and I_{ion} is the total ionic current across the membrane. I_{ion} is given by

$$I_{ion} = I_{Na} + I_{CaL} + I_{to} + I_{Kr} + I_{Ks} + I_{K1} + I_{NCX} +$$

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$$I_{NaK} + I_{pK} + I_{pCa} + I_{Nab} + I_{Cab} \quad (2)$$

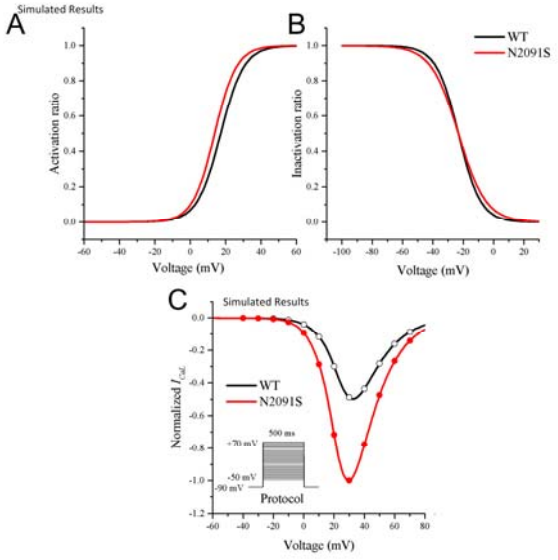

Figure 1. Functional $Ca_V1.2$ in human ventricular cells. Activation (**A**), inactivation (**B**) and current-voltage (**C**) curves under wild-type (WT) and N2091S $Ca_V1.2$ conditions.

In the cellular model, we determined the modifications to the original I_{CaL} model to reproduce the behavior of the mutant I_{CaL} during the same voltage-clamp employed in experiments[7]. Theoretical steady-state activation and inactivation curves which were used to simulate WT and N2091S I_{CaL}, are shown in **Figure 1A&B.** Second, based on current-voltage relationships, mathematical models of I_{CaL} were constructed (**Figure 1C**). This was achieved by simulating the experimental voltage-clamp protocol and scaling relative current proportions. *CACNA1C* mutations-induced changes in I_{CaL} channel kinetics include $V_{a,0.5}$ the half-activation voltage, S_a the slope of the steady-state activation of I_{CaL} channel, $V_{ina,0.5}$ the half-inactivation voltage, S_{ina} the slope of the steady-state inactivation of I_{CaL} channel, and *CSF* a scaling factor of the maximal conductance of I_{CaL} (for details see **Table 1**).

Table 1. The N2091S mutation-induced changes in I_{CaL} channel kinetics.

Parameters	WT	N2091S
CSF	1	1.73
$V_{a,0.5}$ (mV)	6.6	6.2
S_a	-23.3	-23.3
$V_{ina,0.5}$ (mV)	7.35	9.09
S_{ina}	6.6	6.2

I_{CaL} is described as

$$I_{CaL} = CSFG_{CaL}dff_2 4\frac{(V-15)F^2}{RT}\frac{0.25Ca_{SS}e^{\frac{2(V-15)F}{RT}} - Ca_o}{e^{\frac{2(V-15)F}{RT}} - 1} \quad (3)$$

$$\frac{dd}{dt} = \frac{(d_\infty - d)}{\tau_d} \quad (4)$$

$$d_\infty = \frac{1}{1 + e^{\frac{V_{a,0.5} - V}{S_a}}} \quad (5)$$

$$\frac{df}{dt} = \frac{(f_\infty - f)}{\tau_f} \quad (6)$$

$$f_\infty = \frac{1}{1 + e^{\frac{V - V_{ina,0.5}}{S_{ina}}}} \quad (7)$$

where G_{CaL} is the maximal conductance of I_{CaL} (μS/pF), d is the activation variable, f is the voltage-dependent inactivation variable and τ_f is the voltage-dependent time constant of inactivation.

3. Results

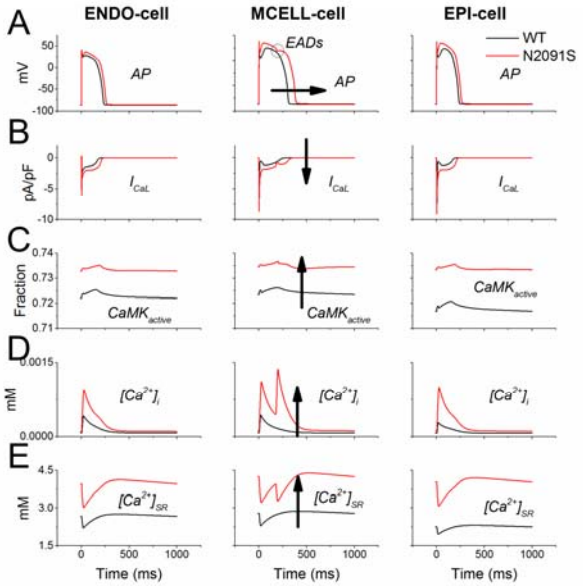

Figure 2. Comparison of action potentials and calcium transients between wild-type (WT) and N2091S $Ca_V1.2$ conditions in endocardial (ENDO), middle (MCELL) and epicardial (EPI) cells. AP (**A**), I_{CaL} (**B**), $CaMKII_{active}$ (**C**), $[Ca^{2+}]_i$ (**D**)and $[Ca^{2+}]_{SR}$(**E**).

I_{CaL} arising from the $Ca_V1.2$ mutations altered intracellular calcium handling and prolonged AP as shown in **Figure 2**. For ENDO cells, the I_{CaL} amplitude was increased by the *CACNA1C* N2091S mutation. Increased inward Ca^{2+} highly activated CaMKII, increased $[Ca^{2+}]_i$, led to SR calcium loading and prolonged APD. In details, the I_{CaL} amplitude was increased from -4.74 pA/pF under the WT condition to -6.06 pA/pF. $CaMKII_{active}$ were 0.725 and 0.735, under WT and N2091S conditions, respectively. The N2091S mutation caused an increase by 128% in $[Ca^{2+}]_i$ (4.15E-4 mM under the WT condition vs. 9.5E-4 mM under the N2091S condition). SR calcium overloading

($[Ca^{2+}]_{SR}$ was increased from 2.76096 mM to 4.13024 mM) was observed under the N2091S condition. The measured APD was prolonged by this mutation from 242 ms to 292 ms.

In addition, the transmural electrical heterogeneity was investigated by producing APs of ENDO, MCELL, and EPI cells. In the three cells, the extent of changes in AP characteristics was most for MCELL cells (for details see **Table 2**). Importantly, under the N2091S condition, an early afterdepolarization (EAD) in the MCELL cells was induced, but no ectopic beats in the EPI and ENDO cells were observed.

Table 2. Electrophysiological characteristics (including I_{CaL}, $CaMKII_{active}$, $[Ca^{2+}]_i$, $[Ca^{2+}]_{SR}$ and APD_{90}) of ventricular cells under wild-type (WT) and N2091S $Ca_V1.2$ conditions.

	I_{CaL} (pA/pF)	$CaMKII_{active}$	$[Ca^{2+}]_i$ (mM)	$[Ca^{2+}]_{SR}$ (mM)	APD_{90} (ms)
ENDO (WT)	-4.74	0.725	4.15E-4	2.76096	242
MCELL (WT)	-7.64	0.726	4.33E-4	2.88162	318
EPI (WT)	-8.07	0.721	2.88E-4	2.32031	243
ENDO (N2091S)	-6.06	0.735	9.5E-4	4.13024	292
MCELL (N2091S)	**-8.72**	**0.737**	**1.36E-3**	**4.39898**	**429**
EPI (N2091S)	-9.09	0.735	9.98E-4	4.21031	295

4. Discussion

This study investigated effects of the *CACNA1C* N2091S mutation on ventricular electrical activity at the cellular level. Our main findings are as follows: (1) Increased I_{CaL} arising from the *CACNA1C* N2091S mutation prolonged APD and caused calcium overload, (2) Increased Ca^{2+} ions due to the *CACNA1C* N2091S mutation highly activated CaMKII and elevated $[Ca^{2+}]_i$ and $[Ca^{2+}]_{SR}$, resulting in spontaneous SR calcium releases during the phase 3 of the AP and thereby EADs, (3) this mutation increased the intrinsic transmural heterogeneity, and (4) the vulnerability of MCELL cell to EADs was increased by this mutation. Consequently, these findings demonstrate that the *CACNA1C* N2091S mutation increases the likelihood of ventricuar arrhythmias due to APD prolongation and triggered activity, which facilitate complex ventricular contractions.

Computational modeling has shed valuable light on the ionic mechanisms underlying complex ventricular contractions. Gain-of-function muations, which include G406R [8], G402S&G406R [9] and R858H [10], were associated with APD prolongation, intracellular calcium handling abnormalities and triggered activity. In additon, alterations in calcium signaling (CaMKII) due to increased $[Ca^{2+}]_i$ arising from these mutation also partly contritubed to triggered activity[11]. In this study, the cellular mechanism is that I_{CaL} due to the N2091S mutation prolongs APD, activates CaMKII and increases SR calcium load, leading to EADs. These simulation data of the present study add to the growing weight of evidence implicating cellular mechanisms of CACNA1C mutations.

5. Conclusion

Thus, these human ventricular models provide powerful tools for drug screening and investigating cardiac dynamics. Our findings of these simualted results suggest the triggered activity due to EADs arising from the *CACNA1C*-N2091S mutation may contribute to the mechanism underlying ventricular arrhythmias.

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