Computational modeling of a spectrum of arrhythmia-associated CACNA1C mutations that prolong cardiac action potential duration

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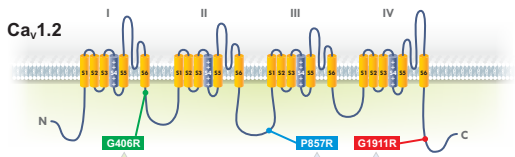
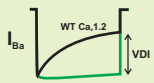
Introduction

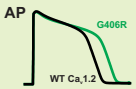
Timothy syndrome (TS) is a congenital long QT disorder caused by a **G406R** mutation in the CACNA1C-encoded $Ca_v1.2$ L-type calcium channel.



Classic Timothy Syndrome
mechanisms of arrhythmogenesis

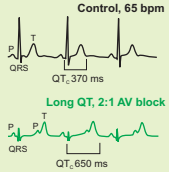


The TS G406R mutation causes a **loss of $Ca_v1.2$ voltage-dependent inactivation** (VDI), increasing Ca^{2+} influx.



Excess Ca^{2+} entry **prolongs cardiac action potential duration (APD)**, providing substrate for arrhythmias.

TS patients have a markedly **prolonged QT interval** and are at high risk for **ventricular tachyarrhythmias.**



EKG adapted from Splawski et al. *Cell*. 2004

Novel mutations in $Ca_v1.2$

causing long QT syndrome and arrhythmias recently reported:

1. **P857R mutation** does not affect channel gating properties but increases I_{Ca} current by increasing channel surface expression.
2. **G1911R mutation** identified in a patient with long QT and recurrent ventricular tachycardia; arrhythmia mechanism unknown.

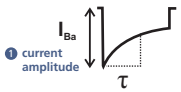
Objective

Determine mechanisms by which the G1911R mutation affects $Ca_v1.2$ function and leads to prolongation of the cardiac action potential.

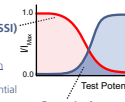
Methods

I. Whole-cell patch clamp: characterize effect of mutation on channel gating.

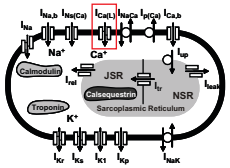
- WT or G1911R $Ca_v1.2$ channels transfected into HEK293T cells
- Ba^{2+} as charge carrier to eliminate Ca^{2+}-dependent inactivation; isolate VDI
- obtain 5 electrophysiological parameters:



1. **current amplitude**
2. **tau of inactivation** measures rate of channel inactivation
3. **steady-state inactivation (SSI)** measures the availability of channels to open as a function of membrane potential
4. **steady-state activation (SSA)** measures the probability of channels to open as a function of membrane potential
5. **"window current"** represents the physiologically relevant membrane potentials at which Ca^{2+} permeates $Ca_v1.2$ channel



II. Computational modeling: predict effect of mutations on APD.

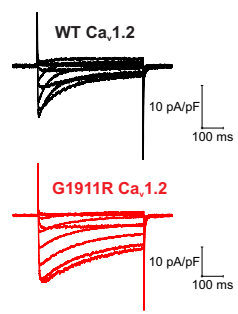


Luo-Rudy myocyte model (Circ. Res. 1994)

- modified $Ca_v1.2$ SSI (f_∞) and SSA (d_∞) gating equations in Luo-Rudy myocyte model to match experimental WT/mutant recordings
- simulated action potentials and I_{Ca} currents for G1911R, G406R, and P857R mutations

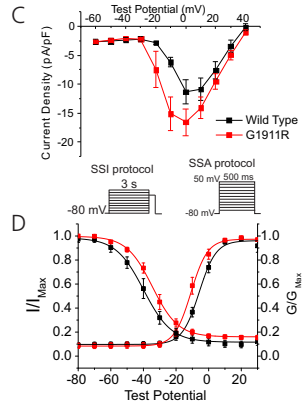
Results

I. Functional analysis of mutant channel: G1911R affects Cav1.2 availability and VDI



Representative I_{Ba} current tracings **show increased current density** and **slower inactivation** in mutant compared to wild-type channels.

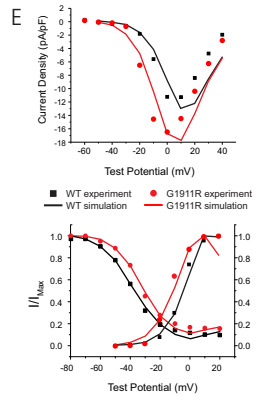
G1911R decreases voltage-dependent inactivation, measured by fitting a single exponential (at 0mV potential) and comparing the tau of inactivation (*$p < 0.05$).



Current-voltage relationship shows an increase in current density in the G1911R mutant compared to WT $Ca_v1.2$.

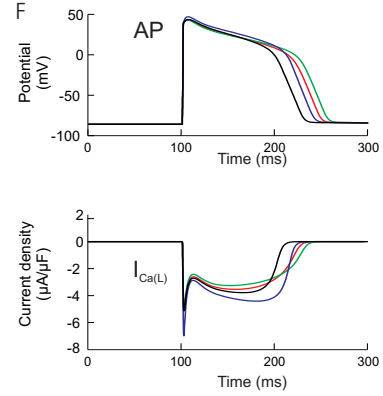
Voltage-dependence of activation and steady-state inactivation curves show a hyperpolarizing and depolarizing shift, respectively, leading to **increased availability and window current.**

II. Computational action potential simulation: G1911R prolongs APD



Simulated current-voltage relationships reflect the increased current density seen experimentally.

Simulated SSA & SSI curves were derived by computationally replicating activation and inactivation experiments to best fit experimental data.



Line	Case	APD80 (ms)	% Control
	Wild-type	122.83	100%
	50% G1911R	141.35	115.0%
	11.5% G406R	148.67	121.0%
	50% P857R	134.16	109.2%

Simulated action potentials (top) and I_{Ca} (bottom) for a heterozygous G1911R mutation (50% mutant channels); the TS G406R mutation (11.5% mutant channel); and a heterozygous P857R mutation show increases in APD80 (APD at 80% repolarization) of 15.0%, 21.0%, and 9.2%, respectively, compared to WT Cav1.2.

Conclusions

- The combined effects of the G1911R mutation to diminish VDI of I_{Ca} and increase window current are predicted to prolong the cardiac APD to a degree nearly as severe as the "classic" Timothy syndrome mutation.
- A mutation that affects current density only, such as the P857R mutation, can also prolong the APD, but to a lesser degree.
- This study broadens our understanding of the mechanisms by which a gain of function of I_{Ca} can contribute to QT prolongation.

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