A Distinct Molecular Mechanism by which Phenytoin Rescues a Novel Long QT 3 Variant

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OBJECTIVES

Mutations in the SCN5A gene, which encodes the primary cardiac Na^+ channel ($Na_V1.5$), can cause arrhythmic disorders such as Type 3 Long QT Syndrome (LQT3). In a pediatric patient with a novel missense mutation in SCN5A (Q1475P), the anticonvulsant drug phenytoin surprisingly proved to be more effective than the conventional anti-arrhythmic drug mexiletine at controlling the patient's arrhythmias.

We sought to uncover:

(1) what defects in gating at the molecular level could account for electrophysiological recordings;

(2) what modified cellular properties could explain the patient's LQT3 phenotype;

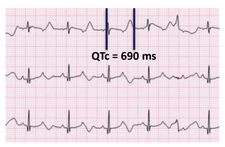
(3) what molecular changes are induced by phenytoin that are able to restore normal cardiac rhythm.

INTRODUCTION

Long QT syndrome (LQTS) is a genetic disease characterized by prolongation of the QT interval manifesting as episodes of syncope, torsades de pointes (TdP) and sudden cardiac death (SCD). The long QT syndrome 3 (LQT3) is the third most common variant and is caused by genetic variants in SCN5A, resulting in gain of function mutations in the $Na_V1.5$ subunit.

Clinical Correlation:

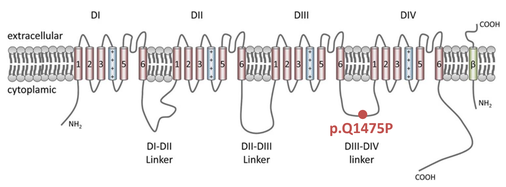
- Case report describing a Full-term female infant presenting with Atrioventricular block, long QT interval, short episodes of TdP [1]



- The patient underwent cardioverter-defibrillator/pacemaker implantation
- Several drugs were trialed on the patient including propranolol, ranolazine, **mexiletine**, diltiazem and phenytoin
- **Phenytoin** alone prevented arrhythmic episodes

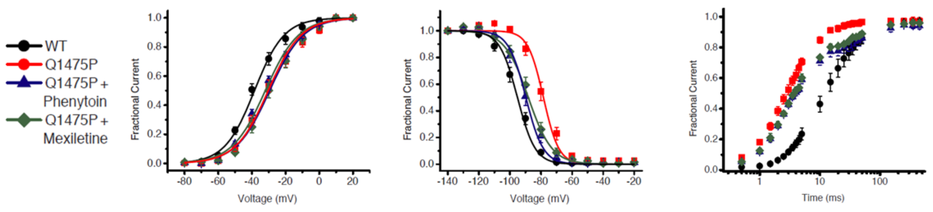
Genetics:

- Genetic testing identified an SCN5A c.A4424C variant resulting in p.Q1475P missense mutation in the $Na_V1.5$ inactivation gate

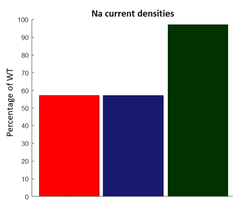


Electrophysiology experiments:

- HEK-293 cells were transfected with wild type (WT) or mutant $Na_V1.5$-Q1475P cDNAs



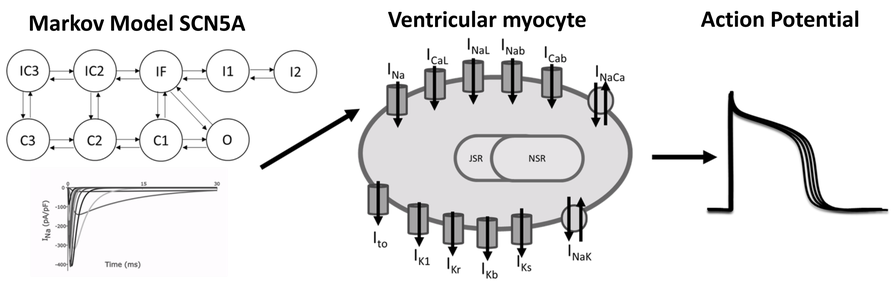
Effects of the Q1475P mutation and phenytoin on the voltage dependence of $Na_V1.5$ kinetic variables. $Na_V1.5$-Q1475P has the following properties: (1) current density was reduced compared to WT; (2) current inactivated faster and activated at more positive voltages; and (3) recovered faster from the inactivation state compared to WT. Phenytoin and mexiletine restore the voltage-dependence of $Na_V1.5$-Q1475P steady-state inactivation nearly to WT values. *Data from Dr. Ivan Gando.*



Phenytoin and mexiletine led to the same electrophysiological remodeling, partially recovering the defective $Na_V1.5$ channels. However, mexiletine increased the magnitude of Na^+ current in Q1475P channels while phenytoin did not.

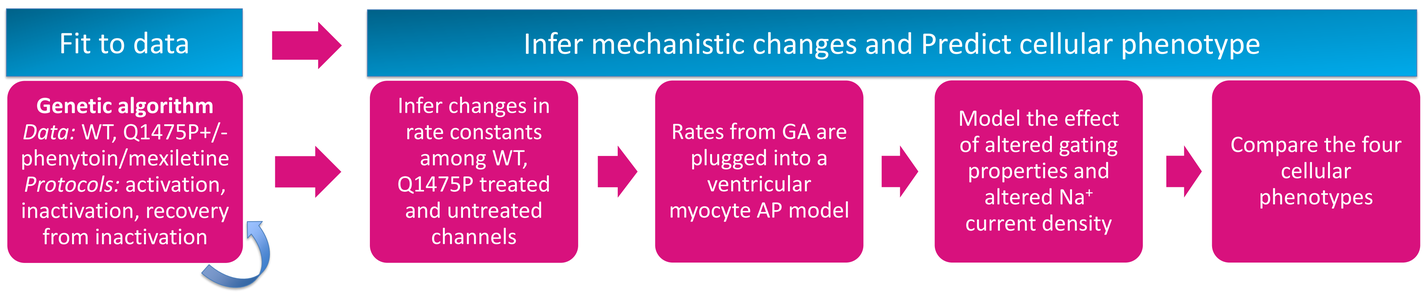
Multiscale Quantitative Systems Pharmacology:

- Molecular properties of WT, mutant, and treated sodium channels were described using Markov models
- Simulations recapitulated the electrophysiology protocols and created a population of variants to be integrated in a mathematical model of the human ventricular action potential to predict cellular phenotype



METHODS

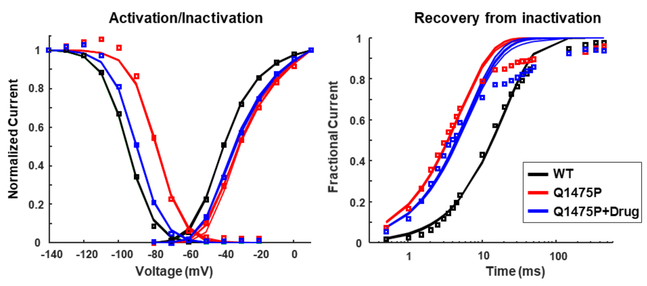
Simulations attempted to recapitulate the electrophysiology results and infer molecular and cellular modifications caused by Q1475P mutation through mathematical modeling of ion channel gating.



RESULTS

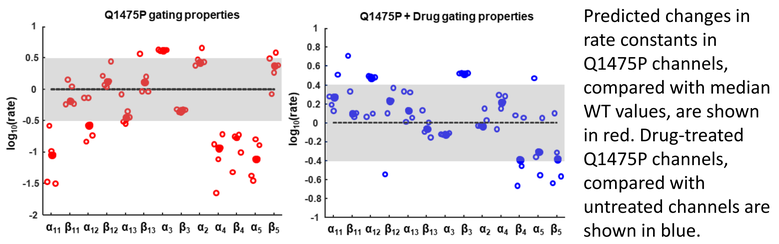
Modeling to infer altered gating transitions in Q1475P and drug-treated Q1475P channels:

- A nine state Markov model was used to model the Na^+ channel. The parameters describing the baseline model were replaced with the ones obtained from the genetic algorithm and the model was used to produce populations of steady-state activation, inactivation and recovery curves.

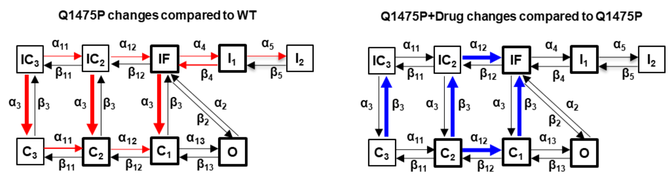


Populations of steady-state activation, inactivation and recovery from inactivation curves produced by the different parameter sets obtained with the genetic algorithm (n=5). The symbols show the experimental data, the solid lines are the solutions from each population.

- Modeling predicts changes in gating parameters induced by Q1475P mutation and by treatment with drugs. Since mexiletine and phenytoin induce the same alterations in Q1475P $Na_V1.5$ gating, in these simulations they are treated as the same drug.



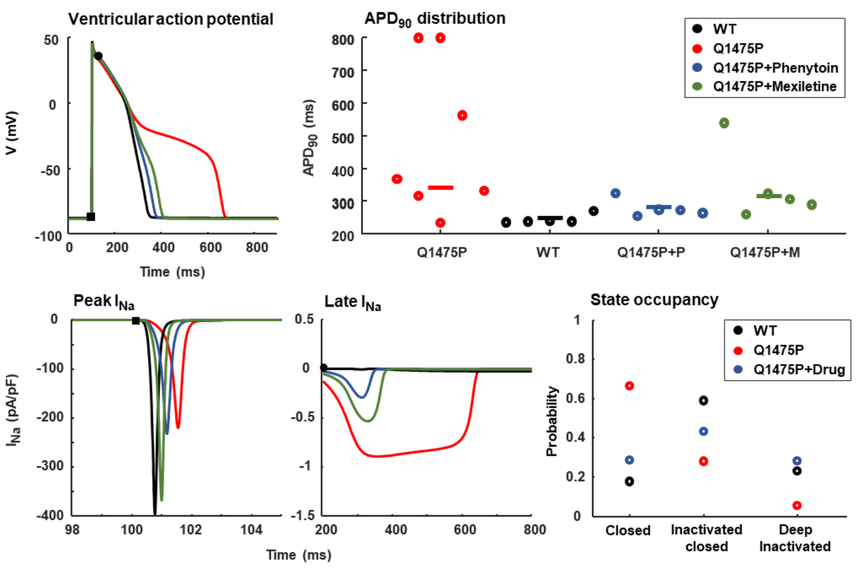
Predicted changes in rate constants in Q1475P channels, compared with median WT values, are shown in red. Drug-treated Q1475P channels, compared with untreated channels are shown in blue.



The rate constants predicted to be increased (thick lines) or decreased (thin lines) are illustrated on the $Na_V1.5$ gating scheme.

Action potential simulations:

- $Na_V1.5$ channels were incorporated into a model of the human ventricular myocyte action potential [3].
- *Na^+ current maximal conductance reflected the trafficking defect in the Q1475P channels, and the rescue of this trafficking defect by mexiletine, but not phenytoin.*
- Q1475P channels were more likely than WT channels to produce arrhythmogenic early afterdepolarizations (EADs) due to increased late Na^+ current that led to prolonged action potential duration (APD);
- Q1475P channels treated with phenytoin led to smaller late Na^+ current and shorter APD than Q1475P channels treated with mexiletine, preventing the occurrence of EADs.
- State occupancy: Channels treated with either phenytoin or mexiletine are more likely to be in inactivated closed states compared to untreated Q1475P channels.



Simulation of human ventricular action potentials incorporating the Na^+ channel kinetics obtained with Markov modeling. When incorporated into a ventricular myocyte action potential model, Q1475P channels compared to WT and phenytoin treated channels show the following: (1) increased APD_{90}; and (2) increased late sodium current. Mexiletine is less efficacious than phenytoin in our AP simulations.

Phenytoin was more effective than mexiletine in treating this patient because it partially restored the kinetic properties of the defective channels without increasing I_{Na} magnitude.

CONCLUSIONS

Mathematical modeling suggested which channel gating steps are most likely to be affected by the mutation and phenytoin and what are the possible mechanisms at the cellular level that can explain the patient's phenotype and the increased efficiency of phenytoin compared to mexiletine.

The results provide important insight into this patient's LQT3 phenotype and demonstrate how multiscale mathematical modeling can generate mechanistic hypotheses about: (1) alterations in ion channel gating that result from mutations; and (2) differential efficacy of drugs in the treatment of cardiac channelopathies.

REFERENCES

[1] Tan RB, Chakravarti S, Busovsky-McNeal M, Walsh A, Cecchin F. Complexity of ranolazine and phenytoin use in an infant with long QT syndrome type 3. HeartRhythm Case Reports. 2017;3(1):104-108. [2] Vladimir E. Bondarenko, Gyula P. Szigeti, Glenna C. L. Bett, Song-Jung Kim, Randall L. Rasmusson. Computer model of action potential of mouse ventricular myocytes. American Journal of Physiology - Heart and Circulatory Physiology. 2004 Vol. 287 no. 3, H1378-H1403. [3] O'Hara T, Virág L, Varró A, Rudy Y (2011) Simulation of the Undiseased Human Cardiac Ventricular Action Potential: Model Formulation and Experimental Validation. PLoS Comput Biol 7(5): e1002061. [4] Clancy, Colleen E., and Yoram Rudy. "Na+ Channel Mutation That Causes Both Brugada and Long-QT Syndrome Phenotypes: A Simulation Study of Mechanism." Circulation 105, no. 10 (March 12, 2002): 1208–13.