Arrhythmia formation in subclinical ("silent") long QT syndrome requires multiple insults: Quantitative mechanistic study using the *KCNQ1* mutation Q357R as example

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BACKGROUND In subclinical or silent long QT syndrome, the QT interval is normal under basal conditions. The hypothesis that insults to the repolarization reserve may cause arrhythmias in silent mutation carriers but not in noncarriers has been proposed as a general principle, yet crucial aspects remain descriptive, lacking quantification.

OBJECTIVE To utilize accurate mathematical models of the human action potential and β-adrenergic stimulation to quantitatively investigate arrhythmia-formation mechanisms peculiar to silent long QT syndrome, using mutation Q357R in *KCNQ1* (α subunit of slow-delayed rectifier I_{Ks}) as a paradigm.

METHODS Markov models were formulated to account for altered I_{Ks} kinetics in Q357R compared with wild type and introduced into a detailed model of the human ventricular myocyte action potential.

RESULTS Dominant negative loss of I_{Ks} available reserve accurately represents Q357R. Action potential prolongation with mutant I_{Ks} was minimal, reproducing the silent phenotype. Partial block of rapid delayed rectifier current (I_{Kr}) was needed in addition to fast pacing and isoproterenol application to cause early afterdepolarizations (EADs) in epicardial cells with mutant I_{Ks}, but this did not produce EADs in wild type. Reduced channel expression at the membrane, not I_{Ks} kinetic differences, caused EADs in the silent mutant. With mutant I_{Ks}, isoproterenol plus partial I_{Kr} block resulted in dramatic QT prolongation in the pseudo-electrocardiogram and EADs formed without I_{Kr} block in mid-myocardial cells during simulated exercise onset.

CONCLUSION Multiple severe insults are needed to evince an arrhythmic phenotype in silent mutation Q357R. Reduced membrane I_{Ks} expression, not kinetic changes, underlies the arrhythmic phenotype.

KEYWORDS Action potential; β-Adrenergic stimulation; Computational models; Electrophysiology; Isoproterenol; Long-QT syndrome; Repolarization reserve; Silent mutation

ABBREVIATIONS **AP** = action potential; **APD** = action potential duration; **CL** = cycle length; **EAD** = early afterdepolarization; **ECG** = electrocardiogram; **epi** = epicardial; **het** = heterozygous; **ISO** = isoproterenol; **LQT1** = long QT syndrome type 1; **LQTS** = long QT syndrome; **M** = mid-myocardial; **ORd** = O'Hara Rudy dynamic human ventricular action potential model; **WT** = wild type

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Introduction

Long QT syndrome (LQTS) can cause cardiac arrhythmia and sudden death in the absence of structural heart disease.[1] LQTS affects as many as 1 in 2000 among Caucasians.[2] The most common form of LQTS is type 1 (LQT1), with *KCNQ1* as the locus of mutations,[3] a gene that transcribes the Kv7.1 protein, forming the α subunit of the repolarizing slow delayed rectifier K^+ current I_{Ks}.

Although LQT1 is less deadly than other forms of LQTS, lethality increases considerably with emotional/physical stress or exercise, conditions associated with β-adrenergic stimulation and fast heart rate.[4] These conditions and also certain drug effects (eg, block of rapid-delayed rectifier I_{Kr}) increase the arrhythmia risk by challenging the genetically compromised repolarization reserve due to LQT1 mutation.[5] It has been conceptualized that physiological or external insults to the repolarization reserve, individually innocuous, combine synergistically to create arrhythmia substrates and cause fatal arrhythmias. As stated by A. Wilde, "a double hit hurts more."[6]

An enigmatic case of subclinical or "silent"[7] LQT1 was identified, missense mutation Q357R near the S6–C-terminal junction of Kv7.1 (Boulet et al[8]). Q357R was discovered in a 40-year-old female proband with a history of syncope,

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but with the corrected QT interval within the normal range (430 ms).[9] The absence of electrocardiogram (ECG) phenotype associated with this silent mutation obscures the connection between phenotype and arrhythmia. Exhaustive measurements by Boulet et al[8] showed that Q357R is characterized by partial loss of I_{Ks} function, a current that is of small magnitude in the human ventricle under basal conditions.

Observations of Boulet et al at the level of I_{Ks} current suggest only a vague possibility of minor action potential (AP) repolarization changes and marginally increased arrhythmia risk for this silent mutation carrier. Thus, several clinical questions are raised: Is it reasonable to suspect that the silent mutation was in fact the cause of syncope in the proband? That is, can known LQT1 insults (emotional/physical stress or exercise) account for arrhythmia formation in this case of silent LQT1? Moreover, is the consequence of the silent mutation so mild that these insults affect mutant and wild-type (WT) I_{Ks} similarly, causing a similar phenotype? If so, the silent mutation may not be the cause of syncope. Finding answers to these questions requires quantification beyond qualitative predictions. Motivated to investigate and explain the possibility of arrhythmia formation in humans with silent LQTS, we investigated Q357R as an instructive paradigm, using newly available, quantitatively accurate mathematical models.

Methods

Using experiments from Boulet et al,[8] human I_{Ks} models for WT and Q357R were developed. AP simulations were conducted by using the O'Hara Rudy dynamic human ventricular action potential model (ORd).[10] Simulations were performed in epicardial (epi) and mid-myocardial (M) cells and in a transmural wedge model from which the pseudo-ECG was computed[11] mimicking experiments.[12] Further details are given in the Supplement. Simulation results for APs or pseudo-ECGs were always preceded by a train of pacing that is not shown. For plotting purposes, time is reset to begin at t = 0, though the true simulation time is much larger.

To determine β-adrenergic stimulation effects on I_{Ks} and resulting changes to its role in the AP, we adapted the model by Heijman et al,[13] including signaling cascade from isoproterenol (ISO) application to compartmentalized protein kinase A concentration and fractional phosphorylation of targets. Equations, validation, and details are given in the Supplement (Supplement Figures S1 and S2).

Unless otherwise stated, black, dashed black, and gray lines are simulation results for WT, Q357R, and the heterozygous (het) case, respectively. Current symbols: fast Na^+ (I_{Na}), L-type Ca^{2+} (I_{CaL}), Na^+/K^+ ATPase (I_{NaK}), ultrarapid K^+ (I_{Kur}, represented by K^+ background, I_{Kb} in ORd).

Results

Q357R I_{Ks} kinetics and behavior

WT I_{Ks} was represented by the Markov model developed by Silva and Rudy[14] shown in Figure 1A, left. The activation of WT channels involves slow transitions from left to right in zone 2 of deep closed states, followed by rapid transitions

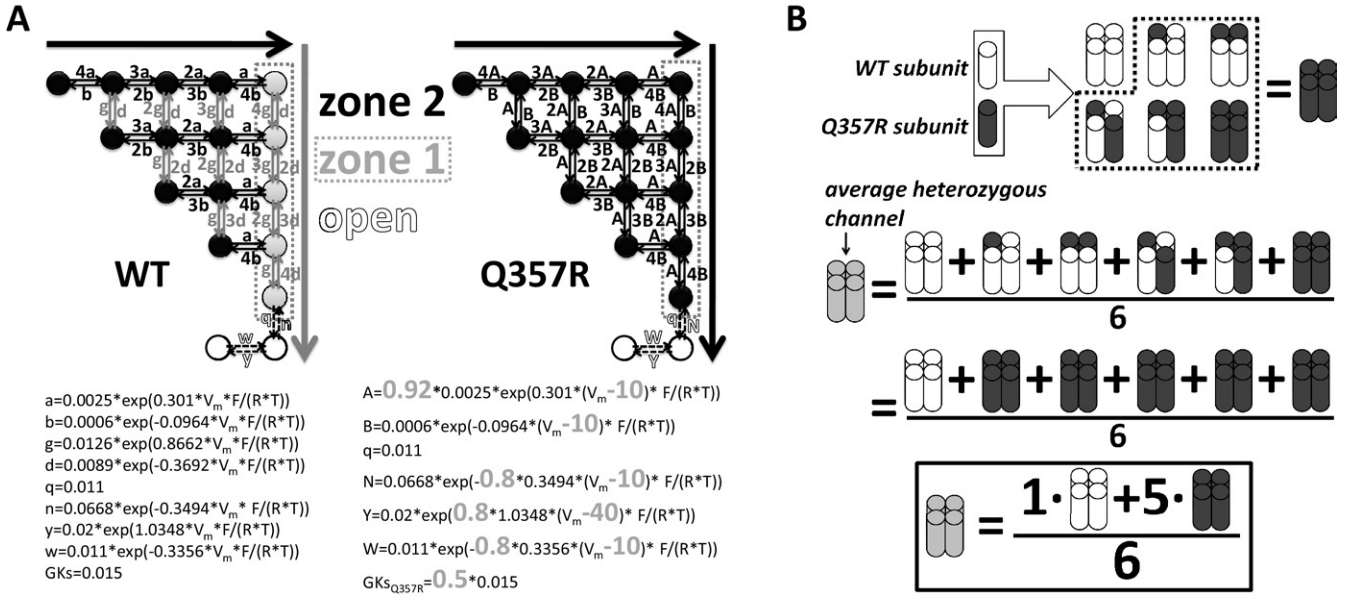

Figure 1 Markov I_{Ks}. **A:** Schematic diagrams and transition rate equations. The WT model is on the left; Q357R is on the right. Equations are below the diagrams (differences from WT are in large bold gray type). WT model activation, as proposed by Silva and Rudy,[14] represents 2 voltage sensor transitions. First transitions are from left to right (large black arrow) and second transitions are from top to bottom (large gray arrow). Channel kinetic states are divided into 2 zones. Different from WT where zone 1 (gray circles) transitions are rapid, for Q357R these transitions are the same as the slower zone 2 (black circles) transitions. **B:** WT (white) and Q357R (black) subunits combine to form 6 tetramer permutations in the heterozygous (het) case (gray). The model considers that the mutation was dominant negative. Thus, the behavior of the average het channel is the average behavior of the permutations: het = (1 × WT + 5 × Q357R)/6. WT = wild type.