Arrhythmogenesis in the Short-QT Syndrome Associated With Combined HERG Channel Gating Defects

— A Simulation Study —

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Background This study aimed to show the mechanism how the HERG channel gating defects causes life-threatening arrhythmia in the short-QT syndrome, using a simulation model of ventricular action potentials (APs). **Methods and Results** To evaluate the electrophysiological consequences of the short-QT syndrome at the level of the cardiac AP, the Markov model of wild-type (WT) KCNH2 channel was modified to obtain a model of the KCNH2 channel with the N588K mutation associated with the short-QT syndrome. Two parameters (β and $\beta\beta$) were changed to reconstruct the N588K mutant Markov model, which successfully reproduced the experimental results of voltage-clamp recordings. The WT and mutant models were then integrated into the Luo-Rudy theoretical model of the cardiac ventricular AP. Unexpectedly, 1 parameter change alone, which caused gain of function, could shorten the AP duration (APD) but failed to induce early after-depolarizations (EADs). Only the condition with the combined gating defects could lead to EAD. **Conclusions** Although the gain of function for KCNH2 shortened APD in the short-QT syndrome, this simulation study suggested that arrhythmogenesis was associated not only with gain of function, but also with accelerated deactivation of KCNH2. (Circ J 2006; 70: 502–508)

Key Words: HERG; Short QT syndrome; Simulation

The short-QT syndrome constitutes a new primary electrical abnormality associated with sudden cardiac death.^{1,2} A part of the disorder has been linked to gain of function mutations in the cardiac channel of rapidly activating delayed rectifier currents (I_{Kr}), HERG (KCNH2).³ Heterogeneous abbreviation of the action potential duration (APD) in different cell types spanning the ventricular wall seems to create the substrate for the genesis of ventricular arrhythmia in the short-QT syndrome, as is proposed for the long-QT syndrome and Brugada syndrome.^{4–8}

Brugada et al identified the first mutation associated with the short-QT syndrome.³ The missense mutation (N588K) involving a substitution of lysine for asparagine in position 588 of KCNH2 was found to cause a remarkable gain of function in the I_{Kr} . We studied how the gain of function mutation N588K in the KCNH2 could affect APD in different cell types of the ventricular walls, and how it could be associated with life-threatening arrhythmia in the Luo-Rudy (LRd) theoretical model of the cardiac ventricular action potential (AP).^{4,5,7,9} Unexpectedly, although the gain of function of KCNH2 resulted in shortening of the APD, arrhythmogenesis was associated not only with the gain of function, but also with accelerated deactivation of the N588K HERG channel.

Methods

To evaluate the electrophysiological consequences of the N588K mutation associated with the short-QT syndrome at the level of the cardiac AP, we constructed a Markov model of the N588K mutant channel, based on the experimental data of voltage-clamp recordings of I_{Kr} heterologously expressed in TSA 201 cells.³ The wild-type (WT) and N588K Markov models were then integrated into the LRd theoretical model of the cardiac ventricular AP.⁵ The simulation programs were encoded in C++ and implemented on a personal computer running Windows XP or an HP AlphaServer running Tru64 UNIX.

Fig 1. Schemes of the Markov models for wild-type (WT) and N588K I_{Kr} channels.⁵ The dotted line shows a decreased rate and the bold line shows an increased rate.

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The Markovian Model of the Cardiac I_{Kr}

As a prototype channel model, we used the previously published Markov model of the cardiac I_{Kr} channel (appendix of reference 4). Hodgkin-Huxley type models do not represent important properties associated with structure-function relationships of individual channels, although the Markov scheme represents distinct channel states and coupling between these states, allowing us to relate state-specific kinetic properties of ion channels to the electrophysiological behavior of the whole cell⁷

The Markov models of the WT and N588K mutant channels are shown in Fig 1. The model includes 3 closed states (C3, C2, C1), an open state (O), and an inactivation state (I). The transition between C2 and C1 is voltage-independent.⁴ We modified the basic Markov model as little as possible to simulate the experimental results (Table 1).³ The simulation for voltage-clamp experiments was calculated using the fourth-order Runge-Kutta method with a fixed time step of 0.020 ms.

AP Simulations

We used the dynamic LRd model of a ventricular cell with recent modifications.⁵ Simulations were conducted for isolated epicardial, endocardial, and M cells, which were simulated by varying the maximum conductance of the slowly activating delayed-rectifier potassium current (I_{Ks})⁹

Table 1 I_{Kr} Formulation

$I_{Kr} = G_{Kr} \cdot P(O) \cdot (V_m - E_K)$	
$P(O) = \text{open probability of } I_{Kr}$	
$G_{Kr} = 0.0135 \cdot [K^+]_{out}^{0.59}$	
$E_K = (R \cdot T / F) \cdot \ln([K^+]_{out} / [K^+]_{in})$	
N588K-mutant rates	
$O \rightarrow I$	$\beta i = 0.015 \cdot 0.656 \cdot e^{(0.000942 \cdot (V))} \cdot 4.5^{0.3} / [K^+]_{out}^{0.3}$
$O \rightarrow CI$	$\beta \beta = 4.5 \cdot 2.9357 \cdot 10^{-3} \cdot e^{(-0.02158 \cdot (V))}$

We labeled the simulated cell types with density ratios GKs:GKr=23:1, 15:1, and 7:1 as epicardial cells, endocardial cells, and M cells, respectively⁹ The absolute value of the maximal repolarization time difference was considered as the maximal transmural dispersion of repolarization (TDR_{max}). All cells were paced over 1,000 times at any basic cycle lengths (BCL). The APD90 (APD50) was defined as APD at 90% (50%) repolarization.

The AP simulation was calculated using the method by Rudy et al¹⁰ (<http://www.cwru.edu/med/CBRTC/LRdOnline/development.htm>) with a flexible time step ranging from 0.002 ms to 0.100 ms.

Fig2. The experimental and simulation results for voltage-clamp protocols. (A) The peak current-voltage relationship. The simulated current-voltage relationship showed that the change of βi alone failed to rectify significantly within a physiological range of potentials as the experimental study⁴ because the current of N588K mutant should be smaller than that of wild-type (WT) at range below 0 mV (arrow). By changing $\beta \beta$ in addition to βi , however, the N588K channel showed smaller currents at voltage clamps below 0 mV. (B) The tail current after repolarization. Although the voltage-clamp recordings of N588K showed significantly reduced tail currents after repolarization, we could not reproduce the mutant effect by only changing βi . The N588K channel showed reduced tail currents by additionally changing $\beta \beta$. The parameters used for simulation were the same as in Clancy and Rudy⁵ except for βi and $\beta \beta$.

Fig 3. (A) Comparison of action potentials (AP) of wild-type (WT) and N588K on epicardial (Epi), M, and endocardial (Endo) model cells (basic cycle lengths (BCL)=1,000 ms). When the I_{Kr} channels were all N588K mutants (thick lines), AP duration (APD) of each wall layers were smaller than WT (thin lines). (B) The frequency dependence of APD in M cells. The APD of N588K (black bar) were shorter than those of WT (white bar) at any BCL tested. Early after-depolarizations (EADs) occurred in the N588K M cell layer at BCL of 2,000 ms.

Results

The Markov Models for *KCNH2*

The N588K mutation, a point mutation in the S5-pore segment of *KCNH2*, dramatically increases I_{Kr} .³ Based on the experimental results of voltage-clamp recordings, we looked for parameters responsible for the gating defects (Table 1).³ First, simulation study revealed that the steady-state current continued to increase linearly at membrane potentials by decreasing the transition rate from O to I (β_i). The current-voltage relationships showed that the N588K model failed to rectify significantly within a physiological range of potentials, similarly as in the experimental study (Fig 2A). A change of d_i also showed the same result as β_i . The changing rates of other gating parameters could not increase I_{Kr} currents (data not shown). These results also agree with other experimental data, which show that N588K channels do not inactivate.^{11,12} Second, although the N588K channel showed small and fast inactivating tail currents after repolarization, and smaller currents at lower membrane potential (Fig 2A, arrow), we could not reconstitute such tail currents by only changing β_i . However, by increasing the transition rate from O to C1 ($\beta\beta$), the tail currents of the mutant channel became smaller and deactivated faster (Fig 2B). The changes of gating parameters other than $\beta\beta$ could not accelerate the deactivation of I_{Kr} currents (data not shown). The simulated N588K showed the acceleration of the rates of activation as compared with the experimental data. Zhou et al reported that high temperature accelerated the rates of activation.¹³ Therefore, we could reasonably well simulate the experimental results of the N588K mutant channel by changing the 2 parameters β_i and $\beta\beta$, because the simulation data were performed at

physiological temperature, whereas experimental data were performed at room temperature.

McPate et al recently showed no significant difference in deactivation time-course between WT and N588K channels at physiological temperature.¹² These results differ from the reports of Brugada et al³ or Cordeiro et al.¹¹ First, these results might be associated with experimental conditions, in which McPate et al¹¹ studied not only at physiological temperature, but without *MiRP1*. Second, the balance of transient rates, which are associated with deactivation and inactivation, could determine the scale and time-course of the tail currents of I_{Kr} currents. Therefore, the changing of 2 parameters β_i and $\beta\beta$ are reasonable, because decelerated transient rate to inactivation could increase the tail currents of I_{Kr} currents, whereas accelerated transient rate to deactivation could affect oppositely. The N588K channels with decelerated transient rate to inactivation, but not significant changes of the tail currents of I_{Kr} currents, necessarily need to have accelerated deactivation at a physiological temperature.

AP Simulations for *LRd* Model

To study the cellular consequences of the mutation, the Markov models of WT and N588K channels were inserted into the *LRd* model of cardiac ventricular cells.^{4,5} The effects of the N588K mutation on AP of epicardial, endocardial, and M cells were studied for the BCL of 1,000 ms. When the I_{Kr} channel was all N588K mutant, the APD of every wall layers were shorter than WT (Fig 3A). The APD of N588K was shorter than WT at any BCL (Fig 3B).

The I_{Kr} currents of WT recorded during AP stimulus displayed a hump-like waveform (Fig 4A). In contrast, N588K currents displayed a dome-like waveform and have a more

Fig4. I_{Kr} of M cell models of wild-type (WT) and N588K. (A) The I_{Kr} currents of WT during action potential (AP) displayed a hump-like waveform. In contrast, N588K currents displayed a dome-like waveform. The change of β_i alone caused larger I_{Kr} currents than the changes of β_i plus $\beta\beta$. (B) The probabilities of residence in the indicated states over the course of AP. I_{Kr} for N588K opens greater because of the decreased open state to inactivation state (O-to-I) transition. Although the WT channel slowly deactivates (from O to C1 transition), the mutant channel deactivates faster than WT. Predictably, the probability on the closed states (is larger) with the changes of $\beta\beta$ plus β_i showed is larger than with the change of β_i alone. WT, thin lines; only changing β_i , dotted lines; changing $\beta\beta$ in addition to β_i , thick lines.

important contribution during the early phases of repolarization of the AP. These simulated I_{Kr} currents showed the same results as experimental studies³. In the WT condition, the channel moves from the leftmost closed state (C3) and passes through C2 and C1 during the AP upstroke. From the C1 state, the channel can pass directly to the O state or to the I state. Once the channel opens, it rapidly inactivates. The equilibrium is reached between the O state and the I

Fig5. Ionic mechanism of early after-depolarizations in the short-QT syndrome. (A) Action potentials. (B) $I_{Ca(L)}$. (C) Calcium transient. (D) A parameter of calcium channel availability, $f \times f_{Ca}$. The larger calcium transient (C) results in a greater initial inactivation of $I_{Ca(L)}$ (B,D). Wild-type, thin lines; N588K, thick lines. Arrow, the faster recovery from inactivation in the short-QT syndrome allows for the reactivation of $I_{Ca(L)}$.

state, and it begins to favor recovery as the AP plateau repolarizes. As recovery from inactivation becomes more prominent, I_{Kr} increases and accelerate late repolarization of the AP⁴. In case of the N588K mutant channel, however, I_{Kr} appears earlier and is larger because of a decreased probability of the O-to-I transition. Although the WT channel slowly deactivates (from O to C1 transition), the mutant channel deactivates faster than WT (Fig4B). Predictably, changing β_i alone caused larger I_{Kr} currents than changing $\beta\beta$ plus β_i (Fig4A), and the probability of populating the closed states is larger with the changes of $\beta\beta$ plus β_i than with the change of β_i alone (Fig4B).

Early After-Depolarizations (EADs) of the Short-QT Syndrome

Although the N588K mutant channel has decreased APD, EADs occur with the BCL of 2,000ms in M cells, but not in epicardial or endocardial cells (Fig5A). The inward currents mediated by the L-type Ca channel ($I_{Ca(L)}$) are a critical determinant of AP and also of EADs. In association with the N588K mutant channel, they show a more prominent dome and greater recovery from inactivation at a membrane potential in the range of $I_{Ca(L)}$ activation

Fig 6. Individual effects of the 2 gating defects. One parameter change alone, which was associated with gain of function ($O \rightleftharpoons I$), shortened the action potential duration (APD), but failed to cause early after-depolarizations (EAD). The accelerated deactivation ($C \rightleftharpoons O$), which was associated with decreased tail currents, did not strongly influence APD, but only the condition of the combined gating defects ($O \rightleftharpoons I + C \rightleftharpoons O$) caused EAD. Wild-type, thin lines; N588K, thick lines.

(Fig 5B). The inactivation of $I_{Ca(L)}$ currents involves voltage-dependent and calcium-dependent processes (Fig 5C and D). In the LRd model, they are represented by 2 gates, f and f_{Ca} , which are voltage- and calcium-dependent, respectively. The product of these 2 gates, $f \times f_{Ca}$, is an important parameter that indicates the recovery from inactivation of $I_{Ca(L)}$ and the availability of calcium channels for subsequent reactivation.⁹ The faster recovery from inactivation at a membrane potential in the range of $I_{Ca(L)}$ activation allows reactivation of $I_{Ca(L)}$, which being an inward current, results in secondary membrane depolarization to generate the EAD (Fig 5D).

Effects of Each Gating Defect for AP Simulations for LRd Model

We investigated how each of the 2 gating defects would affect the myocardial model (Fig 6). Unexpectedly, changes of 1 parameter alone, which was associated with gain of function or accelerated deactivation, could change the APD but failed to cause EAD. The increased transient rate from the O state to the closed state, which was associated with decreased tail currents, was strongly associated with EAD of the short-QT syndrome. Only the combination of the 2 gating defects could cause EAD (Fig 6). $I_{Ca(L)}$ reactivation also occurred only with the combination of the 2 gating defects.

Discussion

The KCNH2 potassium channel has a shaker-like tetrameric structure composed of homologous core units each containing 6 membrane-spanning segments. Co-assembly

Fig 7. The contribution of transient potassium current (I_{to}) to phase I repolarization in epicardial (Epi) and endocardial (Endo) cells. Wild-type, thin lines; N588K, thick lines.

with the β -subunit MiRP1 (KCNE2) is required to fully reproduce the biophysical and pharmacological properties of the native I_{Kr} .¹⁴ KCNH2 has previously been linked to a decrease in outward repolarizing current responsible for the hereditary long-QT syndrome (LQT2)¹⁵ and for acquired forms of long-QT syndrome (LQTS).¹⁶ KCNH2 is also the primary target of class III antiarrhythmic agents, many of which contribute to generation of an acquired form of the LQTS. Brugada et al described the first mutation associated with the short-QT syndrome.³ The short-QT syndrome is genetically heterogeneous. It can also be caused by mutations in the KCNQ1 gene that encodes the KVLQT1 K^+ channel, which forms I_{Ks} in association with the β subunit IsK ,¹⁷ and by mutations in the KCNJ2 gene that encodes the inwardly rectifying $Kir2.1$ channel.¹⁸

We studied how gain of function of KCNH2 would cause life-threatening arrhythmia in the short-QT syndrome. The N588K mutation of KCNH2 was found to cause a remarkable gain of function in the I_{Kr} in short-QT syndrome.³ Based on the voltage-clamp experiments, the present simulation study revealed that N588K had 2 gating defects. Unexpectedly, 1 parameter change alone, which was associated with gain of function could shorten the QT intervals, but could not cause EAD by itself. The increased transition rate from the O state to a closed state (C1), which was associated with decreased tail currents, was strongly associated with EAD of the short-QT syndrome, and only the condition with both gating defects caused EAD. The previous report showed that the accelerated deactivation is related with LQTS.⁴ The accelerated deactivation for N588K mutant does not decrease APD, but increases APD. However, the balance between impaired inactivation and the accelerated deactivation would determine APD, and gain of function in the I_{Kr} might be a strong determinant of APD in the case of N588K. McPate et al and Cordeiro et al reported that N588K channels alter the ion selectivity.^{11,12} Reversal potentials for the N588K mutant are significantly higher. We also studied how the ion selectivity could affect EAD for

N588K channels. Higher reversal potential could easily cause EAD for N588K channels with double gating defects (data not shown).

$I_{Ca(L)}$ reactivation seems to be a universal mechanism of EAD from plateau potentials. It is well known that EAD cause ventricular tachycardia in the LQTS^{4,9}. Prolongation of the post-pause AP plateau because of a smaller I_{Ks} or I_{Kr} and an enhanced inward I_{NaCa} , on the background of the LQTS effects, provides time for the recovery and reactivation of $I_{Ca(L)}$, which generates the depolarizing charge for EAD formation. However, the faster recovery from inactivation in the short-QT syndrome allows for the reactivation of $I_{Ca(L)}$.

The group of Antzelevitch presented the first experimental evidence of the role of transmural dispersion of repolarization (TDR) in arrhythmogenesis associated with short-QT intervals in the ECG⁶. Pinacidil was used to mimic a gain of function of a potassium current in the canine arterially perfused wedge preparation. Pinacidil activates adenosine triphosphate-sensitive potassium currents (I_{K-ATP}), leading to an abbreviation of APD^{19,20}. In the wedge preparation, pinacidil abbreviated the QT interval, leading to a heterogeneous abbreviation of the APD of the 3 principal cell types spanning the ventricular wall. The pinacidil model of the short-QT syndrome, although mechanistically related, is phenotypically different from the clinical syndrome caused by a gain of function of HERG.

The computer simulation is a good tool to predict APD of genetic arrhythmogenic disorders, such as the LQTS and Brugada syndrome^{4,5,7}. We could successfully reproduce these alterations of gating properties in the short-QT syndrome by changing 2 parameters in the Markov model of the KCNH2. When the I_{Kr} channels were all N588K mutants, APD of each wall layer was smaller than that of WT. The TDR_{max} of N588K was also smaller than WT at any BCL (data not shown). These results are opposed to the wedge models. However, Brugada et al showed that the mid- and endocardial regions of the ventricle would repolarize more rapidly than the epicardium, because currents mediated by mutated HERG channels electronically follow the membrane potential. The presence of a notch during phase I therefore lowers the amplitude of the N588K currents in the epicardium vs endocardium and increase TDR. We tested this hypothesis by modifying the LRd model to take into account the contribution of transient potassium current (I_{to}) to phase I repolarization of the epicardium, according to modifications published earlier by Dumaine et al²¹. Although mutant APD for endocardial cells shorten in the presence of I_{to} , shortening of the APD for epicardial cells was slight, and these results lead to increase TDR (Fig 7). Cordeiro et al recently reported that the dispersion of repolarization between the Purkinje fibers and ventricles induced by N588K might create a substrate for premature re-excitation of the endocardium¹¹. In the short-QT syndrome, ventricular tachycardia might occur in the substrate of both EAD in M cells and the dispersion of repolarization between the Purkinje fibers and ventricles. We might need to analyze the transmural AP mapping by applying a validated optical mapping technique²².

Clinically, we have not clearly understood what triggers ventricular tachycardia in the short-QT syndrome unlike the LQTS^{23,24} because adequate genotype-phenotype correlation data are not yet available for the short-QT syndrome. A latest report shows that shortening of the QT interval is prominent at lower heart rates²⁵. Therefore, we speculate

that a potential trigger might occur in ventricular tachyarrhythmias at rest and during sleep in patients with a short-QT syndrome.

In summary, a part of the short-QT syndrome has been linked to gain of function mutation of KCNH2. Although the gain of function for KCNH2 causes shortened APD of the short-QT syndrome, arrhythmogenesis might be associated not only with gain of function, but also with accelerated deactivation of HERG.

Limitations

First, the simulation studies are generally simplified. However, the simulation studies lead to understand complex and interactive phenomena, and relate them to membrane ionic currents and to dynamic changes in the intracellular ionic environment⁷. Second, the simulation studies were conducted in isolated cell models. The ionic mechanism in the single cell helps us to understand the basis of its behavior in the multicellular tissue with complex interactions. In the intact myocardium, cells are interconnected through gap-junctions and we need to study its ionic mechanism in the multicellular tissue level²⁶.

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