Link Between SCN5A Mutation and the Brugada Syndrome ECG Phenotype

— Simulation Study —

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Background The specific changes in the gating kinetics of the sodium current (I_{Na}) responsible for its phenotype have remained to be elucidated. In the present study the effect of changes in the gating kinetics of I_{Na} on early repolarization (ER) and initiation of phase 2 reentry (P2R) were evaluated in a theoretical epicardial ventricular fiber model.

Methods and Results Miyoshi- I_{CaL} was incorporated into the modified Luo-Rudy dynamic (LRd) model. Dispersion at I_{to} -density was set within a theoretical fiber composed of serially arranged epicardial cells with gap junctions. The following changes in I_{Na} kinetics were made: (1) a -10 mV shift in steady-state inactivation, (2) a $+10$ mV shift in steady-state activation curve, (3) a small inactivation time constant (DEC); P2R and ER were observed. A conduction disturbance within the fiber was simulated and only when the I_{Na} -density was decreased did DEC, especially, show a marked increase in the likelihood of causing ER and P2R. Conduction disturbance significantly increased the likelihood causing ER or P2R.

Conclusions In this one-dimension model with I_{to} -density dispersion, DEC- I_{Na} precipitates I_{Na} -blocker inducible ER. This suggests that the characteristic ST-segment elevation in the Brugada syndrome with SCN5A mutation can be interpreted in part by DEC- I_{Na} . Concomitant conduction disturbance may be required to cause P2R at physiological I_{to} density. (Circ J 2005; 69: 567–575)

Key Words: Arrhythmia; Computers; Electrocardiogram; Electrophysiology; Ventricles

Brugada syndrome (BS) is characterized by ST-segment elevation in the right precordial lead and sudden cardiac death in the absence of obvious organic heart disease.¹ The temporal correlation between increased ST-segment elevation and the occurrence of ventricular fibrillation (VF) originating in the right ventricle (RV) suggests that the mechanism of the ST-segment elevation must be the key to the initiation of VF in BS.^{2,3} Since I_{to} is prominent in the RV epicardium,⁴ I_{to} -mediated early repolarization (ER) of the action potential can be observed preferentially in RV epicardium, leading to ST-segment elevation via an increasing transmural gradient of the membrane potential.^{3,5} This is thought to be one of the possible cellular mechanisms of the ST-segment elevation in BS.^{3,5,6} The transmural dispersion of repolarization in the RV is capable of precipitating phase 2 reentry (P2R) and subsequent circus movement reentry,^{5,7–9} but because it does not always do so, the precise mechanisms of P2R and VF in BS have remained unclear.

The finding of enhanced ST-segment elevation by fast sodium current (I_{Na}) blockers^{3,10} and of the SCN5A-muta-

tions in BS^{11–16} suggests that I_{Na} as well as I_{to} plays a major role in the pathogenesis of BS. Although various abnormalities in the current phenotype have been reported in SCN5A mutations, it is difficult to identify the abnormality in kinetics that causes specific phenotypes of the disease in experimental models. Various computer simulations have shown that the current phenotype of the mutant currents can cause ER, but they failed to simulate P2R^{6,12,17} and thus the insight for the arrhythmogenic mechanism in BS was limited.

The marked cell-to-cell variance in I_{to} -density within the RV-epicardium can generate significant dispersion in action potential duration, which has been thought to cause P2R within the RV.⁴ Therefore previously, we composed new 1-dimensional epicardial fiber model in which we set the dispersion in I_{to} -density to simulate P2R.¹⁸ In the previous model, we re-measured the voltage dependent kinetics of the L-type Ca current (I_{CaL}) precisely and then composed new mathematical model of I_{CaL} that was essential to simulate P2R. In the present study we evaluated how such kinetic changes affect the occurrence of P2R and ER in our model by modifying the voltage-dependent kinetics of I_{Na} according to observations that have been reported in the Brugada-related SCN5A mutations.^{11–16} We also investigated the effect of I_{Na} blockade in each simulated SCN5A mutation to identify the mechanism of the I_{Na} -blocker-induced ST-segment elevation only in patients with BS.^{3,10}

On the other hand, because prolongation of the His-ventricular (HV) interval^{1,3} and a delayed potential detected in

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Fig 1. Occurrence of P2R and ER by dispersion at an I_{to} -density. (A) The vertical axis represents the I_{to} -density at the cells on the left side of the theoretical epicardial fiber, and the horizontal axis represents the right side. Each circle symbol represents a pixel from which 3 representative sets of traces (B, C, D) were obtained. See text for details. Please note that the time scale of panel D is longer than for B & C.

the signal-averaged electrocardiogram (ECG)^{3,19,20} are commonly observed in BS, the presence of diseased myocardium with a conduction disturbance has been thought to be a cellular basis for the syndrome.²¹ Isolated RV myocytes are usually smaller in diameter than isolated left ventricular myocytes,^{22,23} and because smaller cell diameter increases intracellular resistance along the longitudinal axis, the conduction disturbance may be caused preferentially in the RV. Therefore, in the present study we simulated a conduction disturbance by changing the cell diameter in order to observe the occurrence of P2R and ER in the model.

Methods

Epicardial Fiber Model

The epicardial fiber model previously described was used.¹⁸ The mathematically defined model of I_{to} devised by Dumaine¹² and our own model of I_{CaL} ¹⁸ were incorporated into the Luo-Rudy dynamic (LRd) model²⁴ to simulate epicardial action potentials. The theoretical fiber²⁵ was composed of 50–150 serially arranged epicardial ventricular cells (total fiber length 0.5–1.5 cm) with gap junctions. No flux boundary conditions were used at either end of the fibers. The intracellular resistance per element unit (R_i) is composed of myoplasmic resistance ($R_{myo}=150 \text{ cm}$) and gap junction resistance ($R_g=1.5 \text{ cm}^2$)²⁵ $\Delta x=100 \mu\text{m}$

(entire cell length) is used as the discretization element, with R_i reflecting the lumped contribution of axial and gap junction resistance ($R_i=R_{myo}+R_g/\Delta x$).²⁵ Differential equations were solved numerically by using the Crank-Nicholson time derivative²⁶ set at $10 \mu\text{s}$, and the space derivative was set at 0.1 mm . Maximal I_{CaL} -conductance was increased 20% over the original Miyoshi- I_{CaL} ¹⁸ value in every experiment for the following reasons. In the previous study, we set the same maximum conductance of the I_{CaL} as in the Dumaine's model in order to compare the Rasmusson's- I_{CaL} model, but the phase 1 magnitude was quite low ($<-30 \text{ mV}$) compared with the experimental data.⁴ In the previous study, the occurrence of P2R was too low to observe the change in the occurrence of P2R induced by the I_{Na} mutation, so empirically we set the I_{CaL} conductance as 120% of our previous paper. We set the dispersion at the I_{to} -density at the both end of the fiber and applied pacing to the cell at the left end so as to generate P2R in the fiber. In order to excite the cell the current was transmembranously injected into the cell with 0.5 ms duration and $2 \times$ threshold intensity. We observed the action potential propagation pattern elicited by the first pacing. We defined a P2R impulse that traveled from left to right as an antidromic P2R, and a P2R from right to left as an orthodromic P2R. As shown previously,¹⁸ it was difficult to distinguish an antidromic P2R from an electrotonic reflection.^{27,28} However,

Fig2. Representative phase 2 reentry trace in the mathematical model and the role of inward currents. (A) Membrane potentials in the fiber at each point in time are shown. The horizontal axis indicates the distance of the cell from the left end of the fiber, and the vertical axis indicates the membrane potential. Each line represents the membrane potential at each point after the start of pacing (denoted along beside). The top bar represents the distribution of cells with each I_{to} -density shown within the bar. The phase 2 dome was conducted from left to right and generated another action potential (*). The striped bars at the bottom represent the range of cells, and their membrane potentials, and the inward currents were precisely observed and are shown in the lower panels. (B) Calculated currents and potentials of each numbered cell are superimposed on each horizontal column. The upper limit in the current column is zero, and the full scale is shown to the left of each column. (C) The currents of the numbered cells (denote the right) are superimposed on each column. The vertical axis is the same as in (B). I_{NaCa} , I_{Na} , I_{CaT} , and I_{CaL} are shown in white, black, dark gray, and light gray, respectively. A peak I_{Na} that was larger than the I_{CaL} and preceded the second action potential of phase 2 reentry (*) was observed in Cells 50–54.

as no antidromic P2R was observed at the end of the fiber, the mechanism of the antidromic P2R differed from that of electrotonic reflection at the dead end of the fiber. Because we preferred to observe the orthodromic P2R, which is usually referred to as P2R²⁷ we changed the I_{to} -density in the 63% cells on the right side of the fiber from that of the 37% cells on the left side to generate the dispersion to observe R2R in the fiber on the right side precisely. The I_{to} -densities were raised from 0.5 to 2 mS/ μ F in increments of 0.09375, and the fiber was observed for the occurrence of P2R and ER. The physiological I_{to} range in the RV epicardium was set at less than 1.1 mS/ μ F in the present study.¹² A P2R was defined as 2 action potentials separated by full repolarization of less than -80 mV generated by a single ventricular pacing. Because the currents through the cell membrane at both dead ends of the fiber are necessarily over-estimated, the P2R data generated from the last 3 cells

at each end of the fiber were discarded. To simulate conduction disturbances, we modified the R_g and monitored the fiber for the occurrence of ER and P2R. The conduction velocity (CV) was measured by the impulse propagation speed between the cell-30 to cell-50 (m/s). A pseudo-ECG²⁹ was calculated as an extracellular field potential along the axis of our theoretical fiber and 4 cm from the right end of the fiber.

Change in Sodium Current Kinetics

Various mutations in the SCN5A channel have been reported^{11–16} in BS. In the present study, we simulated several types of mutations that can decrease I_{Na} conductance. We set the I_{Na} mutation as follow: (1) a -10 mV shift in steady-state inactivation curve ($I_{Na}ACT$),^{13,14,16} (2) a $+10$ mV shift in steady-state activation curve (ACT),^{13,16} and (3) a small inactivation time constant (half of the original LRD)

Fig3. Effect of an I_{Na} blocker on the ECG. (A) Superimposed representative ECG (V₂) tracings from a patient with Brugada syndrome before and after pilsicainide administration. Time after pilsicainide and the calculated QTc interval are shown beside each trace. The effect of a change in I_{Na} -density on the extracellular field potential (Pseudo-ECG) calculated from the theoretical epicardial fiber with DEC- I_{Na} (B) and with wild-type I_{Na} (C) are shown. I_{Na} -density was varied between 30% to 100% (noted beside the trace) of the original LRd model.

(DEC)^{12,16} We incorporated the simulated SCN5A mutations in our epicardial model, and then monitored the fiber for the occurrence of ER and P2R.

ECG From a Patient With BS

An ECG was obtained from a patient with BS during a pilsicainide test (150mg po). The patient was a 45-year-old Japanese male with a family history of sudden death at 41 years of age. The patient gave informed consent for the challenge test, and the institutional review board approved the protocol. The mutations of ion channels in the patient are unknown, because he did not agree to genetic analysis.

Results

The spatial dispersion in I_{to} density within the theoretical fiber (0.8 cm) sometimes generated P2R and ER, and sometimes did not (Non-ER). The relationship between the P2R and the I_{to} densities in the fiber is shown in the matrix sheet (Fig 1A). The data are symbolized as colored pixels on a coordinate plane the vertical axis of which represents I_{to} density in the fiber on the left side (Left fiber) and the horizontal axis represents I_{to} density in the fiber on the right side (Right fiber). In Fig 1B, the right cell showed accentuation of the phase 1 notch and a slight increase in the action potential duration; however, no cell in the fiber showed ER or P2R. The pseudo-ECG showed mild accentuation of the R'-wave (J-wave). A small inverted T-wave

was observed, but it may have been masked by the large T-wave of LV origin because the action potential duration is longer in the LV than in the RV. The pseudo-ECG showed a coved-type ST-segment elevation when P2R occurred in the fiber (Fig 1C). When ER occurred (Fig 1D) there was marked shortening of the QT interval. The present model is an epicardial 1-D fiber model, so there is no transmural axis (along the axis from endo-epi). Gima and Rudy reported that epicardial ER was observed as a saddle-back type ST-segment elevation on the ECG in a transmural 1-D fiber model⁶.

To evaluate the likelihood of arrhythmogenesis, we calculated the pixel area of P2R or ER+P2R. It is known that I_{to} varies beat to beat as a function of the preceding diastolic interval, so we assumed if the pixel area was large, P2R would occur more easily by chance. Furthermore only the P2R observed in the physiological range is significant. Because P2R usually occurs at the margin between the area of ER and non-ER, the large net pixel area of ER and P2R corresponds to the occurrence of P2R at the low end close to physiological I_{to} density. Therefore it is important to note that both the large net pixel area of ER+P2R and a large pixel area of P2R are necessary to generate P2R in BS. Furthermore, the net pixel area of ER and P2R also corresponds to the probability of causing a saddle-back type and coved-type ST-segment elevation, respectively.

In the latter part of the present study, we calculated the pixel area in the matrix data. A representative P2R in the

Wild-type I_{Na}

DEC I_{Na}

Fig 4. Occurrence of P2R and ER as a result of dispersion at an I_{to} -density with wild-type I_{Na} (Upper panels) or DEC- I_{Na} (Lower panels) at various I_{Na} -densities are shown. Each matrix is shown in the same manner as in Fig 1. I_{Na} density is shown at the top of each matrix, and the calculated CV is shown at the bottom of each matrix. The numbers in the matrix are the numbers of white and black pixels. Decreases in I_{Na} density did not increase the area of P2R and ER greatly in the matrix with the wild-type I_{Na} , but they increased the pixel area dramatically in the matrix with DEC- I_{Na} . CV, conduction velocity.

fiber (0.8cm) is shown in Fig2A. The action potential at the right fiber has lost its dome and has repolarized. The phase 2 dome in the left fiber gradually propagated to the right, and finally generated a P2R (*) in an “all or none” manner. The “all or none” excitation of P2R propagated to the right side of the fiber. The I_{CaL} , I_{Na} , T-type Ca current (I_{CaT}), and the NaCa exchanger current (I_{NaCa}) at the time of the onset of P2R are shown in Fig2B,C. The major inward current in the cells to the left of Cell 50 was I_{CaL} , which passed into the cell on the right fiber via a gap junction and caused slow depolarization. As discussed in a previous paper,¹⁸ this current is mainly caused by reactivation after deactivation of I_{CaL} , which is described as the “d-gate” in the mathematical model. However, the P2R did not always occur in an “all or none” manner only by this reactivation of I_{CaL} . Both I_{NaCa} and I_{Na} may play a role in this slow depolarization; however, because I_{NaCa} itself does not cause self-reproducing depolarization, it must not play a major role in triggering P2R. It is noteworthy that in Cells 50–54, the peak I_{Na} , which was greater than the I_{CaL} , preceded the initiation of P2R (*) and I_{CaL} activation, suggesting that the I_{Na} must play an important role in the “all or none” activation of the second action potential of the P2R.

A representative ECG trace during the pilsicainide test in the patient with BS is shown in Fig3A. No genetic data

were available for his ion channel mutation. The V_2 -lead tracings before and after pilsicainide administration have been digitized and superimposed. Pilsicainide unmasked the coved-type ST-segment elevation, whereas the baseline ECG showed a saddleback-type ST-segment elevation. The amplitudes of the R'-wave and negative T-wave increased as a function of time after administration of pilsicainide. At 60 min, when the response to pilsicainide was maximal, QTc was markedly prolonged and the isoelectrical line of the ST-segment disappeared. In the simulation with DEC- I_{Na} , I_{Na} blockade had a similar effect on the pseudo-ECG (Fig3B). Increases in amplitude of the R'-wave and negative T-wave and QT-prolongation were observed as clear functions of decreasing I_{Na} -density. If the P2R was blocked within the local RV epicardium, we would not observe it as PVC, but might observe it as a coved-type ST-segment elevation in the right precordial leads. Blockade of I_{Na} , on the other hand, had little effect on the pseudo-ECG in the simulation with wild-type I_{Na} .

The effect of mutation in I_{Na} on ER and P2R is shown in Fig4 (0.8cm). DEC- I_{Na} decreased the conduction velocity and the pixel area of P2R, but the net pixel area of P2R and ER did not change much. When I_{Na} -density was decreased in the simulation with DEC- I_{Na} , however, the net pixel area of P2R and ER increased dramatically, and there was only

Fig 5. Effect of reducing I_{Na} density on the calculated pixel area of ER+P2R (Left panel) and P2R (Right panel) in the presence of various simulated I_{Na} mutations. At an I_{Na} density of 100%, the ER+P2R pixel area did not change very much. However, ER+P2R increased dramatically as a function of decreases in I_{Na} -density only in DEC- I_{Na} , and not with the other simulated wild-type and mutant I_{Na} . The area of P2R seems to decrease as a function of decreases in I_{Na} -density. ER, early repolarization; P2R, phase 2 reentry.

Fig 6. Occurrence of P2R and ER as a result of dispersion at an I_{to} -density with wild-type I_{Na} in various lengths of epicardial fiber. Each matrix is shown in the same manner as in Fig 1. Fiber length is shown at the top of each matrix. The pixel area of P2R increased dramatically as a function of increases in fiber length, but ER did not change very much.

a small change in the wild-type I_{Na} . Interestingly, the pixel area of the P2R was decreased by the I_{Na} -reduction. The pixel areas of ER and P2R calculated for various simulated SCN5A mutations and I_{Na} -densities are shown in Fig 5. Neither ACT nor InACT increased the ER pixel area very much compared with the wild-type I_{Na} . Although DEC- I_{Na} increased the ER pixel area only slightly at normal I_{Na} -density, it increased dramatically at the lower I_{Na} -density.

We then investigated the effect of changes in the total length of the fiber on the ER and P2R (Fig 6). The pixel area of P2R increased markedly as a function of increases in total fiber length. Conduction disturbance also plays an important role in the occurrence of P2R (Fig 7) in the

0.8 cm epicardial fiber. The pixel areas of P2R and ER in the matrix increased as a function of decreases in the conduction velocity induced by changing the radius of the cell. At the baseline I_{Na} -density, the net pixel areas of ER and P2R were similar in the simulation with both wild-type I_{Na} and DEC- I_{Na} , whereas, at decreased I_{Na} -density, the pixel areas of ER and P2R were markedly increased only when simulated with DEC- I_{Na} , especially in the smaller diameter cells, and thus the P2R occurred much closer to the physiological I_{to} -density (dotted box) in the simulation with a cell radius of 5 μ m and a DEC- I_{Na} . Despite the conduction velocity being slower in the simulation with wild-type I_{Na} , $r=5 \mu$ m, and 40% I_{Na} density than it with DEC- I_{Na} , $r=9 \mu$ m,

Fig7. Occurrence of P2R and ER as a result of dispersion at an I_{to} -density with wild-type I_{Na} (upper 2 columns) or DEC- I_{Na} (lower 2 columns) at various cell radii (r =radius of the cell along the short axis). Each matrix is shown in the same manner as in Fig 1. The radius of the cell is shown at the top of each matrix, and the calculated CV is shown at the left of each matrix. Conduction velocity decreased significantly and the area of the P2R and ER increased as a function of smaller cell size. The area of P2R approached the physiological I_{to} range (within the dotted square) particularly closely when the conditions were $r=5\mu m$, a 40% decrease in I_{Na} , and DEC- I_{Na} . CV, conduction velocity.

and 40% I_{Na} density, the area of P2R was located closer to the physiological I_{to} range in DEC- I_{Na} . This suggests that not the decrease in conduction velocity but the rapid inactivation in the kinetics directly increased the likelihood of causing P2R at near physiological I_{to} density.

Discussion

As stated, ER does not necessarily cause P2R, and thus it was very important to observe P2R in our model to evaluate the incidence of lethal arrhythmia in BS. The present results show that I_{Na} as well as I_{CaL} is important in initiating P2R. The finding that I_{Na} blockade decreased the pixel area of P2R is consistent with the fact that I_{Na} blockers have often been used empirically to prevent VF clinically^{3,30,31} in patients with BS. However, care should be exercised when prescribing I_{Na} channel blockers for patients with the SCN5A mutation of the DEC- I_{Na} type, because the area of the P2R approaches the physiological I_{to} -range,

leading to a proarrhythmic effect.

The degree of ST-segment elevation often changes dynamically during the clinical follow-up of BS. As a result, in some populations of patients, the ST-segment often becomes normal when there is no VF^{3,32,33}. One of the cellular bases for this ST-segment change is the change in I_{CaL} -density¹⁸. On the other hand, I_{Na} blockers are commonly used to unmask the latent ST-segment elevation in BS and this has been explained by a greater decrease in I_{Na} conductance than decrease under the baseline conditions of BS. However, if that were true, strong I_{Na} blockade should cause nonspecific ST-segment elevation, even in normal subjects, and thus there must be another underlying mechanism. Our finding that the rapid inactivation in I_{Na} may be one of the cellular mechanisms might explain why the I_{Na} blocker challenge test can unmask ST-segment elevation in BS but not in normal subjects^{3,10}. As shown in the present and previous studies^{18,34,35}, a prolonged QT interval is often observed in the right precordial lead of symptomatic BS

patients. Our simulation suggested that a prolonged QT interval, which represents the occurrence of P2R within the epicardial RV, is an important ECG sign for predicting P2R and subsequent VF. The previous studies of the mutant SCN5A channel^{6,12} simulated ER and a saddle-back type ST-segment elevation, but did not elucidate how the I_{Na} channel blocker induced ST-segment elevation, especially of the coved-type.

The P2R pixel area increased dramatically as a function of fiber length, which suggests that not only the SCN5A mutation but also the area of the epicardium with I_{to} -dispersion is important for the genesis of P2R. This finding is consistent with the clinical observation of a close correlation between the degree of ST-segment elevation and the likelihood of VF in BS;^{3,32,33} because marked ST-segment elevation may reflect a large amount of epicardial tissue with I_{to} -dispersion. The conduction disturbance must play an important role in the etiology of P2R. The smaller cell size in the RV myocyte compared with those in the LV is commonly observed in the patch clamp experiment. We speculate that the conduction disturbance might occur more easily in the RV than in the LV because the cross-sectional area along the short axis is smaller in RV myocytes than in LV myocytes;^{22,23} which would preferentially increase the intracellular resistance along the longitudinal axis in the RV. Because the conduction disturbance simulated by an increase in R_g had the same effect on the occurrence of ER and P2R (data not shown), we simulated a conduction disturbances in the present study by changing the cell diameter. The smaller cell diameter combined with a conduction disturbance in RV epicardium increased the pixel area of ER and P2R, and in turn increased the likelihood of generating P2R closer to the physiological range of I_{to} -density, especially in DEC- I_{Na} . Increased intracellular resistance may cause the loss of electrical coupling, which may exaggerate dispersion and generate ER and P2R within a limited length of fiber. Regional differences in the size of ventricular myocytes, as well as differences in I_{to} -density^{4,36} may play a role in the RV dominant occurrence of the electrophysiological features of BS. It is noteworthy that conduction disturbance was necessary to cause P2R by I_{to} -dispersion at the physiological range in the simulation with DEC- I_{Na} .

The overlap between BS and long QT syndrome-3 has been reported to be attributable to a single SCN5A mutation.^{6,15,37,38} The Markov model of I_{Na} of this mutant channel reportedly caused ER in the mathematical model.⁶ Because P2R was also observed in our model, which was based on the Markov model of I_{Na} (our preliminary observation), the selection of the I_{Na} model does not seem to have caused any essential changes in our results. The Markov model is a more physiological model than the classic Hodgkin-Huxley model;³⁹ however, we did not select the Markov model as the I_{Na} model for the present study because it is difficult to change the voltage dependent kinetics of I_{Na} without changing the amplitude of the simulated current.

Study Limitations

The incidence of the SCN5A gene mutation in BS is reported as 10–20%. Furthermore only 2 mutations (S1710L¹⁶ and T1620M¹²) have been reported to show a small inactivation time constant. Therefore, our present study did not show that all BS patients have the DEC- I_{Na} mutation, but does show that this current phenotype can easily cause the

BS phenotype. Moreover, it has been reported that a patient with the S1710L mutation did not show Brugada-specific ST-T abnormality.¹⁶ On the other hand, there was not a clear description of the T1620M mutation. However, as the occurrence of the P2R depends on the conductance of I_{CaL} , as shown in the previous study, the sensitivity of the drug challenge test might not be very high. Furthermore, the ECG change in BS is observed only in a localized area⁴⁰ in the right precordial lead. So even if a Brugada-specific ECG change is observed in an electrode, sometimes no change is observed in an adjacent electrode. In particular, the amplitude of the ECG in V_{3R} or V_{4R} is usually low so electrophysiological change in the localized RV free wall might not be observed as a clear Brugada-type ECG change. The other type of change in the current phenotype may contribute to the ECG-phenotype of BS patients who have persistent ST-segment elevation. Finally, although our results from the simulation assist greatly in understanding the mechanism of P2R, they cannot be applied directly to the clinical situation.

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