Subepicardial phase 0 block and discontinuous transmural conduction underlie right precordial ST-segment elevation by a SCN5A loss-of-function mutation

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Brugada syndrome was first described in 1992 as a distinct clinical entity with a high risk of sudden arrhythmic death (10). It is considered a primary electrical cardiomyopathy (4, 32), characterized by coved-type or saddle-back ST-segment elevations in the right precordial ECG leads, as well as conduction disturbances in any of the cardiac compartments and conduction system without clinically identifiable structural abnormalities. In 18–30% of patients, the Brugada phenotype correlates with mutations of the SCN5A gene encoding the α-subunit of the cardiac Na+ channel (4). The mechanistic basis for ST-segment elevation and T-wave inversion in the right precordial ECG leads (the Brugada phenotype) is still a subject of controversy and remains to be fully elucidated.

Two mechanisms are generally proposed to explain right precordial ST-segment elevation: 1) right ventricular (RV) subepicardial action potential shortening and/or loss of the dome; and 2) RV conduction delay, particularly in the RV outflow tract. Both favor reentrant excitation (34). According to the first mechanism, during phase 1 of the subepicardial action potential, a reduction of Na+ current (INa) permits transient outward K+ current (IK1) to repolarize the membrane to potentials at which L-type Ca2+ current (ICa,L) cannot activate properly. This causes subepicardial action potential shortening and/or loss of the dome, which does not occur at the subendocardium, because IK1 expression is very low there (5). The resultant transmural gradient of action potential magnitudes causes ST-segment elevation on the ECG. According to the second mechanism, subepicardial and subendocardial cells activate asynchronously, which can also cause transmural voltage gradients and ST-segment elevation. Furthermore, a delay in subepicardial activation may contribute to inversion of the T wave. Under these conditions, steepening ST-segment elevation or accentuation of the J wave often appears as an R′, suggesting the presence of right bundle branch block (3).

In the present study, we describe a novel mechanism of ST-segment elevation associated with a INa loss-of-function mutation. The heterozygous missense SCN5A mutation p.Phe2004Leu (F2004L) encodes Na+ channels with decreased peak and persistent current amplitudes, increased closed-state and slow inactivation, and decelerated recovery from inactivation. Our data stress the importance of the final part of the SCN5A COOH-terminus in both open- and closed-state inactivation of the Na+ channel. In a RV multicellular one-dimensional fiber model, assembled from Luo-Rudy model cells incorporating the mutant INa, the endo- to epicardial INa-dependent excitation wave is decremental at slow rate [cycle length (CL) 1,000 ms]. Propagation is discontinuous across the midmyocardial (M) to epicardial transition region, exhibiting a long local delay due to phase 0 block. This causes transmural voltage gradients during early repolarization. Propagation continues by ICa,L-dependent phase 2 conduction, causing long delays of excitation and subsequently slow upstroke action potentials at the subepicardium. These conduction abnormalities produce ST-segment elevation and T-wave inversion in the computed pseudo-ECG waveform.

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METHODS

Clinical and genetic diagnosis. This study conforms with the principles outlined in the Declaration of Helsinki and was approved by the Medical Ethics Committee of the Academic Hospital Maastricht, The Netherlands. Subjects were genotyped using standard molecular techniques. Briefly, genomic DNA was extracted from peripheral blood lymphocytes (Wizard genomic DNA purification kit; Promega, Madison, WI). All exons of the SCN5A gene were amplified (AmpliTaq polymerase; Invitrogen, Carlsbad, CA), and both sense and antisense DNA strands were analyzed by direct sequencing (Big-Dye terminator ready reaction kit version 3.1, ABI-3100 Genetic Analyzer; Applied Biosystems, Foster City, CA). Sequences were aligned and compared with the SCN5A reference sequence (NM_198056-SCN5A, transcript variant 1; software Vector NTI suite 8, Invitrogen). DNA sequence variations were registered as recommended (www.genomic.unimelb.edu.au/mgi/mutnomen).

Site-directed mutagenesis, cell transfection, and electrophysiology. The SCN5A mutation was generated by site-directed mutagenesis using the quick-change II XL system (Stratagene, La Jolla, CA). Oligonucleotide design and reaction conditions were essentially as suggested by the manufacturer, except for the extension time (2 min/kb). The construct was completely sequenced to confirm the presence of the mutation and the absence of unwanted changes. Plasmid DNA for mammalian expression was grown in Top10F min/kb). The construct was completely sequenced to confirm the suggested by the manufacturer, except for the extension time (2 min/kb). The construct was completely sequenced to confirm the presence of the mutation and the absence of unwanted changes. Plasmid DNA for mammalian expression was grown in Top10F min/kb). The construct was completely sequenced to confirm the suggested by the manufacturer, except for the extension time (2 min/kb). 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with saddle-back configuration appeared in lead V2 (Fig. 1D, right). No arrhythmias were induced. Similar to II:8, the other carriers, II:1, II:3, and III:1, did not exhibit typical Brugada (type 1) ECG characteristics under baseline conditions. They did not consent to being tested with ajmaline.

We analyzed the ECGs of the blood relatives of the proband, comparing mutant carriers (II:1, II:3, II:8, III:1, III:3; Fig. 1D) and noncarriers (II:4, II:5, II:6, III:2). QRS duration was significantly longer in the carriers: 115 ± 5 vs. 86 ± 7 ms in the noncarriers (P < 0.05). PR interval did not differ: 165 ± 9 vs. 170 ± 13 ms (P = not significant).

**Biophysical characterization of F2004L channels.** The F2004L mutation resulted in significantly decreased \( I_{Na} \) (Fig. 2, A and B). Peak mutant \( I_{Na} \) was ~54% of the WT current, showing a slight deceleration of the time-to-peak activation and a decelerated fast phase (\( \tau_f \)) of fast inactivation. The fraction of channels inactivating with \( \tau_i \) and the slow phase (\( \tau_s \)) of fast inactivation were not different from WT (Fig. 2C; Table 1). The sensitivity of peak \( I_{Na} \) to 30 \( \mu \)m/l tetrodotoxin was similar in F2004L and WT channels (Fig. 2D). Tetrodotoxin-sensitive late \( I_{Na} \) of mutant channels was 56 and 59% of WT current for square-pulse and action potential voltage clamps, respectively (Fig. 2E; Table 1). The membrane potential for \( V_{1/2} \) was similar in mutant and WT, whereas the slope factor \( k \) was significantly higher in F2004L channels, corresponding to a 3.8-mV positive shift in the voltage dependence of peak \( I_{Na} \) activation (Fig. 3A; Table 1). Voltage dependence of steady-state inactivation of F2004L channels was shifted to the negative direction by ~7.5 mV and was less steep than that of WT channels (Fig. 3A; Table 1). These findings prompted us to focus on closed-state inactivation of F2004L channels. The development of closed-state inactivation was not changed. However, the fraction of channels entering this channel state was significantly larger in the case of F2004L (Fig. 3B; Table 1), corresponding to the negative shift of the voltage dependence of steady-state inactivation. The development of slow inactivation was accelerated in F2004L channels, whereas the fraction of channels entering the slow inactivated state was not different between mutant and WT (Fig. 3C; Table 1). \( I_{Na} \) recovery from inactivation was best fitted by a single-exponential function for conditioning pulses of 25 ms (holding potential ~120 mV), whereas the process became biexponential for conditioning pulses of 1,000 or 5,000 ms (Table 1). The time constant of the fast component of recovery \( (\tau_{rec,f}) \) was slower in mutant conditions, whereas the slow time constant \( (\tau_{rec,s}) \) was not different between WT and F2004L (Fig. 4A; Table 1). At holding potentials ~100 and ~80 mV, \( \tau_{rec,f} \) became slower for both mutant and WT \( I_{Na} \), although still different between the two conditions (Fig. 4B).

**Computational modeling of electrophysiological consequences of F2004L mutation.** To investigate the electrophysiological consequences of the F2004L mutation, we used mathematical modeling at three levels, as illustrated in Fig. 5. The characteristics of WT and F2004L current were first introduced into a Markov model of the Na\(^+\) channel adapted to fit the experimentally acquired results (Fig. 5A). Subsequently, the models of Na\(^+\) channels were introduced into a Luo-Rudy dynamic ventricular cell model (Fig. 5B), and 165 such cells (with distinct characteristics corresponding to the particular layers of RV wall) were interconnected into multicellular one-dimensional fiber model of transmural conduction (Fig. 5C). Correspondence of the main characteristics of the experimental and model \( I_{Na} \) is shown in the online data supplement.

Propagation of the excitation wave from endo- to epicardium was simulated in both WT and F2004L models at CLs of 300 and 1,000 ms (Fig. 6A). In the WT model, action potentials were physiologically constituted, and propagation was continuous and uniform. Conduction velocity (CV) was within physiological limits: 45.3 and 44.4 cm/s at CLs of 300 and 1,000 ms, respectively. In the F2004L model, propagation was continuous but slow (CV = 25.2 cm/s) at CL 300 ms. In contrast, at CL 1,000 ms, propagation was discontinuous across the M-cell-to-epicardial transition region, where conduction was extremely slow (CV = 9.2 cm/s) and phase 0 block occurred. The magnitude of the action potential dome was higher than
the peak $I_{Na}$-dependent upstroke potential in M cells (e.g., cell 80) and subepicardial layers (e.g., cell 115; Fig. 6A). Beyond this transition region, after a long delay of 116.5 ms, the axillary excitatory current was provided by phase 2 (dome) of the M-cell action potentials and depended on $I_{Ca}$ ("phase 2 conduction"). The upstroke velocity was very low (4.08 mV/ms for cell 150) in the case of this secondary excitation. In Fig. 6B, a surface plot of both spatial and temporal parameters of action potentials at CL 1,000 ms is shown. When the conductance of $I_{Ca}$ was reduced to 50% in the F2004L model, the delay at CL 1,000 ms was eliminated, whereas no change of CV was observed at CL 300 ms (Fig. 6C). For a given cell, both the peak inward and outward axial currents ($I_{axial}$) decreased with cell number along the F2004L fiber at CL 1,000 ms (Fig. 6D). Thus, as the propagation proceeded, cells received less depolarizing $I_{axial}$ from upstream neighbors.

We tested the sensitivity of the phase 0 block phenomenon to $I_{TO}$ density. For an epicardial $I_{TO}$ reduction by 15% (same as in the M-cell region) or a 12.5% increase, the same phase 0 block and transition to phase 2 conduction were observed. Thus there was a window of 27.5% variation in $I_{TO}$ density over which the phenomenon occurred, which shows that it is reasonably robust and does not depend on a very limited $I_{Na}$-to-$I_{TO}$ ratio.

We also examined the potential contribution of decreased gap junction coupling. In a fiber with 50% reduction of gap junction conductance at the transition region (i.e., the subepicardium), phase 0 block and phase 2 conduction did occur. Further reduction of gap junction expression caused complete conduction block beyond this region, which, of course, resulted in membrane potential gradients and ST-segment elevation.

Table 1. Biophysical characteristics of WT and F2004L channels

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WT</th>
<th>F2004L</th>
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<tbody>
<tr>
<td>Current-voltage relationship</td>
<td>$n = 16$</td>
<td>$n = 17$</td>
</tr>
<tr>
<td>$I_{Na}$, pA/pF</td>
<td>$-336.3 \pm 28.2$</td>
<td>$-182.7 \pm 19.5^a$</td>
</tr>
<tr>
<td>At voltage, mV</td>
<td>$-15.9 \pm 1.2$</td>
<td>$-12.1 \pm 1.4^a$</td>
</tr>
<tr>
<td>Kinetics of $I_{Na}$ at $-15$ mV</td>
<td>$n = 16$</td>
<td>$n = 17$</td>
</tr>
<tr>
<td>Time to peak, ms</td>
<td>$0.66 \pm 0.02$</td>
<td>$0.75 \pm 0.03^b$</td>
</tr>
<tr>
<td>$\tau_1$, ms</td>
<td>$0.49 \pm 0.02$</td>
<td>$0.61 \pm 0.03^b$</td>
</tr>
<tr>
<td>$\tau_2$, ms</td>
<td>$2.70 \pm 0.12$</td>
<td>$2.94 \pm 0.7$</td>
</tr>
<tr>
<td>Fast fraction</td>
<td>$0.84 \pm 0.01$</td>
<td>$0.83 \pm 0.01$</td>
</tr>
<tr>
<td>Late $I_{Na}$ square pulse</td>
<td>$n = 5$</td>
<td>$n = 4$</td>
</tr>
<tr>
<td>$A$, pA/pF</td>
<td>$-1.02 \pm 0.26$</td>
<td>$-0.57 \pm 0.03$</td>
</tr>
<tr>
<td>Late/peak $I_{Na}$</td>
<td>$0.0064 \pm 0.0009$</td>
<td>$0.0061 \pm 0.0008$</td>
</tr>
<tr>
<td>$I_{Na}$, action potential waveform</td>
<td>$n = 4$</td>
<td>$n = 4$</td>
</tr>
<tr>
<td>$A$, pA/pF</td>
<td>$-0.91 \pm 0.36$</td>
<td>$-0.54 \pm 0.06$</td>
</tr>
<tr>
<td>Late/peak $I_{Na}$</td>
<td>$0.0128 \pm 0.0051$</td>
<td>$0.0097 \pm 0.0009$</td>
</tr>
<tr>
<td>Voltage dependence of activation</td>
<td>$n = 16$</td>
<td>$n = 17$</td>
</tr>
<tr>
<td>$V_{1/2}$, mV</td>
<td>$-31.8 \pm 1.2$</td>
<td>$-28.5 \pm 1.3$</td>
</tr>
<tr>
<td>$k$</td>
<td>$6.0 \pm 0.3$</td>
<td>$7.2 \pm 0.3^a$</td>
</tr>
<tr>
<td>Voltage dependence of inactivation</td>
<td>$n = 15$</td>
<td>$n = 14$</td>
</tr>
<tr>
<td>$V_{1/2}$, mV</td>
<td>$-66.6 \pm 0.8$</td>
<td>$-74.1 \pm 1.0^a$</td>
</tr>
<tr>
<td>$k$</td>
<td>$-5.6 \pm 0.2$</td>
<td>$-6.3 \pm 0.1^a$</td>
</tr>
<tr>
<td>Closed-state inactivation</td>
<td>$n = 16$</td>
<td>$n = 15$</td>
</tr>
<tr>
<td>$A$</td>
<td>$0.16 \pm 0.02$</td>
<td>$0.30 \pm 0.03^b$</td>
</tr>
<tr>
<td>$\tau$, ms</td>
<td>$72.9 \pm 5.6$</td>
<td>$73.1 \pm 3.2$</td>
</tr>
<tr>
<td>Slow inactivation</td>
<td>$n = 4$</td>
<td>$n = 7$</td>
</tr>
<tr>
<td>$A$</td>
<td>$0.45 \pm 0.05$</td>
<td>$0.52 \pm 0.04$</td>
</tr>
<tr>
<td>$\tau$, s</td>
<td>$24.6 \pm 2.6$</td>
<td>$12.9 \pm 1.6^b$</td>
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</table>

Values are means ± SE; $n$, no. of cells. WT, wild type; $I_{Na}$, Na$^+$ current; $V_{1/2}$, membrane potentials for half-maximal activation or inactivation; $k$, slope factors; $A$, $A_1$, and $A_2$: amplitude, fast amplitude, and slow amplitude, respectively; $\tau$, $\tau_1$, $\tau_2$, $\tau_{rec}$, $\tau_{rec,f}$, and $\tau_{rec,s}$: time constant, fast phase time constant, slow phase time constant, fast component of recovery time constant, and slow component of recovery time constant, respectively; $^aP < 0.05$, $^bP < 0.01$, and $^cP < 0.001$ between WT and F2004L channels; $^dP < 0.05$, $^eP < 0.01$, and $^fP < 0.001$ between parameters of recovery from inactivation studied with 1,000- and 5,000-ms conditioning pulses.
The mechanism of the F2004L conduction delay at CL 1,000 ms and its pseudo-ECG manifestation are illustrated in Fig. 7. The inward and outward I_{axial} were large for endocardial (cell 15) and M cells (cell 80) due to low closed inactivated states occupancy (IC2 + IC3) preceding the action potential upstroke, which corresponds to high channel availability. In contrast, two small inward peaks (marked as peaks 1 and 2 in Fig. 7A) were observed in epicardial cell 150. These two peaks corresponded to the failed continuous excitation (excitation from upstream cells) and successful delayed (phase 2) excitation of cell 150, respectively. It is evident that peak 2 was about twice as large as peak 1. Peak 1 was accompanied by a relatively large increase in IC2 + IC3 and failed I_{Na} activation (C3 occupancy remained high). During peak 2, the C3 occupancy rapidly decreased to zero, whereas IC2 + IC3 occupancy significantly increased due to inactivation during slow depolarization by the small I_{axial}. It resulted in a very small I_{Na} unable to generate a complete action potential (phase 0) upstroke. The action potential upstroke in cell 150 was biphasic with two different upstroke velocities: the first phase mediated by I_{Na} and the second by I_{CaL} (Fig. 7B). The conduction delay in the epicardium caused a spatial gradient of the transmembrane voltage during the action potential dome in the F2004L model. This gradient generated ST-segment elevation and T-wave inversion in the computed ECG waveform (Fig. 7C), while no conduction delay and thus also no ST-segment elevation was observed in the WT model or in the F2004L model at CL 300 ms.

DISCUSSION

In the present study, we describe a novel mechanism of right precordial ST-segment elevation and T-wave inversion in Brugada syndrome. The missense mutation F2004L in the SCN5A COOH-terminus resulted in Na+ channels with decreased peak and persistent current amplitudes, increased closed-state and slow inactivation, and decelerated recovery from inactivation. Incorporation of these I_{Na} characteristics in a one-dimensional model of right precordial tissue improved the simulation of ST-segment elevation and T-wave inversion in Brugada syndrome.

![Graphs](image1.png)

**Fig. 3.** Voltage dependence of I_{Na} steady-state activation and inactivation and development of closed-state and slow inactivation. A: voltage dependence of steady-state activation (protocol as in Fig. 2A) and inactivation. Curves were fitted with a Boltzmann equation, \( G/G_{\text{max}} = 1/(1 + \exp(V_{1/2} - V/k)) \), to determine the membrane potential for half-maximal activation or inactivation (\( V_{1/2} \)) and slope factor (\( k \)), where \( G \) is Na+ conductance, \( G_{\text{max}} \) is maximal \( G \), \( I \) is peak I_{Na} during depolarizing step, and \( t_{\text{max}} \) is maximal I. For steady-state activation, \( V_{1/2} \) was similar in mutant and WT, whereas \( k \) was higher in F2004L channels. Voltage dependence of steady-state inactivation of F2004L channels was shifted to the negative direction by ~7.5 mV and was less steep. Data are from \( n = 14 \) and 15 cells for F2004L and WT, respectively. However, the fraction of channels entering this state was higher in the mutant condition. B: closed-state inactivation. Averaged data were fitted with a single-exponential function \( I/I_{\text{max}} = A \times [1 - \exp(-t/\tau)] \) to determine amplitude \( A \) and time constant \( \tau \). The temporal development of closed-state inactivation was not different between F2004L and WT channels (\( n = 15 \) and 16 cells, respectively). However, the fraction of channels entering this state was higher in the mutant condition. C: the development of slow inactivation accelerated in F2004L channels. Same fitting as in B; \( n = 7 \) and 4 cells for F2004L and WT, respectively. *\( P < 0.05 \), †\( P < 0.01 \), and ‡\( P < 0.001 \).

![Graphs](image2.png)

**Fig. 4.** Recovery from inactivation. A, left: representative examples for mutant and WT I_{Na}. Right: averaged data of \( I/I_{\text{max}} \) for F2004L and WT; \( n \) cell values are indicated in Table 1. Data were fitted with a double-exponential function \( I/I_{\text{max}} = A_s \times [1 - \exp(-t/\tau_{\text{rec,f}})] + A_f \times [1 - \exp(-t/\tau_{\text{rec,s}})] \), where \( A_s \) and \( A_f \) are the fractions of fast and slow recovering components, \( \tau_{\text{rec,f}} \) and \( \tau_{\text{rec,s}} \) are their time constants, and \( t \) is recovery interval. At holding potential ~120 mV, \( \tau_{\text{rec,f}} \) was significantly longer in F2004L channels. B: recovery from inactivation at various holding potentials. Compared with ~120 mV, recovery was slower at more depolarized holding potentials, although still different between the two conditions. The \( \tau_{\text{rec,s}} \) was not different. *\( P < 0.05 \) and †\( P < 0.01 \).
model of transmural RV conduction revealed decremental excitation from endo- to epicardium at slow, but not at fast, rate. Discontinuous conduction by subepicardial phase 0 block and delayed phase 2 propagation produced the typical Brugada phenotype in the computed pseudo-ECG waveform. Success or failure of conduction was determined by the delicate balance between inward $I_{Na}$ and outward $I_{CaL}$ at a fast rate due to incomplete recovery from inactivation between consecutive beats. Therefore, compromised F2004L $I_{Na}$ was insufficient to sustain conduction. At slow rate, $I_{TO}$ fully recovered, and, consequently, F2004L $I_{Na}$-supported conduction failed, explaining the slow-rate manifestation of the Brugada syndrome. Figure 6A (bottom) demonstrates that reduction of $I_{TO}$ (e.g., by drug block) can restore $I_{Na}$-supported conduction in F2004L. Furthermore, our data suggest that chronotropic interventions aimed at keeping the heart rate well above 60 beats/min prevent discontinuous RV transmural conduction in this genotype, potentially avoiding proarrhythmia under adverse conditions.

The multicellular one-dimensional model was designed to represent transmural plane-wave conduction for the specific simulations of this study. It was not meant to represent the complex three-dimensional structure and anatomy of the RV wall, nor propagation of curved wavefronts or reentry. The ECG waveform computed from the voltage gradient is a pseudo-ECG at a site close to the epicardial surface along the direction of the action potential propagation. It is not a body surface ECG, although there are striking similarities between the pseudo- and body surface ECGs in this study. Pseudo-ECGs have been used extensively in previous studies in cardiac wedge preparations and many other tissue and tissue culture preparations, as well as in computer models (19). They have proven extremely useful in relating ECG waveforms to the action potential and its underlying ionic processes. This is very difficult to achieve in less tractable, higher dimension complex models. In the case of Brugada syndrome, the right precordial ECG leads, in which the ST segment is elevated and the T wave in V2 is often inverted, record mostly isolated activity from a RV section. These leads are close to the RV outflow tract, where the voltage gradients arise. During the ST segment, there is only a small contribution from activity in other regions of the heart. Such conditions increase the correspondence between the pseudo-ECG simulated here and the body surface ECG in the right precordial leads.

The mutant $I_{Na}$ model implements channel kinetic properties specific to the F2004L mutation, as characterized quantitatively in the experiments. Thus the experimental and modeling aspects of the study are tightly coupled, as the experiments provide the data for constructing the $I_{Na}$ model and the model allows integration of $I_{Na}$ into the whole cell environment, where electrophysiological consequences of this specific mutation are simulated and studied.

In general, ST-segment elevation in the ECG reflects a spatial gradient of the ventricular transmembrane potential during the action potential plateau and/or repolarization phases (19). As such, it is a nonspecific marker of spatially distributed phenomena in the ventricular repolarization pattern. In acute ischemia, it results from spatially dependent action potential shortening caused by the opening of ATP potassium current channels, whereas, in Brugada syndrome, it is a consequence of loss of $I_{Na}$ function (19). Reduced $I_{Na}$ could potentially alter the spatial repolarization pattern in two ways: 1) by directly affecting action potential repolarization; or 2) by affecting action potential conduction, which, in turn, alters the sequence of repolarization. A previous study of the SCN5A mutation p.1795insD (7, 13, 44) demonstrated the first mechanism. The 1795insD mutation slows recovery from inactivation of $I_{Na}$, causing reduction of $I_{Na}$ at a fast rate due to incomplete recovery between beats. On the background of repolarizing $I_{TO}$, this results in premature action potential repolarization. Because $I_{TO}$ density varies transmurally (27, 29, 30, 48), a spatial repolarization gradient is established, causing ST-segment elevation. Here, we demonstrate the second conduction-dependent mechanism. The F2004L mutant channels accumulate in the hyperabsorbing closed-inactivation states during slow pacing, which reduces $I_{Na}$ to cause slow conduction and conduction delays. These, in turn, generate spatial membrane potential gradients that induce ST-segment elevation. This behavior is specific for the F2004L mutation, because it depends on the details of the mutant $I_{Na}$ kinetic properties, which are characterized by decreased peak and persistent current amplitudes, increased closed-state and slow inactivation, and accelerated recovery from inactivation, compared with WT. The slow-rate dependence of the phenotype is consistent with the observation that episodes of ventricular fibrillation in Brugada patients occur typically during bradycardia (4, 23, 33, 35, 38, 43). Both mechanisms described above involve an interplay between mutant $I_{Na}$ and $I_{TO}$. The latter is expressed heterogeneously across the ventricular wall (27, 29, 30, 48). In contrast to $I_{TO}$, $I_{CaL}$ does not show transmural heterogeneity (28) and is transmurally homogeneous in the simulations. However, as shown previously (22, 24, 25, 41, 47), $I_{CaL}$ plays an important role in supporting conduction, where the action poten-

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**Fig. 5. Model schematic. A:** WT and F2004L $I_{Na}$ models share the same structure. C, closed state; IC, inactivated-closed state; O, open (conducting) state; IF, fast inactivation state; IM, intermediate inactivation state; $\alpha$ and $\beta$ are transition rates between states. Transition rate increases and decreases in the mutant relative to the WT model are represented as thick solid or thin dashed arrows, respectively. B: $I_{Na}$ models were incorporated into the Luo-Rudy (LRd) ventricular cell model. C: a one-dimensional model containing 165 LRd cells connected through gap junctions was used to simulate transmural RV conduction and compute pseudo-ECG waveforms.

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tial encounters long conduction delays, as occurs here with the F2004L mutation.

A consensus report on Brugada syndrome attests that $I_{\text{T}}$ antagonists, $I_{\text{CaL}}$ agonists, or a combination of both can be therapeutic (4). This fact highlights the need to study electrophysiological effects of the Brugada syndrome in the context of the whole cell action potential, where $I_{\text{T}}$ and $I_{\text{CaL}}$ play an important role, rather than only studying mutant SCN5A channels in isolation. The results of our simulations indeed suggest that reduction of $I_{\text{T}}$ can compensate for arrhythmogenic effects of F2004L that manifest at slow rate.

Cardiac conduction disturbances in Brugada syndrome. Besides the typical coved-type pattern of right precordial ST segments, the proband exhibited atrioventricular and intraventricular conduction delays. This is in agreement with previous studies reporting cardiac conduction disease in Brugada syndrome, particularly in patients with a SCN5A mutation (e.g., Refs. 26, 42). In addition, Bezzina et al. (9) showed that QRS prolongation in Asian individuals with Brugada syndrome was similar in right and left precordial ECG leads. They also documented that PR and QRS intervals were more prolonged in SCN5A-mutation-positive than in -negative Brugada patients (9). Collectively, these data indicate that the original supposition that Brugada syndrome is “an electrical disease of the RV” is oversimplified and that the presence of more generalized cardiac conduction disease appears to correlate with the presence of a SCN5A mutation, as also found in the present study.

Microscopic analyses of RV endomyocardial biopsies of 18 consecutive Brugada patients with an apparently normal cardiac structure and function on noninvasive examinations revealed distinct structural abnormalities in all (18). This and other observations has led to the speculation that fibrosis (8, 15) and myocarditis (18) may exacerbate the Brugada phenotype and trigger tachyarrhythmic events. The presence of SCN5A mutations appears to be particularly associated with structural derangements (8, 15, 18), possibly due to deleterious consequences of an altered myocardial Na$^+$ homeostasis when Na$^+$ channel function is impaired. Our RV modeling studies indicate that the functional defects of F2004L channels themselves can account for the typical right precordial ECG features in the proband, even without structural abnormalities being incorporated.
New insights into genotype-phenotype relationships. After the initial description of a missense, a splice donor and a frame-shift mutation in three families in 1998 (11), over 80 different mutations of the SCN5A gene have been identified in Brugada syndrome to date (www.fsm.it/cardmoc). However, the percentage of Brugada patients carrying a SCN5A mutation is relatively low [18–30% (4)]. Until recently, mutations were the percentage of Brugada patients carrying a SCN5A mutation final part of the SCN5A COOH-terminus is involved in inactivation gating.

The F2004L variant has been recently reported in patients with sudden infant death syndrome (46), sudden cardiac death (2), and surprisingly also in apparently healthy individuals (1). Thus the question arises why the pathogenic potential of this SCN5A variant is so variable. Undoubtedly, modifier genes could play a role.

Noticeably, the ion current properties that we have recorded in CHO cells differ markedly from those of Wang et al. (46). These investigators characterized F2004L in tsA201 cells and found an unaltered peak amplitude and an increased persistent $I_{Na}$, suggesting that F2004L is a long-QT type 3 variant. Large differences exist in the endogenous expression of β-subunits and other molecular characteristics between CHO and tsA201 cells, which could explain some of the functional discrepancies between these studies. Importantly, the loss of function of F2004L-mutant current that we describe matches with the Brugada ECG of the proband. Such direct comparison of ion current characteristics and ECG data is overtly lacking in the published reports mentioned above. We know of one other F2004L carrier, unrelated to the family of this study, who
expressed right bundle branch block and T-wave negativity in lead V2 under baseline conditions (E. Schulze-Bahr, personal communication). This person was admitted because of complete atrioventricular block, indicating severe cardiac conduction disease. Based on this combination of data, we believe that the F2004L loss-of-function variant is a disease-associated mutant with characteristics of Brugada syndrome and/or cardiac conduction disease in carriers expressing the phenotype. In the present study, we focused primarily on the mechanisms of right precordial ST-segment elevation and T-wave negativity in lead V2. Whether these mechanisms apply also to other SCN5A loss-of-function variants, especially those with a high penetrance of the Brugada phenotype, remains to be determined.

Conclusions. Our results explain right precordial ST-segment elevation in Brugada syndrome on the basis of RV transmural gradients of membrane potentials during early repolarization caused by subepicardial phase 0 block and discontinuous transmural conduction. Resultant late slow-upstroke action potentials at the subepicardium produce T-wave inversion in the computed ECG waveform, in line with the clinical ECG.

APPENDIX

The structure of the $I_{Na}$ Markov model used in this study, which includes fast and intermediate inactivated states, as well as closed-inactivated states, was adopted from previous work (13). Model parameters defining the voltage dependence of transition rates between kinetic states were determined separately for WT and F2004L $I_{Na}$ using the Nelder-Mead simplex algorithm (37) and the Asynchronous Parallel Pattern Search Package (21) running on a cluster of Intel Xeon processors. We incorporated the WT and F2004L $I_{Na}$ models into the Luo-Rudy (LRd) ventricular cell model (17), as was first described in Ref. 12. F2004L maximum conduction was modulated to yield peak current that was 54% of WT peak current, as measured in the experiments. LRd models were then assembled, according to previous work (19), into a multicellular one-dimensional fiber model of RV transmural conduction (Fig. 5C). Use of this preparation allowed us to study the effects of the mutation on action potential conduction and the pseudo-ECG waveform (19).

Equations governing the WT and F2004L $I_{Na}$ models are provided below. They represent behavior at 23°C and are used for validation by comparison to measurements at this temperature. For simulations in the LRd model at 37°C, transition rates are adjusted by the appropriate Q_{10} (36).

$I_{Na}$ Model

See Fig. 5A.

**WT $I_{Na}$ Formulation**

$$I_{Na,WT} = G_{Na,WT} \cdot P(O) \cdot (V - E_{Na})$$

$$G_{Na,WT} = 60.0 \text{ mS/\mu F}$$

$$E_{Na} = \frac{RT}{F} \ln \left( \frac{[\text{Na}^+]_{o}}{[\text{Na}^+]_{i}} \right)$$

where $I_{Na,WT}$ is WT $I_{Na}$; $G_{Na,WT}$ is WT Na$^+$ conductance ($G_{Na}$); $P(O)$ is open probability; $E_{Na}$ is Na$^+$ potential; $R$ is ideal gas constant; $T$ is temperature; $F$ is Faraday constant; $[\text{Na}^+]_{o}$ is extracellular Na$^+$ concentration; and $[\text{Na}^+]_{i}$ is intracellular Na$^+$ concentration.

**WT $I_{Na}$ Transition Rates**

$$\alpha_{11} = 1.0 \cdot \left\{ 0.02701 \cdot \exp \left[ \frac{-(20.0 + V)}{17.0} \right] + 0.3568 \cdot \exp \left[ \frac{-(20.0 + V)}{100.0} \right] \right\}$$

$$\alpha_{12} = 1.0 \cdot \left\{ 0.02701 \cdot \exp \left[ \frac{-(20.0 + V)}{22.75} \right] + 0.006049 \cdot \exp \left[ \frac{-(20.0 + V)}{100.0} \right] \right\}$$

$$\alpha_{13} = 1.0 \cdot \left\{ 0.02701 \cdot \exp \left[ \frac{-(20.0 + V)}{12.0} \right] + 0.3190 \cdot \exp \left[ \frac{-(20.0 + V)}{100.0} \right] \right\}$$

$$\beta_{11} = 1.333 \cdot \left\{ \exp \left[ \frac{-(13.438 + V)}{20.3} \right] + \exp \left[ \frac{-(13.438 + V)}{15.0} \right] \right\}$$

$$\beta_{12} = 0.1208 \cdot \left\{ \exp \left[ \frac{-(19.5 + V)}{20.3} \right] + \exp \left[ \frac{-(13.438 + V)}{15.0} \right] \right\}$$

$$\beta_{13} = 0.5276 \cdot \left\{ \exp \left[ \frac{-(19.0 + V)}{20.3} \right] + \exp \left[ \frac{-(13.438 + V)}{10.0} \right] \right\}$$

$$\alpha_{3} = 1.0254 \cdot 10^{-6} \cdot \exp \left( \frac{-V}{7.7} \right)$$

$$\beta_{3} = 0.0084 + 1.209 \cdot 10^{-5} \cdot V$$

$$\alpha_{2} = 18.356 \cdot \left\{ \exp \left( \frac{V - 10.0}{45.0} \right) + 1.0 \right\} \left( 0.01 + \exp \left( \frac{V + 30.0}{10.0} \right) \right)$$

$$\beta_{4} = 1.0 \left\{ 3.523 \cdot \exp \left( \frac{V}{182.73} \right) + 2.583 \cdot \exp \left( \frac{V + 64.31}{18.324} \right) \right\}$$

$$\alpha_{5} = \frac{\alpha_{13} \cdot \alpha_{2} \cdot \alpha_{3}}{\beta_{13} \cdot \beta_{3}}$$

$$\alpha_{4} = \alpha_{2}$$

$$\gamma_{4} = 65610.0$$

$$\beta_{4} = \frac{\beta_{4}}{14.11}$$

where $\alpha$ and $\beta$ are transition rates between states.

**F2004L Mutant $I_{Na}$ Formulation**

$$I_{Na,F2004L} = G_{Na,F2004L} \cdot P(O) \cdot (V - E_{Na})$$

$$G_{Na,F2004L} = 20.0 \text{ mS/\mu F}$$

$$E_{Na} = \frac{RT}{F} \ln \left( \frac{[\text{Na}^+]_{o}}{[\text{Na}^+]_{i}} \right)$$

where $I_{Na,F2004L}$ and $G_{Na,F2004L}$ are F2004L $I_{Na}$ and $G_{Na}$, respectively.

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