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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Departments of ¹Cardiology, ²Molecular Cell Biology and Genetics, ³Clinical Genetics, Cardiovascular Research Institute Maastricht, Academic Hospital Maastricht and Maastricht University, Maastricht, The Netherlands; and ⁴Cardiac Bioelectricity and Arrhythmia Center, Washington University in St. Louis, St. Louis, Missouri

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Bébarová M, O’Hara T, Geelen JL, Jongbloed RJ, Timmermans C, Arens YH, Rodriguez LM, Rudy Y, Volders PG. Subepicardial phase 0 block and discontinuous transmural conduction underlie right precordial ST-segment elevation by a SCN5A loss-of-function mutation. Am J Physiol Heart Circ Physiol 295: H48–H58, 2008. First published May 2, 2008; doi:10.1152/ajpheart.91495.2007.—Two mechanisms are generally proposed to explain right precordial ST-segment elevation in Brugada syndrome: 1) right ventricular (RV) subepicardial action potential shortening and/or loss of dome causing transmural dispersion of repolarization; and 2) RV conduction delay. Here we report novel mechanistic insights into ST-segment elevation associated with a Na⁺ current (I_{Na}) loss-of-function mutation from studies in a Dutch kindred with the COOH-terminal SCN5A variant p.Phe2004Leu. The proband, a man, experienced syncope at age 22 yr and had coved-type ST-segment elevations in ECG leads V1 and V2 and negative T waves in V2. Peak and persistent mutant I_{Na} were significantly decreased. I_{Na} closed-state inactivation was increased, slow inactivation accelerated, and recovery from inactivation delayed. Computer-simulated I_{Na}-dependent excitation was decremental from endo- to epicardium at cycle length 1,000 ms, not at cycle length 300 ms. Propagation was discontinuous across the midmyocardial to epicardial transition region, exhibiting a long local delay due to phase 0 block. Beyond this region, axial excitatory current was provided by phase 2 (dome) of the M-cell action potentials and depended on L-type Ca²⁺ current ("phase 2 conduction"). These results explain right precordial ST-segment elevation on the basis of RV transmural gradients of membrane potentials during early repolarization caused by discontinuous conduction. The late slow-upstroke action potentials at the subepicardium produce T-wave inversion in the computed ECG waveform, in line with the clinical ECG.

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 (I_{Na}) permits transient outward K⁺ current (I_{TO}) to repolarize the membrane to potentials at which L-type Ca²⁺ current (I_{Ca,L}) cannot activate properly. This causes subepicardial action potential shortening and/or loss of the dome, which does not occur at the subendocardium, because I_{TO} 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, downsloping 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 I_{Na} 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 I_{Na}, the endo- to epicardial I_{Na}-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 I_{Ca,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’ cells (Invitrogen) and then isolated from the bacterial cells using an endotoxin-free midiprep kit (PureYield; Promega). Chinese hamster ovary (CHO) cells were cultured in Ham’s F-12 medium supplemented with 10% fetal calf serum and 0.005% gentamycin. SCN5A (0.25 μg) was cotransfected with an enhanced green fluorescent protein expression vector (pIRE2-EGFP, 0.6 μg) using Fugene 6 (Roche Applied Science, Basel, Switzerland).

Biophysical characterization of SCN5A channels. CHO cells were harvested at 20–24 h after transfection by brief trypsinization, washed twice with culture medium, and placed in a perfusion chamber on an inverted microscope that was continuously perfused with external solution containing the following (in mmol/l): 145 NaCl, 4 CsCl, 1 MgCl2, 1.8 CaCl2, 10 HEPES, and 11.1 glucose (pH 7.4 with CsOH). EGFP-positive cells were used for patch clamping at room temperature (23 ± 1°C) using an Axopatch-1D amplifier and pCLAMP8 software (Axon Instruments, Union City, CA). Pipette resistances ranged from 1 to 2.5 MΩ when filled with internal solution containing the following (in mmol/l): 145 NaCl, 4 CsCl, 1 MgCl2, 1.8 CaCl2, 10 HEPES, and 11.1 glucose (pH = 7.4 with CsOH). Series resistance was measured by the ramp method. For each measurement, the holding potential was −120 mV, and the stimulation frequency was 0.2 Hz, unless otherwise indicated. For steady-state activation, the voltage dependence of normalized Na+ conductance $G/G_{max}$, with $G$ calculated as $I/(V - E_{rev})$, $I$ the peak $I_{Na}$ at a depolarizing step, $V$ the command potential, and $E_{rev}$ the reversal potential of $I_{Na}$, was fitted with a Boltzmann equation, $G/G_{max} = 1/[1 + \exp(V - V_{1/2})/k]$, to determine the membrane potential for half-maximal activation ($V_{1/2}$) and the slope factor (k).

The same equation was applied to determine $V_{1/2}$ and k for steady-state inactivation using $I_{maxal}$ and the slope factor (k). In the case of the closed state and inactivation, the averaged data were fitted with a single exponential function $I_{maxal} = A \times [1 - \exp(-t/\tau_{1})]$ where $t$ is time (duration of prepulse or conditioning pulse), to determine amplitude A and time constant $\tau_{1}$.

Computational modeling. Markov models of wild-type (WT) (12) and mutant Na+ channels were incorporated in Luo-Rudy model cells assembled into a one-dimensional fiber to simulate plane-wave transmural RV conduction (19). Formulation of the Markov models, including rate constants of transitions between kinetic channel states, is provided in the Appendix. The theoretical fiber is composed of 165 model cells connected through gap junctions. Transmural heterogeneous ion channel densities are introduced to represent the three RV cell layers: endocardial (cells 1–60), M (cells 61–105), and epicardial (cells 106–165). Density of slowly activating delayed-rectifier K+ current is varied, as described previously (35), with the lowest in the M cells. $I_{Ks}$ is introduced in epicardial and M cells after a modified version of the formulation of Dumaine et al. (16). The maximum conductance of $I_{Ks}$ is set to 3.50956619 mS/μF and 4.1289014 mS/μF in M and epicardial cells. $I_{Ks}$ is not expressed in endocardial cells. Gap junction conductance (1.73 μS) is normal and uniform throughout the fiber. A 0.5-ms suprathreshold current stimulus is applied to cell 1 to initiate action potential propagation from endocardium to epicardium.

For parameters that differ between WT and F2004L models, we also reduced the differences by 15% in test simulations. Effectively, this yielded a “weaker” mutant model that we used to conservatively test the influence of parameter choice within reasonable experimental variability. Our results were not qualitatively altered by the reduction. Specifically, ST-segment elevation at slow pacing persisted and for the same mechanistic reason (“subepicardial phase 0 block and phase 2 conduction”). This demonstrates the robustness of the simulations in the presence of biological and experimental variability.

Data analysis. Group data are expressed as means ± SE. Inter-group comparisons between mutant carriers and noncarriers (for ECG parameters), and between F2004L and WT $I_{Ks}$ were made with an unpaired Student’s t-test. A two-tailed $P < 0.05$ was considered statistically significant.

Results

Clinical and genetic characterization. The proband, a man, was recognized with Brugada syndrome at age 22 yr after a syncopal episode while driving his car, which led to a crash. Two years before, he had suffered another such episode without prodromes while taking a shower. No signs of structural heart disease were present. Serum electrolytes were normal. As illustrated in Fig. 1A, accentuated J waves (suggesting right bundle branch block) and negative T waves in ECG lead V2, as well as ST-segment elevation in V3, were present at baseline. After administration of flecainide (100 mg iv), coved-type ST-segment elevation was noted in V2. Under baseline conditions, conduction was prolonged at all cardiac levels: PR interval was 200 ms, and QRS width 135 ms. Distal atrioventricular conduction was slow with a HV interval of 56 ms during intracardiac recording. Corrected QT was 382 ms. Flecainide prolonged the PR interval to 240 ms and the QRS duration to 160 ms. No arrhythmias were induced by programmed electrical stimulation during flecainide. The patient received an implantable cardiac defibrillator.

Sequence analysis showed a heterozygous missense mutation of nucleotide c.6010T>C, which results in an amino acid change p.Phe2004Leu (abbreviated as F2004L) in the final part of the COOH-terminus of the Na+ channel (Fig. 1, B and C). Position 2,004 of the altered human amino acid lies in a stretch of at least 20 amino acids, which is conserved in monkey (Pan troglodytes), cow (Bos taurus), rat (Rattus norvegicus), and mouse (Mus musculus).

Four other family members carried the mutation (Fig. 1D, left). Among them, the patient’s father (II:8) demonstrated nonsustained monomorphic ventricular tachycardia during 24-h ambulatory ECG monitoring at age 52 yr. He has never experienced cardiac symptoms. His baseline ECG showed minimal ST-segment elevation in lead V1. After provocation with ajmaline (80 mg iv), significant ST-segment elevations.
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 (τ_f) of fast inactivation. The fraction of channels inactivating with τ_f and the slow phase (τ_s) of fast inactivation were not different from WT (Fig. 2C; Table 1). The sensitivity of peak I_{Na} to 30 μmol/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 (τ_{rec,f}) was slower in mutant channels, whereas the slow time constant (τ_{rec,s}) was not different between WT and F2004L (Fig. 4A; Table 1). At holding potentials −100 and −80 mV, τ_{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 axial 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 \( V_{1/2} \)).

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 25.7% 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></th>
<th>WT</th>
<th>F2004L</th>
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<tr>
<td>Current-voltage relationship</td>
<td>( n = 16 )</td>
<td>( n = 17 )</td>
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<tr>
<td>Peak ( I_{Na} ), pA/pF</td>
<td>( -336.3 \pm 28.2 )</td>
<td>( -182.7 \pm 19.5 )</td>
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<tr>
<td>At voltage, mV</td>
<td>( -15.9 \pm 1.2 )</td>
<td>( -12.1 \pm 1.4 )</td>
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<tr>
<td>Kinetics of ( I_{Na} ) at (-15 ) mV</td>
<td>( n = 16 )</td>
<td>( n = 17 )</td>
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<tr>
<td>Time to peak, ms</td>
<td>( 0.66 \pm 0.02 )</td>
<td>( 0.75 \pm 0.03 )</td>
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<tr>
<td>( \tau_{r} ), ms</td>
<td>( 0.49 \pm 0.02 )</td>
<td>( 0.61 \pm 0.03 )</td>
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<tr>
<td>( \tau_{m} ), ms</td>
<td>( 2.70 \pm 0.12 )</td>
<td>( 2.94 \pm 0.7 )</td>
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<tr>
<td>Fast fraction</td>
<td>( 0.84 \pm 0.01 )</td>
<td>( 0.83 \pm 0.01 )</td>
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<tr>
<td>Late ( I_{Na} ) square pulse</td>
<td>( n = 5 )</td>
<td>( n = 4 )</td>
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<tr>
<td>( A_{Na} ), pA/pF</td>
<td>( -1.02 \pm 0.26 )</td>
<td>( -0.57 \pm 0.03 )</td>
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<tr>
<td>Late/peak ( I_{Na} )</td>
<td>( 0.0064 \pm 0.0009 )</td>
<td>( 0.0061 \pm 0.0008 )</td>
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<tr>
<td>Late ( I_{Na} ), action potential waveform</td>
<td>( n = 5 )</td>
<td>( n = 4 )</td>
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<tr>
<td>( A_{Na} ), pA/pF</td>
<td>( -0.91 \pm 0.36 )</td>
<td>( -0.54 \pm 0.06 )</td>
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<tr>
<td>Late/peak ( I_{Na} )</td>
<td>( 0.0128 \pm 0.0051 )</td>
<td>( 0.0097 \pm 0.0009 )</td>
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<td>Voltage dependence of activation</td>
<td>( n = 16 )</td>
<td>( n = 17 )</td>
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<tr>
<td>( V_{1/2} ), mV</td>
<td>( -31.8 \pm 1.2 )</td>
<td>( -28.5 \pm 1.3 )</td>
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<tr>
<td>( k )</td>
<td>( 6.0 \pm 0.3 )</td>
<td>( 7.2 \pm 0.3 )</td>
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<td>Voltage dependence of inactivation</td>
<td>( n = 15 )</td>
<td>( n = 14 )</td>
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<tr>
<td>( V_{1/2} ), mV</td>
<td>( -66.6 \pm 0.8 )</td>
<td>( -74.1 \pm 1.0 )</td>
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<tr>
<td>( k )</td>
<td>( -5.6 \pm 0.2 )</td>
<td>( -6.3 \pm 0.1 )</td>
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<tr>
<td>Closed-state inactivation</td>
<td>( n = 16 )</td>
<td>( n = 15 )</td>
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<tr>
<td>( A_{2} )</td>
<td>( 0.16 \pm 0.02 )</td>
<td>( 0.30 \pm 0.03 )</td>
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<td>( \tau_{s} ), ms</td>
<td>( 72.9 \pm 5.6 )</td>
<td>( 73.1 \pm 3.2 )</td>
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<td>Slow inactivation</td>
<td>( n = 4 )</td>
<td>( n = 7 )</td>
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<tr>
<td>( A_{s} )</td>
<td>( 0.45 \pm 0.05 )</td>
<td>( 0.52 \pm 0.04 )</td>
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<tr>
<td>( \tau_{s} ), ms</td>
<td>( 24.6 \pm 2.6 )</td>
<td>( 12.9 \pm 1.6 )</td>
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<td>Recovery from inactivation at (-120 ) mV</td>
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<td>25-ms conditioning pulses</td>
<td>( n = 11 )</td>
<td>( n = 11 )</td>
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<tr>
<td>( A_{rec} ), ms</td>
<td>( 0.99 \pm 0.01 )</td>
<td>( 0.99 \pm 0.01 )</td>
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<td>( t_{rec,f} ), ms</td>
<td>( 2.20 \pm 0.17 )</td>
<td>( 3.17 \pm 0.27 )</td>
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<td>1,000-ms conditioning pulses</td>
<td>( n = 16 )</td>
<td>( n = 14 )</td>
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<tr>
<td>( A_{r} )</td>
<td>( 0.88 \pm 0.02 )</td>
<td>( 0.85 \pm 0.01 )</td>
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<td>( t_{rec,f} ), ms</td>
<td>( 2.67 \pm 0.20 )</td>
<td>( 3.92 \pm 0.28 )</td>
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<tr>
<td>5,000-ms conditioning pulses</td>
<td>( n = 8 )</td>
<td>( n = 11 )</td>
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<tr>
<td>( A_{r} )</td>
<td>( 0.80 \pm 0.02 )</td>
<td>( 0.70 \pm 0.02 )</td>
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<tr>
<td>( t_{rec,f} ), ms</td>
<td>( 2.78 \pm 0.41 )</td>
<td>( 4.84 \pm 0.38 )</td>
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| 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; \( A_{r} \), \( A_{s} \), and \( A_{2} \), amplitude, fast amplitude, and slow amplitude, respectively; \( \tau_{r} \), \( \tau_{m} \), \( \tau_{rec} \), \( t_{rec,f} \), and \( t_{rec,s} \), \( \tau_{rec} \) time constant, fast phase time constant, slow phase time constant, fast component of recovery time constant, and slow component of recovery time constant, respectively; \( *P < 0.05 \), \( \#P < 0.01 \), and \( ^{\dagger}P < 0.001 \) between WT and F2004L channels; \( ^{\dagger}P < 0.05 \) and \( ^{\dagger}P < 0.01 \) between parameters of recovery from inactivation studied with 1,000- and 5,000-ms conditioning pulses.

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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 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 $I_{To}$. At fast rate, $I_{To}$ was reduced because of incomplete recovery from inactivation between consecutive beats. Therefore, compromised F2004L $I_{Na}$ was sufficient 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 transmural potential during the action potential plateau and/or repolarization phases (19). As such, it is a nonspecific marker of spatially distributed changes 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 decelerated 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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### Table 1: Characterization of $I_{Na}$ Models

<table>
<thead>
<tr>
<th>Model</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>Wild-type model with normal $I_{Na}$ properties.</td>
</tr>
<tr>
<td>F2004L</td>
<td>Mutant model with altered $I_{Na}$ properties.</td>
</tr>
<tr>
<td>LRd</td>
<td>Multicellular model containing 165 LRd cells connected through gap junctions</td>
</tr>
</tbody>
</table>

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

A consensus report on Brugada syndrome attests that I_{TO} antagonists, I_{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_{TO} and I_{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_{TO} 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 myocellular Na^+/H^+ 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.
Fig. 7. Mechanism underlying the F2004L conduction delay at CL 1,000 ms and its pseudo-ECG manifestation. A: cells 15 (endo), 80 (M), and 150 (epi) represent three important behaviors. Both inward and outward $I_{	ext{Na}}$ are large for cells 15 and 80. The two inward peaks in $I_{	ext{Na}}$ for cell 150 are numbered 1 and 2, which correspond to failed continuous excitation and successful delayed excitation, respectively. Peak 1 is provided by the suppressed upstroke of upstream cells; peak 2 by their (greater magnitude) dome. The inset is clipped above 0 pA/pF to show the inward peaks on an enlarged scale. Inactivated-closed state occupancy (IC2 + IC3) is low for cells 15 and 80 preceding the action potential upstroke, indicating minimal inactivation and high channel availability. In these cells, closed state C3 empties, indicating $I_{	ext{Na}}$ activation, which generates the upstroke. In contrast, in cell 150, peak 1 of $I_{	ext{Na}}$ corresponds to a relatively large rise in IC2 + IC3 and failed $I_{	ext{Na}}$ activation (C3 occupancy remains high). When peak 2 of $I_{	ext{Na}}$ is reached, C3 empties, but IC2 + IC3 occupancy increases due to slow depolarization by the small $I_{	ext{Na}}$. This suppresses $I_{	ext{Na}}$. B: cell 150 is first depolarized by the suppressed $I_{	ext{Na}}$, which is too small to generate a complete action potential upstroke. It depolarizes the membrane to activate L-type Ca$^{2+}$ current, which then causes full depolarization. Thus the upstroke is biphasic with two different upstroke velocities (dV/dt): maximal dV/dt of the first phase is 3.66 mV/ms and of the second 4.08 mV/ms, compared with 48.00 mV/ms of the $I_{	ext{Na}}$-supported upstroke in cell 15. C: the conduction delay causes a spatial gradient of transmembrane voltage ($V_{m}$) during early repolarization in F2004L (gray). This gradient generates ST-segment elevation and T-wave inversion in the computed ECG waveform $\Phi$ (top). Note that in WT (black), there is no conduction delay and a related $V_{T,m}$. The ECG waveform returns to baseline between the QRS-complex and the upright T wave (no ST-segment elevation). au, Arbitrary unit.

**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 only detected in the encoding region of the SCN5A gene. However, intronic alterations and variations in the promoter region have also been reported now in some families (20, 40). Both exonic and intronic mutations are characterized by a partial or complete loss of function of the Na$^{+}$ channel, caused either by shifts in the voltage- and time-dependent features of activation, inactivation, or recovery from inactivation, or by an impaired expression of channels at the membrane. Combinations of these mechanisms are common. Here, we identified the missense mutation F2004L in a coding region of the SCN5A gene. The biophysical characteristics of this mutation mimicked those of previously reported ones, which suggests that, based on modeling, the described electrophysiological mechanisms may be more widely applicable in Brugada syndrome due to a SCN5A channelopathy. F2004L channels had an increased closed-state inactivation, corresponding to a negative shift in the voltage dependence of steady-state inactivation. Similar changes were observed in at least two other COOH-terminal mutants of the Na$^{+}$ channel: p.1795insD (6) and p.Tyr1795His (39). In line with this, artificial deletions of the distant half of the SCN5A COOH-terminus encompassing the predicted sixth helix and the final unstructured region were shown to cause a negative shift in the voltage dependence of steady-state inactivation [p.Lys1888stop (31); p.Ser1885stop (14)]. Our data point out that the unstructured region of the 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 $I_{\text{Na}}$, 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 conductance 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

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

$$G_{Na,WT} = 60.0 \text{ mS/µF}$$

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

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; [Na$^+$]$_o$ is extracellular Na$^+$ concentration; and [Na$^+$]$_i$ is intracellular Na$^+$ concentration.

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

$$\alpha_1 = 1.0 \cdot \left( 0.02701 \cdot \exp \left( \frac{-(20.0 + V)}{17.0} \right) + 0.3568 \cdot \exp \left( \frac{-(20.0 + V)}{100.0} \right) \right)$$

$$\alpha_2 = 1.0 \cdot \left( 0.02701 \cdot \exp \left( \frac{-(20.0 + V)}{12.0} \right) + 0.3190 \cdot \exp \left( \frac{-(20.0 + V)}{100.0} \right) \right)$$

$$\alpha_3 = 1.333 \cdot \left( \exp \left( \frac{-(13.438 + V)}{20.3} \right) + \exp \left( \frac{-(13.438 + V)}{15.0} \right) \right)$$

$$\beta_1 = 1.333 \cdot \left( \exp \left( \frac{-(13.438 + V)}{20.3} \right) + \exp \left( \frac{-(13.438 + V)}{15.0} \right) \right)$$

$$\beta_2 = 0.1208 \cdot \left( \exp \left( \frac{-(19.0 + V)}{20.3} \right) + \exp \left( \frac{-(13.438 + V)}{15.0} \right) \right)$$

$$\beta_3 = 0.5276 \cdot \left( \exp \left( \frac{-(19.0 + V)}{20.3} \right) + \exp \left( \frac{-(13.438 + V)}{15.0} \right) \right)$$

$$\beta_4 = 1.0 \cdot \left( 13.438 \cdot \exp \left( \frac{V - 10.0}{45.0} \right) + 0.01 + \exp \left( \frac{V + 30.0}{10.0} \right) \right)$$

$$\beta_5 = \frac{\alpha_{11} \cdot \alpha_2 \cdot \alpha_3}{\beta_1 \cdot \beta_3}$$

$$\alpha_2 = \alpha_2$$

$$\beta_4 = 1.0 \cdot \left( 3.523 \cdot \exp \left( \frac{V}{182.73} \right) + 2.583 \cdot \exp \left( \frac{V + 64.31}{18.324} \right) \right)$$

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

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

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

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

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

where $I_{Na,F2004L}$ and $G_{Na,F2004L}$ are F2004L $I_{Na}$ and $G_{Na}$, respectively.
F2004L Mutant Ina Transition Rates

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

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

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

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

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

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

\[ \alpha_3 = 3.418 \cdot 10^{-7} \cdot \exp\left(-\frac{V}{7.7}\right) \]

\[ \beta_3 = 0.0084 + 4.03 \cdot 10^{-7} \cdot V \]

\[ \alpha_5 = 9.178 \left[ \exp\left(\frac{V}{45.0}\right) + 1.0 / \exp\left(0.01 + \exp\left(\frac{V}{10.0}\right) + \exp\left(-\frac{V + 30.0}{1000.0}\right) \right) \right] \]

\[ \beta_2 = \frac{\alpha_{11} \cdot \alpha_{13} \cdot \alpha_3}{\beta_{11} \cdot \beta_{13} \cdot \beta_1} \]

\[ \alpha_1 = \frac{\alpha_2}{100.0} \]

\[ \beta_4 = 1.0 \left[ 3.523 \cdot \exp\left(\frac{V}{609.1}\right) + 25.83 \cdot \exp\left(\frac{V}{0.4581}\right) \right] \]

\[ \alpha_2 = \frac{\alpha_2}{65610.0} \]

\[ \beta_5 = \frac{\beta_4}{14.11} \]

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