L-type Ca\(^{2+}\) channel mutations and T-wave alternans: a model study

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Zhu ZI, Clancy CE. L-type Ca\(^{2+}\) channel mutations and T-wave alternans: a model study. *Am J Physiol Heart Circ Physiol* 293: H3480–H3489, 2007. First published October 12, 2007; doi:10.1152/ajpheart.00476.2007.—A number of mutations have been linked to diseases for which the underlying mechanisms are poorly understood. An example is Timothy Syndrome (TS), a multisystem disorder that includes severe cardiac arrhythmias. Here we employ theoretical simulations to examine the effects of a TS mutation in the L-type Ca\(^{2+}\) channel on cardiac dynamics over multiple scales, from a gene mutation to protein, cell, tissue, and finally the ECG, to connect a defective Ca\(^{2+}\) channel to arrhythmia susceptibility. Our results indicate that 1) the TS mutation disrupts the rate-dependent dynamics in a single cardiac cell and promotes the development of alternans; 2) in coupled tissue, concordant alternans is observed at slower heart rates in mutant tissue than in normal tissue and, once initiated, rapidly degenerates into discordant alternans and conduction block; and 3) the ECG computed from mutant-simulated tissue exhibits prolonged QT intervals at physiological rates and with small increases in pacing rate, T-wave alternans, and alternating T-wave inversion. At the cellular level, enhanced Ca\(^{2+}\) influx due to the TS mutation causes electrical instabilities. In tissue, the interplay between faulty Ca\(^{2+}\) influx and steep action potential duration restitution causes arrhythmogenic discordant alternans. The prolongation of action potentials causes spatial dispersion of the Na\(^{+}\) channel excitability, leading to inhomogeneous conduction velocity and large action potential spatial gradients. Our model simulations are consistent with the ECG patterns from TS patients, which suggest that the TS mutation is sufficient to cause the clinical phenotype and allows for the revelation of the complex interactions of currents underlying it.

VOLTAGE-GATED ION CHANNELS underlie critical physiological processes like cardiac excitation, muscle contraction, and nerve impulses. In the last two decades, a number of genetic defects in ion channels have been identified and causally linked to a wide variety of clinical diseases, including cardiac arrhythmias, epilepsy, and disorders of muscle contraction. However, the question of exactly how a genetic mutation causes, or if it is sufficient to explain, the clinically observed disease state has been difficult to answer. This is largely due to limitations in the available investigative techniques that permit examination of derangements at individual scales, such as experiments to identify how specific mutations alter kinetic properties of channels but rarely provide information about the emergent effects of the mutations. It is precisely the emergent effects that result from nonlinear interactions within cells, between cells, and among various tissue components that cause disease. In the clinic, the ECG provides information about the organ level manifestation of disease but reveals nothing about the underly-lying mechanisms. Here we employ theoretical modeling to understand the process of disease development that leads to arrhythmia susceptibility that occurs over multiple scales, from gene to molecule, to cell, to tissue, and finally to the ECG clinical observations. This allows us to simultaneously examine individual ionic contributors to arrhythmia development, which enables the revelation of mechanisms for how genetic defects propagate as emergent dynamics in higher dimensions and trigger disordered rhythms. In the present work, we examine the consequences of L-type Ca\(^{2+}\) channel (LTCC) mutations linked to Timothy Syndrome (TS) (41, 42) on the dynamic and electrical properties in cells and coupled tissue. In doing so, we aim to reveal the ionic mechanism underlying the electrocardiographic phenotypes observed in TS patients.

Recently, two single amino acid mutations in the cardiac LTCC, Cav1.2 (CACN\(_{1C}\)) and G406R or G402S in the COOH-terminus end of the sixth transmembrane (S6) segment of domain I (D1) (D1/S6), were identified as causes of TS, a childhood disorder characterized in part by severe cardiac arrhythmia including QT prolongation, atrioventricular (AV) block, T-wave alternans, and ventricular tachycardia (41, 42). How T-wave alternans develops is of particular interest, since T-wave alternans is observed in a variety of clinical and experimental conditions associated with ventricular arrhythmias (31).

Voltage-gated LTCCs inactivate upon the passage of Ca\(^{2+}\) current or membrane depolarization, known as Ca\(^{2+}\)-dependent inactivation (CDI) and voltage-dependent inactivation (VDI), respectively, which are critical in shaping the action potential (AP). The two mutations underlying TS result in the same aberration of channel function, namely the elimination of VDI of the channel. Despite the loss of VDI, the CDI remains fully intact. Hence, these naturally occurring mutations provide an opportunity to investigate and define a role for the inactivation processes in cardiac electrical dynamics. Since the effects of the mutations on channel gating are the same (41), we refer to them hereafter as the TS mutation.

The structural determinants of the VDI and CDI have been the subject of numerous recent investigations (14, 26, 32, 40, 43, 47), which have suggested that at least two forms of VDI are important. Mutations in S6 pore regions in all four domains have been shown to disrupt fast VDI (including the TS mutations in D1/S6 that we investigate here), suggesting that C-type inactivation is present (39). Other experiments have shown that the linker between D1 and D11 in Cav1.2 is important for the VDI of the channels (2, 43). The linker peptide contains a highly conserved \(\alpha_1\)-subunit interaction domain (AID) that forms a high-affinity binding site for the Ca\(_{\text{V}}\) \(\beta\)-subunit. This interaction is presumed to result in an additional hinged-lid

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inactivation mechanism, common in Na⁺ and K⁺ channel inactivation. Interestingly, there is also evidence that this region coordinates interaction between the VDI and CDI processes (19, 21), although the mechanisms of this coupling have not yet been fully revealed.

Previous studies have shown that T-wave alternans on the ECG arises from repolarization alternans at the level of the single cell (30, 31). In contrast to the well-studied mechanism of reentrant arrhythmias associated with T-wave alternans (5, 30, 34), the ionic mechanisms underlying T-wave alternans is poorly understood. Theoretical studies (17, 18, 20, 28) showed that alternans (and the resulting arrhythmia) can arise from purely dynamical instabilities in the heart that exist even in normal healthy tissue. How mutations and other abnormalities might increase the occurrence of such instability is a topic of much interest and many experimental and theoretical studies might increase the occurrence of such instability is a topic of much interest and many experimental and theoretical studies (8, 10, 27, 36). Here we employ theoretical methodology to understand the process of disease development that occurs over multiple scales, from a gene mutation to altered protein, cell, and tissue function to the ECG. This approach allows us to simultaneously examine individual ionic contributors to arrhythmia development at different scales and gain new insight into how genetic defects propagate as emergent dynamics in higher dimensions and trigger disordered rhythms.

**MATERIALS AND METHODS**

**Canine ventricular myocyte model.** The canine ventricular (CV) myocyte (CVM) model developed by Fox et al. (12) was used for cell and tissue simulations in the study.

**Voltage clamp.** Protocols for the simulated channel are the same as those used experimentally (41, 42). Current-voltage (I-V) relationships were determined by measuring the peak inward current elicited by 2-s test pulses applied in 10-mV increments to membrane potentials ranging from −70 to +40 mV. The voltage dependence of the Ba²⁺ current inactivation was determined with a two-pulse protocol. The relative magnitude of the current elicited during a second pulse (to +10 mV) was plotted as a function of the prepulse voltage during the conditioning 2-s prepulse. The relative currents are the values of the peak currents measured at different potentials normalized to the maximum peak current, which in Fig. 1C is at 0 mV and in Fig. 1E is at −70 mV. Figure 1, D and E, shows experimental and simulated LTCC VDI curves with Ba²⁺ used as the charge carrier. Experimentally, Ba²⁺ has been widely used in lieu of Ca²⁺ to uncouple Ca²⁺ and voltage-dependent processes in the LTCC. Ba²⁺ mimics Ca²⁺ entry through the channel while allowing for investigation of voltage-dependent properties without Ca²⁺ dependent inactivation (4, 6, 25, 39, 41, 42). Using Ba²⁺ allows for a measure of purely VDI. In the simulated VDI protocols, the CDI is not permitted (achieved by clamping the CDI gate, fca, to 1). The VDI of the TS mutation is simulated based on experiments, suggesting that the mutation abolishes VDI, observed as full-channel availability regardless of the holding potential. This feature is simulated by clamping the VDI gate, f, of the TS mutant channel to 1.

**TS mutation.** TS mutation was simulated using the Hodgkin-Huxley (H-H) model of the Ca²⁺ channel from Fox et al. (12) by eliminating the VDI in the model by clamping the VDI gate, f, for the TS mutant channel to 1. Because the effect of the mutation was limited to a single inactivation process (VDI), while leaving the other inactivation process apparently fully intact (CDI), the H-H model was sufficient to reproduce the altered gating observed experimentally.

**Computational measure of AP duration.** AP duration (APD) was measured as the time between the time of the maximum AP upstroke velocity and 95% cellular repolarization. Two restitution measurements were used in the study. The first was the S1S2 protocol (Fig. 3A). Cells were paced at a basic cycle length (BCL) = 1,000 ms to

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**Fig. 1.** Mutations in the L-type Ca²⁺ channel (LTCC) underlie Timothy Syndrome (TS) and disrupt voltage-dependent inactivation (VDI) of the channel. A: schematic of the LTCC (Ca1.2) and the two inactivation processes, VDI and Ca²⁺-dependent inactivation. I-II link and C-terminal Ca²⁺-dependent inactivation. B: experimentally recorded peak Ca²⁺ currents in response to indicated depolarizing test pulses are shown for the wild-type (WT) (○) and the TS mutations (△). C: corresponding simulated currents for WT and TS mutant channels. D: the TS mutation completely disrupted VDI. With Ba²⁺ used as the charge carrier, WT channels displayed clear VDI, as indicated by the channel availability curve (●). The inactivation was absent in the TS mutant channels. E: simulated WT and mutant channels display voltage-dependent channel availability comparable with that observed experimentally. Insets: patch-clamp protocols. B and D are from Ref. 42.
steady state, after which a second stimulus was applied after a varying interval, as indicated. The APD, in response to the second stimulus, was then plotted as a function of the diastolic interval (DI). A dynamic restitution measurement was also used (Fig. 3, B–H) (20, 29). The cells were paced at a range of BCLs (from 800 to 100 ms) to steady state. The APD was recorded and plotted as a function of the BCL.

One-dimensional simulations. We simulated a one-dimensional fiber composed of CVM cells connected by resistances to simulate gap junctions, as described previously (13). Current flow is described by the following equation:

\[
\frac{\partial V(x, t)}{\partial t} = D \frac{\partial^2 V(x, t)}{\partial x^2} - \frac{I_{\text{ion}} + I_{\text{stim}}}{C_m}
\]

where \(V\) is the membrane potential, \(t\) is time, \(D\) is the tissue diffusion coefficient, \(I_{\text{ion}}\) is the sum of the transmembrane ionic currents (using the algebraic and differential equations described in Ref. 24), \(I_{\text{stim}}\) is the stimulus current (800 mA/cm² for 1 ms), and \(C_m\) is the membrane capacitance (1 μF/cm²). The length of the one-dimensional fiber was set to 220 cells with a cell length = 100 μm and a diffusion coefficient = 0.001 cm²/ms (13).

The fiber was paced at a BCL = 500 ms to steady state (100 beats), and the BCL was then gradually shortened, first by 50-ms decrements and then by 10 ms, until alternans occurred, at which point the BCL was shortened by 1-ms intervals, to determine the threshold heart beat for each event, until 2:1 block. The number of pacing cycles at each BCL has little effect on the characteristic behavior of the fiber (data not shown). In the present study, we paced the fiber for 100 beats at each BCL. The stimulus was applied to the first cell.

Numerical methods. Simulations were run on Macintosh G5 computers with a C/C++ code. Integration in the fiber was carried out with 4th-order Runge-Kutta. The time step was 0.005 ms.

ECG computation. Extracellular unipolar potentials (\(\Phi_e\)) generated by the fiber in an extensive medium of conductivity \(\sigma_e\), were computed from the transmembrane potential \(V_m\) using the integral expression as in Plonsey and Barr (32a):

\[
\Phi_e(x', y', z') = \frac{a^2 \sigma_i}{4 \sigma_e} \int \left[ -\nabla V_m \cdot \frac{1}{r} \right] dx
\]

\[
r = \left( (x - x')^2 + (y - y')^2 + (z - z')^2 \right)^{1/2}
\]

where \(\nabla V\) is the spatial gradient of \(V_m\), \(a\) is the radius of the fiber, \(\sigma_i\) is the intracellular conductivity, and \(r\) is the distance from a source point \((x, y, z)\) to a field point \((x', y', z')\). \(\Phi_e\) was computed at an electrode site 2.0 cm away from the distal end along the fiber axis.

RESULTS

A number of models of cardiac cells have been developed for the simulation of cardiac dynamics in species from mouse to human (3, 12, 23, 33, 44). In this study, we chose the CVM model developed by Fox et al. (12), because it was specifically developed to reproduce experimentally observed electrical and dynamical properties of cells during rapid pacing. Importantly, the model develops alternans within the experimentally observed range (12).

In Fig. 1B, experimentally recorded Ca²⁺ I-V curves [reproduced from Ref. 31 by Semplski et al. (41)] are shown for the wild-type (WT) and TS mutant channels. Figure 1C shows the simulated Ca²⁺ current I-V curve for WT and TS mutant channels. Figure 1D shows that, in the absence of Ca²⁺ (recorded as Ba²⁺ currents), the WT channels display clear VDI, which is absent in the TS mutant channels (42). Figure 1E shows the simulated WT and mutant channels in the absence of Ca²⁺, with voltage-dependent channel availability comparable with experiments (see METHODS for details).

The TS mutation prolongs APD. We next incorporated the TS mutation into the CVM cell model to examine the effects of the mutation on the morphology of the AP. The TS mutation results in a 30% increase in APD compared with WT, despite an apparently modest increase in the Ca²⁺ current (heart rate, 120 beats/min) (Fig. 2A). Because we were surprised by the extent of mutation-induced AP prolongation, we compared it with a simulation where we increased the conductance of the LTCC twofold (Fig. 2B). Interestingly, despite the fact that doubling the channel conductance increases the peak Ca²⁺ current even more than the TS mutation, it has a smaller effect on APD. This simulation demonstrates the importance of ion channel kinetics in determining outcomes at the cellular scale. The effect of the mutation is pronounced throughout the duration of the plateau, a very low conductance (high resistance) phase of the AP. As a result, the increase in the Ca²⁺ current, albeit modest, has a large effect on APD due to the timing of the late Ca²⁺ current (Fig. 2C; arrow). In contrast, when only the conductance is increased, the larger peak Ca²⁺ current triggers a larger calcium-induced calcium release, which inactivates the Ca²⁺ channels.

The TS mutation alters single-cell dynamics. Due to the TS mutation effects on APD, we postulated that APD restitution, a quantity that describes the adaptation of APD to the preceding DI, and the propensity of the cell to develop arrhythmogenic rhythms such as alternans (the alternating long-short pattern of APD) might be affected. To examine the effect of the mutation on the rate dependence of cellular dynamics, we carried out an S1S2 restitution protocol (see MATERIALS AND METHODS) for both the WT and the mutant cell. As shown in Fig. 3A, the virtual cell containing the TS mutation has a much steeper APD restitution curve compared with the WT cell, indicating increased susceptibility to alternans development.

We next paced the virtual cells at a range of BCLs from 100 to 500 ms. Steady-state APDs for WT (Fig. 3B) and TS mutants (Fig. 3C) (after 1,000 beats) were recorded and plotted as a function of BCL. The WT cell develops 1:2 alternans at fast pacing rates, between BCL 150 and 210 ms (Fig. 3B), which is consistent with experimental observations (12). However, the TS mutant cells develop alternans at slower rates, between 244 and 262 ms (Fig. C, inset), which rapidly degenerates into complicated rhythms at BCL < 244 ms, indicating increased susceptibility to abnormal rhythm. These dynamics are summarized as a staircase plot of response-to-stimulus (R:S) ratios versus the BCL in Fig. 3C. From fast-pacing rates (starting from BCL ~140 ms) to slower rates, the R:S ratio varies sequentially from 1:6, 1:5, 1:4, 1:3, 2:4, and finally to 1:2 regular alternans. Examples of complicated activation patterns of R:S ratios including 1:3, 2:4, 1:2, and 1:1 are shown at their respective pacing rates in Fig. 3, D–H. Note that at BCLs from 213 to 262 ms, there are three distinct patterns, a 2:4 response and distinct 1:2 responses, as illustrated in Fig. 3, E–G, respectively. The cell fails to repolarize after the long AP in Fig. 3F but repolarizes after the subsequent short AP, which allows for a long subsequent DI. In Fig. 3G, the BCL is long enough for repolarization before the activation of the short beat, corresponding to “true” alternans. For the WT cell, throughout the whole range of BCLs at which alternans de-
velop (from BCL 150 to 210 ms), the cell fully repolarizes between each AP.

In coupled tissue, the TS mutation increases susceptibility to abnormal rhythms. We next constructed a fiber composed of 220 CVM model cells to approximate a linear strand of tissue. A pseudo-ECG, representing a signal average of spatial and temporal gradients of APs, is computed for the fibers of the WT or the TS mutant cells paced at 120 beats/min (Fig. 4). Compared with WT (WT, QT = 290 ms), the mutant exhibits prolonged QT intervals (TS, QT = 350 ms) (Fig. 4C). The simulations are consistent with the observation that 100% of the TS mutation carrier exhibits QT interval prolongation (22, 41, 42).

We showed in Fig. 3 that TS mutant cells are more vulnerable to electrical instabilities (i.e., alternans). This instability is further complicated by the electrotonic coupling in tissue. Using the theoretical cardiac fibers described above, we investigated the effect of the mutation on tissue dynamics. We began by pacing the fibers at a physiological rate (120 beats/min) and then subjected them to gradually accelerating rates (protocol described in detail in MATERIALS AND METHODS). The TS mutant fiber develops concordant alternans at BCL = 259 ms. An example of such a pattern generated at BCL = 258 ms is shown in Fig. 5A. With a modestly increased pacing rate, spatial gradients of repolarization rapidly gain magnitude large enough to generate discordant alternans at BCL 257 ms. In other words, when the TS mutation is present, the discordant alternans almost immediately degenerates into discordant alternans. An example of representative discordant alternans at BCL 250 ms is shown (Fig. 5B). Further acceleration to BCL = 249 ms leads to 2:1 block (Fig. 5C), where the fiber only responds to every other stimulus. The fiber with the WT channels responds normally (Fig. 5D) until a concordant alternans develops at the much faster BCL of 210 ms. A discordant alternans is not observed until pacing is accelerated to BCL 188 ms (Table 1). In the WT fiber, considerable acceleration is required for the degeneration into discordance, suggesting a margin of safety.

The TS mutant causes T-wave anomalies in computed ECGs at physiological rates. To examine manifestation of temporal and spatial dispersion of APD on the ECG, we simulated ECG waveforms from the gradients of transmembrane potential in the fiber (Fig. 6). When there is no alternans (shown in Fig. 6A at BCL = 262 ms), the ECG waveforms are uniform from beat to beat, including the direction and amplitude of the T-wave. During concordant alternans (Fig. 6B), the T-wave amplitude alternates. For the first beat, the APs are prolonged, whereas on the next beat they are relatively short but also heterogeneous in duration along the fiber (note the lengthening of the APD down the fiber). This heterogeneity results in larger repolarization gradients, reflected in the larger T-wave amplitude on the ECG. All T-waves are downturned, suggesting that the repolarization of the fiber proceeds from the proximal to the distal end.

During discordant alternans (Fig. 6C), the T-wave inverts on alternate beats. Figure 6D illustrates how temporal and spatial APD dispersion leads to alternating T-wave inversion. Three APs along the fiber during four consecutive beats are shown. In
beat a, the cells at the beginning of the fiber activate first but, due to long APD, repolarize last (line 2). The cells at the end activate last but have short APD and repolarize first (line 1). The ECG displays an upright T-wave, indicative of the repolarization that occurs in a direction opposite to depolarization. In beat b, the cells at the beginning depolarize first and, due to short APD, repolarize first (line 3), whereas the cells at the distal end depolarize and repolarize last (line 4). This results in a reverse in the direction of repolarization potential gradients, which causes the T-wave to invert. Note that the T-wave of beat b merges with the QRS complex of the next beat, because cells at the beginning of the fiber activate before the repolarization of cells at the end is complete.

In the clinical studies (22, 41, 42), extreme T-wave alternans (with an inversion on opposite beats) is observed in TS patients with a heart rate of 120 beats/min (an example is shown in Fig. 6E, which was reproduced from Ref. 42), which represents a 1.7-fold increase in heart rate compared with a normal adult resting rate of 70 beats/min. In our simulation with the mutant CVM model, the T-wave alternans occurs at \( \sim 235 \) beats/min (BCL, 255 ms) in tissue containing the TS mutation compared with a normal canine rate of 120 beats/min, corresponding to a 1.9-fold increase in heart rate. Hence, the relative increases in heart rate at which degeneration into discordant alternans between our model simulations and the clinical observations are in good agreement.

Mechanisms underlying facilitated spatial dispersion of APD alternans in mutant fiber. The TS mutation has two important effects on tissue dynamic. First, it facilitates the development of abnormal rhythms at much slower, more physiologically likely heart rates than observed in WT. Second, once the concordant alternans is initiated in the mutant fiber, it rapidly degenerates into highly arrhythmogenic discordant alternans.

In Fig. 7A, we summarize the mutation effects on the spatial gradients of temporal alternans. The alternans magnitude is the difference in APD between two consecutive beats, calculated as \( \text{APD}_{\text{ALT}} - \text{APD}_{\text{REST}} \). For the fiber of the mutant cells, a small acceleration of pacing (from BCL 260 to 257 ms) causes the alternans amplitude to increase dramatically in cells near the stimulus end (from \( \sim 0 \) at BCL 260 ms to 90 ms at BCL 257 ms). However, the temporal alternans damps rapidly along the fiber. The steepness of the curve indicates how fast this damping occurs. When the value of alternans magnitude is equal to zero, there is no alternans in that cell. This is a so-called node. Cells downstream with a negative alternans magnitude are alternating out of phase with the cells that have a positive alternans magnitude. Hence, a line that crosses into a negative region indicates that a fiber has discordant alternans.

In contrast, in the WT fiber, the alternans magnitude near the stimulus end increases slowly with an accelerated pacing (from BCL 210 to 190 ms). Importantly, the spatial gradients of alternans are much smaller compared with the mutant fiber.

The ionic mechanism for the rapid degeneration into discordant alternans in the mutant case is as follows. As shown in Fig. 7B, after the first AP (indicated as 1), the short DI at the stimulus end does not allow for complete recovery of Na\(^+\) channels from inactivation and results in slow propagation of the next impulse. The slow propagation means that downstream cells have a longer DI and therefore more time to...
recover. Hence, as the second AP (marked as 2) propagates down the fiber, the cells downstream have a longer APD. This is why the alternans damps along the fiber, and this occurs in both WT and mutant fibers. But the effect is more pronounced in the mutant fiber because in the TS fiber, the APD prolongation of the second AP in response to the lengthening of the preceding DI along the fiber is magnified by the loss of LTCC inactivation, which increases the inward current flowing during the AP plateau (resulting in steeper APD restitution). This increase in APD restitution of the TS mutant cell (as observed in single cells in Fig. 3) results in the observed larger damping of temporal alternans down the fiber.

The steepness of the alternans magnitude slope (Fig. 7A) indicates the propensity of the tissue to develop discordant alternans in a tissue of a given size. In other words, if the slope is steep enough (damping is sufficiently pronounced) to indicate the formation of a node (crossing zero) and the presence of the negative alternans region, then a discordant alternans is present. A very steep slope indicates that a discordant alternans can develop in a small piece of tissue (the steeper the slope, the smaller the necessary tissue size). The mutation reduces the length scale for node development. As the slope becomes increasingly shallow, the tissue size must be larger for a discordant alternans to occur. In summary, the TS mutation

Fig. 4. The TS mutation resulted in QT interval prolongation. Fibers composed of 220 CVM cells were paced to steady state at BCL of 00 ms. A: APs of fiber of WT cells. B: APs of fiber of TS mutant cells had prolonged APD. C: computed electrocardiogram (ECG), which represents a signal average of spatial and temporal gradients of APs. When compared with the WT fiber (black line), TS mutants displayed a prolonged QT interval (red line).

Fig. 5. A discordant alternans occurs at slow pacing rates in the TS mutant fiber. Fibers composed of WT or TS mutant cells were paced at various cycle lengths, as indicated. The mutant fiber develops concordant alternans from BCL of 259 to 258 ms (A). With increasing pacing rate, the discordant alternans rapidly develops at BCL of 257 ms and continues to BCL of 250 ms (B). Further acceleration to BCL of 249 ms leads to 2:1 block (C). For the fiber comprising WT cells, no dynamic instability occurs at the same range of pacing rates. Normal response of the fiber of WT cells is shown in D.
shortens the tissue size (wavelength) required for the development of discordant alternans and, presumably, lethal arrhythmia.

Node development and conduction block. We next wanted to address the question, Is alternans wavelength sufficient to predict conduction block?

The simulations above were carried out in a fiber of 220 cells, which approximates the length of a transmural wall of heart chamber. To further explore the effects of the TS mutation on the multiple node development in a larger tissue, we constructed fibers of 900 cells (WT or mutant) and paced them as described for the previous simulations. The threshold pacing frequencies for alternans and conduction block were unchanged in the longer fiber (Table 1). However, when a discordant alternans developed in the mutant tissue, we observed an abrupt formation of two nodes (from BCL 260 to 259 ms), which quickly degenerated into three nodes (BCL, 256 ms) (Fig. 8B). In the WT fiber, the pacing had to be accelerated over a considerably wider range (from BCL 212 to 189 ms, Fig. 8A) to see the progression from normal dynamics to the formation of three nodes.

As shown in Fig. 8B, in the mutant fiber, the first node develops at a position very close to the stimulus end (Fig. 8B, arrow; BCL, 259 ms). We also shortened the BCL by smaller 0.2-ms increments from 260 to 259 ms, to be sure that the node indeed formed close to the stimulus (and not that we failed to notice the migration of the node from the distal end). Indeed, when a discordant alternans appears (at BCL 259.6 ms), there are two nodes at exactly the same position as BCL of 259 ms, but the magnitude of alternans is very small (not shown). In the

<table>
<thead>
<tr>
<th>Threshold BCL, ms</th>
<th>Concordant Alternans</th>
<th>Discordant Alternans</th>
<th>2:1 Block</th>
</tr>
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<tbody>
<tr>
<td>Wild-type</td>
<td>210</td>
<td>188</td>
<td>185</td>
</tr>
<tr>
<td>Mutant</td>
<td>259</td>
<td>257</td>
<td>249</td>
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Table 1. Threshold BCL of arrhythmic events for fibers of normal and mutant fiber

BCL, basic cycle length.

Fig. 6. Computed ECG from TS mutant tissue. A: a normal ECG from a fiber of TS mutant cells at BCL of 262 ms with no dynamic instability. B: with increasing rate, a concordant alternans develops, and the computed ECG exhibits T-wave amplitude alternans. C: an increased pacing rate results in discordant alternans, causing alternating T-wave inversion (T-waves are indicated with arrows). D: gradients underlying T-wave alternans were APs of the whole fiber (i); the corresponding behavior of 3 cells representing different points in space along the fiber (ii); and events observed in computed ECG (iii). In beat a, cells at the distal end of the fiber repolarized first (line 1) and cells at the stimulus end repolarized last (line 2). In beat b, due to temporal and spatial dispersion, cells at the stimulus end repolarized first (line 3) and cells at the distal end repolarized last (line 4), creating an opposite direction of potential gradients, which causes the T-wave to invert. E: an example ECG recorded from TS patients with a heart rate of 120 beats/min (reproduced from Ref. 42).
WT fiber, when a discordant alternans develops, the first node appears (Fig. 8A; BCL, 208 ms) far away from the stimulus end (Fig. 8A, arrow). In other words, in the mutant tissue, a discordant alternans develops at first in a smaller piece of tissue. However, it is notable that in the tissue with TS mutant cells, only three nodes develop before conduction block occurs (BCL, 250 ms). The WT fiber, on the other hand, can support four nodes before conduction block (BCL, 186 ms). Hence, even though the initial development of discordant alternans occurs in a smaller tissue in the presence of the mutation, as the pacing frequency is increased, the WT tissue can actually support more nodes. This suggests that the relationship between node development, the number of nodes that can be supported within a particular tissue size, and the onset of conduction block are complex.

Echebarria and Karma (7) developed a quantitative description of the spatiotemporal dynamics of wave propagation in a one-dimensional fiber. Their theory provides a tool for predicting the alternans wavelength (i.e., the distance between two nodes), and they proposed a number of parameters that determine the wavelength for node development, including CV restitution, APD restitution, and diffusion. They also demonstrated that nodes formed via the steep CV restitution mechanism emerge from the boundary (7). In the present study, where the TS mutation does not fundamentally change the CV restitution in the paced fiber, the discordant alternans arises from a different mechanism, namely an increased slope of APD restitution. In this case, the first node directly appears at a position close to the stimulus end of the fiber, instead of gradually migrating from the boundary on the distal end.

Clearly, the prediction of conduction block is determined by factors in addition to the distance between nodes. Our simulations point to the importance of large temporal alternans magnitude (the fluctuation of APD on 2 consecutive beats, for example, in cell 200 of the mutant fiber during pacing at 250 ms; Fig. 8B), which leads to the enormous spatial dispersion of alternans magnitude along the fiber. Although the distance between nodes may be constant, temporal APD dispersion...
between them varies significantly, depending on APD restitution. Based on our simulations, we are encouraged to speculate that the interplay between temporal alternans magnitude and alternans wavelength may be an important determinant in predicting the onset of conduction block.

**DISCUSSION**

Here we demonstrate the potential for simulations in cardiac model cells and tissue to aid in the elucidation of arrhythmia development over multiple scales, from a mutation in a gene to its effect on protein cell and tissue function and the corresponding ECG. We use as an example a mutation in the LTCC linked to TS. The importance of making such connections is clear, given that despite the wealth of genetic information that now exists implicating mutations in disease, a paucity of available tools prevents the revelation of how mutations actually cause dynamic abnormalities indicative of an arrhythmia. Here we use mathematical modeling to reveal the ionic mechanisms underly the arrhythmogenic rhythms in both isolated cardiac cells and coupled cardiac tissue. The manifestations are the emergent behaviors resulting from complex nonlinear interactions stemming from a mutation that disrupts the inactivation of the LTCC.

LTCC inactivation is an important determinant of the morphology of the current time course during the cardiac AP and also determines the amplitude and morphology of the Ca\(^{2+}\) transient. LTCCs exhibit highly regulated VDI and CDI. The molecular basis and dynamic regulation of the two mechanisms have been investigated, but their interactions are unclear (1, 11, 15, 16, 19, 26, 46). For the TS mutations we explored, we benefited from the fact that the mutation has a clear-cut effect on exactly one of these processes, namely, the elimination of VDI, whereas CDI remains intact. As a result, a multistate Markov model of the Ca\(^{2+}\) channel was not a requirement. Nonetheless, more complex mutations may require a more detailed model of the Ca\(^{2+}\) channel that recapitulates the complex observed processes of inactivation and facilitation like the model developed recently by Faber et al. (9).

Our study serves as an example of the applicability of nonlinear dynamics and restitution analysis in understanding arrhythmia susceptibility. In Fig. 3, we demonstrated the effect of the TS mutation on APD adaptation and APD restitution, which describe the single cell dynamics and are suggestive of the propensity for arrhythmogenic rhythms such as alternans to develop. When compared with the WT cell, the TS mutant develops a alternans at much slower rates. Moreover, the alternans rapidly degenerates into complicated and highly arrhythmogenic rhythms with small increases in pacing rate.

Previous studies have shown that a discordant alternans can form spontaneously in spatially homogeneous tissue through the interaction of conduction velocity and APD restitution at high-pacing frequencies (13, 45). Here, we demonstrate a practical example of this theory for the mechanism of the development of discordant alternans because of a naturally occurring mutation. In a fiber, the mutation has distinct effects at different pacing frequencies (Figs. 6–8), resulting in four primary phenomena that underlie the increased arrhythmia susceptibility in TS patients including 1) the formation of discordance at slower BCLs; 2) node formation closer to the pacing site; 3) reduced distances between nodes; and 4) significant increases in $da/dx$, where $a$ is the alternans magnitude. All of these properties have been implicated in arrhythmogenicity by promoting wavebreak (13, 30, 34, 45). At slow pacing rates, despite prolonged APD in TS cells due to enhanced Ca\(^{2+}\) influx, the DI following each APD is long enough to allow high conduction velocity, guaranteeing dynamic homogeneity along the fiber. When pacing is accelerated, although still in a physiologically relevant range, the mutation-induced prolonged APD leads to a shorter subsequent DI. Because the tissue with the mutation has steeper APD restitution, large gradients of APD result, leading to the onset of a discordant alternans. Our model simulations also suggest that the TS mutation reduces the size of the tissue necessary for a discordant alternans to develop. However, when the tissue is large enough for multiple nodes to develop, the mutant tissue can actually support the development of fewer stable nodes before the onset of conduction block compared with WT. The complex interplay between repolarization gradients resulting from large temporal alternans and the wavelength of alternans requires further investigation to develop a predictive methodology for ascertaining the likelihood of conduction block.

Whereas the TS mutation example that we investigated here is a rare inherited disorder, abnormalities in Ca\(^{2+}\) handling and linkage to lethal arrhythmias are not. The investigations of inherited mutations are opportunities to consider electrical abnormalities in the absence of other complicating factors like structural abnormalities due to infarction or remodeling. Nonetheless, it stands to reason that disorders of Ca\(^{2+}\) handling that occur during pathological conditions like heart failure share some common arrhythmiasusceptibility mechanisms with those described here.

We chose to use the Fox et al. (12) model because it was specifically developed to accurately reproduce experimental observations during rapid pacing where alternans and other complex rhythms develop. The model develops a alternans via a voltage-driven mechanism that is coupled to and synchronizes with the Ca\(^{2+}\)-cycling system. Lately, much effort has been put forth to delineate the situations in which the voltage versus Ca\(^{2+}\) is the so-called conductor that directs the initial degeneration into alternans. In our study, a voltage alternans arises from the mutation-induced steepening of APD restitution. A Ca\(^{2+}\)-transient alternans is coupled to and driven by the voltage instability and hence are always observed in the same phase. Indeed, although several elegant studies by Sato et al. (37, 38) have shown that a steep relationship between Ca\(^{2+}\) release and Ca\(^{2+}\) load can result in a Ca\(^{2+}\)-driven alternans, the TS mutations we present here do not, to our knowledge, directly affect this relationship. This should, however, be the subject of a future study, since there may be an important increase in sarcoplasmic reticulumCa\(^{2+}\) load due to larger influx of Ca\(^{2+}\) through the defective channel that could affect the formation of a Ca\(^{2+}\)-driven alternans. At present, our study demonstrates how the direct effects of a genetic mutation lead to increased susceptibility to voltage alternans and affect higher dimensional dynamics.

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