Modeling of $I_{K1}$ mutations in human left ventricular myocytes and tissue

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1Institut für Biomedizinische Technik, Universität Karlsruhe (TH), Karlsruhe, Germany; 2Nora Eccles Harrison Cardiovascular Research and Training Institute and 3Bioengineering Department, University of Utah, Salt Lake City, Utah; 4Howard Hughes Medical Institute, Department of Neurology, University of California, San Francisco, California; and 5Division of Pediatric Cardiology, University of Utah, Salt Lake City, Utah

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Seemann G, Sachse FB, Weiss DL, Ptáček LJ, Tristani-Firouzi M. Modeling of $I_{K1}$ mutations in human left ventricular myocytes and tissue. Am J Physiol Heart Circ Physiol 292: H549–H559, 2007.—Elucidation of the cellular basis of arrhythmias in ion channelopathy disorders is complicated by the inherent difficulties in studying human cardiac tissue. Thus we used a computer modeling approach to study the mechanisms of cellular dysfunction induced by mutations in inward rectifier potassium channel (Kir)2.1 that cause Andersen-Tawil syndrome (ATS). ATS is an autosomal dominant disorder associated with ventricular arrhythmias that uncommonly degenerate into the lethal arrhythmia torsade de pointes. We simulated the cellular and tissue effects of a potent disease-causing mutation D71V Kir2.1 with mathematical models of human ventricular myocytes and a bidomain model of transmural conduction. The D71V Kir2.1 mutation caused significant action potential duration prolongation in subendocardial, midmyocardial, and subepicardial myocytes but did not significantly increase transmural dispersion of repolarization. Simulations of the D71V mutation at shorter cycle lengths induced stable action potential alternans in midmyocardial, but not subendocardial or subepicardial cells. The action potential alternans was manifested as an abbreviated QRS complex in the transmural ECG, the result of action potential propagation failure in the midmyocardial tissue. In addition, our simulations of D71V mutation recapitulate several key ECG features of ATS, including QT prolongation, T-wave flattening, and QRS widening. Thus our modeling approach faithfully recapitulates several features of ATS and provides a mechanistic explanation for the low frequency of torsade de pointes arrhythmia in ATS.

Andersen-Tawil syndrome; Kir2.1 mutation; ion channel modeling

The inward rectifier potassium current $I_{K1}$ is the major determinant of the resting membrane potential in the heart and participates in the most terminal phase of action potential repolarization (10). $I_{K1}$ is conducted by homo- and/or heterotetrameric channels formed by coassembly of the inward rectifier potassium channel (Kir)2.x subfamily of proteins (Kir2.1, Kir2.2, and Kir2.3). Message and protein expression studies indicate that Kir2.1 is the most abundant subfamily member in ventricular tissue (19, 23). Mutations in KCN2, the gene encoding Kir2.1, cause Andersen-Tawil syndrome (ATS), an autosomal dominant disorder associated with ventricular arrhythmias, periodic skeletal muscle paralysis, and dysmorphic skeletal features (7). ATS is a disorder of ventricular repolarization that is manifested by mild QTc interval prolongation but marked prolongation of the QUC interval (15, 22). Prominent U waves are a frequent finding in this disorder. Arrhythmias in ATS patients include frequent premature ventriculart contractions, bigeminy, and nonsustained polymorphic and bidirectional ventricular tachycardia (15). Although tachycardia burden is often high in ATS subjects (2), degeneration into lethal ventricular arrhythmias is relatively uncommon (17).

The vast majority of KCN2 mutations cause loss of function when expressed alone and variable degrees of dominant-negative suppression of wild-type (WT) Kir2.1 channel function (1, 15). Disease-causing mutant Kir2.1 subunits also exert dominant-negative effects on Kir2.2 and -2.3 channel function (8). One mutant, D71V Kir2.1, exerts a strong dominant-negative effect on all Kir2.x family members, consistent with the idea that one or more mutant subunits within the tetrameric complex are sufficient to eliminate channel function (7). Thus the functional current is carried only through tetramers containing four WT subunits representing 1/16 (6.25%) of control values. Recently, in vivo adenoaviral gene transfer of a dominant-negative Kir2.1 construct in guinea pig left ventricle induced a variable cellular phenotype depending on the degree of $I_{K1}$ suppression (5, 6). Action potential duration (APD) prolongation was noted in isolated cardiomyocytes with moderate degrees of $I_{K1}$ suppression. More severe $I_{K1}$ suppression resulted in spontaneous electrical activity, the so-called genetically engineered “biological pacemaker.”

The ionic mechanisms underlying biological pacemaker activity have been reported with computational models of guinea pig and human ventricular myocytes. With the Luo-Rudy guinea pig ventricle model, stable spontaneous pacemaker activity developed in the setting of reduced $I_{K1}$ conductance ($g_{K1}$) to 19% of control values (12). The primary pacemaker current was conducted by the Na$^+$/Ca$^{2+}$ exchanger (INaCa). Using a modified Priebe-Beuckelmann model of human ventricle, Kurata and colleagues (3) reported that spontaneous activity developed at 15% $g_{K1}$ and that INaCa was critical for spontaneous electrical activity. However, they reported that the major role for INaCa in the maintenance of stable pacemaker activity was not simply as a depolarizing current during phase 4, but rather maintaining low intracellular Ca$^{2+}$ concentration to prevent Ca$^{2+}$-dependent inactivation of L-type Ca$^{2+}$ current.

Rather than focusing on the ionic mechanisms of biological pacemaker activity, we were interested in the mechanisms of cellular dysfunction induced by ATS mutations in Kir2.1. We used an electrophysiological model of human ventricular myocytes (ten Tusscher et al., Ref. 14) to simulate the cellular effects of reduced $I_{K1}$ caused by a potent dominant-negative
mutant, D71V K\textsubscript{ir}2.1. The myocyte model includes all major ionic currents and is based on data from human ventricular myocytes or expression of human cardiac ion channels. Our modification of the model incorporates electrophysiological data from multiple myocyte types across the ventricular wall, allowing for the reconstruction of transmural heterogeneity in the left ventricle. We applied a bidomain approach of electrical conduction to calculate transmural ECGs. Thus the objective of this study was to determine the electrophysiological consequences of a K\textsubscript{ir}2.1 diseasing-causing mutation at the cellular and tissue levels.

**METHODS**

**Modeling of I\textsubscript{K1} channels.** The inwardly rectifying potassium current I\textsubscript{K1} is controlled by tetrameric ion channels formed by coassembly of the K\textsubscript{ir}2.x subfamily of proteins. We assume that the probability of subunit combinations underlying an individual ion channel can be approximated by multinomial distributions. The probability $P(X_i = x_i, \ldots X_k = x_k)$ is given by the multinomial distribution:

$$P(X_i = x_i, \ldots X_k = x_k) = \frac{n!}{x_1! \cdots x_k!} p_1^{x_1} \cdots p_k^{x_k}$$  \hspace{1cm} (1)

with the nonnegative integers $x_1, \ldots, x_k$, single subunit probabilities $p_1, \ldots, p_k$, the number of subunits of a channel $n = \sum x_i$, and the number of subunit types $k$. I\textsubscript{K1} channels are tetrameric; thus $n = 4$. The single-subunit probabilities $p_i$ are constants. These probabilities are measurable by studies of protein densities and dependent on tissue type and species.

The current $I_{K1}$ through an ensemble of channels can be defined as:

$$I_{K1} = N \sum_{(x_1, \ldots, x_k)} P(X_1, \ldots X_k) I_{K1}(x_1, \ldots x_k)$$  \hspace{1cm} (2)

with the number of channels in the ensemble $N$ and the current $I_{K1}(x_1, \ldots x_k)$, which is for a specific subunit combination.

Simplification of Eq. 1 can be applied to characterize $I_{K1}$ and reconstruct measurement results from electrophysiological studies. For example, in the case of only two subunit types, the probability for a subunit combination $(x_1, x_2)$ can be described with a binomial distribution:

$$P(X_1 = x_1, X_2 = x_2) = \frac{n!}{x_1!(n-x_1)} p_1^{x_1}(1-p_1)^{n-x_1}$$  \hspace{1cm} (3)

A simplification of Eq. 2 results from properties of dominant-negative mutations in subunit combinations. Here, only mutation-free combinations allow current flow through channels, i.e., $I_{K1}(x_1, \ldots, x_k) = 0$ for combinations with mutated subunits.

**Myocyte and tissue modeling.** An electrophysiological model of human left ventricular subepicardial myocytes (ten Tusscher et al., Ref. 14) was used to reconstruct the cellular electrophysiological properties. The model is based on coupled nonlinear first-order ordinary differential equations, which describe ion concentrations, fluxes through dynamically changing ion channels, pumps, and exchangers, as well as the transmembrane voltage ($V_m$). This model was adapted similarly as described previously to reconstruct transmural heterogeneity (11). In short, the rapidly activating, voltage-gated potassium current $I_{K1}$ and the slowly activating, delayed potassium current $I_{Ks}$ were modeled as a function of left ventricular free wall location. The conductance $g_{Ks}$ is changing over space as described originally by ten Tusscher et al. (14), and $g_{K1}$ is varying equivalent to the approach used in Ref. 11. Myocyte models were created for various wall locations (e.g., subendocardial, midmyocardial, and subepicardial) and different expressions of I\textsubscript{K1} channels, applying Eqs. 1 and 2. Simulations with the cell models were carried out with the Euler method for numerical integration of ordinary differential equations and a time step of 10 ms (9). Results after the 100th stimulation were analyzed for each simulation.

A strand model of the left ventricular free wall was created based on a bidomain approach to reconstruct anisotropic electrical conduction (20). The bidomain model allowed for calculation of current flow through gap junctions and the intracellular space as well as through the extracellular space. The current flow was reconstructed with Poisson’s equation for both intra- and extracellular domains for stationary electrical fields. An iterative Gauss-Seidel method with a time step of 10 ms was applied to solve the system of partial differential equations with the finite differences method (9). The strand model was inserted virtually into a bath medium in which two electrode positions were selected to extract the transmural ECG during electrophysiological activity (Fig. 1A). Electrophysiological heterogeneity in the strand was reconstructed by wall location-specific assignment of the myocyte models. The midmyocardial region was located at 20% of the distance from endocardium to epicardium, thus in the deep subendocardium. The orientation of muscle fibers leading to the anisotropic conduction was inserted into the model in such a way that the depolarization propagated always transverse to the fibers. Propagation was initiated by injecting an appropriate electrical current into the intracellular domain of cells at the endocardial end of the strand. This reconstructs the activation of the tissue due to the Purkinje fiber network. Figure 1B displays a normal excitation and repolarization process given by this model at different time instances. Data after the 10th stimulation were used for analyzing the simulations.

**RESULTS**

Estimation of functional I\textsubscript{K1} induced by D71V mutation. ATS is an autosomal dominant disorder, and therefore affected individuals express 50% mutant and 50% WT K\textsubscript{ir}2.1 protein. Assuming random association of mutant and WT subunits, the combinatorial possibilities of tetrameric channels are described by a binomial distribution (Eq. 3). The probability of a homomorphic WT channel is 6.25%. We elected to study the dominant-negative ATS mutant D71V given that coexpression experiments indicated that functional current was conducted only by WT homomorphic channels. Cardiac I\textsubscript{K1} is conducted by the sum of $K_{ir}2.1, -2.2$, and -2.3 channels. From mRNA expression in human ventricle, $K_{ir}2.1$ subunits constitute 92% of total $K_{ir}2.x$, with $K_{ir}2.2$ and -2.3 contributing 7% and 1%, respectively (Table 1). Four distinct subunit types (i.e., WT $K_{ir}2.1$, D71V $K_{ir}2.1$, $K_{ir}2.2$, and $K_{ir}2.3$) were considered for the calculation of subunit combinations. Using Eq. 1, we estimated that the probability of homomorphic channels consisting of only WT $K_{ir}2.1, -2.2$, or -2.3 subunits is 8.5%. In the simulations that follow, we use 8.5% $g_{K1}$ as an estimate of the effect of D71V on total ventricular I\textsubscript{K1}. Further simulations with 20% $g_{K1}$ serve for prediction of the effects of $K_{ir}2.1$ mutations that cause less severe degrees of dominant-negative suppression of I\textsubscript{K1} (15).

Effect of g\textsubscript{K1} reduction in single ventricular myocytes. Simulated $V_m$ for subendocardial, midmyocardial, and subepicardial human left ventricular myocytes in response to pacing frequencies of 1 and 2 Hz are demonstrated in Fig. 2.
The APD of the midmyocardial cell was longer than that of subendocardial and subepicardial cells, an effect attributable to the relative decrease in $I_{Ks}$ density in midmyocardium (21) (Table 2 and Fig. 2). A progressive reduction in $g_{K1}$ resulted in a progressive decrease in resting $V_m$ in all three cell layers (Table 2), consistent with the role of this current in setting diastolic $V_m$. Decreasing $g_{K1}$ to 20% of control values caused APD prolongation for all three cell types; however, the degree of APD prolongation was greater in midmyocardial cells compared with subendocardial and subepicardial cells (Table 2 and Fig. 2). A further reduction in $g_{K1}$ to 8.5% of control values caused a modest amount of additional APD prolongation compared with 20% $g_{K1}$, but produced a reduction of the maximal diastolic $V_m$, and in the phase 1 notch. Increasing the pacing frequency to 2 Hz did not markedly alter the action potential parameters in the setting of reduced $g_{K1}$ (Table 2 and Fig. 2).

The effects of reduced $g_{K1}$ on transmembrane currents in a subendocardial myocyte during 1-Hz stimulation are demonstrated in Fig. 3. The peak amplitude of sodium current ($I_{Na}$) and $I_{to}$ progressively decreased as $g_{K1}$ decreased (Fig. 3B), accounting for the reduced maximal diastolic $V_m$ and reduced phase 1 notch in simulated action potentials (Fig. 2). Rapidly activating delayed rectifier potassium current ($I_{Kr}$) is increased substantially and to a greater degree than $I_{Ks}$ in the setting of reduced $g_{K1}$ (Fig. 3, C and D) and provided the majority of repolarizing current during phase 3. L-type calcium current ($I_{Ca}$) was essentially unaffected by reduced $g_{K1}$. Decreasing $g_{K1}$ to 20% and 8.5% of control values decreased the amplitude of $I_{Na-Ca}$ during phases 1, 3, and 4.

A further increase in pacing frequency to 3 Hz in the setting of reduced $g_{K1}$ caused significant perturbations in action potential parameters from all cell types (Fig. 4). Subendocardial and subepicardial cells behaved similarly, with 20% $g_{K1}$ causing a reduced phase 1 notch and 8.5% $g_{K1}$ causing marked slowing of the action potential upstroke. The upstroke in the setting of 8.5% $g_{K1}$ was mediated primarily by the stimulus current followed by $I_{Ca}$ (Fig. 5E). The effect of increased pacing frequency was most dramatic in the midmyocardial cell, where a stable pattern of action potential alternans developed at 20% and 8.5% $g_{K1}$ with marked abbreviation of the action potential amplitude and duration. The transmembrane currents underlying action potential alternans at 3-Hz stimulation are presented in Fig. 5. Reduction of $g_{K1}$ led to absence of $I_{Na}$ and $I_{to}$ (Fig. 5, D and G). Also, $I_{Ca}$ was markedly reduced during the abbreviated action potential (Fig. 5E). The majority of

### Table 1. Absolute and relative mRNA levels in human cardiac tissue

<table>
<thead>
<tr>
<th>$K_w$</th>
<th>Atrium mRNA, amol/µg total RNA</th>
<th>Ventricle mRNA, amol/µg total RNA</th>
<th>Atrium rel., %</th>
<th>Ventricle rel., %</th>
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<tr>
<td>2.1</td>
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</tr>
<tr>
<td>2.2</td>
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<td>9</td>
<td>7</td>
</tr>
<tr>
<td>2.3</td>
<td>7.1</td>
<td>0.6</td>
<td>10</td>
<td>1</td>
</tr>
</tbody>
</table>

Absolute mRNA levels are from Ref. 19.

Fig. 1. Transmural strand model for the calculation of excitation propagation. A: the heterogeneous strand model with the excitable tissue in blue is inserted in a bath (turquoise). The positions for measurement of the transmural ECG are marked with red circles. B: excitation propagation and repolarization at different times for the physiological model. Excitation was initiated at the subendocardium representing Purkinje fiber activation. The depolarization is conducted toward subepicardium. Repolarization propagates from subepicardium toward subendocardium.
repolarizing current during phases 2 and 3 of the abbreviated action potential was provided by $I_{K_{r}}$, with a smaller contribution of $I_{K_{s}}$ (Fig. 5, C and D). $I_{NaCa}$ was reduced in concomitant fashion during the abbreviated action potential.

To explore the role of reduced $g_{K_{1}}$ in triggering spontaneous activity, cells were stimulated at 1, 2, and 3 Hz in settings of 0% to 7% $g_{K_{1}}$ and $V_m$ was recorded following a pause in pacing rate. This protocol assessed the capacity for spontaneous activity as might occur during a sinus pause (Table 3). No spontaneous activity was detected after a pause preceded by 1-, 2-, and 3-Hz stimulation for $g_{K_{1}}$ larger than a threshold value of 6%. However, for $g_{K_{1}}$ less than or equal to the threshold, spontaneous action potentials were triggered. Action potentials and the underlying transmembrane currents for $g_{K_{1}}$ values near

**Table 2.** Diastolic transmembrane voltage and action potential duration at different levels of $g_{K_{1}}$ in the three cell types at different frequencies

| Frequency, Hz | $g_{K_{1}}$, % | Subendo | | Mid | | Subepi |
|---------------|----------------|---------|---|---|---|---|---|
|               | $V_m$, mV | APD<sub>90</sub>, ms | $V_m$, mV | APD<sub>90</sub>, ms | $V_m$, mV | APD<sub>90</sub>, ms |
| 1  | 100  | -86  | 278   | -86  | 327   | -87  | 270   |
|    | 20   | -84  | 307   | -84  | 365   | -84  | 299   |
|    | 8.5  | -80  | 317   | -79  | 379   | -80  | 308   |
| 2  | 100  | -86  | 273   | -86  | 315   | -86  | 264   |
|    | 20   | -83  | 302   | -83  | 352   | -83  | 294   |
|    | 8.5  | -80  | 312   | -80  | 365   | -80  | 304   |
| 3  | 100  | -86  | 252   | -85  | 274   | -86  | 245   |
|    | 20   | -82  | 282   | -82  | 276   | -82  | 276   |
|    | 8.5  | -77  | 297   | -78  | 288   | -78  | 288   |

Subendo, subendocardial; Mid, midmyocardial; Subepi, subepicardial; $g_{K_{1}}$, inward rectifier potassium current conductance; $V_m$, transmembrane voltage; APD<sub>90</sub>, action potential duration at 90% repolarization.

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Fig. 2. Transmembrane voltages ($V_m$) of subendocardial (A, B), midwall (C, D), and subepicardial (E, F) myocytes for stimulation frequencies of 1 (A, C, E) and 2 (B, D, F) Hz and 8.5%, 20%, and 100% inward rectifier potassium current ($I_{K_{1}}$) conductance ($g_{K_{1}}$). The stimulus was applied at $t = 50$ ms. Reduction of $g_{K_{1}}$ led to increased action potential durations (APDs) and depolarized resting voltages.
the threshold for spontaneous activity are shown in Fig. 6. A pause after 3-Hz pacing induced a single spontaneous action potential after a delay of 1.48 s in the setting of 6% $g_{K1}$. The dominant inward current during phase 4 depolarization was $I_{NaCa}$, which depolarized $V_m$ to the threshold of $I_{Ca}$ activation, responsible for phase 0 upstroke (Fig. 6, F and G). Further reductions in $g_{K1}$ resulted in an increased frequency of spontaneous action potential generation (Table 3).

**Tissue electrophysiology-simulated ECGs.** Electrical conduction and transmural ECGs were simulated within the transmural strand model (Fig. 1A). The depolarization and repolarization process in the case of 100% $g_{K1}$ is depicted in Fig. 1B at different time instances for a stimulation frequency of 1 Hz. The depolarization propagated from subendocardium to subepicardium, leading to a positive QRS complex in the transmural ECG (Fig. 7A). The repolarization propagated in the opposite direction, and thus the T wave is also positive.

Simulated transmural ECGs in the setting of reduced $g_{K1}$ are compared with control values in Fig. 7. Both 20% and 8.5% $g_{K1}$ caused flattening and widening of the T wave as well as QRS and QT prolongation, a consequence of APD prolongations at the cellular level (Figs. 2 and 4); 8.5% $g_{K1}$ prolonged the QT duration to 387 ms for a stimulus frequency of 1 Hz, compared with 357 ms in control conditions. QRS prolongation from 31 ms for control to 36 ms for 8.5% $g_{K1}$ was due to reduced Na channel availability as a consequence of reduced membrane repolarization (Fig. 7D). This leads to a reduction in conduction velocity in the strand model. Alternans in the transmural ECG was observed for reduced $g_{K1}$ at a stimulus frequency of 3 Hz (Fig. 7C).
DISCUSSION

Elucidation of the cellular basis of arrhythmias in ion channelopathy disorders is complicated by the inability to study isolated human ventricular myocytes from healthy subjects or those with inherited diseases. Thus the use of mathematical models of human ventricular tissue to simulate the effects of ion channel dysfunction at the cellular and tissue levels is of paramount importance. Moreover, studies of the cellular consequences of Kir2.1 mutations associated with ATS are complicated by the lack of specific Kir2.1 channel blockers, further underscoring the importance of computational modeling. To this end, we used a modified ten Tusscher et al. model of human ventricular myocytes and a bidomain model of electrical conduction to study the cellular and tissue effects of a potent disease-causing mutation, D71V Kir2.1.

The D71V mutant Kir2.1 subunit behaves in heterologous expression systems like a “poison pill” (7, 8). That is, one mutant subunit within the tetrameric channel complex is sufficient to render the channel nonfunctional. ATS is an autosomal dominant disorder, and therefore an affected individual expresses a WT and a mutant allele. Assuming a random likelihood of subunit association, the probability of a tetramer containing one or more D71V subunits is 15/16 (93.75%) and the probability of containing four WT Kir2.1 subunits is 1/16 (6.25%). Indeed, coexpression of WT and D71V Kir2.1 induced a current that was ~5% of control values, implying that functional current was conducted through channels consisting only of WT subunits. From the relative abundance of Kir2.x transcripts in human ventricle (19) and the dominant-negative properties of D71V, we calculated that homotetrameric Kir2.x channels constitute 8.5% of the total number of channels that comprise human I_K1. This approximation is based on the assumption that Kir2.x protein levels mirror that of mRNA and that mutant mRNA and protein are as stable as WT. With these caveats, we simulated the electrophysiological consequences of a Kir2.1 disease-causing mutation in human left ventricular tissue.

Our simulations revealed that reduced g_K1 exerted differential effects on APD. Although reduced g_K1 caused APD prolongation in all three cell types, APD prolongation was greatest in the midmyocardial cell. However, the differential APD prolongation in midmyocardial cells was diminished when cells were coupled in a transmural fiber, such that no significant increase in transmural dispersion of repolarization was detected. These findings are consistent with those reported by Tsuboi and Antzelevitch (16), who used BaCl2 to suppress I_Kr in the canine wedge preparation. Thus the low frequency of torsade de pointes arrhythmia in ATS patients may be a consequence of the lack of transmural dispersion of repolarization in the setting of reduced I_K1, despite the fact that APD is prolonged. Interestingly, the contribution of I_Kr to phase 3 repolarization increased dramatically in the setting of reduced g_K1. The consequences of this observation are that patients with ATS may be at a substantially increased risk of arrhythmias in the setting of reduced I_Kr associated with hypokalemia or the wide variety of medications known to block hERG channels.
The effects of reduced $g_{K1}$ were more dramatic at faster pacing rates. A stimulation frequency of 3 Hz at 8.5% $g_{K1}$ induced a stable pattern of action potential alternans in the midmyocardial cell, characterized by alternating action potentials of reduced peak amplitude and duration. The reduced peak amplitude and duration were a consequence of markedly reduced $I_{to}$ and $I_{Ca}$. The APD alternans resulted in the failure of action potentials to propagate through the midmyocardial portion of the simulated ventricular strand. Such a unidirectional block might provide a substrate for reentrant tachycardia.

Our simulations of the D71V mutation were unable to recapitulate some of the cardinal ECG abnormalities described in ATS patients, that is, prominent U waves and QU interval prolongation (see Limitations). Notwithstanding this, the presented transmural ECGs reproduced several important ATS features, including QT prolongation, flattening of the T wave, and QRS prolongation. QT prolongation was a prominent feature in the initial cohort of ATS probands, representing the most highly affected individuals (15). Low-amplitude or inverted T waves were described in 19% of ATS patients (22). An example of low-amplitude T waves in a patient with the D71V mutation is shown in Fig. 10. Similar T-wave changes were reported by Tsuboi and Antzelevitch (16) in their canine wedge preparation model of ATS. Our simulations indicate that flattening and widening of the T wave occur as a consequence of APD.

Table 3. Number of spontaneous beats in 10-s pause and delay of first beat

<table>
<thead>
<tr>
<th>Frequency, Hz</th>
<th>$g_{K1}$, %</th>
<th>Subendocardial</th>
<th>Midmyocardial</th>
<th>Subepicardial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beats</td>
<td>Delay, ms</td>
<td>Beats</td>
<td>Delay, ms</td>
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<tr>
<td>1 2 0</td>
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<tr>
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<td>657</td>
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<tr>
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<td>998</td>
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<td>12 781</td>
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prolongation at the cellular level. Conduction disorders were identified in 23% of ATS patients, with a bundle branch block pattern documented in 14% (22). The simulated D71V mutation resulted in a 16% increase in QRS duration in the one-dimensional transmural strand. We anticipate that this degree of prolongation would be maintained in a three-dimensional heart, consistent with the QRS prolongation seen in some ATS patients. Our simulations indicate that QRS prolongation is due to partial inactivation of $I_{Na}$ as a consequence of depolarized $V_m$ in the setting of reduced $g_{K1}$.

Miake and colleagues (5, 6) used in vivo gene transfer of a dominant-negative $K_{ir}2.1$ construct in guinea pig left ventricle and induced a cellular phenotype that varied with the degree of $I_{K1}$ suppression. For moderate levels of $I_{K1}$ suppression, APD prolongation was detected in isolated myocytes, similar to that seen in our simulations of 20% and 8.5% $g_{K1}$. Spontaneous action potentials were detected when $I_{K1}$ density in transfected myocytes was <0.4 pA/pF, corresponding to ~13% of control $g_{K1}$ (6). Spontaneous pacemaker activity was elicited in the Luo-Rudy and Priebe-Beuckelmann models at 19% and 15% $g_{K1}$, respectively (3, 12). With the ten Tusscher et al. model, spontaneous action potential generation required 6% $g_{K1}$ and a pause preceded by 3-Hz pacing. Spontaneous action potential generation was found for smaller $g_{K1}$ for stimulus frequencies of 1–3

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Fig. 6. Transmembrane voltage and currents of subendocardial myocyte paced with 6% and 7% $g_{K1}$ after stimulation with 3 Hz with $V_m$ (A), $I_{K1}$ (B), $I_{to}$ (C), $I_{KS}$ (D), $I_{Ca}$ (E), $I_{NaCa}$ (G), and $I_{Na}$ (H). A spontaneous beat was triggered 1,483 ms after the last stimulus in the myocyte with 6% $g_{K1}$.
Hz. The differences in \( g_{K1} \) necessary for simulated pacemaker activity are related to underlying differences in the ventricular models. The Luo-Rudy formalism is based on guinea pig experimental data and would be predicted to best recapitulate the in vivo gene transfer results. The Priebe-Beuckelmann human ventricle model is a modified version of the Luo-Rudy phase II model, but several ionic currents are based on animal, not human, data.

Although our simulated D71V mutation did not trigger spontaneous pacemaker activity, the model tissue was near the threshold for spontaneous activity. Perhaps other triggers or comodifiers tip the balance toward local changes in \( I_{K1} \) that trigger spontaneous generation of action potentials. Indeed, ATS patients alternate between sinus rhythm and ventricular arrhythmias throughout the day, implying that spontaneous activity is not incessant. We propose that the D71V-induced
reduction in \( g_{K1} \) provides the substrate for arrhythmia by altering the balance between inward and outward currents during phase 4 such that \( I_{NaCa} \) reaches a threshold to trigger spontaneous action potentials. Additional factors, such as hypokalemia, could promote arrhythmia in ATS patients by further reducing \( I_{K1} \) and \( I_{Kr} \) [both dependent on the concentration of extracellular potassium ([K\(^+\)]\_o)]. Our simulations indicate an increased contribution of \( I_{Kr} \) during phase 3 in the setting of reduced \( g_{K1} \). Thus ATS patients may be particularly susceptible to changes in \( I_{Kr} \) (such as hERG channel blockers or decreased [K\(^+\)]\_o) given the baseline reduced repolarization reserve and the dependence on \( I_{Kr} \) as a substitute for reduced \( I_{K1} \).

**Limitations.** A primary limitation of this study is the inability to properly model the U wave in the simulated transmural ECGs and thereby test whether reduced \( g_{K1} \) reproduces QU prolongation and prominent U waves typical of ATS. This limitation is a consequence of our incomplete understanding of the electrophysiological basis of the U wave. Although several theories have been proposed to explain the genesis of the U wave, controversy remains as to its cellular mechanism (13, 18). The mechanoelectrical coupling theory postulates that activation of mechanosensitive (stretch activated) cation channels during ventricular filling generates a small depolarizing current that produces the U wave (13, 18). The molecular identity of a mechanosensitive channel expressed in human heart was recently reported as a member of the transient receptor potential channel (TRPC) family (4). As more experimental evidence becomes available, the properties of mechanosensitive channels will be incorporated into the existing computational modeling framework to test the effects of ATS mutations on cardiac repolarization. Irrespective of our limited understanding of the U wave, any depolarizing influence during phase 4 will be augmented in the setting of reduced \( g_{K1} \), resulting in accentuation of the U wave as typically seen in ATS.

In summary, we propose that the D71V Kir2.1 mutation provides a substrate for arrhythmia by altering the balance between inward and outward currents during phase 4 such that \( I_{NaCa} \) can trigger the generation of spontaneous action potentials. Our simulations of D71V mutation recapitulate several key ECG features, including QT prolongation, T wave flattening, and QRS widening. Elucidation of the cellular and ionic basis of the U wave will allow simulation of the ECG features typical of ATS, such as prominent U waves and QU prolongation.

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Fig. 9. \( V_m \) of subendocardial, midmyocardial, and subepicardial cells inside the strand model for 8.5% \( g_{K1} \) and a stimulus frequency of 1 (A), 2 (B), and 3 (C) Hz.

Fig. 10. Einthoven III lead of patient with D71V mutation. Note the low-amplitude T wave.
REFERENCES


