Special Report

Mutation in the *KCNQ1* Gene Leading to the Short QT-Interval Syndrome

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Background—The electrocardiographic short QT-interval syndrome forms a distinct clinical entity presenting with a high rate of sudden death and exceptionally short QT intervals. The disorder has recently been linked to gain-of-function mutation in *KCNH2*. The present study demonstrates that this disorder is genetically heterogeneous and can also be caused by mutation in the *KCNQ1* gene.

Methods and Results—A 70-year man presented with idiopathic ventricular fibrillation. Both immediately after the episode and much later, his QT interval was abnormally short without any other physical or electrophysiological anomalies. Analysis of candidate genes identified a g919c substitution in *KCNQ1* encoding the K^+ channel KvLQT1. Functional studies of the KvLQT1 V307L mutant (alone or coexpressed with the wild-type channel, in the presence of IsK) revealed a pronounced shift of the half-activation potential and an acceleration of the activation kinetics leading to a gain of function in I_{Ks}. When introduced in a human action potential computer model, the modified biophysical parameters predicted repolarization shortening.

Conclusions—We present an alternative molecular mechanism for the short QT-interval syndrome. Functional and computational studies of the *KCNQ1* V307L mutation identified in a patient with this disorder favor the association of short QT with mutation in *KCNQ1*. **(*Circulation*. 2004;109:2394-2397.)**

Key Words: death, sudden ■ genetics ■ arrhythmia ■ ion channels ■ fibrillation, ventricular

In recent years, extensive progress has been made in unraveling the pathophysiology of the monogenic arrhythmia syndromes among which are long-QT syndrome, Brugada syndrome, and catecholaminergic polymorphic ventricular tachycardia.[1] The latest addition to this class of disorders is the description of families with a high rate of sudden death and exceptionally short QT intervals,[2] recently attributed to gain-of-function mutation in *KCNH2*.[3] In this study, we demonstrate that this disorder is genetically heterogeneous and can also be caused by mutation in the *KCNQ1* gene that encodes the KvLQT1 K^+ channel, which, in association with the β-subunit IsK, forms the slow component of the cardiac delayed rectifier K^+ current (I_{Ks}).[4]

Methods

Patient Characteristics

A 70-year-old man was successfully resuscitated after a ventricular fibrillation episode. He had been without complaints up until then, and his family history was unremarkable. Physical examination revealed no abnormalities. His ECG is presented in Figure 1. Sinus rhythm was present with normal conduction intervals and a QT interval of 290 ms (QTc, 302 ms). Similarly short QT intervals were observed on every ECG up to 3 years of follow-up. Biochemical analysis at the time of admission, including echocardiography, exercise testing, coronary angiography, left (LV) and right ventricular (RV) angiography, scintigraphy, and ergonovine coronary spasm test, revealed no abnormalities. Nuclear LV ejection fraction was 49%. During electrophysiological study, no arrhythmias could be induced. The electrophysiology protocol used was ventricular stimulation from two sites (RVA, RVOT), with 2 cycle length (600, 430 ms) and up to 2 extras (coupling interval $\geq$180 ms with capture, effective refractory period $\leq$180 ms). LV myocardial biopsies revealed no abnormalities.

Genetic Studies

Genetic studies were approved by the Medical Ethical Committee of the Academic Medical Center. Mutation screening of the coding region of the *KCNE1* (minK, IsK), *KCNE2* (MIRP1), *KCNH2* (HERG), and *KCNQ1* (except for exon 1) genes was performed either by direct sequencing, single-strand conformation polymorphism analysis followed by sequencing of aberrant conformers, or denaturing high-performance liquid chromatography analysis followed by direct sequencing of fragments displaying an abnormal elution profile.

The *KCNQ1* gene was analyzed in 200 unrelated control individuals to exclude the possibility that the identified *KCNQ1* mutation (V307L) represented a common polymorphism. This was done by direct sequencing (n=86) or by denaturing high-performance liquid chromatography analysis (n=114) of the exon concerned.

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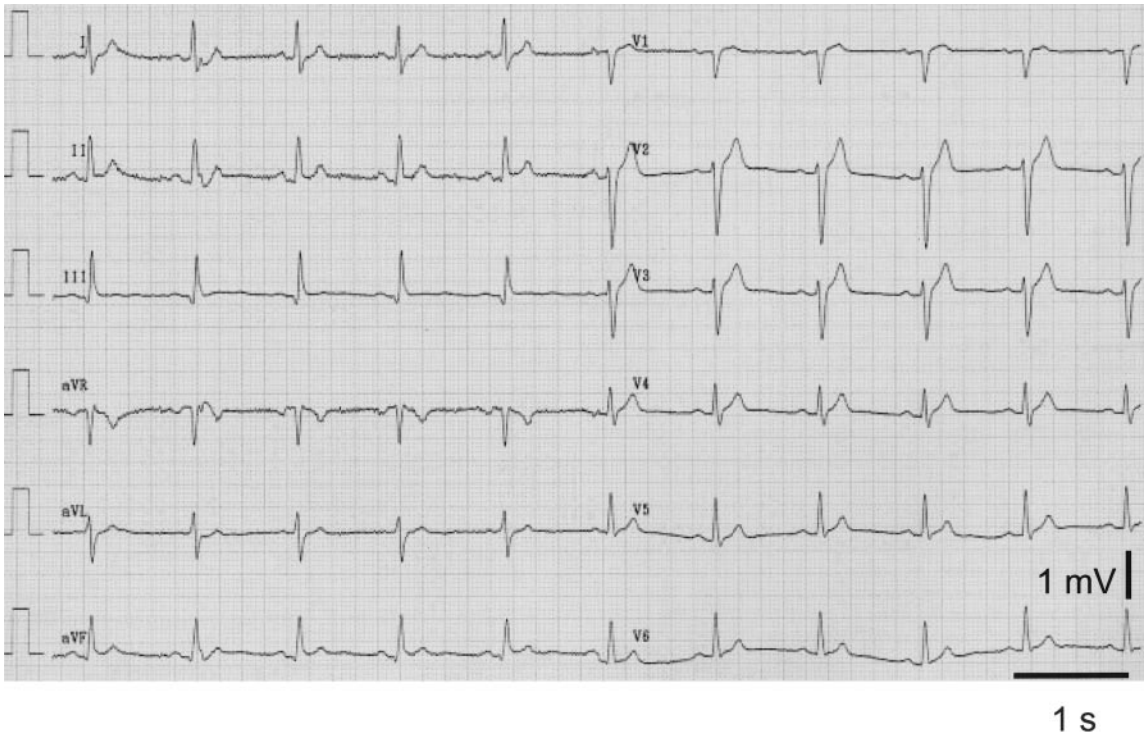

Figure 1. ECG of the patient obtained a few days after the successful resuscitation from VF. Note the short QT interval.

Electrophysiological Analysis

The V307L KvLQT1 cDNA was subcloned from the pSGEM vector (a kind gift from Klaus Steinmeyer, Aventis Pharma Deutschland GmbH) into pCI-CMV. COS-7 cells were transfected with pRC-CMV-IsK and wild-type (WT) or V307L KvLQT1 construct in a ratio of 2:1. To mimic the heterozygous state, COS cells were transfected with pRC-CMV-IsK, and WT and V307L KvLQT1 constructs in a ratio of 2:0.5:0.5. To investigate further IsK-V307L KvLQT1 interactions, a ratio of pRC-CMV-IsK to V307L of 1:2 was also studied. In all cases, pEGFP (BD Biosciences Clontech) was cotransfected as reporter. Twenty-four hours after transfection, whole-cell currents were recorded at 35°C using the permeabilized-patch configuration. The pipette solution contained (in mmol/L) potassium gluconate 145, EGTA 1, and HEPES 10, and 0.2 to 0.4 μg/mL amphotericin B added extemporarily, pH 7.3 with KOH. The extracellular medium contained (in mmol/L) sodium gluconate 145, potassium gluconate 4, hemi–calcium gluconate 7 (free Ca^{2+}: 1), hemi–magnesium gluconate 4 (free Mg^{2+}: 1), HEPES 5, and glucose 5, pH 7.4 with NaOH. Patch-clamp data are presented as mean±SEM. Statistical significance was assessed by means of the Student *t* test or 2-way ANOVA when needed.

Computer Modeling

To investigate the impact of the altered characteristics of the V307L mutation on the ventricular action potential (AP), we used the human ventricular cell model of Priebe and Beuckelmann.[5] Intracellular $[Na^+]$ and $[K^+]$ were kept constant, and steady-state values and time constants of activation and inactivation of all currents were precalculated and stored in look-up tables. Steady-state activation of I_{Ks} was replaced by a Boltzmann equation to incorporate the findings with respect to half-activation voltage ($V_{0.5}$) and slope factor. For the calculations we used a fixed time step of 200 ms. The last AP of each 10-s run was analyzed.

Results

Genetic studies on the patient's DNA excluded mutation in *KCNE1*, *KCNE2*, and *KCNH2*. Analysis of the *KCNQ1* gene identified a G>C substitution at nucleotide 919 (GTG>CTG) leading to the substitution of the valine at position 307 by leucine (V307L), which was not detected in 400 normal chromosomes.

Illustrative WT and V307L KvLQT1 K^+ currents recorded in the presence of IsK are shown in Figure 2A (a and c). The V307L channel produced a K^+ current with an amplitude similar to WT (tail current density at −40 mV elicited by a prepulse from −80 mV to +40 mV for 1 s, 13.93±2.01 pA/pF for V307L and 10.58±1.67 pA/pF for WT [n=16 cells in each group; NS, *t* test]). As illustrated in Figure 2B, compared with WT, $V_{0.5}$ for the mutant channel was more negative ($P<0.001$) and the slope of the voltage sensitivity was unchanged (17.40±1.19 and 16.09±1.00 for WT and V307L, n=9 and 14 cells, respectively; NS). In addition, activation kinetics were accelerated at each potential as depicted in Figure 2A and 2C. When the activating current traces were fitted using a double exponential function, both fast ($\tau_{fast\ act}$) and slow ($\tau_{slow\ act}$) activation time constants were significantly decreased for the mutant ($P<0.01$, 2-way ANOVA for repeated measurements). The fast activating component contributed to ≈75% of the WT or V307L current at each potential. Kinetics of deactivating currents at various test-pulse potentials after depolarization to +60 mV were not significantly different between mutant and WT (not shown). Altogether, the shift in activation and the faster activation kinetics imply a gain of function for the mutant.

The effects of the V307L mutation were also evaluated in conditions mimicking the heterozygous state of the patient. As compared with WT alone, coexpression of WT

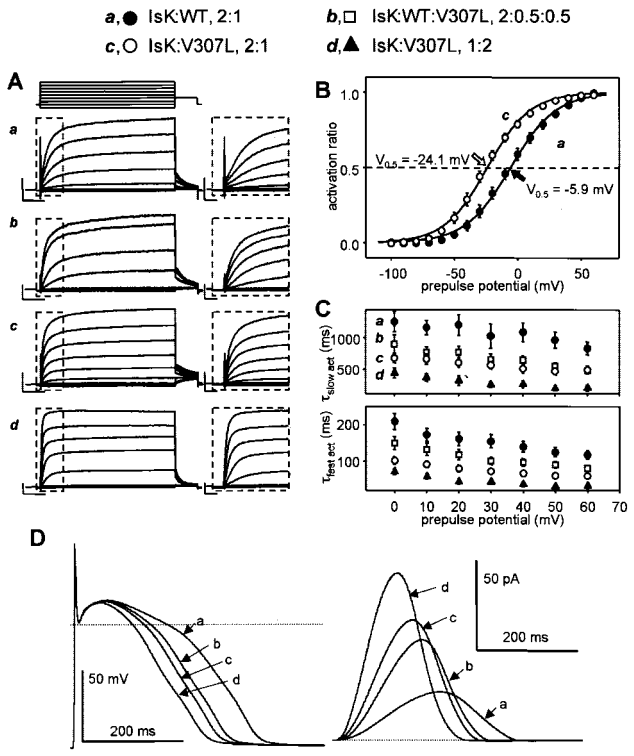

Figure 2. Characteristics of WT, V307L, and WT+V307L KvLQT1/IsK currents. A, Superimposed currents activated by a 3-s prepulse to various potentials (from −100 to +60 mV, 20-mV increments) and repolarized by a 500-ms test-pulse to −40 mV (holding potential: −80 mV; 0.14 Hz). Current scale, 10 (a and c) or 20 (b and d) pA/pF. Time scale, 500 ms (left), 100 ms (right, time-expended framed zone). B, Current-voltage relation for the tail current of WT (a, ●; n=9) and V307L (c, ○; n=14). C, Fast ($\tau_{fast\ act}$; bottom) and slow ($\tau_{slow\ act}$; top) activation time constants of WT (a, ●; n=6 to 9), WT+V307L (b, □; n=9), and V307L (c, ○; n=12 to 13, and d, ▲; n=9). D, Computer model of the ventricular AP. Stimulus pulse, 1 Hz, 2 ms, 2.9 nA. Steady-state activation was calculated by a Boltzmann function $1/\{1+\exp[(V_{0.5}-Vm)/s]\}$. Left panel, APs of WT ($V_{0.5}=-5.9$ mV, $s=17.4$ mV; a), heterozygous expression ($V_{0.5}=-20.62$ mV, s=10.96 mV, activation time constant tau set at 70% of WT; b), homozygous mutant expression ($V_{0.5}=-24.05$ mV, $s=16.0$ 9 mV, tau set at 52% of WT; c), and homozygous mutant expression with reduced IsK ($V_{0.5}$ and s as in c; tau set at 32% of WT [d]). AP durations at 50% repolarization were 280, 223.5, 206, and 177 ms, respectively. Right panel, I_{Ks} currents during APs in left panel.

and mutant channels (Figure 2Ab and 2C) led to an accelerated activation of K^+ current ($P<0.01$ for both time constants), whereas the current density was not different (tail current density at −40 mV, 11.79±0.80 pA/pF; n=13). In addition, compared with WT alone, activation was shifted toward more negative potentials ($V_{0.5}=-20.62\pm$ 2.23 mV; n=9; $P<0.001$) and the slope of activation was changed (10.96±1.15; n=9; $P<0.001$).

KvLQT1 channel activity is strongly regulated by the IsK subunit. The effect of different IsK and V307L channel ratios (namely 2:1 and 1:2) was also evaluated. As illustrated in Figure 2Ad and 2C, at a 1:2 ratio, K^+ current activation was accelerated with respect to the 2:1 ratio ($P<0.01$; n=9 for both time constants). Surprisingly, the current density was not significantly decreased (11.44±0.078 pA/pF; n=19), whereas, in cells expressing a 2:1 WT:IsK ratio, we failed to observe any K^+ current in 8 of 19 cells, resulting in a mean tail current density reduction to 2.63±0.44 pA/pF (n=19).

In the Priebe–Beuckelmann[5] computer model of the human AP, the observed changes in $V_{0.5}$, slope and time constants were implemented. Figure 2D shows that the combined shift of activation and decrease in activation time constant diminished AP duration and that their effects were additive. The figure also shows the change in amplitude of I_{Ks} during the APs.

Discussion

Herein we present an alternative molecular substrate for the short QT-interval syndrome. We demonstrate that gain-of-function mutation affecting I_{Ks} also results in shortening of the QT interval. Abbreviation of the AP, particularly when occurring inhomogeneously, should provide a substrate for reentrant arrhythmias. Because the contribution of I_{Ks} is different in the various cell layers of the heart, such inhomogeneity in AP duration is to be expected.

The V307L mutation has previously been investigated in *Xenopus* oocytes, although in the absence of IsK.[6] V307L led to suppression of KvLQT1 inactivation, a characteristic that is exclusively observed in the absence of IsK.[7] In the presence of IsK, we show that the major effects of the V307L mutation, even in the heterozygous state, are an acceleration of the activation kinetics and a shift in the $V_{0.5}$ of activation, both effects leading to a gain of function. As a result, the K^+ current resulting from association of WT and V307L subunits can be recruited earlier during the AP. The studies involving different ratios of V307L to IsK suggest that V307L subunit trafficking is less dependent on the presence of IsK than WT even if its activation kinetics remain under the control of IsK.

A S140G *KCNQ1* mutation with gain-of-function characteristics was recently linked to familial atrial fibrillation.[8] It is not immediately clear why the S140G mutation is not associated with shortened QT intervals. However, a major difference between the S140G and the V307L mutations is that the former leads to a time-independent current in the presence of IsK, whereas the latter conserves its time dependency. A time-independent K^+ current is reminiscent of the current activated by acetylcholine in the atria that is known to precipitate atrial tachyarrhythmias.

Although the present report does not definitely prove a causal relation between the short QT interval, the occurrence of ventricular fibrillation, and the *KCNQ1* mutation, circumstantial evidence for a causal relationship is provided on several levels. Firstly, the mutation has not been found in 200 control individuals. Secondly, it concerns a residue that is well conserved in different species. Thirdly, the observed basic electrophysiological characteristics associated with the mutation are expected to lead to enhanced repolarization with shortening of the QT interval.

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