A *KCNQ1* mutation contributes to the concealed type 1 long QT phenotype by limiting the Kv7.1 channel conformational changes associated with protein kinase A phosphorylation

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BACKGROUND Type 1 long QT syndrome (LQT1) is caused by loss-of-function mutations in the *KCNQ1*-encoded Kv7.1 channel that conducts the slowly activating component of the delayed rectifier K^+ current (I_{Ks}). Clinically, the diagnosis of LQT1 is complicated by variable phenotypic expressivity, whereby approximately 25% of genotype-positive individuals present with concealed LQT1 (resting corrected QT [QTc] interval $\leq$460 ms).

OBJECTIVE To determine whether a specific molecular mechanism contributes to concealed LQT1.

METHODS We identified a multigenerational LQT1 family whereby 79% of the patients genotype-positive for p.Ile235Asn-*KCNQ1* (I235N-Kv7.1) have concealed LQT1. We assessed the effect I235N-Kv7.1 has on I_{Ks} and the ventricular action potential (AP) by using in vitro analysis and computational simulations.

RESULTS Clinical data showed that all 10 patients with I235N-Kv7.1 have normal resting QTc intervals but abnormal QTc interval prolongation during the recovery phase of an electrocardiographic treadmill stress test. Voltage-clamping HEK293 cells coexpressing wild-type Kv7.1 and I235N-Kv7.1 (to mimic the patients' genotypes) showed that I235N-Kv7.1 generated relatively normal functioning Kv7.1 channels but were insensitive to protein kinase A (PKA) activation. Phosphomimetic and quinidine sensitivity studies suggest that I235N-Kv7.1 limits the conformational changes in Kv7.1 channels, which are necessary to upregulate I_{Ks} after PKA phosphorylation. Computational ventricular AP simulations predicted that the PKA insensitivity of I235N-Kv7.1 is primarily responsible for prolonging the AP with β-adrenergic stimulation, especially at slower cycle lengths.

CONCLUSIONS *KCNQ1* mutations that generate relatively normal Kv7.1 channels, but limit the upregulation of I_{Ks} by PKA activation, likely contribute to concealed LQT1.

KEYWORDS Long QT syndrome; *KCNQ1*; Kv7.1; PKA activation; I_{Ks}; Treadmill stress test

ABBREVIATIONS **AKAP9** = A-kinase anchoring protein 9 (Yotiao); **AP** = action potential; **APD_{90}** = steady-state action potential duration at 90% repolarization; **ECG** = electrocardiogram/electrocardiographic; **HEK293** = human embryonic kidney 293; **I_{Ks}** = slowly activating delayed rectifier K^+ current; **I_{MAX}** = maximally activated **I_{Ks}**; ***k*** = slope factor; **LQT1** = type 1 long QT syndrome; **LQTS** = long QT syndrome; **PKA** = protein kinase A; **QTc** = corrected QT; **$V_{½}$** = midpoint potential for half-maximal activation of I_{Ks}; **WT** = wild-type Kv71.

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Introduction

Congenital long QT syndrome (LQTS) is a condition of abnormal cardiac repolarization that affects approximately 1 of every 2000 live births and is characterized clinically by prolongation of the heart rate–corrected QT (QTc) interval on a resting 12-lead electrocardiogram (ECG). Patients with LQTS are at an increased risk of syncope, seizures, and

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sudden cardiac death secondary to polymorphic ventricular tachyarrhythmias.[1] Type 1 long QT syndrome (LQT1) is caused by loss-of-function missense, nonsense, frameshift, or splice-site altering mutations in the *KCNQ1*-encoded Kv7.1 α subunit and accounts for an estimated 40% of all genotype-positive LQTS cases.[1–4] Kv7.1 α subunits tetramerize to form the pore of the slowly activating delayed rectifier K^+ current (I_{Ks}) channel complex, and in the human heart, I_{Ks} is upregulated by protein kinase A (PKA) activation to prevent ventricular action potential (AP) prolongation during β-adrenergic stimulation.[5,6] Consequently, many patients with LQT1 tend to experience life-threatening symptoms while exercising or swimming.[7,8] Unfortunately, rendering a diagnosis of LQT1 on the basis of a 12-lead ECG alone presents a significant challenge as an estimated 25% of genotype-positive individuals with LQT1 fail to display an abnormal QTc interval at rest, commonly referred to as a concealed LQT1 phenotype.[9,10]

Patients with a concealed LQTS phenotype may remain at risk of cardiac events during exercise owing to inappropriate adaptation of repolarization.[11] As such, developing a deeper understanding of the molecular mechanisms underlying a concealed LQT1 phenotype might improve personalized diagnostic and management approaches to lower the risk of life-threatening arrhythmias. In this study, we tested the hypothesis that some mutations contribute to a high incidence of the concealed LQT1 phenotype by a specific molecular mechanism.

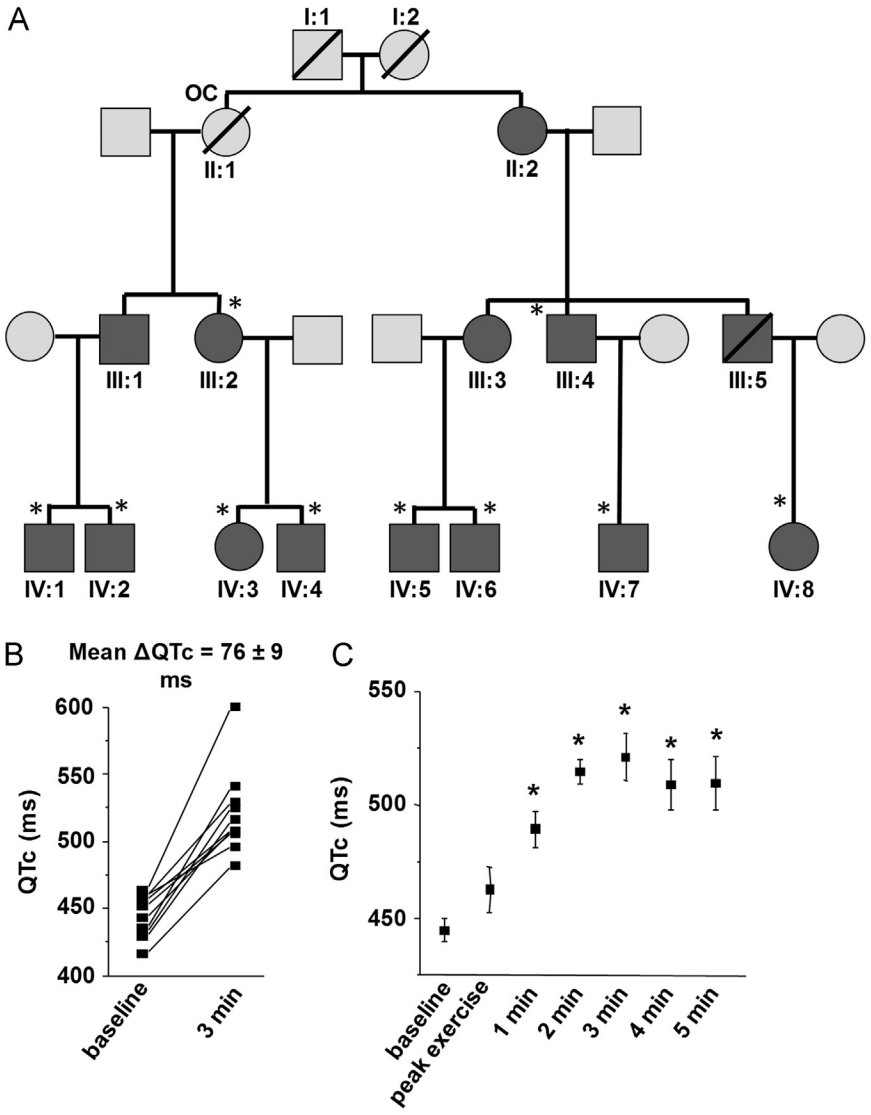

Figure 1 I235N-Kv7.1 causes concealed LQT1. **A:** A pedigree of a LQT1 family with I235N-Kv7.1 who underwent an ECG treadmill stress test. Patients with the I235N-Kv7.1 mutation are denoted by filled gray symbols. Individual males and females are denoted by squares and circles, respectively; each generation is denoted by a Roman numeral; OC denotes an obligate carrier; and asterisks signify individuals who underwent an ECG treadmill stress test. **B:** The QTc interval values recorded at baseline and after 3 minutes of recovery used to calculate ΔQTc of the 10 patients positive for I235N-Kv7.1 who underwent an ECG treadmill stress test are plotted. **C:** The mean QTc interval values recorded during the exercise treadmill stress test were plotted at baseline, peak exercise, and 1, 2, 3, 4, or 5 minutes during the recovery phase ($^*P < .05$ vs baseline). ECG = electrocardiogram; LQT1 = type 1 long QT syndrome; QTc = corrected QT; ΔQTc = difference in QTc interval between baseline recording and 3 minutes after the ECG treadmill stress test.