concealed LQT1 at rest (QTc, 452 ± 5 ms) but shows marked paradoxical QTc prolongation (534 ± 16 ms) during the recovery phase of treadmill stress testing. This abnormal recovery response in the independent LQT1 patients taking flunarizine blocks (487 ± 11 ms). Voltage-clamping experiments using HEK293 cells expressing WT or I235N with the Kv7.1 β-subunit KCNE1 showed that I235N decreased KCNQ1 current (IKCNQ1) by 93% and caused a large positive shift in the midpoint potential for activation (V1/2). However, cells co-expressing WT and I235N (to mimic the patients’ genotypes) showed only a small decrease in IKCNQ1 (±30%) and shift in V1/2. Since excessive QTc prolongation in I235N patients appears secondary to β-adrenergic stimulation, we tested whether I235N prevented PKA activation of IKCNQ1. In cells expressing WT, PKA stimulation with forskolin and IBMX increased IKCNQ1 by 64%, but it did not increase IKCNQ1 in cells co-expressing WT and I235N. Computational simulations using a ventricular action potential (AP) model showed that reducing the IKs component by 30% increased the AP duration at 90% repolarization (APD90) by only 1.6%. However, incorporating β-adrenergic signaling showed that reducing the IKs component by 30% and preventing its activation by PKA increased the APD90 by 7.1%. We conclude I235N modestly affects basal QTc, IKs, and APD90, but it prevents PKA activation of IKs to cause a dangerous prolongation in the QTc/APD90 during β-adrenergic stimulation.

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Malignant Long QT Syndrome KCNQ1 Mutations in the Pore Disrupt the Molecular Basis for Rapid K+ Permeation
Don E. Burgess1, Daniel C. Bartos1, Allison R. Reloj1, Kenneth S. Campbell2, Jonathan N. Johnson2, David J. Tester2, Pascale Guicheney3, Arthur J. Moss4, Seiko Ohno5, Minoru Horie5, Brian Delisle6
1University of Kentucky, Lexington, KY, USA; 2Mayo Clinic, Rochester, MN, USA; 3UMPC Université, Paris, France; 4University of Rochester Medical Center, Rochester, NY, USA; 5Shiga University of Medical Science, Ohtsu, Japan.

Type I long QT syndrome (LQT1) syndrome is caused by loss-of-function mutations in the KCNQ1-encoded K+ channel (Kv7.1) that underlies the slowly activating delayed rectifier K+ current (IKs) in the heart. Intrinsic rate stratification suggests LQT1 mutations that disrupt conserved amino acid residues in the pore are an independent risk factor for LQT1-related cardiac events. The purpose of this study is to determine possible molecular mechanisms that underlie the loss-of-function for these higher risk mutations. Extensive genotype-phenotype analyses of LQT1 patients showed that the pore mutations T322M, T322A, or G325R-Kv7.1 confer a higher risk for LQT1-related cardiac events. Heterologous expression of these mutations with KCNE1 suggested that they generated non-functional channels and caused dominant negative suppression of WT-Kv7.1. We performed molecular dynamic simulations (MDS) for the analogous mutations in KcsA (T85M, T85A, and G88R-KcsA). MDS of WT, T85M, T85A, and G88R-KcsA channels showed the selectivity filters are contiguous and could bind to K+ ions and water molecules in an alternating manner. For the most part, the oxygen atoms that line the WT-KcsA selectivity filter faced the central pore axis; however, the T85M-, T85A-, or G88R-KcsA simulations showed they stabilized a configuration where the carbonyl oxygen atom between S2 and S3 was “flipped” away from the lumen of the selectivity filter. T85M- and T85A-KcsA stabilized the flipped configuration for two adjacent z-subunits. The stabilization of the flipped configuration upset the balance between the strong attractive and K+–K+ repulsive forces required for rapid K+ permeation. We conclude that the T322M-, T322A-, or G325R-Kv7.1 cause a loss-of-function by disrupting the architectural and physical properties of the selectivity filter.