Action Potential Clamp as a Tool for Risk Stratification of Sinus Bradycardia due to Mutations in *HCN4*

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The hyperpolarization-activated 'funny' current (I_f) is an important modulator of the spontaneous diastolic depolarization underlying the sinus node pacemaker activity. The ion channels carrying I_f are built of HCN4 proteins, which are encoded by the *HCN4* gene. Heterozygous loss-of-function mutations in *HCN4* have been associated with sinus bradycardia.

We carried out computer simulations using the comprehensive Fabbri–Severi model of a human sinus node cell to assess whether action potential clamp experiments on cells from a cell line transfected with wild-type or mutant *HCN4* may function as a useful tool for risk stratification of sinus bradycardia due to loss-of-function mutations in *HCN4*.

First, we used action potentials that we had previously recorded from isolated human sinus node cells to simulate action potential clamp experiments on transfected cells expressing wild-type or heterozygously mutant HCN4 channels and computed the charge carried by the HCN4 channels during the diastolic depolarization. For each of the mutations tested, we next incorporated the mutation-induced changes in fully-activated conductance and kinetics of I_f into the Fabbri–Severi model and computed the cycle length in the presence of the specific mutation at different levels of autonomic tone, corresponding with minimum heart rate, average heart rate, and maximum heart rate.

At each level of autonomic tone, the beating rate of the model cell showed a close correlation with the charge carried by the HCN4 channels in the simulated action potential clamp experiments: r^2 amounted 0.99 under vagal tone, 0.99 under normal autonomic tone, and 0.98 under adrenergic tone.

We conclude that action potential clamp on transfected cells is a promising tool for risk stratification of sinus bradycardia due to loss-of-function mutations in *HCN4*. In combination with an I_f blocker, this tool may also prove useful when applied to human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) obtained from mutation carriers and non-carriers.