Knock-in gain-of-function sodium channel mutation prolongs atrial action potentials and alters atrial vulnerability

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BACKGROUND Patients with long QT syndrome (LQTS) are at increased risk not only for ventricular arrhythmias but also for atrial pathology including atrial fibrillation (AF). Some patients with "lone" AF carry Na^+-channel mutations.

OBJECTIVE The purpose of this study was to determine the mechanisms underlying atrial pathology in LQTS.

METHODS In mice with a heterozygous knock-in long QT syndrome type 3 (LQT3) mutant of the cardiac Na^+ channel (ΔKPQ-SCN5A) and wild-type (WT) littermates, atrial size, function, and electrophysiologic parameters were measured in intact Langendorff-perfused hearts, and histologic analysis was performed.

RESULTS Atrial action potential duration, effective refractory period, cycle length, and PQ interval were prolonged in ΔKPQ-SCN5A hearts (all $P < .05$). Flecainide (1 μM) reversed atrial action potential duration prolongation and induced postrepolarization refractoriness ($P < .05$). Arrhythmias were infrequent during regular rapid atrial rate in both WT and ΔKPQ-SCN5A but were inducible in 15 (38%) of 40 ΔKPQ-SCN5A and 8 (29%) of 28 WT mice upon extrastimulation. Pacing protocols generating rapid alterations in rate provoked atrial extrasystoles and arrhythmias in 6 (66%) of 9 ΔKPQ-SCN5A but in 0 (0%) of 6 WT mice ($P < .05$). Atrial diameter was increased by nearly 10% in ΔKPQ-SCN5A mice >5 months old without increase in fibrotic tissue.

CONCLUSION Murine hearts bearing an LQT3 mutation show abnormalities in atrial electrophysiology and subtle changes in atrial dimension, including an atrial arrhythmogenic phenotype on provocation. These results support clinical data suggesting that LQTS mutations can cause atrial pathology and arrhythmogenesis and indicate that murine sodium channel LQTS models may be useful for exploring underlying mechanisms.

KEYWORDS Antiarrhythmic drugs; Atrial remodeling; Electrophysiology; Genetic models; Nav1.5

ABBREVIATIONS **AF** = atrial fibrillation; **APD** = action potential duration; **ECG** = electrocardiogram; **ERP** = effective refractory period; **LA** = left atrium; **LQT3** = long QT syndrome type 3; **LQTS** = long QT syndrome; **MAP** = monophasic action potential; **RA** = right atrium; **WT** = wild-type

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Introduction

Na^+-channel mutations are found in patients with lone atrial fibrillation (AF).[1,2] Genetic alterations are predicted to cause either loss-of-function[3,4] or gain-of-function changes.[5,6] Furthermore, patients with long QT syndrome (LQTS),[7-9] including long QT syndrome type 3 (LQT3),[10] may be at increased risk for AF. Recognized ventricular arrhythmia-promoting factors in LQTS include prolonged repolarization, bradycardia, pauses and rapid rate accelerations,[11,12] and possibly giant TU waves reflecting afterdepolarizations.[13] Prolonged atrial action potentials and atrial arrhythmias have been observed in patients with LQTS,[8,14] particularly following rapid pacing,[15] but the effects of LQT3-causing mutations on atrial structure and function have not been systematically studied.

The present study was designed to characterize the atrial phenotype of mice with the classic LQT3 mutation ΔKPQ-SCN5A, which interferes with cardiac Na^+-chan-

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nel inactivation and results in increased late sodium current.[12,16,17]

Materials and methods

Animal model

Sex- and age-matched pairs of wild-type (WT) littermates and LQT3 mice with a heterozygous ΔKPQ-SCN5A knock-in mutation on a Swiss Agouti background were studied.[11,12] All experiments and animal care procedures were approved by the local animal care and use committee (G61/99, G83/2004).

Doppler echocardiography

Doppler echocardiography was performed in sedated (1% isoflurane plus oxygen) mice with high-rate transducers (15 MHz, Sonos 5500, Philips, Hamburg, Germany; 30–70 MHz, Vevo 770 and 2100, Visualsonics, Toronto, Canada).[18] The left atrium (LA) was visualized in the parasternal long-axis view in the plane of the aortic root. LA diameter was measured during ventricular systole at its maximal dimension for five two-dimensional and motion(M)-mode images per animal at comparable heart rate. Atrial function was assessed by calculating atrial fractional shortening and using color Doppler-guided transmitral Doppler flow measurements.

Electrophysiologic study in the isolated heart

The experimental setup is shown in Figure 1A. Isolated, beating Langendorff-perfused mouse hearts (mean age 34 weeks, range 22–53 weeks) were studied as previously reported (heart temperature 35.0° ± 0.2°C, perfusion flow rate 3.2 ± 0.2 mL/min).[17] An octapolar murine electrophysiology catheter was inserted into right atrium (RA) and right ventricle (Figure 1A). Atrial and ventricular electrograms, tissue bath ECG, and monophasic action potential (MAP) from the LA were simultaneously recorded. Hearts were subjected to a stabilization period of 10 minutes, followed by observation of spontaneous sinus rhythm for 5 minutes. The RA was paced at twice diastolic threshold at a cycle length of 100, 120, 140, or 150 ms for 1.5 minutes each via the octapolar catheter. RA extrastimulation was performed with eight-beat trains for determination of effective refractory period (ERP). In some experiments, mechanical AV block was performed to facilitate analysis of atrial signals.[17,19] To generate rapid alterations in atrial rhythm, atria were subjected to repetitive intermittent high-rate burst stimulation with cycle lengths of 80 to 40 ms resulting in short–long–short sequences due to pauses in high-rate burst pacing.

LA APD, activation times, ERP, and PQ intervals were assessed in WT and ΔKPQ-SCN5A hearts at baseline. Protocols were repeated in other experiments at baseline and during infusion of flecainide (1 μM), orciprenaline (1.7 μM), or carbachol (0.1–1 μM). Order of baseline and drug were varied to control for time-dependent effects.

To test arrhythmogenesis associated with rapid alterations in heart rate, intermittent short periods of high-rate burst pacing was performed at 1.5 times diastolic threshold with and without infusion of 1 μM carbachol and low-dose catecholamines (orciprenaline or isoproterenol, 0.1 μM).

Experiments were accepted for analysis of action potential duration (APD) and activation time if they met published quality criteria.[17,19] If MAPs were stable, signals >1 mV were accepted for action potential analysis and signals >0.5-mV amplitude were accepted for evaluation of arrhythmias. Recordings were manually screened for arrhythmias. Atrial extrasystole was defined as premature atrial action potentials. Atrial tachycardia was defined as ≥5 consecutive atrial extrasystoles with a cycle length shorter than that in sinus rhythm.

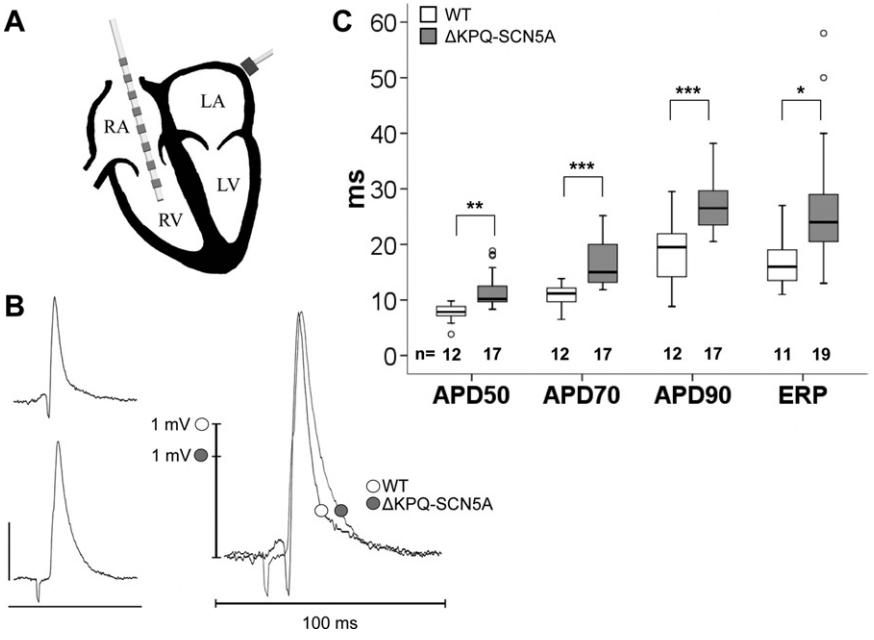

Figure 1 Prolonged atrial action potential duration (APD) and effective refractory period (ERP) in long QT syndrome type 3 (LQT3) atria. **A:** Position of the octapolar mouse catheter in the right atrium (RA) and right ventricle (RV) and position of monophasic action potential (MAP) electrode on the left atrium (LA). LV = left ventricle. **B:** Representative left atrial MAPs recorded in wild-type and ΔKPQ-SCN5A during steady-state pacing at 100 ms, separate and as an overlay. Bars indicate 100 ms and 1 mV. *White dots* indicate wild-type (WT); *gray dots* indicate ΔKPQ-SCN5A. **C:** Pacing at fixed rate of 120 ms confirmed that atrial APD and ERP were prolonged in ΔKPQ-SCN5A compared to WT. *P <.05; **P <.01; ***P <.001. Box plots indicate smallest observation *(lower horizontal line),* interquartile range (X75%–X25%, *rectangle height),* median *(thicker horizontal line),* largest observation *(higher horizontal line),* and potential outliers *(blank circles).* (See Online Supplemental Figure 1 for data at 100-ms pacing cycle length and Online Supplemental Figure 2 for values during spontaneous sinus rhythm.)