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CARDIOVASCULAR PHYSIOLOGY

Atrial arrhythmogenicity in aged *Scn5a*+/ΔKPQ mice modeling long QT type 3 syndrome and its relationship to Na^+ channel expression and cardiac conduction

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Abstract Recent studies have reported that human mutations in Nav1.5 predispose to early age onset atrial arrhythmia. The present experiments accordingly assess atrial arrhythmogenicity in aging *Scn5a*+/ΔKPQ mice modeling long QT3 syndrome in relationship to cardiac Na^+ channel, Nav1.5, expression. Atrial electrophysiological properties in isolated Langendorff-perfused hearts from 3- and 12-month-old wild type (WT), and *Scn5a*+/ΔKPQ mice were assessed using programmed electrical stimulation and their Nav1.5 expression assessed by Western blot. Cardiac conduction properties were assessed electrocardiographically in intact anesthetized animals. Monophasic action potential recordings demonstrated increased atrial arrhythmogenicity specifically in aged *Scn5a*+/ΔKPQ hearts. These showed greater action potential duration/refractory period ratios but lower atrial Nav1.5 expression levels than aged WT mice. Atrial Nav1.5 levels were higher in young *Scn5a*+/ΔKPQ than young WT. These levels increased with age in WT but not *Scn5a*+/ΔKPQ. Both young and aged *Scn5a*+/ΔKPQ mice showed lower heart rates and longer PR intervals than their WT counterparts. Young *Scn5a*+/ΔKPQ mice showed longer QT and QTc intervals than young WT. Aged *Scn5a*+/ΔKPQ showed longer QRS durations than aged WT. PR intervals were prolonged and QT intervals were shortened in young relative to aged WT. In contrast, ECG parameters were similar between young and aged *Scn5a*+/ΔKPQ. Aged murine *Scn5a*+/ΔKPQ hearts thus exhibit an increased atrial arrhythmogenicity. The differing Nav1.5 expression and electrocardiographic indicators of slowed cardiac conduction between *Scn5a*+/ΔKPQ and WT, which show further variations associated with aging, may contribute toward atrial arrhythmia in aged *Scn5a*+/ΔKPQ hearts.

Keywords LQT3 syndrome · Atrial arrhythmogenicity · Genetically modified mice · Na channels · Age · Arrhythmia · Sodium channel · Mouse · Electrophysiology · Excitation

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Introduction

Atrial arrhythmia, of which the most common form is atrial fibrillation (AF), is particularly common in the elderly. Genetically normal patients show a median age of onset of AF of 75 years with ~70% of such patients aged between 65 and 85 years [13]. There have been recent studies implicating an involvement of cardiac ion channels in the development of AF [5–7]. Thus, long QT syndrome (LQTS) patients show an earlier than normal onset of AF [20], typically at age ~50±14 years [9]. In particular, variants of the *SCN5A* gene encoding the cardiac Na^+ channel (Nav1.5) have been clinically

associated with the presence of AF. A study resequencing the *SCN5A* coding regions reported that 6% of 375 patients with AF showed variations in *SCN5A* [9]. Roles for Nav1.5 in the development of atrial arrhythmia are further implicated by the *SCN5A* polymorphism, H558R, prevalent in 20% of the population, also being a risk factor for AF [8].

Of the >200 such *SCN5A* mutations reported [40], the gain of function mutation *Scn5a*+/ΔKPQ involves deletion of the three conserved amino acids, KPQ1505–1507. It has been clinically associated with long QT syndrome type 3 (LQT3). This in turn is associated with a potentially fatal ventricular arrhythmogenic tendency that increases with age and that becomes evident within the first four decades of life [50, 51]. Loss of function mutations in *SCN5A* are associated with Brugada syndrome (BrS), a cardiac disorder characterized by an elevated ST segment in electrocardiographic (ECG) waveform [18]. Furthermore, a proportion of patients that harbor various losses and gain of function *SCN5A* mutations all demonstrate AF. Thus, 10–30% of BrS patients show increased propensities to atrial fibrillation [4, 26, 31, 32]. In contrast, Benito et al. [3] described a family, with eight members across three generations, showing both early onset AF and LQT3 associated with a mutant *SCN5A* gene involving the Y1795C mutation. Similarly, early onset AF in a Japanese family has been related to a novel gain-of-function, M1875T, mutation in *SCN5A* [45].

Such similarities in clinical outcome as a result of differing *SCN5A* mutations are consistent with reports of overlap syndrome in patients with LQT3 and BrS; conversely, some mutations can be associated with more than one phenotype in particular patients [42, 45]. For example, a single Na^+ channel mutation involving deletion of lysine 1500, ΔK1500, is associated with not only LQTS but also BrS and conduction system disease [16]. Also the insertion D1795 induces both LQTS and BrS and has been shown to result in a 62% reduction of channel expression [2]. Finally, clinical phenotypes that overlap with those observed in BrS have also been reported to occur in patients carrying the *SCN5A*+/ΔKPQ mutation [35].

The physiological relationships between the underlying *SCN5A*+/ΔKPQ mutation and such related ventricular arrhythmic phenotypes have been investigated using a murine *Scn5a+/ΔKPQ* model [44]. Murine systems have also been used to study atrial arrhythmogenicity [25, 48]. However, very few experimental studies have related *SCN5A* mutations to alterations in atrial arrhythmogenicity. One such report described a reduced atrial arrhythmogenicity in *Scn5a +/ΔKPQ* murine hearts modeling LQT3 syndrome [10]. However, it did not investigate the effects of aging. Nevertheless, this is an important factor contributing to the variable penetrance of LQT3 [15].

This study accordingly proceeds to explore for the development of atrial arrhythmogenic properties with aging, comparing these to action potential waveform and refractory periods, Nav1.5 expression, and electrocardiographic properties in *Scn5a*+/ΔKPQ and WT mice for the first time. We demonstrated that Nav1.5 protein expression was affected by age and genotype in a manner that would be compatible with a phenotypic overlap and attributed these findings to evidence for a compromised Nav1.5 function.

Methods

Experimental animals Wild-type (WT) and *Scn5a*+/ΔKPQ mice, with an inbred 129/Sv genetic background, were housed in cages at 21±1°C with 12 h light/dark cycles. The mice, studied at ages of either 3 months (young) and 12 months (aged), were killed by cervical dislocation, in compliance with the UK Animals (Scientific Procedures) Act 1986.

Isolation, cannulation, and perfusion of mouse hearts For the purpose of Na^+ channel extraction and experiments on isolated Langendorff preparations, hearts were excised and immediately placed in ice-cold bicarbonate Krebs–Henseleit (KH) buffer solution containing (mM): 119 NaCl, 25 $NaHCO_3$, 4.0 KCl, 1.2 KH_2PO_4, 1.0 $MgCl_2$, 1.8 $CaCl_2$, 10 glucose, and 2.0 sodium pyruvate (pH 7.4). The KH buffer was bubbled with 95% O_2/5% CO_2 (British Oxygen Company, Manchester, UK) for 20 min prior to addition of 1.8 mM $CaCl_2$. The bubbling with 95% O_2/5% CO_2 was continued during the experiments themselves. A 2-mm length of the aorta was cannulated and held in place with an aneurysm clip. The hearts were perfused with KH buffer warmed to 37°C until they regained a healthy pink appearance and resumed spontaneous activity. Perfusion was continued for a further 10 min before either electrophysiological studies or protein extraction.

Monophasic action potential recording Hearts were excised and cannulated as described above, and AV nodes were ablated. Left atrial monophasic action potentials (MAPs) were recorded using a miniaturized MAP electrode tip (Linton Instruments, Harvard Apparatus, UK). A platinum stimulating electrode (1 mm inter-pole spacing) was placed on the right atrium. Square wave stimuli (Grass S48 stimulator, Grass-Telefactor, Slough, UK) of 2 ms duration and ×1.5 threshold were applied at a cycle length of 125 ms for 10 min until MAP waveforms had stable baselines, rapid upstroke phases, and smooth repolarization phases. Action potential durations were determined from regular pacing. Programmed electrical stimulation (PES)

was used to assess arrhythmogenicity and refractoriness. This consisted of cycles of stimulus trains of eight S1 beats delivered at 8 Hz at ×1.5 threshold followed by an S2 extrastimulus, at progressively shorter S1S2 coupling intervals until the S2 no longer elicited an action potential. Arrhythmia was defined as three or more consecutive premature atrial waveforms.

Nav1.5 protein preparation After perfusion, left atrial appendages were removed and clamp-frozen. The tissue was homogenized in liquid nitrogen with a mortar and pestle on dry ice. Homogenates were resuspended in a solution consisting of (in mM) 50 Tris–HCl, 10 NaCl, 320 sucrose, 5 EDTA, and 2.5 EGTA (pH 7.4) and placed into Eppendorf tubes and gently resuspended with a pipette tip. Once thawed, but when still cool, the samples were placed on ice. Samples were blade homogenized for 20 s each, rinsing the blade thoroughly between samples. The preparation was lysed with 1% Triton X100 and rotated for 1 h at 4°C. After incubation, the samples were subjected to centrifugation at 12,000×*g* and 4°C. The supernatant was decanted into a sample loading buffer (NuPAGE, Invitrogen, Paisley, UK) to obtain a 1:4 dilution. Samples were boiled at 70°C for 10 min, flash frozen and stored at −80°C.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis and Western blots Five micrograms of total protein was loaded per lane (NuPAGE gels) as was measured in triplicate with Bradford Assay (Bio-Rad Laboratories Ltd, Herts, UK) alongside a molecular weight marker (SeeBlue Plus prestained standard, Paisley, UK). Duplicate gels were subjected to Imperial Blue Protein stain (Thermo Scientific, Rockford, IL, USA) to confirm equal loading. NuPAGE electrophoresis tanks were used to run sodium dodecyl sulfate polyacrylamide gel electrophoresis at 100 mA for 3 h. Protein bands were transferred onto nitrocellulose membrane by a NuPAGE blotting system for 2 h. Membranes were blocked overnight in 5% milk–Tris-buffered saline–1% Tween, followed by several rinses prior to incubation with Nav1.5 antibody (1:500, ASC005, Caltag Medisystems, Alomone, Israel). The membranes were incubated with secondary antibody conjugated with horseradish peroxidase from Sigma-Aldrich (Poole, Dorset, UK) and rinsed five times. Western blot development was performed with Amersham ECL reagents (Amersham Biosciences, Amersham, Bucks, UK).

Electrophysiological study Mice were anesthetized with Avertin (Sigma) 240 mg/kg at a dose rate of 0.1 ml/10 g body weight. Injection was given intraperitoneally with a 27G hypodermic needle into the left peritoneal cavity. Lead II ECG recordings were acquired using LabChart software (ADI Instruments, Chalgrove, Oxfordshire, UK). This incorporated algorithms for determinations of ECG parameters, including RR intervals, P wave durations, PR intervals, QRS intervals and QT durations. It also included QTc intervals derived from the QT interval corrected for variations in the RR interval [19, 33]

Data analysis and statistics Data was acquired using a model 1401 interface, analysed with Spike version 5.2 (Cambridge Electronic Design, Cambridge, UK) and are presented as means ± standard errors of the means. Image J was used for densitometry (NIH, Bethesda, MD, USA). Comparisons were performed by a one-way analysis of variance (ANOVA) or Fisher's exact test where appropriate, with SPSS software (SPSS UK, Woking, Surrey, UK). For all tests, young and aged mice of the same genotype were compared followed by tests between WT and *Scn5a*+/ΔKPQ of the same age. Statistical significance was assumed at $P<0.05$.

Results

The *Scn5a*+/ΔKPQ mutation abolishes normal increases in atrial refractory periods with age and results in high action potential duration/atrial effective refractory period (APD/AERP) ratios

Figure 1 shows representative traces of atrial MAPs from young (3 months old) WT (a), young *Scn5a*+/ΔKPQ (b), aged (12 month) WT (c), and aged *Scn5a*+/ΔKPQ (d) atria, which show fast upstroke and smooth repolarization phases. APDs were measured from the interval between upstroke

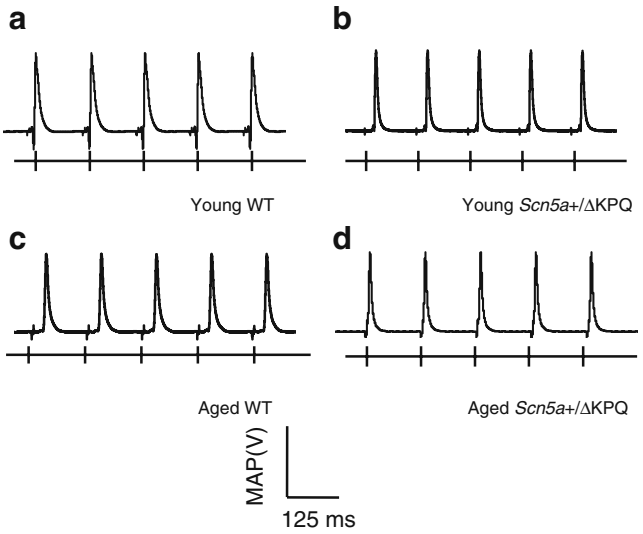

Fig. 1 Atrial monophasic action potentials (MAPs). Representative traces of hearts young (3 months old) WT (**a**), young *Scn5a*+/ΔKPQ (**b**), aged (12 month) WT (**c**) and aged *Scn5a*+/ΔKPQ (**d**) paced at 8 Hz

peak and 90% repolarization. WT and *Scn5a*+/ΔKPQ atrial preparations showed similar action potential durations (Fig. 2a). Thus, there were no significant differences in APD between young and aged hearts of the same genotype or between WT and *Scn5a*+/ΔKPQ hearts of the same age.

Figure 2b demonstrates that the AERP was prolonged in aged WT compared to young WT ($P<0.001$), consistent with previous findings in murine hearts that atrial refractory periods increase with advancing age. This would be expected to offset any arrhythmogenic effects of corresponding decreases in conduction velocity known to occur with age [22, 23, 30]. In contrast, young and aged *Scn5a*+/ΔKPQ atria showed similar AERPs. Young WT and young Scn5a+/ΔKPQ hearts showed indistinguishable AERPs. However, AERPs in aged WT hearts were significantly prolonged compared to aged *Scn5a*+/ΔKPQ ($P<0.01$; $n=7$ in each group).

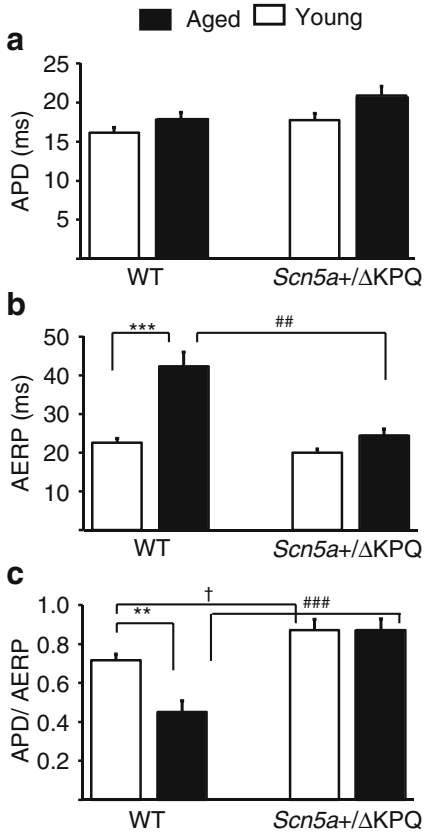

Fig. 2 Action potential durations (APDs) and atrial effective refractory periods (AERPS) (**a**) APD_{90} values of young WT, young *Scn5a*+/ΔKPQ, aged WT, and aged *Scn5a*+/ΔKPQ atria. **b** The effect of age and *Scn5a*+/ΔKPQ mutation on AERPs in WT and *Scn5a*+/ΔKPQ hearts. **c** Mean APD_{90}/AERP ratios in WT and *Scn5a*+/ΔKPQ hearts. The symbols *, †, and # indicate significant differences between young WT and aged WT (*), young *Scn5a*+/ΔKPQ compared to young WT (†) and aged *Scn5a*+/ΔKPQ compared to aged WT (#) respectively. The notation *, †, and # denote $P<0.05$; **, ††, and ## denote $P<0.01$; and ***, †††, and ### denote $P<0.001$

Figure 2c summarizes APD/AERP ratios, previously used to assess atrial arrhythmogenicity [10], derived from the APD and AERP findings summarized above. Young *Scn5a*+/ΔKPQ hearts showed increased APD/AERP ratios compared to young WT hearts ($P<0.05$). However, whereas APD/AERP ratios decreased with age in WT ($P<0.01$), these remained the same in *Scn5a*+/ΔKPQ hearts, with values approaching unity and significantly high ratios in aged *Scn5a*+/ΔKPQ relative to aged WT ($P<0.001$). This was consistent with the increased arrhythmic tendency found specifically in aged *Scn5a*+/ΔKPQ observed in the experiments that followed.

Significant increases in atrial arrhythmogenic tendency are specific to aged *Scn5a*+/ΔKPQ mice

The presence or otherwise as well as the frequency of arrhythmic tendency in both WT and *Scn5a*+/ΔKPQ atrial preparations were compared using PES in isolated heart Langendorff-perfused hearts. The latter involved impositions of extrasystolic S2 stimuli at the end of trains of eight pacing S1 stimuli. The S1 stimuli were delivered at 8 Hz, and the S2 stimuli were delivered at S1S2 intervals that were progressively decremented by 1 ms with each successive pacing cycle. The protocols were terminated when hearts either became refractory or went into self-terminating arrhythmias. Figure 3a shows typical results from an aged *Scn5a*+/ΔKPQ heart in the course of such a procedure. Figure 3b exemplifies typical action potentials at the end of such a procedure showing refractoriness (arrowed). Figure 3c shows episodes of arrhythmias (arrowed).

Table 1 summarizes the results of such experiments in which the observed incidences of atrial arrhythmia in young and aged, WT and *Scn5a*+/ΔKPQ hearts were compared using Fisher's exact tests, assuming significance with $P<0.05$. It demonstrates that both young and aged WT hearts did not show significant incidences of arrhythmia. Similarly, both young WT and young *Scn5a*+/ΔKPQ showed indistinguishable incidences of arrhythmogenesis. In contrast, aged *Scn5a*+/ΔKPQ hearts showed significantly greater incidences of arrhythmia compared to both young *Scn5a*+/ΔKPQ ($P<0.001$) and aged WT ($P<0.001$).

The *Scn5a*+/ΔKPQ mutation results in abnormal changes in Na^+ channel expression levels with age

Expression levels of the cardiac Na^+ channel, Nav1.5, from atria of young and aged WT and *Scn5a*+/ΔKPQ mice were examined by Western blotting of randomized blinded samples. Nav1.5 was extracted from WT and *Scn5a*+/ΔKPQ hearts, and Western blots were performed with 5 μg of total protein per lane. Figure 4a shows blots of Nav1.5

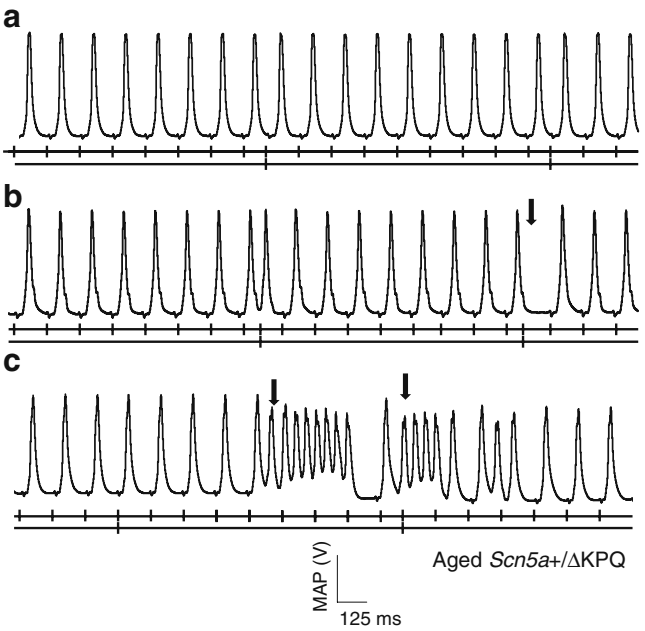

Fig. 3 Assessment for arrhythmogenicity during S1S2 pacing of the left atria. A representative trace from an aged *Scn5a*+/ΔKPQ heart, paced at 8 Hz (*S1*), delivered at ×1.5 threshold (**a**). An example of atrial refractoriness with programmed electrical stimulation (PES). The extrastimuli (*S2*) are delivered following a train of eight S1 stimuli at progressively shorter S1S2 coupling intervals until the S2 no longer elicits an action potential (*arrowed*). The *short lines* below the traces indicate S1 timings, and the *longer lines* indicate S2 stimuli (**b**). Arrhythmic episode during PES (*arrowed*) (**c**)

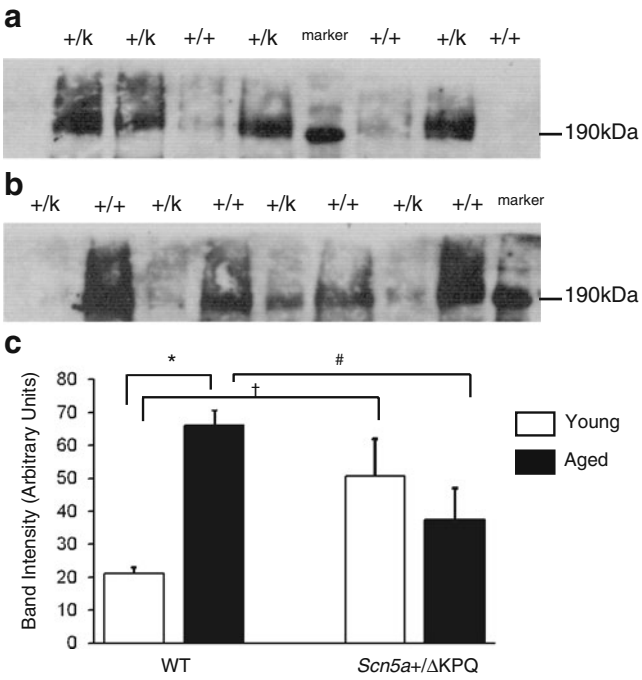

Fig. 4 Cardiac Na^+ channel, Nav1.5, expression. Western blots of Nav1.5 obtained from atrial tissue of young (3 months) WT (labeled +/+) and *Scn5a*+/ΔKPQ hearts (labeled +/k) (n=3 and 4, respectively). Five micrograms of total protein was loaded per lane (**a**). Anti-Nav1.5 antibody specific binding to samples from aged (12 months) WT (n=4) and *Scn5a*+/ΔKPQ (n=4) hearts (**b**). Densitometric analysis demonstrates that the *Scn5a*+/ΔKPQ mutation results in a loss of the normal aging pattern showed by WT (**c**). The symbols *, †, and # indicate significant differences between young WT and aged WT (*), young *Scn5a*+/ΔKPQ compared to young WT (†), and aged *Scn5a*+/ΔKPQ compared to aged WT (#) respectively. The notation *, †, and # denote P<0.05; **, ††, and ## denote P<0.01; and ***, †††, and ### denote P<0.001

obtained from atrial tissue of the young WT (labeled +/+; n=3) and *Scn5a*+/ΔKPQ hearts (labeled +/k; n=4 respectively). Figure 4b shows antibody specific binding to murine Nav1.5 from aged WT (n=4) and *Scn5a*+/ΔKPQ (n=4) hearts. Figure 4c shows the results of densitometric analysis that provided a relative quantification of Nav1.5.

The young WT mice showed lower levels of Nav1.5 expression than did the aged WT, indicating increases in expression with aging (P<0.01). In contrast, both young and aged *Scn5a*+/ΔKPQ atria contained similar levels of Nav1.5. Comparisons between genotypes similarly demonstrated that young *Scn5a*+/ΔKPQ mice showed higher Nav1.5 expression than did young WT (P<0.05). Conversely, aged *Scn5a*+/ΔKPQ atria showed decreased Nav1.5 expression compared to aged WT (P<0.05). Thus, *Scn5a*+/ΔKPQ atria failed to show the age dependent expression patterns demonstrated in WT mice.

Table 1 Incidence of atrial arrhythmia

	WT	*Scn5a*+/ΔKPQ
Young	3 of 7[c]	0 of 6[a]
Aged	0 of 7[b]	6 of 6[a,b,c]

Numbers of hearts that showed arrhythmic episodes when subjected to PES at 8 Hz. The superscripted letters indicate significant differences obtained from successive pairwise tests P<0.05

Scn5a+/ΔKPQ hearts show altered conduction properties

Figure 5 shows typical results from experiments that compared in vivo ECG features of WT and *Scn5a*+/ΔKPQ hearts in intact mice anesthetized with Avertin. Each recording period followed a 10-min period permitting the preparation to stabilize and lasted 5 min. The PQRST complexes in the lead II ECG readings confirmed normal sequences of atrial activation and conduction, atrioventricular (AV) conduction and ventricular depolarization as well as recovery in all hearts whether in young (a) or aged WT (b), or young (c) or aged *Scn5a*+/ΔKPQ (d). No spontaneous arrhythmia was observed in any of the ECG recordings.

Table 2 summarizes differences in electrocardiographic parameters and therefore in cardiac pacing and conduction function, expressed as means ± SEMs, between the different

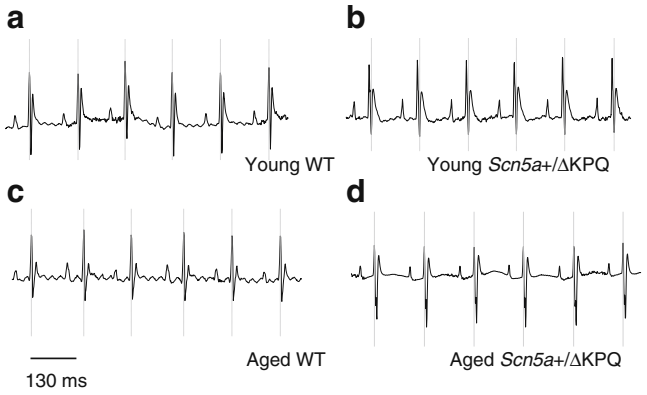

Fig. 5 Representative ECG recordings from young WT (**a**), young *Scn5a*+/ΔKPQ (**b**), aged WT (**c**), and aged *Scn5a*+/ΔKPQ (**d**) hearts respectively

murine populations examined. It also summarizes the results of ANOVA tests of the electrocardiographic parameters, sorted by the single variables of genotype and age. This was followed by statistical tests for significant differences when these findings were sorted by both these variables. These demonstrated the following differences in pacemaker function and intracardiac conduction that corroborated the present findings, extending some of them to ventricular properties of the heart. Thus, (1) the *Scn5a*+/ΔKPQ mutation results in an inhibited SA node function. Thus, RR intervals were greater in young *Scn5a*+/ΔKPQ compared to young WT mice and aged *Scn5a*+/ΔKPQ compared to the aged WT mice. (2) *Scn5a*+/ΔKPQ and WT mice showed similar action potential durations as reflected in their P wave durations in agreement with MAP results. (3) AV conduction as assessed by PR intervals was consistently slower in *Scn5a*+/ΔKPQ than WT, when comparisons were made by age. It was also slower in aged *Scn5a*+/ΔKPQ compared to aged WT. (4) Intraventricular conduction, reflected in QRS intervals, was prolonged in the aged *Scn5a*+/ΔKPQ compared to aged WT but was similar between young *Scn5a*+/ΔKPQ and young WT. Finally, (5) ventricular action potential durations, assessed by QT intervals and QTc intervals, were longer in the young *Scn5a*+/ΔKPQ compared to the young WT hearts. Such intervals were similar between young and aged *Scn5a*+/ΔKPQ but increased with age in WT.

Discussion

The present experiments compared changes in basic electrophysiological properties and their relationship to the development or otherwise of atrial arrhythmogenic properties in aging murine *Scn5a*+/ΔKPQ and WT hearts. They extend previous reports on atrial [10] and ventricular [41, 44] arrhythmogenicity in *Scn5a*+/ΔKPQ hearts that, however, did not explore effects of aging on these properties. An initial determination of the normal changes in WT provided controls against which to assess the presence or absence of abnormal changes in the *Scn5a*+/ΔKPQ hearts. MAP records were first obtained from Langendorff-perfused hearts to explore for arrhythmogenic properties in response to both regular and extrasystolic imposed stimulation. These properties were then compared with the features of action potential recovery as reflected in their durations and refractory periods. The presence or absence of arrhythmogenicity was then related to the results of Western blot determinations of cardiac Na^+ channel (Nav1.5) expression levels performed for the first time in the *Scn5a*+/ΔKPQ system. These in turn were related to electrocardiographic assessments of in vivo cardiac conduction properties in anesthetized animals.

The MAP studies in isolated Langendorff-perfused hearts assessed the frequencies of atrial arrhythmogenesis in young and aged, WT and *Scn5a*+/ΔKPQ, hearts using a PES procedure. They demonstrated for the first time that aged *Scn5a*+/ΔKPQ hearts showed an increased atrial arrhythmogenicity compared to any of the other three groups. This took place despite an absence of any detectable differences in atrial action potential durations to 90% recovery (APD). The latter finding rules out arrhyth-

Table 2 ECG parameters compared in WT and *Scn5a*+/ΔKPQ

	WT		*Scn5a*+/ΔKPQ	
	Young	Aged	Young	Aged
RR interval, ms	141.18±3.03 (6)[a]	129.87±4.49 (5)[b]	161.12±1.32 (5)[a]	152.54±8.18 (6)[b]
Heart rate	426.51±8.73 (6)[c]	464.54±16.73 (5)[d]	372.75±3.05 (5)[c]	399.03±20.71 (6)[d]
PR interval (ms)	45.00±1.93 (5)[e,f]	37.31±1.92 (6)[e,g]	50.45±0.83 (5)[f]	48.09±1.46 (6)[g]
P wave duration (ms)	11.40±0.77 (6)	13.42±1.19 (5)	16.59±2.60 (5)	15.74±1.50 (6)
QRS interval (ms)	11.48±0.93 (6)	10.75±0.24 (5)[h]	12.88±0.68 (5)	15.70±1.91 (6)[h]
QT interval (ms)	33.03±1.44 (6)[i,j]	44.08±9.79 (5)[i]	45.89±2.49 (5) [j]	47.81±2.79 (6)
QTc interval (ms)	27.95±1.40 (6)[k,l]	37.56±4.07 (5)[k]	37.31±1.45 (5)[l]	38.83±2.21 (6)

The superscripts a–l indicate significant differences between the designated values ($P<0.05$; n values given in parenthesis)

mogenic mechanisms involving action potential recovery previously implicated in the ventricular arrhythmogenicity shown by *Scn5a*+/ΔKPQ on earlier occasions [46]. Whereas refractory periods in young WT and young *Scn5a*+/ΔKPQ were closely similar, they sharply increased with age in WT, consistent with previous reports [23]; however, they did not do so in *Scn5a*+/ΔKPQ. Consequently, *Scn5a*+/ΔKPQ hearts showed shorter refractory periods and greater APD/AERP ratios than aged WT. Both of these have been previously introduced as indices of ventricular [41] as well as atrial [10] arrhythmogenicity, respectively. These results were thus consistent with the higher inducibility of atrial arrhythmias by programmed atrial stimulation that was observed in aged Scn5a/ΔKPQ mice.

These findings were then compared with results from complementary examinations of Nav1.5 protein expression in the experimental groups. Such Western blot studies directly measure specific protein levels as opposed to indirect measures involving messenger RNA (mRNA). These demonstrated that Nav1.5 channel expression increased with age and genotype, complementing previous electrophysiological reports of altered cardiac Ca^{2+} and K^+ channel expression with age in WT [11, 21]. Thus, young WT showed relatively low levels of Nav1.5 expression, but these markedly increased with normal aging. In contrast, young *Scn5a*+/ΔKPQ showed higher Nav1.5 expression than young WT but then showed no further increases with age. Aged *Scn5a*+/ΔKPQ consequently showed substantially lower Nav1.5 expression than aged WT.

The latter findings were compatible with the final, electrocardiographic, results. These similarly revealed genotype- and age-specific phenotypic alterations in intact anesthetized WT and *Scn5a*+/ΔKPQ mice. Firstly, young *Scn5a*+/ΔKPQ mice showed longer QT and QTc intervals than did young WT, consistent with the LQT3 condition. Secondly, even young WT and young *Scn5a*+/ΔKPQ mice differed in those ECG features that bore on atrial pacemaker and AV conduction properties. Young and aged *Scn5a*+/ΔKPQ mice had lower heart rates and slower AV conduction than their respective WT counterparts. In addition, aged *Scn5a*+/ΔKPQ mice showed depressed intra-ventricular conduction relative to aged WT. Finally, WT and *Scn5a*+/ΔKPQ also showed differing alterations with age. Thus, while PR and QT intervals change with age in WT mice, this was not evident in *Scn5a*+/ΔKPQ hearts.

Comparisons between these findings were consistent with a hypothesis in which small APD/AERP ratios together with high levels of Nav1.5 expression might balance the arrhythmic effects of a slowing of conduction with age. This could be related to a progressive interstitial fibrosis that has been shown to occur in the atria of humans [12, 43] and animals [1, 17]; the latter includes mice with mutations involving *Scn5a* [39]. Such fibrosis is also associated with clinical AF [24, 29]. In addition, localized alterations in AERP shown to increase with age would be expected to accentuate dispersions of intra-atrial refractoriness [49]. All these processes would be expected to cause a heterogeneous slowing of atrial conduction and consequent age-related increases in atrial arrhythmic tendency.

In such a situation, the observed increases in Nav1.5 could protect against atrial arrhythmia in aged WT. Thus, WT hearts were not arrhythmogenic: APD/AERP ratios were smaller for aged WT than young WT, while there was a higher Nav1.5 expression in aged WT relative to young WT. In contrast, aged *Scn5a*+/ΔKPQ were more arrhythmogenic than the remaining groups. This was consistent with their higher APD/AERP ratios and reduced Nav1.5 expression levels relative to aged WT. Finally, these results additionally relate the greater arrhythmogenecity of aged *Scn5a*+/ΔKPQ compared to young *Scn5a*+/ΔKPQ, despite similar APD/AERP ratios and AERP values, to their failure to increase Nav1.5 expression with age unlike WT. Thus, despite possessing a gain of function mutation, aged *Scn5a*+/ΔKPQ atria showed reduced Nav1.5 expression levels and high APD/AERP ratios. This may have resulted in an atrial arrhythmogenicity that was prevented by the increase in Nav1.5 expression with age shown by the WT.

Such changes could also involve altered expression of a range of genes and ion channels, besides Nav1.5. Thus, *ventricular* mRNA expression profiles in studies of human BrS associated with Na^+ channelopathy suggested remodeled K^+ and Ca^{2+} in addition to Na^+-channel expression [14]. For example, their reduced expression of Kv4.3 encoding the principal I_{to} subunit, increased transcript expression of Nav2.1, and increased 2P-channel expression would tend to compensate for the Nav1.5 underexpression observed in BrS-affected individuals. Conditions of *atrial* fibrillation or tachycardia results in reductions of transient outward K^+ current (I_{to}), downregulation of I_{CaL} pore-forming α-subunit mRNA, and increased expression of Kir2.1 mRNA and protein [36]. Furthermore, there is evidence associating BrS with such atrial remodeling [47].

In demonstrating that aged *Scn5a*+/ΔKPQ mice show an increased propensity to atrial arrhythmia, the findings in this study thus recapitulate clinical situations of phenotypic overlap between LQTS and BrS. A loss of Nav1.5 function may reflect haploinsufficiency and impaired intracellular trafficking reducing surface level expression [38]. The reduction in Nav1.5 protein expression observed here is compatible with such a possibility. This in turn may be the cause of a situation resembling the case of the Brugada mouse model in which a loss of Na^+ channel expression results in both ventricular [37] and atrial arrhythmogenic properties [27, 28]. Such a notion would be compatible with the electrocardiographic evidence for reduced pace-

maker activity and delayed conduction in both young and aged *Scn5a*+/ΔKPQ relative to WT hearts. These also parallel reduced SA node activity and slowed AV conduction that has been reported in BrS patients [34]. Taken together, these findings could therefore account for phenotypic overlaps between LQT3 and BrS patients [42] besides demonstrating the existence of a complex dynamic interplay between aging, ion channel expression, and cardiac tissue excitability.

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Conflicts of interest The authors declare that they have no conflict of interest.

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