Diversity and complexity of cardiac sodium channel (dys)function
Rivaud, M.

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A common co-morbidity modulates disease expression and treatment efficacy in inherited cardiac sodium channelopathy


*these authors contributed equally

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Abstract

Aims: Management of patients with inherited cardiac ion channelopathy is hindered by variability in disease severity and sudden cardiac death (SCD) risk. We here investigated the modulatory role of hypertrophy on arrhythmia and SCD risk in sodium channelopathy.

Methods and Results: Follow-up data was collected from 164 carriers of the SCN5A-1795insD founder mutation and 247 related non-carriers. A total of 38 (obligate) mutation carriers died suddenly or suffered life-threatening ventricular arrhythmia. Of these, 18 were aged >40 years, a high proportion of which had a clinical diagnosis of hypertension and/or cardiac hypertrophy. While pacemaker implantation was highly protective in preventing bradycardia-related SCD in young mutation carriers, 7 mutation carriers aged >40 experienced life-threatening arrhythmic events despite pacemaker treatment. Of these, 6 had a diagnosis of hypertension/hypertrophy, pointing to a modulatory role of this co-morbidity. Induction of hypertrophy in adult mice carrying the homologous mutation (Scn5a1798insD/) caused SCD and excessive conduction disturbances, confirming a modulatory effect of hypertrophy in the setting of the mutation. The deleterious effects of the interaction between hypertrophy and the mutation were prevented by genetically impairing the pro-hypertrophic response and by pharmacological inhibition of the enhanced late sodium current associated with the mutation.

Conclusion: This study provides the first evidence for a modulatory effect of co-existing cardiac hypertrophy on arrhythmia risk and treatment efficacy in inherited sodium channelopathy. Our findings emphasize the need for continued assessment and rigorous treatment of this co-morbidity in SCN5A mutation carriers.
**Translational perspectives**

SCN5A mutations present with a broad spectrum of clinical phenotypes, including sudden cardiac death (SCD). Disease penetrance and severity varies between individuals carrying the same SCN5A mutation, and common co-morbidities may modulate arrhythmia and SCD risk with potential implications for anti-arrhythmic treatment efficacy. This study conducted in patients and mice, provides strong evidence for a modulatory role of hypertension/hypertrophy in modulating arrhythmic risk of the SCN5A-1795insD mutation. Our findings support the concept that SCN5A mutation carriers should be carefully monitored for the development of cardiac hypertension and hypertrophy during follow up, and that their clinical management adjusted where necessary to also include the management of this co-morbidity.


**Introduction**

The genetic basis of the Mendelian cardiac rhythm disorders associated with sudden cardiac death (SCD) has been brought into focus over the last 20 years with the discovery of a large spectrum of causal mutations in genes encoding components of cardiac ion channels.\(^1\) Although this enabled genetic testing and consequently improvements in clinical care, patient management is still hindered by the reduced penetrance and substantial variability in disease severity and SCD risk among mutation carriers.\(^1\) Although co-morbidities are expected to modulate disease severity, these remain unexplored. Identification of disease modifiers in these disorders is however hindered by the substantial genetic heterogeneity across patients, as different mutations may be associated with different effects and thus also contribute to inter-individual variability.\(^2\) Studies in large families that harbour founder mutations (where all affected individuals carry the same familial mutation) circumvent this limitation and therefore offer opportunities for the identification of modulatory factors.

We have previously described a large Dutch family harboring a founder mutation, p.Tyr1795_Glu1796insAsp (previously called ‘SCN5A-1795insD’), in the SCN5A gene which encodes the major sodium channel isoform in heart (Na\(_V\)1.5).\(^3,4\) This mutation displays multiple biophysical defects and causes ‘overlap’ sodium channelopathy with features of long QT syndrome, Brugada syndrome, and conduction disease.\(^5,6\) While variability in disease severity among mutation carriers in this family is long recognized,\(^3\) predictors of arrhythmia and SCD risk have remained elusive. By combining clinical observations in the family with experimental studies in Scn5a\(^{1798insD/+}\) mice carrying the mouse homolog of the mutation, we here uncovered a modulatory effect of hypertension and cardiac hypertrophy on disease severity and expression. In particular, the co-occurrence of cardiac hypertrophy was found to exacerbate cardiac conduction slowing and arrhythmia risk in the setting of the mutation, and was associated with a decreased efficacy of pacemaker treatment in preventing SCD. Our findings furthermore provide evidence for a potential therapeutic role of pharmacological late sodium current inhibition.
Methods

Study approval
Human studies conformed to the principles outlined in the Helsinki Declaration of the World Medical Association, and written consent was obtained from patients or relatives where appropriate. Animal studies were in accordance with governmental and institutional guidelines for animal use in research.

SCN5A-1795insD mutation study population
The study population consisted of a large Dutch family of European decent segregating the SCN5A-1795insD mutation associated with manifestations of Long-QT syndrome type 3, Brugada syndrome and cardiac conduction disease occurring either in isolation or in combinations thereof.\textsuperscript{3,4} Available clinical data (patient and family history, ECGs, Holter and pacemaker recordings, exercise tests, echocardiography, resuscitation records, time and circumstance of death) was collected from cardiologists, cardiogeneticists, and general practitioners involved in patient care. Post-mortem examination reports were obtained from local pathology departments, and cardiac tissue sections were subsequently investigated by a specialized cardiac pathologist to confirm diagnosis of cardiac hypertrophy.

Generation of Scn5a\textsuperscript{1798insD/+} mice
Male chimeras were crossed with 129P2/OlaHsd females to give F1 heterozygous offspring (129P2- Scn5a\textsuperscript{1798insD/+} and maintained in this genetic background by backcrossing with wild-type 129P2 mice (invariably purchased from Harlan). 129P2-Scn5a\textsuperscript{1798insD/+} mice were outcrossed with wild-type FVB/N mice (invariably purchased from Charles River Laboratories) for \( \geq 10 \) generations, establishing a second line on the FVB/N background (FVB/N-Scn5a\textsuperscript{1798insD/+} Mice were genotyped as described previously.\textsuperscript{5} All experiments were performed on adult (3-5 months old) and aged (8-22 months old) male and female Scn5a\textsuperscript{1798insD/+} mice with their wild-type littermates as control.

Generation of Scn5a\textsuperscript{1798insD/+}Nfatc2\textsuperscript{-/-} mice
F1 offspring were heterozygous for the Nfatc2 gene and wild-type or Scn5a\textsuperscript{1798insD/+} for the Scn5a gene. F2 mice were obtained by brother-sister mating of the F1 generation mice (WT x Scn5a\textsuperscript{1798insD/+} giving WT-Nfatc2\textsuperscript{+/-}, Scn5a\textsuperscript{1798insD/+}-Nfatc2\textsuperscript{+/-}, WT-Nfatc2\textsuperscript{-/-} and Scn5a\textsuperscript{1798insD/+}-Nfatc2\textsuperscript{-/-} mice, which were genotyped as previously described.\textsuperscript{5,7} All experiments were performed on adult males (3 to 5 month old) Scn5a\textsuperscript{1798insD/+}-Nfatc2\textsuperscript{+/-} and Scn5a\textsuperscript{1798insD/+}-Nfatc2\textsuperscript{-/-} with WT-Nfatc2\textsuperscript{+/-} and WT-Nfatc2\textsuperscript{-/-} littermates serving as controls.
Transverse aortic constriction (TAC)

Mice were anaesthetized by isoflurane (mean 2.5% in oxygen), intubated with a 20G polyethylene catheter, ventilated (Minivent, Hugo Sachs Electronics, Germany) and placed on a heating pad to maintain body temperature at 37°C. Carprofen (0.05 mg/kg) was injected subcutaneously for postsurgical analgesia. The thoracic cavity was accessed through a small incision at the left upper sternal border in the second intercostal space. A 7-0 silk suture was passed around the aorta between the right innominate and left common carotid arteries. Constriction of the transverse aorta was performed by tying against a 27 G needle, which was subsequently removed. The same procedure was followed in sham animals, except for the constriction. Adequacy of the constriction was confirmed using either continuous Doppler echocardiography or pulsed Doppler measurements. Using echocardiography, blood flow velocities were measured across the aortic valve and across the transverse aortic constriction, and these velocities values were transformed into pressure values using Bernoulli’s formula \((4\times v^2)\). A pressure gradient (defined as the difference between the developed pressure across the constriction subtracted by the pressure across the aortic valve) of approximately 50 mmHg was considered appropriate. In the pulsed Doppler measurements, the ratio between the maximum blood flow velocity of the right carotid and the left carotid artery was determined as described in Reddy et al.\(^8\) and a pressure gradient ratio of at least 5 was required for a successful TAC procedure. In a subset of mice, a radiotelemetry transmitter (Data Sciences International) was surgically inserted into the peritoneal cavity. After 2 days of recovery, ECG signals were measured continuously until mice were sacrificed (2 weeks after TAC).

Electrophysiological assessments in Langendorff-perfused hearts

Mice were anesthetized by an i.p. injection of pentobarbital, after which the heart was excised, cannulated, mounted on a Langendorff perfusion set-up, and perfused at 37°C with Tyrode’s solution (128 mmol/l NaCl, 4.7 mmol/l KCl, 1.45 mmol/l CaCl\(_2\), 0.6 mmol/l MgCl\(_2\), 27 mmol/l NaHCO\(_3\), 0.4 mmol/l NaH\(_2\)PO\(_4\), and 11 mmol/l glucose \([\text{pH maintained at 7.4 by equilibration with a mixture of 95% O}_2 \text{ and 5% CO}_2]\) ). All data was acquired with the BioSemi system.

Assessment of atrio-ventricular conduction. Hearts were paced from the right atrium at a basic cycle length (BCL) of 150 ms for the aged animals and 120 ms for the TAC animals. Atrial-ventricular delay (AV-delay) was determined by calculating the difference between the time of atrial stimulation and the onset of ventricular activation.

Assessment of ventricular arrhythmia inducibility. The ventricles were paced at a BCL of 120 ms (2 ms pulse duration, twice diastolic threshold). Arrhythmia
inducibility was tested using a premature stimulation protocol, and applying 16 stimuli at BCL, followed by up to 3 extra stimuli (S1-S2-S3) with progressively shortened coupling intervals of 10 ms above the ERP of each extra stimulus. In addition, burst pacing was applied using 36 stimuli at BCL, followed by 40 shortly coupled stimuli at progressively shortened coupling intervals.

**Optical mapping.** Hearts were incubated in 10 ml Tyrode’s solution containing 15 μM Di-4 ANEPPS and subsequently placed in an optical mapping setup. Hearts were perfused at 37°C with Tyrode’s solution containing blebbistatin to prevent movement artifacts. Excitation light was provided by a 5-watt power LED (filtered 510 ± 20 nm). Fluorescence (filtered >610 nm) was transmitted through a tandem lens system on CMOS sensor (100 × 100 elements; MICAM Ultima). Hearts were paced at BCL of 120 ms (unless stated otherwise) at twice the diastolic stimulation threshold from the center of the ventricular epicardial surface. Optical action potentials were analyzed with custom software. Local activation was defined as the maximum positive slope of the action potential, and local activation times were used to construct ventricular activation maps. To calculate conduction velocity (CV) in longitudinal and transversal directions, the difference in activation time was determined between at least three consecutive electrode terminals, separated by a known distance and located parallel (longitudinal) or perpendicular (transversal) to the direction of propagation. The direction of propagation was determined using isochronal maps. Three parallel values of CV in each of the two directions were acquired and averaged.

**Quantitative PCR assay and analysis**
Total mRNA was isolated from left ventricular tissue from sham or TAC hearts using Trizol (Ambion, Life Technologies). 250 μg of mRNA was used as template for reverse transcription using the Reverse Transcriptase-SuperScript II kit (Invitrogen, Life Technologies). Real time RT-PCR was carried out in a Light Cycler apparatus (Roche) using SYBR Green and the following primers: Rcan1-4_F AGCTCCCTGATTGCTTGTGG, Rcan1-4_R GTTGCTGAAGTTTATCCGGACAC, Gapdh_F GTCAGCAATGCATCCTGCA, and Gapdh_R CCGTTCAAGCTCTGGATGA. Data was analysis with the LinReg program.9

**Whole-mount in situ hybridization**
Nonradioactive in situ hybridization using an Anf cDNA probe was performed on cardiac PFA sections as previously described.10
Statistical analysis
Data are presented as mean ± SEM, unless otherwise specified. Differences between groups were analyzed by ANOVA or non-parametric Kruskal Wallis test, followed by LSD post hoc test. If one or more of the assumptions were violated, a Log or rank transformation was applied prior to analysis. Genotype, mouse strain, age and/or procedure (TAC versus Sham) and their interactions were included in the model. The level of statistical significance was set to p<0.05, unless stated otherwise. Differences in arrhythmia incidence between groups were analyzed using a chi-square test. Differences in survival between groups were assessed using a log rank (Mantel-Cox) test. The proportional (i.e. constant) hazard assumption of pacemaker implantation was tested using the Schoenfeld residuals from a Cox regression model.
Results

Hypertension and hypertrophy in older SCN5A-1795insD mutation carriers who died suddenly

Through an extensive genealogical search, we reconstructed the pedigree of the family with the SCN5A-1795insD mutation back to the 18th century, linking 164 mutation carriers and 247 non-carriers (Figure 1A). Carriers displayed (atrio-)ventricular conduction slowing, sinus node dysfunction, excessive ventricular repolarization abnormalities at slow heart rates, and SCD occurring predominantly at night.3,4 A total of 38 individuals died suddenly or suffered life-threatening ventricular arrhythmia (13 males, 25 females; average age at event 38±18 years, range 13-76) (Figure 1A,B). Twenty-five of these individuals were confirmed or obligate carriers of the mutation; genetic testing was not possible in the rest as these were deceased individuals from past generations. Eighteen individuals who suffered SCD or a life-threatening arrhythmia were older than 40 at the time of the event (4 males, 14 females, average age 54±9 years, range 41-76) (Figure 1A,B). Recent clinical information was available for 10 of these (all confirmed mutation carriers); 9 of them had a clinical diagnosis of hypertension and/or evidence for the presence of left ventricular hypertrophy on MRI or post-mortem examination (Table 1), suggesting a modulatory effect of cardiac hypertrophy on arrhythmia risk.

Decreased pacemaker treatment efficacy in SCN5A-1795insD mutation carriers older than 40

Mutation carriers died suddenly predominantly during the night, and excessive QT-prolongation during (nocturnal) bradycardic episodes was frequently documented on Holter recordings.3,4,12 Hence, a bradycardia-dependent trigger for arrhythmia was originally suspected, and pacemaker implantation has been routinely employed in the family to prevent SCD.4 While this approach initially proved successful,4 in the last decade 7 mutation carriers suffered ventricular tachyarrhythmias and/or SCD despite pacemaker implantation (Figure 1A). All of them were older than 40 (age range 41-58 years), and in the majority, ventricular fibrillation or tachycardia was documented (Table 1), in addition to ventricular extrasystoles recorded during Holter monitoring or exercise testing in several cases. Figure 1C shows a pacemaker readout showing a nocturnal, fast ventricular tachycardia (presumably polymorphic) in a 58-year old female mutation carrier; following this episode, her pacemaker was replaced with an ICD. These observations suggested an age-dependent shift towards a different arrhythmia triggering mechanism at older age with arrhythmias occurring despite prevention of bradycardia. Indeed, comparing the occurrence of SCD/life-threatening
Figure 1. Life-threatening cardiac events in SCN5A-1795insD mutation carriers. (A) The pedigree of the SCN5A-1795insD founder population. Circles indicate SCN5A-1795insD mutation carriers suffering sudden cardiac death (SCD), ventricular fibrillation (VF), and/or ventricular tachycardia (VT) in the presence or absence of pacemaker (PM) treatment and hypertension and/or left ventricular hypertrophy (HYP). (B) Survival from SCD and/or ventricular arrhythmias was significantly reduced in SCN5A-1795insD mutation carriers (n=164) versus non-carriers (n=247) (p<0.0001). (C) Overall survival from SCD and/or ventricular arrhythmias in SCN5A-1795insD mutation carriers was significantly higher in carriers with (n=87) as compared to without (n=77) pacemaker (p<0.0001), but pacemaker efficacy was significantly reduced in mutation carriers above the age of 40 years (p=0.028; Cox regression model). (D) Pacemaker readout (A: atrial lead; V: ventricular lead; paper speed 25 mm/sec) of a 58-year female mutation carrier showing a nocturnal, fast (up to 300/min) and apparently polymorphic ventricular tachycardia despite adequate anti-bradycardia treatment.
Hypertension modulates SCD risk in sodium channelopathy

Pacemaker showed that while pacemaker implantation was highly protective in young mutation carriers, its efficacy in preventing arrhythmias and SCD was significantly decreased above the age of 40 years ($p=0.028$; proportional hazard assumption of pacemaker implantation tested using the Schoenfeld residuals from a Cox regression model) (Figure 1C). Importantly, in 6 of the 7 carriers who suffered a life-threatening event despite pacemaker treatment, a history of hypertension was confirmed and/or the presence of left ventricular hypertrophy documented on MRI or post-mortem examination (Table 1). These observations led us to hypothesize that the co-occurrence of the mutation with cardiac hypertrophy, developing with age secondary to for instance hypertension, plays a pivotal role in modulating arrhythmia risk.

<table>
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<tr>
<th>Gender</th>
<th>Age</th>
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<th>Event</th>
<th>Hypertension</th>
<th>LV dysfunction</th>
<th>Postmortem</th>
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<td>LVH</td>
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<td>yes</td>
<td>LVH</td>
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<tr>
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<td>Yes</td>
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<td>yes</td>
<td>LVH</td>
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<tr>
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<td>SCD</td>
<td>yes</td>
<td>LVH</td>
<td>Yes</td>
<td>SCD (VF documented); LVH on postmortem</td>
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</tbody>
</table>

DCM: dilated cardiomyopathy; ICD: implantable cardioverter defibrillator; LVH: left ventricular hypertrophy; SCD: sudden cardiac death; VF: ventricular fibrillation; VT: ventricular tachycardia

* Patient did not have documented hypertension prior to VF, but developed clinically relevant hypertension one year later.

The co-occurrence of cardiac hypertrophy is pro-arrhythmic in aged Scn5a$^{1798insD/+}$ mice

We further explored the modulatory role of cardiac hypertrophy in Scn5a$^{1798insD/+}$ mice carrying the exact mouse homolog of the human SCN5A-1795insD mutation. We have previously generated two distinct mouse lines harbouring the Scn5a$^{1798insD/+}$ mutation, with respectively the FVB/N and 129P2 inbred genetic background, enabling investigation of the effect of the mutation on different genetic backgrounds. In young adult mice we previously demonstrated
strain-dependent variable disease severity, with more pronounced conduction slowing and prolongation of repolarization in mutant mice of the 129P2 strain.\textsuperscript{13} We now studied aged wild-type (WT) and $Scn5a^{1798insD/+}$ mutant (MUT) mice of both strains. The 129P2 strain was found to develop more cardiac hypertrophy with age as compared to the FVB/N strain. This feature was intrinsic to the 129P2 strain and independent of the mutation, since both aged WT and MUT 129P2 mice displayed greater heart weight and higher expression levels of pro-hypertrophic markers as compared to aged WT and MUT FVB/N mice (Figure 2A,B). These intrinsic strain-dependent differences in susceptibility to hypertrophy enabled a comparison of the pro-arrhythmic effect of the mutation in the presence (i.e. aged 129P2 MUT mice) and absence (i.e. aged FVB/N MUT mice) of hypertrophy. We therefore conducted ECG studies in anesthetized mice and electrophysiological studies in Langendorff-perfused hearts, comparing aged (8-22 months old) WT and MUT mice of both strains. This indeed uncovered a genotype x strain interaction, where aged 129P2-MUT mice displayed significantly more pronounced ventricular conduction slowing \textit{in vivo} (QRS-duration on surface ECG analysis; genotype x strain interaction $p=0.012$; Figure 2C,D; Supplementary Table 1) and \textit{ex vivo} (LV activation time in isolated Langendorff-perfused hearts; genotype x strain interaction $p=0.036$; Figure 2F,G). Moreover, 129P2-MUT mice exhibited significantly more spontaneous ventricular extrasystoles and arrhythmias \textit{in vivo} (Figure 2C,E) and an increased inducibility of ventricular arrhythmias \textit{ex vivo} (Figure 2H,I). While these observations do not provide a causal link, they are in line with a pro-arrhythmic interaction between hypertrophy and the mutation, similar to our observations in the $SCN5A$-1795insD family.

\textbf{Figure 2. Pro-arrhythmic effects of cardiac hypertrophy in aged $Scn5a^{1798insD/+}$ mice.} Mice of the 129P2 strain develop more severe cardiac hypertrophy with age (wild type, WT and MUT, $Scn5a^{1798insD/+}$ to a similar extent) than mice of the FVB/N strain, as indicated by increased heart weight to body weight ratio (A) and increased Anf expression on \textit{in situ} hybridization (B). (C) Typical examples of surface ECGs and arrhythmias. (D,E) Aged 129P2-MUT mice show significantly increased QRS-duration (genotype x strain interaction $p=0.005$) and significantly more spontaneous arrhythmias (Pearson Chi-square overall $p<0.0005$; *$p<0.0005$; threshold for significance $p=0.00625$, Bonferroni adjusted) on surface ECG than aged 129P2-WT, FVB/N-WT and FVB/N-MUT mice (SND: sinus node dysfunction; AVB: atrio-ventricular block; VPBs: ventricular premature beats; VT: ventricular tachycardia). (F) Typical examples of left ventricular (LV) activation maps (central stimulation at 120 ms) obtained by optical mapping in isolated hearts. (G) Aged 129P2-MUT mice display significantly longer LV activation times, indicating more pronounced ventricular conduction slowing. (H) Typical example of a VT induced by 1 short-coupled extra stimulus (S1) in an isolated aged 129P2-MUT heart, and non-inducibility in an aged FVB/N-MUT heart with up to 3 extra stimuli (S1-S3). (I) Isolated aged 129P2-MUT hearts display significantly higher inducibility of ventricular arrhythmias \textit{ex vivo} (Pearson Chi-square overall $p<0.05$; †$p<0.005$; threshold for significance $p=0.00625$, Bonferroni adjusted).
Hypertension modulates SCD risk in sodium channelopathy

A

Heart weight/body weight

WT: n=19, MUT: n=21, Aged FVB/N: n=14, Aged 129P2

B

Aged FVB/N

Ant

Aged 129P2

C

Aged FVB/N

WT

MUT

100 ms

MUT

100 ms

MUT

MUT

D

QRS duration (ms)

WT: n=20, MUT: n=40, Aged FVB/N: n=29, Aged 129P2

WT: n=32, MUT: n=56, Aged FVB/N: n=37, Aged 129P2

E

Arrhythmia incidence (%)

SND/AVB, VPBs, VT

WT

MUT

F

Arrhythmia inducibility (%)

Aged FVB/N: n=10, Aged 129P2: n=19

Aged 129P2: n=12, MUT: n=11
Chronic pressure overload elicits conduction delay and sudden death in Scn5a<sup>1798insD/+</sup> mice

To provide direct evidence for a modulatory effect of hypertrophy in the setting of the mutation, we subjected adult FVB/N-WT and FVB/N-MUT mice (10-12 weeks old) to transverse aortic constriction (TAC; duration of 2 weeks), an intervention which leads to chronic pressure overload and consequent development of cardiac hypertrophy. TAC induced similar extent of hypertrophy in WT and MUT mice, as illustrated by equal increases in heart mass and upregulation of hypertrophic genes (Figure 3B,C; Supplementary Table 2). However, approximately 35% of MUT-TAC mice died suddenly during the 2-week post-TAC period, while all WT-TAC and sham mice survived (Figure 3A). Continuous 24-hour telemetric ECG recordings in a subset of MUT-TAC mice revealed progressive bradycardia and excessive (atrio-)ventricular conduction abnormalities prior to SCD (Supplementary Figure 2). ECG analysis in surviving MUT mice post-TAC uncovered a more pronounced increase in QRS-duration compared to WT (genotype x TAC interaction \( p=0.016 \); Supplementary Table 3). Moreover, \textit{ex vivo} measurements in isolated Langendorff-perfused hearts post-TAC showed atrio-ventricular delay and exacerbated ventricular conduction slowing in MUT but not WT mice (genotype x TAC interaction \( p<0.0001 \) and \( p=0.025 \), respectively; Figure 3D-G; Supplementary Table 2). Hence, TAC elicited SCD and conduction abnormalities in MUT mice only, indicating a synergistic, deleterious interaction between cardiac hypertrophy and the mutation.

Figure 3. Cardiac hypertrophy induced by transaortic constriction (TAC) causes SCD and conduction disturbances in Scn5a<sup>1798insD/+</sup> mice. (A) Kaplan-Meier survival curves of WT and MUT mice subjected to Sham or TAC. (B,C) Magnitude of cardiac hypertrophy (measured by HW/TL, heart weight/tibia length ratio (B) and mRNA expression levels of the pro-hypertrophic marker \textit{Rcan1-4} (C)) secondary to TAC is similar in WT and MUT mice. (D) Typical examples of atrio-ventricular (AV) delay measurements (atrial stimulation at 120 ms). (E) MUT-TAC mice display more severe AV-conduction delay as compared to WT-TAC (genotype x TAC interaction \( p<0.0005 \)). (F) Typical examples of LV activation maps in isolated hearts (ventricular stimulation at 120 ms). (G) MUT-TAC mice display increased LV activation time, indicating slowed ventricular conduction (genotype x TAC interaction \( p<0.05 \)). Additional data is presented in Supplementary Table 2.
Hypertension modulates SCD risk in sodium channelopathy

**A**

![Graph showing survival percentage over duration of TAC (days)]

- **WT Sham** (n=16)
- **WT TAC** (n=16)
- **MUT Sham** (n=13)
- **MUT TAC** (n=27)

**B**

![Bar charts showing HW/TL ratio for WT and MUT groups]

- **Sham** (n=10)
- **TAC** (n=12)
- **Sham** (n=8)
- **TAC** (n=11)

**C**

![Bar charts showing Rcan1-4/Gapdh for WT and MUT groups]

- **Sham** (n=12)
- **TAC** (n=10)
- **Sham** (n=8)
- **TAC** (n=9)

**D**

![Diagram showing AV-delay for WT and MUT groups]

**E**

![Bar charts showing AV-delay for WT and MUT groups]

**F**

![Images showing LV activation time for WT and MUT groups]

**G**

![Bar charts showing LV activation time for WT and MUT groups]

- **Sham** (n=6)
- **TAC** (n=5)
- **Sham** (n=6)
- **TAC** (n=4)
TAC-induced conduction abnormalities and SCD in Scn5a<sup>1798insD/+</sup> mice are attenuated by decreasing the hypertrophic response through genetic inhibition of the calcineurin-Nfat pathway

Activation of the calcineurin-Nfat (Nuclear Factor of Activated T-cells) signaling pathway is known to play a major role in mediating the pro-hypertrophic consequences of chronic pressure overload of the heart. To investigate whether the more severe electrophysiological abnormalities in FVB/N-MUT mice post-TAC are the direct consequences of cardiac hypertrophy and not to other (indirect) effects of pressure overload, we abrogated the hypertrophic response by inducing genetic deletion of the main downstream effector of the calcineurin-Nfat pathway by crossing Scn5a<sup>1798insD/+</sup> mice with mice lacking Nfatc2 (Nfatc2<sup>−/−</sup>).<sup>7,14</sup> WT and MUT animals with unaltered Nfatc2 expression (Nfatc2<sup>+/+</sup>), were subjected to TAC for a period of 2 weeks. As expected, cardiac hypertrophy in response to pressure overload was attenuated in WT and MUT mice deficient for Nfatc2 (WT-Nfatc2<sup>−/−</sup> and MUT-Nfatc2<sup>−/−</sup>), as illustrated by lower heart weights and lower expression of hypertrophic genes as compared to WT-Nfatc2<sup>+/+</sup> and MUT-Nfatc2<sup>+/+</sup> (Figure 4B,C, Supplementary Table 4). No SCD was observed in MUT-Nfatc2<sup>−/−</sup> mice subjected to TAC (Figure 4A), and the (atrio-)ventricular conduction abnormalities secondary to TAC observed in MUT mice with intact Nfatc2 expression were rescued in MUT-Nfatc2<sup>−/−</sup> mice (Figure 4D-F; Supplementary Table 4). Hence, blocking the downstream, pro-hypertrophic signaling cascade prevented the TAC-induced conduction abnormalities and SCD in MUT mice, providing support for a direct interaction between the mutation and cardiac hypertrophy.

Figure 4. Rescue of TAC-induced SCD and conduction disturbances in Scn5a<sup>1798insD/+</sup> mice by genetic deletion of Nfatc2 or late sodium current inhibition by Ranolazine. (A) Kaplan-Meier survival curves of WT and MUT mice (Nfatc2<sup>−/−</sup>; with genetic deletion of Nfatc2; RAN: fed Ranolazine chow) subjected to Sham or TAC. (B,C) Magnitude of cardiac hypertrophy (measured by HW/TL, heart weight/tibia length ratio (B) and mRNA expression levels of the pro-hypertrophic marker Rcan1-4 (C)) secondary to TAC is similar in WT and MUT (+/-Ranolazine chow) mice, but lower in WT and MUT mice on a Nfatc2<sup>−/−</sup> background. (D) Typical examples of atrio-ventricular (AV) delay measurements (atrial stimulation at 120 ms; left panel). The AV-conduction delay induced by TAC in MUT mice is reversed by genetic inhibition of Nfatc2 and by Ranolazine treatment. (E) Typical examples of LV activation maps in isolated hearts. (F) Ventricular conduction slowing (indicated by increased LV activation time) induced by TAC in MUT mice is reversed by genetic inhibition of Nfatc2 and by Ranolazine treatment. In panels B, C, D and F, data is presented as ratio for TAC versus Sham; actual values for each group are listed in Supplementary Tables 2 and 4.
Hypertension modulates SCD risk in sodium channelopathy
Chronic late sodium current inhibition prevents TAC-induced conduction abnormalities and SCD in Scn5a<sup>1798insD/+</sup> mice

We have previously demonstrated that the SCN5A-1795insD mutation is associated with multiple biophysical defects including a gain of channel function due to sustained (late) inward sodium current. This mutation-induced enhanced late sodium current (I\textsubscript{Na,late}) can be blocked through pharmacological inhibition. We therefore explored whether blocking this biophysical defect of the mutation would prevent the exacerbation of electrophysiological abnormalities associated with TAC in MUT mice. For this we administered the I\textsubscript{Na,late} inhibitor Ranolazine by feeding WT and MUT mice either control or Ranolazine chow for the 2-week period of TAC or sham (starting 2 days after the TAC or sham procedure). Food intake and body weights were constant throughout the duration of the experiment and did not differ between groups (Supplementary Figure 1). I\textsubscript{Na,late} inhibition decreased QTc-duration in sham mice, and moreover prevented TAC-induced QTc-prolongation (Supplementary Table 3). Blocking I\textsubscript{Na,late} did not affect the magnitude of TAC-induced cardiac hypertrophy, as indicated by a similar increase in pro-hypertrophic markers in WT-TAC and MUT-TAC mice that were fed Ranolazine chow (Figure 4B,C; Supplementary Table 2). Yet, I\textsubscript{Na,late} blockade prevented SCD and attenuated markers in WT-TAC and MUT-TAC mice that were fed Ranolazine chow (Figure 4B,C; Supplementary Table 2). Yet, I\textsubscript{Na,late} blockade prevented SCD and attenuated (atrio-)ventricular conduction abnormalities in MUT mice subjected to TAC (Figure 4D-F, Supplementary Table 2). Hence, pharmacological inhibition of the mutation-induced enhanced I\textsubscript{Na,late} rescued the TAC-induced deleterious interaction between hypertrophy and the Scn5a<sup>1798insD/+</sup> mutation.

Discussion

Our findings point to a modulatory effect of hypertension and consequent cardiac hypertrophy on age-dependent risk for sudden arrhythmic death and pacemaker treatment efficacy in carriers of the SCN5A-1795insD mutation. This is supported by our observations in Scn5a<sup>1798insD/+</sup> mice carrying the homologous mutation, where cardiac hypertrophy (either occurring with age or induced by TAC) led to severe conduction disturbances and an increased risk for ventricular arrhythmias and/or SCD. This study for the first time provides evidence for a modulatory role of co-morbidity in modulating disease severity of an inherited arrhythmic disease, demonstrating a pro-arrhythmic gene-environment interaction.
Modulatory effect of cardiac hypertrophy on (age-dependent) arrhythmic phenotype

Cardiac hypertrophy, which commonly occurs as a consequence of hypertension, develops over time and progressively remodels the myocardium. Its impact is therefore expected to increase with age, potentially altering disease severity in mutation carriers in an age-dependent manner. The observed age-dependent shift from bradycardia-induced (prevented by pacemaker therapy) to apparent bradycardia-independent arrhythmias and SCD in older SCN5A-1795insD mutation carriers moreover indicates a modulatory effect on disease expression. Our clinical findings are further supported by our observations in Scn5a<sup>1798insD/+ </sup>129P2-Scn5a<sup>1798insD/+ </sup>mice) developed a more pronounced arrhythmic phenotype with significantly more spontaneous and inducible ventricular arrhythmias. Although other factors besides hypertrophy, that differ between the patients and between the two mouse strains may also contribute, collectively this human and mouse data support the concept that age-dependent development of cardiac hypertrophy interacts with sodium channel dysfunction to predispose the heart to ventricular arrhythmias and SCD. Data obtained in the TAC studies in young-adult Scn5a<sup>1798insD/+ </sup>mice provide direct proof of the modulatory role of hypertrophy on disease expression. Despite the fact that wild type and Scn5a<sup>1798insD/+ </sup>mice developed similar extent of cardiac hypertrophy secondary to TAC, SCD and conduction abnormalities were observed only in Scn5a<sup>1798insD/+ </sup>mice with chronic pressure overload. In contrast to aged 129P2 Scn5a<sup>1798insD/+ </sup>mice however, no spontaneous or inducible ventricular arrhythmias were observed in mutant mice following TAC (data not shown). The young-adult age of the mice subjected to TAC, the relatively abrupt development of hypertrophy secondary to TAC (in contrast to gradual progression with age), and the short duration of TAC (two weeks) may underlie this apparent discrepancy. One should note however that as for aged Scn5a<sup>1798insD/+ </sup>mice, young-adult Scn5a<sup>1798insD/+ </sup>mice developed ventricular conduction slowing post-TAC, a phenomenon that is well established to promote ventricular arrhythmias.

Potential mechanisms underlying modulatory role of hypertrophy

The mechanisms underlying the modulatory, pro-arrhythmic effects of hypertension and consequent hypertrophy may be numerous and complex. Hypertrophy is associated with progressive electrical (alterations in sodium current and other ion channels), homeostatic (dysregulation of intracellular calcium homeostasis) and structural (collagen deposition) remodelling.<sup>15–18</sup> These alterations may be further exacerbated in the setting of an SCN5A mutation, acting synergistically with the biophysical defects caused by the SCN5A mutation in
creating a highly arrhythmogenic environment.\textsuperscript{19,20} In support of this, we found that similar levels of hypertrophy induced a pro-arrhythmic phenotype in \textit{Scn5a}\textsuperscript{1798insD/+} but not wild type mice, indicating a synergistic interaction between hypertrophy and the mutation. Moreover, TAC-induced conduction disturbances and SCD were prevented by either blocking the downstream pro-hypertrophic response (by genetic deletion of \textit{Nfatc2}) or by pharmacological inhibition of the detrimental consequences of the mutation (i.e. through late sodium current inhibition). Enhanced late sodium current increases sodium influx, which may secondarily lead to increased intracellular calcium concentrations. The latter is a well-established pro-arrhythmic feature of hypertrophy and heart failure, and may be exacerbated in the \textit{SCN5A}-1795insD mutation carriers in the presence of hypertrophy.\textsuperscript{21} The involvement of the enhanced late sodium current defect of the mutation in mediating, at least in part, the observed interaction with hypertrophy is supported by the fact that targeting this molecular defect by late sodium current blockade prevented SCD and attenuated (atrio-)ventricular conduction abnormalities in mutant mice post-TAC.

\textit{Implications for age-dependent treatment efficacy}

The observed age-dependent change in arrhythmia phenotype had crucial consequences for treatment efficacy in \textit{SCN5A}-1795insD mutation carriers: while pacemaker implantation remained 100\% efficacious in preventing SCD in young carriers, it no longer afforded complete protection over the age of 40. Our combined observations in the family and in mice implicate a role for hypertension and consequent hypertrophy in disease expressivity with consequences for treatment efficacy. Other co-morbidities may also play an as yet unidentified modulatory role. These findings therefore underline the necessity to consider additional treatment strategies in older \textit{SCN5A} mutation carriers, including prevention of cardiac hypertrophy through rigorous blood pressure control as well as careful monitoring of co-morbidities. Our observations in \textit{Scn5a}\textsuperscript{1798insD/+} mice furthermore identify pharmacological late sodium current inhibition as a potential target for preventing the pro-arrhythmic sequence of events in the setting of hypertrophy.

\textit{Limitations}

While the homogeneity in genetic cause in the extended \textit{SCN5A}-1795insD family allowed for detection of this interaction, it is associated with the limitation that transferability of these observations to other \textit{SCN5A} mutations, although considered likely, will need to be explored. It is possible that our current findings are specific for “overlap syndrome” mutations associated with both a loss and a gain of sodium channel function. Furthermore, while hypertension and/or
hypertrophy was observed in the majority of older SCN5A-1795insD mutation carriers who suffered a life-threatening event despite pacemaker treatment, we currently do not know the exact prevalence of these co-morbidities in all (non-) carriers in the family.

**Conclusion**

Our findings show that a common co-morbidity (e.g. hypertension) may significantly affect arrhythmia risk and survival in inherited sodium channelopathy. The impact of such unrelated co-morbidities varies with age, leading to potential age-dependent changes in arrhythmia mechanism. As a consequence, efficacy of treatment strategies to prevent SCD in the setting of inherited arrhythmic disease may vary over time, underscoring the need for continuous diagnosis and monitoring of relevant co-morbidities and their rigorous treatment.
Funding

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Acknowledgments

The authors thank Prof. Leon de Windt (Maastricht University Medical Center, The Netherlands) for kindly providing the Nfatc2−/− mice, and Dr. Jan Ruijter (Department of Anatomy, Embryology and Physiology, Academic Medical Center, Amsterdam, The Netherlands) for expert assistance with quantitative RT-PCR data analysis.
Hypertension modulates SCD risk in sodium channelopathy

References

### Supplementary Tables and Figures

#### Supplementary Table 1. ECG parameters of aged (>8 months) wild type (WT) and SnSca<sup>2886delΔ</sup> (MUT) mice of the FVB/N and 129P2 mouse strains

<table>
<thead>
<tr>
<th></th>
<th>Age (months)</th>
<th>Heart rate (BPM)</th>
<th>PR-interval (ms)</th>
<th>QRS-interval (ms)</th>
<th>QTc-interval (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVB/N-WT aged (n=20)</td>
<td>16.0 ± 1.2</td>
<td>473.7 ± 9.3</td>
<td>34.7 ± 0.7</td>
<td>8.4 ± 0.2</td>
<td>44.2 ± 1.1</td>
</tr>
<tr>
<td>FVB/N-MUT aged (n=40)</td>
<td>16.6 ± 0.9</td>
<td>460.5 ± 9.1</td>
<td>38.0 ± 0.9†</td>
<td>10.0 ± 0.3#</td>
<td>47.3 ± 0.6#</td>
</tr>
<tr>
<td>129P2-WT aged (n=29)</td>
<td>14.2 ± 0.8</td>
<td>457.6 ± 8.4</td>
<td>44.2 ± 1.0‡</td>
<td>12.8 ± 0.3‡</td>
<td>43.9 ± 0.8</td>
</tr>
<tr>
<td>129P2-MUT aged (n=56)</td>
<td>13.9 ± 0.6</td>
<td>434.4 ± 7.3§</td>
<td>50.0 ± 0.7§</td>
<td>16.9 ± 0.4§</td>
<td>46.5 ± 0.9**</td>
</tr>
</tbody>
</table>

2-way ANOVA

* Data presented as mean±SEM.
† Data presented as mean±SEM genotype (WT, MUT) X strain (FVB/N, 129P2) interaction.
‡ Data presented as mean±SEM genotype (WT, MUT) X strain (FVB/N, 129P2) interaction.
# Data presented as mean±SEM genotype (WT, MUT) X strain (FVB/N, 129P2) interaction.

#### Supplementary Table 2. Hypertrophic and ex vivo electrophysiological parameters of wild type (WT) and SnSca<sup>2886delΔ</sup> (MUT) mice subjected to Sham or TAC with or without late sodium current inhibition (RAN)

<table>
<thead>
<tr>
<th></th>
<th>HW/TL</th>
<th>Rcan1.4/Gapdh (×10^-3)</th>
<th>AV-delay (ms)</th>
<th>LV AT (ms)</th>
<th>LV CV-Long (cm/s)</th>
<th>LV CV-Trans (cm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT-Sham</td>
<td>84.4 ± 4.7 (n=10)</td>
<td>8.9 ± 1.6 (n=12)</td>
<td>38.1 ± 1.1 (n=11)</td>
<td>7.5 ± 0.2 (n=6)</td>
<td>63.5 ± 1.4 (n=6)</td>
<td>33.5 ± 2.0 (n=6)</td>
</tr>
<tr>
<td>MUT-Sham</td>
<td>85.6 ± 2.9 (n=10)</td>
<td>9.1 ± 2.9 (n=10)</td>
<td>40.6 ± 1.8 (n=9)</td>
<td>7.8 ± 0.4 (n=6)</td>
<td>57.9 ± 1.7 (n=6)</td>
<td>26.9 ± 1.8** (n=6)</td>
</tr>
<tr>
<td>WT-TAC</td>
<td>109.7 ± 5.5* (n=12)</td>
<td>20.0 ± 3.4* (n=6)</td>
<td>37.1 ± 1.3 (n=12)</td>
<td>7.3 ± 0.2 (n=5)</td>
<td>65.2 ± 2.8 (n=5)</td>
<td>39.5 ± 3.1 (n=5)</td>
</tr>
<tr>
<td>MUT-TAC</td>
<td>107.4 ± 4.8† (n=11)</td>
<td>26.1 ± 3.5† (n=8)</td>
<td>59.1 ± 3.0‡ (n=12)</td>
<td>9.3 ± 0.5‡ (n=4)</td>
<td>52.0 ± 1.7†‡ (n=4)</td>
<td>33.2 ± 0.5†‡ (n=4)</td>
</tr>
</tbody>
</table>

2-way ANOVA

2-way interaction

* Data presented as mean±SEM.
† Data presented as mean±SEM genotype (WT, MUT) X strain (FVB/N, 129P2) interaction.
‡ Data presented as mean±SEM genotype (WT, MUT) X strain (FVB/N, 129P2) interaction.
§ Data presented as mean±SEM genotype (WT, MUT) X strain (FVB/N, 129P2) interaction.

2-way interaction denotes SnSca<sup>2886delΔ</sup> genotype (WT, MUT) X procedure (sham, TAC) interaction.

### Notes

- **p<0.05 vs. FVB/N-MUT aged; †p<0.05 vs. FVB/N-WT aged; ‡p<0.005 vs. FVB/N-WT aged; ††p<0.005 vs. 129P2-WT aged; †∗p<0.05 vs. FVB/N-MUT aged; **p<0.05 vs. 129P2-WT aged.
- †Kruskal Wallis test
### Supplementary Table 3. ECG parameters of wild type (WT) and SnsSa+/SnsSa+ (MUT) mice subjected to Sham or TAC with or without late sodium current inhibition (RAN)

<table>
<thead>
<tr>
<th></th>
<th>Heart rate (bpm)</th>
<th>PR interval (ms)</th>
<th>QRS duration (ms)</th>
<th>QTc interval (ms)</th>
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<tr>
<td>WT-Sham (n=30)</td>
<td>482.1 ± 15.0</td>
<td>33.0 ± 0.5</td>
<td>8.4 ± 0.1</td>
<td>42.3 ± 0.6</td>
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<tr>
<td>MUT-Sham (n=20)</td>
<td>471.0 ± 16.2</td>
<td>36.2 ± 0.4†</td>
<td>9.1 ± 0.2†</td>
<td>43.8 ± 0.8</td>
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<tr>
<td>WT-TAC (n=25)</td>
<td>464.2 ± 14.8</td>
<td>33.4 ± 0.8</td>
<td>8.8 ± 0.1</td>
<td>44.6 ± 0.5</td>
</tr>
<tr>
<td>MUT-TAC (n=34)</td>
<td>403.5 ± 13.4††</td>
<td>38.0 ± 0.8§§</td>
<td>10.5 ± 0.3§§</td>
<td>45.4 ± 0.5</td>
</tr>
</tbody>
</table>

2-way ANOVA

- WT-Sham RAN (n=12): 410.4 ± 21.6 (36.6 ± 0.7, 9.1 ± 0.3, 39.4 ± 0.6***
- MUT-Sham RAN (n=12): 361.3 ± 23.0 (37.6 ± 0.9, 9.6 ± 0.2, 41.5 ± 0.6**
- WT-TAC RAN (n=7): 359.4 ± 21.1 (36.0 ± 0.6, 9.4 ± 0.4, 40.9 ± 1.1**
- MUT-TAC RAN (n=12): 344.6 ± 23.2 (36.8 ± 0.9, 9.7 ± 0.3**, 42.3 ± 0.8**

Data presented as mean±SEM; RAN: ranolazine chow.

2-way interaction denotes SnsSa genotype (WT, MUT) X procedure (sham, TAC) interaction.

* p<0.005 vs. WT-TAC; † p<0.005 vs. MUT-Sham; ‡ p<0.005 vs. WT-Sham; § p<0.0005 vs. WT-TAC; ‖ p<0.05 vs. WT-Sham; ⋆ p<0.0005 vs. MUT-Sham; ⋆⋆ p<0.05 vs. similar experimental group without RAN

### Supplementary Table 4. Hypertrophic and ex vivo electrophysiological parameters of wild type (WT) and SnsSa+/SnsSa+ (MUT) mice subjected to Sham or TAC with (Nfatc2+−) or without (Nfatc2−−) genetic deletion of Nfatc2

<table>
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<tr>
<th></th>
<th>HW/TL</th>
<th>Ran14/Gapd (10^-3)</th>
<th>AV-delay (ms)</th>
<th>LV AT (ms)</th>
<th>LV CV-Long (cm/s)</th>
<th>LV CV-Trans (cm/s)</th>
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<tbody>
<tr>
<td>WT-Nfatc2−−-Sham</td>
<td>77.6 ± 3.8 (n=6)</td>
<td>9.1 ± 2.3 (n=7)</td>
<td>40.5 ± 2.2 (n=4)</td>
<td>8.3 ± 0.3 (n=2)</td>
<td>72.8 ± 0.3 (n=2)</td>
<td>33.2 ± 0.7 (n=2)</td>
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<tr>
<td>MUT-Nfatc2−−-Sham</td>
<td>74.6 ± 3.6 (n=6)</td>
<td>6.6 ± 1.5 (n=7)</td>
<td>43.0 ± 2.8 (n=3)</td>
<td>8.5 ± 0.2 (n=3)</td>
<td>64.2 ± 4.3 (n=3)</td>
<td>33.1 ± 5.6 (n=3)</td>
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<tr>
<td>WT-Nfatc2−−-TAC</td>
<td>103.9 ± 6.5* (n=10)</td>
<td>31.3 ± 3.2* (n=13)</td>
<td>44.3 ± 1.5 (n=5)</td>
<td>8.5 ± 0.3 (n=3)</td>
<td>76.8 ± 2.0 (n=3)</td>
<td>37.0 ± 3.3 (n=3)</td>
</tr>
<tr>
<td>MUT-Nfatc2−−-TAC</td>
<td>104.1 ± 4.1† (n=13)</td>
<td>22.4 ± 2.9† (n=18)</td>
<td>58.9 ± 3.8†† (n=9)</td>
<td>10.6 ± 0.4†† (n=5)</td>
<td>55.6 ± 2.7†† (n=5)</td>
<td>31.3 ± 0.8 (n=5)</td>
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</table>

2-way ANOVA

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>WT-Nfatc2−−-Sham</td>
<td>p&lt;0.0005 NS</td>
<td>p&lt;0.0005 NS</td>
<td>p&lt;0.0005 NS</td>
<td>p&lt;0.0005 NS</td>
<td>p&lt;0.05 NS</td>
<td>NS</td>
</tr>
<tr>
<td>MUT-Nfatc2−−-Sham</td>
<td>81.9 ± 3.2 (n=7)</td>
<td>10.8 ± 3.0 (n=10)</td>
<td>45.7 ± 4.8 (n=3)</td>
<td>8.5 ± 0.9 (n=3)</td>
<td>67.2 ± 3.9 (n=3)</td>
<td>34.6 ± 5.2 (n=3)</td>
</tr>
<tr>
<td>WT-Nfatc2−−-TAC</td>
<td>91.5 ± 5.7 (n=6)</td>
<td>14.2 ± 2.3 (n=11)</td>
<td>44.1 ± 1.7 (n=9)</td>
<td>8.4 ± 0.3 (n=8)</td>
<td>74.6 ± 1.6 (n=8)</td>
<td>32.3 ± 2.5 (n=8)</td>
</tr>
<tr>
<td>MUT-Nfatc2−−-TAC</td>
<td>93.1 ± 2.6 (n=17)</td>
<td>18.9 ± 2.9 (n=19)</td>
<td>49.0 ± 1.5 (n=12)</td>
<td>9.1 ± 0.3 (n=10)</td>
<td>71.7 ± 2.0 (n=10)</td>
<td>34.9 ± 1.3 (n=10)</td>
</tr>
</tbody>
</table>

Data presented as mean±SEM; AV-delay: atrio-ventricular delay (at BCL of 120 ms); HW/TL: heart weight/tibia length; LV AT: left ventricular activation time; LV CV-Long or Trans: left ventricular conduction velocity in longitudinal or transversal direction; TAC: transverse aortic constriction.

2-way interaction denotes SnsSa genotype (WT, MUT) X procedure (Sham, TAC) interaction.

* p<0.05 vs. WT-Nfatc2−−-Sham; † p<0.005 vs. MUT-Nfatc2−−-Sham; ‡ p<0.05 vs. WT-Nfatc2−−-TAC
Supplementary Figure 1. Body weight and Ranolazine chow intake in Sham and TAC mice. 
(A) Average values for body weight in Sham (left panel) and TAC (right panel) measured during the 14 day duration of the experiment with control or Ranolazine treatment (RAN) in wild type (WT) or Scn5a\textsuperscript{1798insD/+} (MUT). (B) Average values for control or RAN chow intake during the 14 day duration of the experiment for Sham or TAC mice.
Supplementary Figure 2. Continuous 24-hour telemetric ECG recordings in Sham and TAC mice. (A) Typical examples of ECG traces obtained by telemetry in a Scn5a<sup>1798insD/+</sup> (MUT-TAC) mouse after TAC surgery and 1-2 days prior to sudden cardiac death (SCD), showing slow heart rate, QRS-widening, and AV-block. (B) Average values for PR-interval and QRS-duration measured 1 to 4 days prior to SCD or end of TAC period in MUT-TAC mice with SCD (n=4) and MUT-TAC mice who survived the 2-week TAC period (n=2).