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A truncating *SCN5A* mutation combined with genetic variability causes sick sinus syndrome and early atrial fibrillation

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Abstract

Background—Mutations in the *SCN5A* gene, encoding the α -subunit of the cardiac Na^+ channel, $\text{Na}_v1.5$, can result in several life-threatening arrhythmias.

Objective—To characterize a distal truncating *SCN5A* mutation, R1860Gfs*12, identified in a family with different phenotypes including sick sinus syndrome (SSS), atrial fibrillation (AF), atrial flutter and atrioventricular-block.

Methods—Patch-clamp and biochemical analysis were performed in HEK293 cells transfected with wild-type (WT) and/or mutant channels.

Results—The mutant channel expressed alone caused a 70% reduction in I_{Na} density compared to WT currents, consistent with its partial proteasomal degradation. It led also to a negative shift of steady-state inactivation and to a persistent current. When mimicking the heterozygous state of the patients by co-expressing WT and R1860Gfs*12 channels, the biophysical properties of I_{Na} were still altered, and the mutant channel α -subunits still interacted with the WT ones. Since the

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proband developed paroxysmal AF at young age, we screened 17 polymorphisms associated with AF risk in this family, and showed that the proband carries at-risk polymorphisms upstream of *PITX2*, a gene widely associated with AF development. In addition, when mimicking the difference in resting membrane potentials between cardiac atria and ventricles in HEK293 cells, or using computer-model simulation, R1860Gfs*12 induced a more drastic decrease in I_{Na} at the atrial potential.

Conclusion—We have identified a distal truncated *SCN5A* mutant associated with gain- and loss-of-function effects, leading to SSS and atrial arrhythmias. A constitutively higher susceptibility to arrhythmias of atrial tissues and genetic variability could explain the complex phenotype observed in this family.

Keywords

Arrhythmia; Atrial fibrillation; $Na_v1.5$; *SCN5A*; Sodium; *PITX2*; polymorphism; SNP

Introduction

Coordinated activity of multiple ion channels tightly controls generation and propagation of cardiac action potentials (AP)¹. Mutations in the *SCN5A* gene, encoding the $Na_v1.5$ α -subunit of the cardiac sodium channel, have been involved in numerous inherited cardiac arrhythmias including long QT syndrome (LQTS), Brugada syndrome (BrS), and rare cases of sick sinus syndrome (SSS) and atrial fibrillation (AF)². Atrial arrhythmias are being increasingly diagnosed in patients with BrS (incidence of 6-38%)³, as well as LQTS⁴. Originally, the various *SCN5A*-related arrhythmias were considered separate clinical entities with distinct phenotypical characteristics. Recently, a wide spectrum of mixed disease phenotypes was reported in these arrhythmias, referred to as overlap syndrome of cardiac Na^+ channelopathy². The reasons why the same *SCN5A* mutation can result in different phenotypes remain unknown, but it raises the possibility that the disease expressivity is influenced by altered biophysical properties and genetic modifiers⁵.

In this study, we characterized the $Na_v1.5$ C-terminal truncating mutation R1860Gfs*12 identified in a family presenting with a complex clinical picture of SSS and AF or atrial flutter. Heterologous expression of the mutant channels alone or with wild-type (WT) channels led to a reduction in I_{Na} density, a persistent current and a drastic alteration of the inactivation properties. Interestingly, because of the constitutively different resting membrane potentials in atrial and ventricular tissues, the atrium of the patients might be more susceptible to the altered biophysical properties of the mutant channels and, thus, more prompt to develop arrhythmias. Moreover, the proband carries at-risk polymorphisms upstream of *PITX2*, a gene widely associated with AF development. Altogether, our results could explain the mixed clinical phenotype of this family.

Methods

Patient

Blood samples were obtained after signed written informed consent for genetic analyses and after approval by the local ethics committee. The study was conducted according to the principles of the Helsinki Declaration.

Mutation and SNP analysis

Genomic DNA was isolated from leukocytes according to standard procedures. Screening for mutations was performed by genomic DNA amplification of all exons and splice junctions of several genes responsible for arrhythmias (Supplemental methods). We also genotyped single nucleotide polymorphisms (SNP) associated with AF^{6,7} (Supplemental Table 1).

PCR products were directly sequenced with the Big Dye Terminator v.3.1 kit on an ABI PRISM 3730 automatic DNA sequencer (Applied Biosystems). Variants were identified by visual inspection of the sequences with Seqscape software (Applied Biosystems).

SCN5A cDNA cloning and mutagenesis

Plasmids pcDNA3.1-hH1a (no tag) and pcDNA3.1-GFP-hH1a (N-terminal-GFP) were the gift of Dr H. Abriel (Bern, Switzerland). The plasmid pRcCMV-FLAG-SCN5A (N-terminal-FLAG) was the gift of Dr N. Makita (Nagasaki, Japan). All these plasmids contain the hH1a isoform of *SCN5A*. Na_v1.5 mutant R1860Gfs*12 was prepared using the QuikChange II XL Site-Directed Mutagenesis Kit (Stratagene) according to the manufacturer's instructions and verified by sequencing. The plasmid pIRES2-acGFP1-FHF1B (C-terminal His₆) was the gift of Dr G. S. Pitt (Durham NC, USA).

HEK293 cell culture and transfection

HEK293 cells were transfected with jetPEI (Polyplus Transfection, New York, USA) according to the manufacturer's instructions. For patch-clamp recordings, HEK293 cells were transfected with pcDNA3.1-GFP-hH1a WT or mutant in 35-mm well dishes, with a total of 0.6 µg of plasmid per 35 mm dish. To mimic the heterozygous state of the patient, cells were co-transfected with 0.3 µg of pcDNA3.1-hH1a (no tag) WT and 0.3 µg of pcDNA3.1-GFP-hH1a mutant. For biochemical analysis, cells were plated in 25-cm² flasks and transfected with 2 µg of pcDNA3.1-GFP-hH1a WT or mutant.

Electrophysiological recordings

Patch-clamp recordings were carried out in the whole-cell configuration at room temperature (~22°C). Solutions for patch-clamp recording are described in the Supplemental methods. Ionic currents were recorded with the amplifier Axopatch 200B (Axon Instruments, CA, USA). Patch pipettes (Corning Kovar Sealing code 7052, WPI) had resistances of 1.5–2.5 MΩ, when filled with pipette medium. Currents were filtered at 10 kHz (–3 dB, 8-pole low-pass Bessel filter) and digitized at 50 kHz (NI PCI-6251, National Instruments, Austin, TX, USA). Data were acquired and analyzed with ELPHY2® software (G. Sadoc, CNRS, Gif/Yvette, France).

Current–voltage relationships (I/V curves) and the steady-state inactivation- V_m protocols were as previously reported⁸. Data for the activation- V_m and steady-state inactivation- V_m relationships of I_{Na} were fitted to the Boltzmann equation as previously reported⁸.

The putative involvement of a persistent Na^+ current was assessed by using 100 μM tetrodotoxine (TTX). Currents were elicited by a 500-ms step to -20 mV from a holding potential of -120 mV. The percentage of persistent current was calculated by dividing the TTX-sensitive I_{Na} amplitude determined at the end of the pulse by the peak I_{Na} amplitude obtained before TTX application.

Computer simulations of atrial and ventricular membrane action potentials were performed using Oxsoft Heart Model V. 4.8 as described in the Supplemental methods.

Protein extraction and Western blot

They were performed as previously reported⁸. Primary antibodies used were: rabbit anti-GFP (1:2000, Torrey Pines Biolabs, USA), mouse anti-Flag (1:500, Sigma, USA), and rabbit anti-GAPDH (1:2000, Abcam, UK). Total protein signals were firstly normalized to GAPDH levels and then to WT levels.

Co-immunoprecipitation

Co-immunoprecipitation experiments were performed as described in the Supplemental methods. Briefly, total cell lysates were incubated with Dynabeads (Dyna, Norway) crosslinked to anti-GFP antibody (Torrey Pines Biolabs, USA) for 2 h at room temperature. Western blots were revealed using either rabbit anti-GFP (Torrey Pines Biolabs, USA), or mouse anti-Flag (Sigma, USA) antibodies.

Statistical analysis

Data are presented as means $\pm$ SEM. Statistical significance was estimated with SigmaPlot® software by Student's t-test or ANOVA, as appropriate. $P < 0.05$ was considered significant.

Results

Identification of a truncating mutation in *SCN5A*

The proband (III.2) was a 26-year-old female diagnosed at 13 with severe SSS associated with severe sinus bradycardia, symptomatic sinus pauses and syncope (Figure 1A and B) which required a pacemaker implantation. At the age of 20, paroxysmal AF was documented in the memories of the pacemaker. She secondarily developed a first degree AV-block (PR = 280 ms, QRS = 80 ms, QTc = 360 ms). Her father (II.2) was diagnosed at 27 with an asymptomatic sinus node dysfunction. At the age of 42, he had one episode of atrial flutter after jogging, and was treated by cardioversion. Moreover, he developed first degree AV-block with left anterior hemi-block (PR = 240 ms, QRS = 80 ms, QTc = 400 ms) (Figures 1A and Ca-b). The proband's uncle (II.1) was diagnosed with SSS which required a pacemaker implantation at 30. None displayed spontaneous Brugada ECG pattern or developed ventricular arrhythmias. The proband's father underwent an ajmaline challenge that excluded BrS ECG pattern (Figure 1Cc-d). It induced bradycardia (40 beats/min),

severe prolongation of the PR interval (300 ms) and the QRS complex (240 ms). In addition, sinus node dysfunction with junctional escape rhythm, and supraventricular premature contractions were observed (Supplemental Figure 1). Echocardiograms ruled out structural heart disease except for mild atrial dilatation in the proband's father. The mother and the proband's sister were asymptomatic (Figure 1A).

We sequenced the coding regions of fourteen genes involved in cardiac arrhythmias and identified a unique mutation in *SCN5A*, a deletion of one base pair (A) at the position 5578 in exon 28 (c.5578delA). This deletion induced a frameshift mutation, p.R1860Gfs*12, which changed the amino acid arginine at position 1860 into glycine followed by 10 frame-shifted amino acids before a premature stop codon (Supplemental Figure 2). The proband, her father and uncle carried this mutation, whereas her mother and sister did not (Figure 1A). This variant has never been described and is absent from publicly available databases.

Suspecting the possible contribution of additional genetic factors for AF development, we genotyped the family members for 17 SNPs that alter AF susceptibility (Supplemental Table 1). Interestingly, the proband, who experienced severe SSS and early onset AF, is the only mutation carrier to have one at-risk allele of rs6817105 and rs2200733 located upstream of the *PITX2* gene, which she received from her mother (Figure 1A and Supplemental Table 1). In addition, the father had 2 copies of the protective allele of rs3853445, another SNP located upstream of the *PITX2* gene, while the proband carried only one copy (Figure 1A and Supplemental Table 1). The multimarker risk score for AF based on combined rs2200733, rs17570669 and rs3853445 genotypes⁹ was higher in the proband (1.74) compared to her father (<1) and uncle (<1).

The R1860Gfs*12 mutation produced a loss- and gain-of-function of Na_v1.5

Na⁺ currents were recorded in HEK293 cells 36 h after transfection with WT or mutant channels. I_{Na} traces and I/V relationships are shown in Figure 2. Peak current densities and *P*-values are given in Table 1. Alone, R1860Gfs*12 showed a drastic reduction of I_{Na} density compared to WT channels (Figure 2A and B). Furthermore, the biophysical characteristics of the C-terminal mutant channels were impaired (Table 1, Figure 2C and D). Indeed, the V_{1/2} of activation for R1860Gfs*12 was shifted by +7.1 mV, and the V_{1/2} of inactivation was shifted by -24.5 mV compared to WT. No change in recovery from inactivation was observed (Supplemental Figure 3). Fast and slow time constants of inactivation (τ_{fast} and τ_{slow}) were significantly increased compared to WT channels (Table 1, Figure 3A-C).

The presence of a TTX-sensitive persistent Na⁺ current was assessed using 100 μ M TTX (Figure 3D and Supplemental Figure 4). The mutant channel generated a persistent current of 4% of the peak current.

Co-expression of WT and R1860Gfs*12 channels also led to a loss- and gain-of-function of Na_v1.5

To mimic the heterozygous state of the patient, cells were co-transfected with the WT channel (no tag) and the R1860Gfs*12 mutant channel (GFP tagged) in a 1:1 ratio. In co-

transfected cells, peak current densities were slightly reduced (Figure 2A and B), but not significantly different from the WT alone, as for the $V_{1/2}$ of activation. In contrast, the $V_{1/2}$ of steady-state inactivation remained significantly shifted by -16.6 mV compared to WT (Table 1, Figure 2C and D). Moreover, $\square_{fast}$ and $\square_{slow}$ were significantly increased at the heterozygous state compared to WT alone (Table 1, Figure 3A and B), and the persistent current was still present (Table 1, Figure 3D and Supplemental Figure 4).

Since the steady-state inactivation of the mutant channels alone or co-expressed with WT channels were drastically shifted, we measured I_{Na} successively from a holding potential (HP) of -86 and -83 mV mimicking the ventricular vs atrial resting membrane potentials^{10,11}. Interestingly, we showed that the 3-mV difference in HPs was sufficient to enhance the reduction of I_{Na} due to the mutation in the atrium compared to the ventricle (Figure 3E). Indeed, the current reduction was 17% for WT channels, 25% for WT + R1860Gfs*12, and 40% for, R1860Gfs*12 at a HP of -83 vs -86 mV.

Differences between WT and heterozygous channel characteristics were included into computer-model simulations of single atrial and ventricular cell membrane action potentials (Supplemental methods). The AP maximum upstroke velocity ($[dV/dt]_{max}$) of the heterozygous state exhibited an important decrease in the atrial (68%) compared to the ventricular myocyte (33%), as well as a marked lengthening of the AP duration in the atria (Figure 4).

The R1860Gfs*12 mutant was partially degraded by the ubiquitin-proteasome system

Western blots of total lysates from transfected HEK293 cells showed a significant decrease of $\approx 70\%$ in the total protein expression of the mutant channels when compared to WT, in accordance with the reduction of the current density (Figure 5A). Moreover, incubation of the cells with the 26S-subunit ubiquitin-proteasome inhibitor, MG132, significantly increased the mutant total expression, suggesting a proteasomal degradation of the R1860Gfs*12 mutant (Figure 5A).

The R1860Gfs*12 mutant interacted with WT α -subunits

We have previously demonstrated by co-immunoprecipitation that $Na_v1.5$ α -subunits interact together⁸. To assess if the R1860Gfs*12 truncating mutant still interacted with the WT channel, we performed co-immunoprecipitation in HEK293 cells transfected with either Flag-WT + GFP-WT or Flag-WT + GFP- R1860Gfs*12, and Flag-WT alone as a negative control. Immunoprecipitations were performed with the anti-GFP antibody, and western blot revealed with the anti-Flag antibody. In cells expressing Flag-WT + GFP-WT or Flag-WT + GFP- R1860Gfs*12, a specific band at the expected size of Flag-WT was present (Figure 5B), indicating that the WT and the R1860Gfs*12 truncated α -subunits interacted with each other.

To test whether the R1860Gfs*12 α -subunits could interact with each other, coimmunoprecipitation was performed in HEK293 cells transfected with Flag-R1860Gfs*12 + GFP-R1860Gfs*12. Figure 5B shows that the interaction between truncated α -subunits still

occurred. Thus, our results suggested that the last 156 amino acids of the C-terminal domain of Na_v1.5 were not essential for the interaction between Na_v1.5 α -subunits.

Discussion

We functionally characterized a novel frameshift deletion in *SCN5A* inducing the most distal truncation of the C-terminus of Na_v1.5 reported so far, identified in a family with early SSS and atrial arrhythmias. We report that the R1860Gfs*12 mutant showed a loss- and gain-of-function phenotype, which, combined with the variable genetic background of the mutation carriers, may explain the patients' clinical features.

Functional characteristics of R1860Gfs*12 channels

Mutations in *SCN5A* are known to cause various types of arrhythmias including BrS, LQTS, sinus node dysfunction, AF, and overlap syndrome². In general, *SCN5A* mutations leading to a decrease in I_{Na} (loss-of-function) have been associated with conduction slowing and BrS. Gain-of-function mutations in *SCN5A* are typical of LQTS. Nevertheless, numerous reports have shown that a single Na_v1.5 mutation may induce various combinations of clinical phenotypes². Here, the R1860Gfs*12 mutant channel expressed alone was mostly degraded, causing a severe reduction in I_{Na} density. Moreover, it induced a 7-mV positive shift of activation, which could delay the action potential, and a dramatic -25 mV shift of the steady-state inactivation, which is likely due to the location of the mutation within a region involved in the inactivation of Na_v1.5 and would decrease I_{Na} and affect the action potential velocity. In addition, we showed an inactivation defect delaying the fast and slow inactivation. This loss-of-function phenotype was associated with gain-of-function characteristics since the mutant produced a significant persistent Na⁺ current. When co-expressing this mutant with WT channels in order to mimic the heterozygosity of the patients, the steady-state inactivation was also importantly left-shifted, and a persistent current was still present. These parameters have been shown to be a substrate for atrial fibrillation by causing repolarization failure, early after-depolarizations and delayed after-depolarizations¹²⁻¹⁴. Moreover, regarding the important role of Na_v1.5 channels in the sino-atrial node function^{15,16}, the negative shift of inactivation and the persistent current could account for the sinus node dysfunction in this family¹⁷.

We have recently shown that Na_v1.5 α -subunits were able to oligomerize, leading to the dominant-negative effect of some BrS *SCN5A* mutations through the retention of WT channels by mutant channels⁸. Here, we showed that the truncated R1860Gfs*12 mutant channels were still interacting with the WT channels. This result suggests that the last 156 amino acids of the C-terminal domain of Na_v1.5 are not the main site for α -subunit interaction. In addition, the significant negative shift of the steady-state inactivation in cells co-expressing mutant and WT channels suggests that Na_v1.5 α -subunits also function as oligomers. Alternatively, the C-terminus of Na_v1.5 is a site of interactions with several proteins that have important roles, not only in channel function but also in the stabilization of the channels within the plasma membrane¹⁸. We verified that the truncated R1860Gfs*12 channel did not interact with FHF1B (Supplemental methods, and Supplemental Figure 5).

A similar loss of interaction with partners such as SAP97 and syntrophin could contribute to a loss of stability and expression of the truncated channels at the cell membrane¹⁸.

R1860Gfs*12 gain- and loss-of-function features underlie a complex clinical phenotype

Since Na_v1.5 channels are expressed in both the atrium and the ventricle, it is unclear why the R1860Gfs*12 mutation carriers showed only atrial but no ventricular arrhythmias. One hypothesis is that the constitutively different electrophysiological properties of atrial and ventricular cells might be an underlying cause. Indeed, it has been shown that the resting membrane potential is more depolarized^{10,11}, and the I_{Na} density is more important with a negative shift in the steady-state inactivation in the atrium compared to the ventricle¹⁰. Moreover, atrial cells are more readily excitable than ventricular cells¹¹. This suggests that the atrium is constitutively more susceptible to develop arrhythmias than the ventricle. This led us to explore the R1860Gfs*12 mutant current density at the atrial vs the ventricular resting membrane potential. Here we showed that a 3-mV difference in resting membrane potentials led to a significant decrease of the peak current by 40% in the atria vs the ventricle for the mutant alone, and by 25% when the mutant was co-expressed with WT channels. These results were confirmed by computer-model simulations showing that co-expression of mutant and WT channels led to a more important decrease in the maximum upstroke velocity ($[dV/dt]_{max}$) in the atrial compared to the ventricular myocytes, as well as a significant increase in the AP duration. Therefore, this *SCN5A* mutation could have different tissue consequences, and could contribute to the predominance of an atrial phenotype in this family.

A very few C-terminal truncating mutations have been identified in Na_v1.5^{19,20}, but their functional consequence have not been studied, except for the C-terminal truncating mutation L1821fs*10. This mutation has been detected in a patient with a complex clinical phenotype of congenital SSS, cardiac conduction disorder with recurrent monomorphic ventricular tachycardia, but no BrS²⁰. Functional characterization of this mutant evidenced biophysical properties very similar to the ones of R1860Gfs*12, displaying a gain- and loss-of-function phenotype. Nevertheless, Tan *et al* only reported the absence of a dominant-negative effect of the mutant on the WT channel I_{Na} density²⁰, while we observed an alteration of the inactivation biophysical parameters and the presence of a persistent current, when R1860Gfs*12 was co-expressed with WT channels. Altogether, these observations suggest that other parameters, such as additional genetic factors, might influence the clinical expression of the R1860Gfs*12 mutation.

Genetic background of the R1860Gfs*12 mutation carriers

The early occurrence of sinus node dysfunction in the proband at the age of 13 years, followed by atrial fibrillation at 20 was suggestive of a specific genetic background in this patient. Thus, we explored the possibility that polymorphisms associated with AF could modulate the phenotypic expression of the R1860Gfs*12 mutation. Interestingly, the proband is the only mutation carrier who received the *PITX2* at-risk alleles for AF (rs6817105, rs2200733)^{6,7}. Furthermore, she received only one *PITX2* protective allele (rs3853445)⁹, while her father had 2 copies. The role of the *PITX2* transcription factor has been reported in the development of the sinus node and pulmonary vein myocardium²¹,

which is an important site for AF initiation. Among the numerous variants associated with AF, the 4q25 variants upstream of the *PITX2* gene have the largest effect in early and late AF^{6,7}. Reduced *PITX2c* expression, the main cardiac isoform, was found in AF patients and was shown to induce atrial electrical and structural remodeling in a murine model with a reduction of mRNA and Na_v1.5 protein levels²¹. Thus, it is tempting to speculate that the presence of 4q25 variants could induce early atrial morphological changes and further loss of function of Na_v1.5 channels in the atrium in the proband, and may thus account for the occurrence of severe SSS with AF at young age compared to her father who had asymptomatic SSS, and her uncle who developed SSS later in his life. Of course, additional factors may also contribute to the clinical phenotypes.

Conclusion

In conclusion, we showed that a C-terminal truncated Na_v1.5 α -subunit interacts with WT α -subunits. Their co-expression induced only a mild reduction of the I_{Na} density but a marked shift in inactivation and gave rise to a persistent current, compatible with SSS and AF. The mutant biophysical defects associated with the difference in resting membrane potentials between atrium and ventricle combined with genetic factors might explain the predominantly atrial expression of this mutant. A better knowledge of the functional effects of mutations in *SCN5A* and the genetic background of the mutation carriers should help in the future to explain the large phenotypical variability of the sodium channelopathies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

AF	atrial fibrillation
AP	action potential
BrS	Brugada syndrome
ECG	electrocardiogram
HP	holding potential
HR	heart rate
I_{Na}	inward sodium current
LQTS	long QT syndrome
SNP	single nucleotide polymorphism
SSS	sick sinus syndrome

TTX	tetrodotoxine
WT	wild type

References

1. Roden DM, Balser JR, George AL Jr, Anderson ME. Cardiac ion channels. *Annu. Rev. Physiol.* 2002; 64:431–475. [PubMed: 11826275]
2. Remme CA. Cardiac sodium channelopathy associated with SCN5A mutations: electrophysiological, molecular and genetic aspects. *J. Physiol.* 2013; 591:4099–4116. [PubMed: 23818691]
3. Francis J, Antzelevitch C. Atrial fibrillation and Brugada syndrome. *J. Am. Coll. Cardiol.* 2008; 51:1149–1153. [PubMed: 18355651]
4. Johnson JN, Tester DJ, Perry J, Salisbury BA, Reed CR, Ackerman MJ. Prevalence of early-onset atrial fibrillation in congenital long QT syndrome. *Heart Rhythm Off. J. Heart Rhythm Soc.* 2008; 5:704–709.
5. Scicluna BP, Wilde AAM, Wilde AW, Bezzina CR. The primary arrhythmia syndromes: same mutation, different manifestations. Are we starting to understand why? *J. Cardiovasc. Electrophysiol.* 2008; 19:445–452. [PubMed: 18284507]
6. Gudbjartsson DF, Arnar DO, Helgadóttir A, et al. Variants conferring risk of atrial fibrillation on chromosome 4q25. *Nature.* 2007; 448:353–357. [PubMed: 17603472]
7. Ellinor PT, Lunetta KL, Albert CM, et al. Meta-analysis identifies six new susceptibility loci for atrial fibrillation. *Nat. Genet.* 2012; 44:670–675. [PubMed: 22544366]
8. Clatot J, Ziyadeh-Isleem A, Maugenre S, Denjoy I, Liu H, Dilanian G, Hatem SN, Deschênes I, Coulombe A, Guicheney P, Neyroud N. Dominant-negative effect of SCN5A N-terminal mutations through the interaction of Nav1.5 α -subunits. *Cardiovasc. Res.* 2012; 96:53–63. [PubMed: 22739120]
9. Lubitz SA, Sinner MF, Lunetta KL, et al. Independent susceptibility markers for atrial fibrillation on chromosome 4q25. *Circulation.* 2010; 122:976–984. [PubMed: 20733104]
10. Burashnikov A, Di Diego JM, Zygmunt AC, Belardinelli L, Antzelevitch C. Atrium-selective sodium channel block as a strategy for suppression of atrial fibrillation: differences in sodium channel inactivation between atria and ventricles and the role of ranolazine. *Circulation.* 2007; 116:1449–1457. [PubMed: 17785620]
11. Golod DA, Kumar R, Joyner RW. Determinants of action potential initiation in isolated rabbit atrial and ventricular myocytes. *Am J Physiol.* 1998; 274:H1902–13. [PubMed: 9841518]
12. Lemoine MD, Duverger JE, Naud P, Chartier D, Qi XY, Comtois P, Fabritz L, Kirchhof P, Nattel S. Arrhythmogenic left atrial cellular electrophysiology in a murine genetic long QT syndrome model. *Cardiovasc Res.* 92:67–74. [PubMed: 21672931]
13. Song Y, Shryock JC, Belardinelli L. An increase of late sodium current induces delayed afterdepolarizations and sustained triggered activity in atrial myocytes. *Am J Physiol Heart Circ Physiol.* 2008; 294:H2031–9. [PubMed: 18310511]
14. Sossalla S, Kallmeyer B, Wagner S, Mazur M, Maurer U, Toischer K, Schmitto JD, Seipelt R, Schöndube FA, Hasenfuss G, Belardinelli L, Maier LS. Altered Na⁺ Currents in Atrial Fibrillation: Effects of Ranolazine on Arrhythmias and Contractility in Human Atrial Myocardium. *J. Am. Coll. Cardiol.* 2010; 55:2330–2342. [PubMed: 20488304]
15. Dobrzynski H, Boyett MR, Anderson RH. New insights into pacemaker activity: promoting understanding of sick sinus syndrome. *Circulation.* 2007; 115:1921–1932. [PubMed: 17420362]
16. Lei M, Goddard C, Liu J, et al. Sinus node dysfunction following targeted disruption of the murine cardiac sodium channel gene *Scn5a*. *J. Physiol.* 2005; 567:387–400. [PubMed: 15932895]
17. Lei M, Huang CL-H, Zhang Y. Genetic Na⁺ channelopathies and sinus node dysfunction. *Prog. Biophys. Mol. Biol.* 2008; 98:171–178. [PubMed: 19027778]

18. Shy D, Gillet L, Abriel H. Cardiac sodium channel Na(V)1.5 distribution in myocytes via interacting proteins: The multiple pool model. *Biochim. Biophys. Acta.* 2013; 1833:886–894. [PubMed: 23123192]
19. Kapplinger JD, Tester DJ, Alders M, et al. An international compendium of mutations in the SCN5A-encoded cardiac sodium channel in patients referred for Brugada syndrome genetic testing. *Heart Rhythm Off. J. Heart Rhythm Soc.* 2010; 7:33–46.
20. Tan B-H, Iturralde-Torres P, Medeiros-Domingo A, Nava S, Tester DJ, Valdivia CR, Tusié-Luna T, Ackerman MJ, Makielski JC. A novel C-terminal truncation SCN5A mutation from a patient with sick sinus syndrome, conduction disorder and ventricular tachycardia. *Cardiovasc. Res.* 2007; 76:409–417. [PubMed: 17897635]
21. Franco D, Christoffels VM, Campione M. Homeobox transcription factor Pitx2: The rise of an asymmetry gene in cardiogenesis and arrhythmogenesis. *Trends Cardiovasc. Med.* 2014; 24:23–31. [PubMed: 23953978]

Figure 1. Pedigree and ECG recordings of the family

(A) Pedigree of the family. The proband (III.2) is indicated by an arrow. Squares represent males, circles females, + and – signs, mutation carriers and non-carriers. The haplotypes for 5 SNPs located upstream of the *PITX2* gene are indicated under each subject. At-risk alleles are written in red and protective ones in blue. (B) ECG recordings of the proband. The pacemaker spikes are indicated by arrows. The proband's ECG showed prolongation of the PR interval, a sign of first degree AV block. (C) ECG recordings of the father (II.2) showing the atrial flutter that he developed at 42 (a), and his normal sinus rhythm after cardioversion with left axis deviation (b). (c and d) ECG recordings of II-2 during the ajmaline test (1mg/kg over 5 min) he underwent at 64. At basal condition (c, time 0), the patient has a regular sinus rhythm with a heart rate (HR) of 60 beats/min, a prolongation of the PR interval of 240 ms, and a QRS duration of 120 ms. After the injection of ajmaline (d, time 7 min), bradycardia was observed (HR= 40 beats/min) with a prolongation of the PR interval (300 ms) and of the QRS duration (240 ms), without sign of BrS. Rapid sodium-bicarbonate infusion was then immediately started.

Figure 2. Electrophysiological characterization of Na_v1.5 channels (1)

(A) Representative families of Na⁺ current traces of WT, R1860Gfs*12 and WT + R1860Gfs*12 channels. (B) Current density-voltage relationships. R1860Gfs*12 current density is significantly reduced compared to WT and WT + R1860Gfs*12 channels. (C) Activation-V_m relationship of R1860Gfs*12 was shifted to more positive potentials compared to WT and WT + R1860Gfs*12 channels. (D) Steady-state inactivation-V_m relationships were shifted towards negative potential for R1860Gfs*12 and WT + R1860Gfs*12 channels compared to WT channels. Numbers of cells and statistical analysis are reported in Table 1.

Figure 3. Electrophysiological characterization of Na_v1.5 channels (2)

(A) and (B) Fast and slow time constants (τ_f and τ_s , respectively) for WT, R1860Gfs*12 and WT + R1860Gfs*12 channels were plotted against the test potential. R1860Gfs*12 and WT + R1860Gfs*12 showed an overall slowing of the fast and slow rates of inactivation compared to the WT. (C) Three superimposed representative I_{Na} traces corresponding to the above conditions. The R1860Gfs*12 and the WT + R1860Gfs*12 current traces were normalized to the peak of the WT current (≈ 1200 pA). (D) Ratios of the persistent Na⁺ current amplitude (end of pulse) over the peak I_{Na} amplitude for WT, WT + R1860Gfs*12 and R1860Gfs*12 channels, reported in percentage. (E) Ratios of the I_{Na} loss between HPs of -86 and -83 mV for WT, WT + R1860Gfs*12 and R1860Gfs*12. Peak current density was calculated at -20 mV. * indicates $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and n the number of tested cells.

Figure 4. Computer model simulations of single atrial cell and ventricular cell membrane action potentials

Computed AP time courses and expanded time-scale inset exhibiting first time derivatives (dV/dt) of the AP upstroke, in A: atrium and in B: ventricle. Computed dV/dt time courses exhibit a higher decrease in $[dV/dt]_{\max}$ for the “heterozygous atrium” condition than for WT (respectively: 68 V/s vs. 212 V/s) whereas the decrease was smaller for “heterozygous ventricle” condition than for WT (respectively: 147 V/s vs. 220 V/s).

Figure 5. Biochemical analysis

HEK cells were transfected with GFP-Nav1.5 (A) and with GFP-Nav1.5 and/or Flag-Nav1.5 (B). Data are represented as arbitrary units. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as total protein loading control. (A) Western blots of total cell lysates show that the R1860Gfs*12 protein quantity is significantly lower than the WT. Incubation of transfected cells with MG132 prevented the degradation of the C-terminal mutant; ****P* 0.001. (B) Co-immunoprecipitation of Nav1.5 α-subunits tagged with either Flag or GFP. Immunoprecipitation was performed with the anti-GFP antibody, and western blot revealed with the anti-Flag antibody. These results demonstrated an interaction between R1860Gfs*12 α-subunits, and between WT and R1860Gfs*12 α-subunits.

Table 1

Biophysical and kinetic properties of I_{Na} from WT and mutant channels

Na ⁺ channels	Peak current density (pA/pF)	Activation			Inactivation				
		$V_{1/2}$ (mV)	P	k (mV)	P	$V_{1/2}$ (mV)	P	k (mV)	P
WT	280.8±22.2 (n=20)	44.2±1.6 (n=17)		7.0±0.4		84.5±1.9 (n=12)		8.1±0.6	
R1860G fsX12	-80.3±1.6 (n=13)	-37.1±1.4 (n=12)	***	6.1±0.6	ns	-108.1±1.8 (n=8)	***	-6.3±0.4	***
1/2 WT+1/2 R1860G fsX12	-237.0±22.5 (n=14)	40.8±2.0 (n=12)	ns	5.9±0.4	ns	101.1±1.7 (n=11)	***	6.3±0.5	***

Data are presented as means ± SEM. Peak current density was calculated at -20 mV. WT indicates wild type

*** P 0.005

*** P 0.001 and ns: not significant compared with WT channel. $V_{1/2}$ indicates half activation or inactivation value, k inverse slope factor, and τ_f and τ_s : fast and slow time constants of I_{Na} inactivation.