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

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BACKGROUND Mutations in the *SCN5A* gene, encoding the α subunit of the cardiac Na^+ channel, $Na_v1.5$, 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, atrial fibrillation (AF), atrial flutter, and atrioventricular block.

METHODS Patch-clamp and biochemical analyses were performed in human embryonic kidney 293 cells transfected with wild-type (WT) and/or mutant channels.

RESULTS The mutant channel expressed alone caused a 70% reduction in inward sodium current (I_{Na}) density compared to WT currents, which was consistent with its partial proteasomal degradation. It also led to a negative shift of steady-state inactivation and to a persistent current. When mimicking the heterozygous state of the patients by coexpressing 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 channels. Since the proband developed paroxysmal AF at a 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 human embryonic kidney 293 cells or when using computer model simulations, 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 sick sinus syndrome 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

ABBREVIATIONS **AF** = atrial fibrillation; **AP** = action potential; **BrS** = Brugada syndrome; **GFP** = green fluorescent protein; **HEK293** = human embryonic kidney 293; **HP** = holding potential; **I_{Na}** = inward sodium current; **LQTS** = long QT syndrome; **SNP** = single nucleotide polymorphism; **SSS** = sick sinus syndrome; **τ_f and τ_s** = fast and slow time constants of inward sodium current inactivation; **TTX** = tetrodotoxine; **WT** = wild type

Introduction

The coordinated activity of multiple ion channels tightly controls the generation and propagation of cardiac action potentials (APs).[1] Mutations in the *SCN5A* gene, encoding the α subunit of the cardiac sodium channel, $Na_v1.5$, 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

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well as LQTS.[4] Originally, 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 an overlap syndrome of cardiac Na^+ channelopathy.[2] The reasons why the same *SCN5A* mutation can result in different phenotypes remain unknown, but it raises the possibility that the disease expressivity is affected by altered biophysical properties and genetic modifiers.[5]

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. The heterologous expression of the mutant channels alone or with wild-type (WT) channels led to a reduction in inward sodium current (I_{Na}) density, a persistent Na+ 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 single nucleotide polymorphism 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 (see the Online Supplemental Methods). We also genotyped single nucleotide polymorphisms (SNPs) associated with AF[6,7] (Online Supplemental Table 1).

The polymerase chain reaction products were directly sequenced with the Big Dye Terminator v.3.1 kit on an ABI PRISM 3730 automated DNA sequencer (Applied Biosystems, Carlsbad, CA, USA). 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 green fluorescent protein (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*. The $Na_v1.5$ mutant R1860Gfs*12 was prepared by using the QuikChange II XL Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA, USA) according to the manufacturer's instructions and verified by sequencing. The plasmid pIRES2-acGFP1-FHF1B (C-terminal His_6) was the gift of Dr G.S. Pitt (Durham, NC).

Human embryonic kidney 293 cell culture and transfection

Human embryonic kidney 293 (HEK293) cells were transfected with jetPEI (Polyplus Transfection, Illkirch, France) 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 well dish. To mimic the heterozygous state of the patient, cells were cotransfected with 0.3 μg of pcDNA3.1-hH1a (no tag) WT (50% WT) and 0.3 μg of pcDNA3.1-GFP-hH1a mutant (50% 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 recordings are described in the Online Supplemental Methods. Ionic currents were recorded with the amplifier Axopatch 200B (Axon Instruments, Foster City, 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). Data were acquired and analyzed with ELPHY2 software (developed by G. Sadoc, CNRS, Gif-sur-Yvette, France).

Current-voltage relationships and steady-state inactivation-V_m relationships were used as reported previously.[8] Data for the activation-V_m and steady-state inactivation-V_m relationships of I_{Na} were fitted to the Boltzmann equation as reported previously.[8]

The putative involvement of a persistent Na^+ current was assessed by using 100 μM tetrodotoxine (TTX). Currents were increased from a holding potential (HP) of −120 to −20 mV in 500-ms steps. 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 APs were performed by using Oxsoft Heart model (Version 4.8, Oxsoft Ltd, Oxford, England) as described in the Online Supplemental Methods.

Protein extraction and Western blot

They were performed as reported previously.[8] Primary antibodies used were as follows: rabbit anti-GFP (1:2000, Torrey Pines Biolabs, Houston, TX, USA), mouse anti-Flag (1:500, Sigma, St Louis, MO, USA), and rabbit anti–glyceraldehyde 3-phosphate dehydrogenase (1:2000, Abcam, Cambridge, UK). Total protein signals were first normalized