Published in final edited form as:

Heart Rhythm. 2009 August ; 6(8): 1170–1175. doi:10.1016/j.hrthm.2009.04.034.

Unique Mixed Phenotype and Unexpected Functional Effect Revealed by Novel Compound Heterozygosity Mutations Involving *SCN5A*

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Abstract

Background—Functional characterization of mutations involving the *SCN5A*-encoded cardiac sodium channel has established the pathogenic mechanisms for type 3 long QT syndrome (LQT3) and type 1 Brugada syndrome and has provided key insights into the physiological importance of essential structure-function domains.

Objective—To present the clinical and biophysical phenotypes discerned from compound heterozygosity mutations in *SCN5A* on different alleles in a toddler diagnosed with QT prolongation and fever induced ventricular arrhythmias.

Methods—A 22-month-old male presented emergently with fever and refractory ventricular tachycardia. Despite restoration of sinus rhythm, the infant sustained profound neurological injury and died. Using PCR, DHPLC, and direct DNA sequencing, comprehensive open reading frame/splice mutational analysis of the 12 known LQTS-susceptibility genes was performed.

Results—The infant had two *SCN5A* mutations: a maternally inherited N-terminal frameshift/deletion (R34fs/60) and a paternally inherited missense mutation, R1195H. The mutations were engineered by site-directed mutagenesis and heterologously expressed transiently in HEK293 cells. As expected, the frame-shifted and prematurely truncated peptide, *SCN5A*-R34fs/60, showed no current. *SCN5A*-R1195H had normal peak and late current but abnormal voltage-dependent gating parameters. Surprisingly, co-expression of *SCN5A*-R34fs/60 with *SCN5A*-

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R1195H elicited a significant increase in late sodium current, while co-expression of SCN5A-WT with SCN5A-R34fs/60 did not.

Conclusions—A severe clinical phenotype characterized by fever-induced monomorphic ventricular tachycardia and QT interval prolongation emerged in a toddler with compound heterozygosity involving SCN5A: R34fs/60, and R1195H. Unexpectedly, the 94-aminoacid “fusion” peptide derived from the R34fs/60 mutation accentuated the late sodium current of R1195H-containing Na_v1.5 channels in vitro.

Keywords

Sudden Death; Sodium Channel; Long QT Syndrome; Channelopathies; Ventricular Tachycardia

INTRODUCTION

Voltage-gated sodium channels are responsible for action potential initiation and propagation in excitable cells. Nine members of the voltage-gated sodium channel family have been characterized in mammals and a 10th member has been recognized as a related protein¹. The cardiac sodium channel isoform or Na_v1.5 encoded by *SCN5A* underlies the rapid upstroke (phase 0) of the action potential recorded in atrial and ventricular cells. Gain-of-function mutations in *SCN5A* increase late or persistent *I*_{Na} and cause type 3 long QT syndrome (LQT3)². Loss-of-function mutations on Na_v1.5 channel decrease peak *I*_{Na} and are responsible of type 1 Brugada syndrome (BrS1)³, progressive cardiac conduction disease (PCCD)⁴, congenital sick sinus syndrome (SSS)⁵ and more rarely, ventricular tachycardia (VT)⁶⁻⁹, atrial fibrillation (AF)¹⁰, and dilated cardiomyopathy (DCM) ¹¹.

Functional analyses of human genetic variants have provided key insights into the physiological importance of essential structure-function domains and represent an opportunity to dissect the mechanistic basis of human arrhythmogenic disease. In this report, we analyze the electrophysiological implications of compound heterozygosity in *SCN5A* involving a frame shift truncation mutation, R34fs/60, at the N-terminal region of Na_v1.5 and the R1195H missense mutation localizing to the interdomain linker II-III in a toddler with an atypical and severe mixed arrhythmia phenotype, characterized by fever-induced monomorphic ventricular tachycardia and QT interval prolongation. Functional characterization of these mutations revealed that the Na_v1.5 N-terminal region may influence channel inactivation and may participate in channel-channel interaction of these monomeric alpha subunits.

METHODS

Mutational analysis

Comprehensive open reading frame/splice site mutational analysis of all known LQTS-susceptibility genes: *KCNQ1*, *KCNH2*, *SCN5A*, *ANKB*, *KCNE1*, *KCNE2*, *KCNJ2*, *CACNA1C*, *CAV3*, *SCN4B*, *AKAP9*, and *SNTA1*, was performed using denaturing high performance liquid chromatography (DHPLC), and direct DNA sequencing. The study was performed according to the terms required by the Research Ethics Committee of the National Institute of Cardiology “Ignacio Chávez”, México City and the Mayo Foundation Institution Review Board; written informed consent was obtained from all participants.

Site-directed mutagenesis and heterologous expression

The R34fs/60 and R1195H mutations were created by site-direct mutagenesis (mutagenesis kit from Stratagene®) using a PCR technique. The appropriate nucleotide changes was engineered into the most common splice variant of human cardiac voltage-dependent Na

channel *SCN5A/hNa_v1.5* [lacking a glutamine at position 1077 (Genbank accession No. AY148488)] in the pcDNA3 vector (Invitrogen; Carlsbad, CA). The integrity of the constructs was verified by DNA sequencing. WT and mutant channels were expressed transiently in HEK-293 cells for functional study as described previously^{12, 13}.

Standard electrophysiological measurements for functional characterization

Macroscopic I_{Na} was measured using a standard whole-cell patch clamp method at a temperature of 22°–24°C. Details have been published previously¹³. The extracellular (bath) solution contained, in mmol/L, NaCl 140, KCl 4, CaCl₂ 1.8, MgCl₂ 0.75 and HEPES 5 (pH 7.4 set with NaOH). The pipette solution contained, in mmol/L, CsF 120, CsCl 20, EGTA 2 and HEPES 5 (pH 7.4 set with CsOH). Pipettes had resistances between 1.0 and 2.0 MΩ when filled with recording solution. The data were acquired using pClamp 8.2 (Axon Instruments Inc., Union City, CA) and analyzed using Clampfit (Axon Instruments Inc.). The standard voltage clamp protocols are presented with the data and as described in detail previously¹⁴.

Statistic Analysis

Data are shown as symbols with standard error of the mean (S.E.M.). Determinations of statistical significance were performed using One-way ANOVA for comparisons of two groups. A *p* value of < 0.05 was considered statistically significant. Curve fits are done using pClamp 8.2 (Axon Instruments). Non-linear curve fitting is performed with Origin 6.0 (Microcal Software).

RESULTS

Clinical Case

The index case was a 22-month-old male with history of febrile seizures, who presented emergently with upper respiratory tract infection, fever, and syncope. A very rapid pulse was noted, the electrocardiogram revealed monomorphic ventricular tachycardia at 250 bpm. After electrical cardioversion, normal sinus rhythm was obtained but there was marked prolongation of the QT interval (QTc, 519 ms, Figure 1). Serum electrolytes were within normal limits. An echocardiogram demonstrated a structurally and hemodynamically normal heart. The toddler continued to develop short episodes of ventricular tachycardia for which propafenone infusion was started. The next day refractory ventricular monomorphic tachycardia occurred. Despite multiple electrical shocks and eventual restoration of normal sinus rhythm, the infant sustained profound neurological injury and died (case examined by AMD and PIT). Necropsy confirmed a structurally normal heart.

Post-Mortem Genetic Testing

Two mutations were detected in *SCN5A*. First, a maternally inherited base pair deletion (G) at position 105 in exon 2 produced a frame-shift mutation, R34fs/60. This annotation indicates that the last normal amino acid is an arginine (R) at position 34 followed by 60 frame-shifted amino acids before truncating prematurely. The final product of this mutant allele ends at amino acid 94, resulting in a severe truncation of nearly the entire protein (Figure 2A, 2C). R34fs/60 is therefore essentially a “fusion” peptide whereby only the first 34 amino acids are identical to the N-terminus of NaV1.5. Notably, the decedent’s mother was asymptomatic at 44 years of age and her ECG was normal. Specifically, there was no suggestion of a Brugada ECG pattern or conduction disease. A second paternally inherited missense mutation was detected, at position 3584 (G>A) in exon 20, producing a histidine (H) instead of an arginine (R) at position 1195 (R1195H, Figure 2B, 2C). This residue is

highly conserved across different species (Figure 2D). The father was also asymptomatic at age of 47 years with a reportedly normal ECG.

Functional Expression

Transfected HEK-293 cells transiently expressing either SCN5A wild type (WT), R34fs/60, R1195H, SCN5A-WT + SCN5A-R34fs/60 or SCN5A-R1195H + SCN5A-R34fs/60 were voltage clamped after 24-h incubation. As anticipated given the nature and severity of the truncation mutation, the R34fs/60 “fusion” peptide showed no current even with an increased incubation time to 48 hours or with co-expression of the *SCNBI*-encoded beta 1 subunit (data not shown). On the other hand, R1195H had normal peak and late current but abnormal voltage-dependent gating parameters (Table 1, Figure 3A-B, Figure 4). Surprisingly, co-expression of SCN5A-R34fs/60 + SCN5A-R1195H significantly accentuated the late (persistent) sodium current compared to SCN5A-R1195H alone (Figure 3C-D), but co-expression of SCN5A-R34fs/60 + SCN5A-WT did not augment the late sodium current compared to SCN5A-WT alone. The activation and inactivation midpoint in mutant channels of SCN5A-R1195H were significantly voltage shifted negatively compared to WT channel (Table 1 and Figure 4A-B), recovery from inactivation for SCN5A-R1195H was slower than WT channel (Table 1 and Figure 4C) and intermediate inactivation was enhanced for SCN5A-R1195H compared to SCN5A-WT (Figure 4D).

DISCUSSION

We present a case of a toddler with severe and atypical mixed phenotype of fever-induced monomorphic ventricular tachycardia and QT interval prolongation secondary to compound heterozygosity involving *SCN5A*. Considering that the trademark dysrhythmia of LQTS is actually *torsades de pointes*, monomorphic ventricular tachycardia is not an expected arrhythmia of LQTS. However, the unique compound heterozygous mutations found in this case accounts for the phenotype. SCN5A- R34fs/60 if translated generates the earliest Nav1.5 truncation mutation reported in the literature and resulted in a natural occurring Nav1.5 genetic haploinsufficiency (i.e. I_{Na} produced solely from the unaffected allele) and theoretically, loss-of-function phenotype. Patients with loss of function mutations, like Brugada Syndrome type 1 (BrS1) and progressive cardiac conduction defects, can exhibit monomorphic ventricular tachycardia⁶⁻⁹. It is known that BrS phenotype is rarely manifested during childhood¹⁵ and several similar cases reported in the literature with Nav1.5 genetic haploinsufficiency due to premature protein truncations exhibit an age-dependent progressive CCD or BrS phenotype¹⁶⁻¹⁹. The proband did display however “febrile seizures” as a consequence of ventricular tachycardia; fever is now a well appreciated arrhythmogenic trigger for patients with BrS²⁰⁻²². The prolonged QT interval observed could be explained by the second *SCN5A* mutation, R1195H, as all “functional” Nav1.5 channels in the decedent would have had this missense mutation that exhibits a mixed cellular phenotype of both gain and loss-of-function alterations in its kinetic properties plus accentuation of its late or persistent sodium current conferred by the R34fs/60 “fusion” peptide if the decedent’s other mutant allele is translated.

The Nav1.5 N-terminal region comprises the first 129 residues of the 2015 or 2016 amino-acid-containing Nav1.5 alpha subunit. The presence of common polymorphisms in Nav1.5’s N-terminus is low. Ackerman et. al. reported the prevalence of *SCN5A* variants in 829 unrelated healthy subjects; just one polymorphism was found in the N-terminal region, R34C and less commonly R34H²³. A second polymorphism has been reported among Japanese subjects, V120I²⁴. The regulatory role(s) of Nav1.5’s N-terminus on channel function have not been explored before, however a significant number of putative disease associated mutations localize to this region. To date, 9 putative channelopathy-associated missense mutations have been reported in the Nav1.5 N-terminus including 3 LQT3-

associated mutations (G9V, R18W, and V125L)²⁵ and 5 BrS1-associated mutations (R27H 26, G35S27, Q55X18, V95I28, R104Q27, K126E29).

Recently, Lee et al.³⁰ reported an interesting approach to test the hypothesis that the carboxy and amino-terminal regions modulate channel gating. A chimeric channel was constructed using Na_v1.2 and Na_v1.6 isoforms and their respective amino and carboxy-terminal regions were swapped. This exchange revealed that the N-terminus can shift the V_{1/2} of inactivation as well as impart isoform-specific voltage-dependence of activation suggesting that the N-terminus is likely involved in the conformational changes coupling activation to inactivation³⁰. In another isoform, Na_v1.8 encoded by *SCN10A*, the interaction of annexin II light chain with the N-terminal domain is essential for the functional expression of the channel³¹, and the interacting residues are highly conserved across voltage gated sodium channel isoforms.

Surprisingly, co-expression of the *SCN5A* mutants, R34fs/60 and R1195H, markedly accentuated the late or persistent sodium current, suggesting that the 94 amino acid, N-terminal mutant “fusion” peptide may be interacting with and modulating the inactivation properties of the full-length, R1195H-containing sodium channel alpha subunit to increase late current. Yet, this 94 amino acid-containing “fusion” peptide only bares identity to the proximal 34 amino acids of the N-terminus. However, similar interaction between previously presumed as *independent* sodium channel monomers has been reported before in other sodium channel isoforms. Kamiya et.al. reported a nonsense heterozygous mutation R102X in the N-terminus of the voltage gated sodium channel isoform Na_v1.2 encoded by *SCN2A*³² in a child with intractable epilepsy and severe mental decline. This 102 amino acid-containing polypeptide exerted a dominant negative effect on the wild-type Na_v1.2 channel increasing the susceptibility of wild-type channels to inactivate.

In our study, however, the 94 amino acid-containing “fusion” peptide generated by the mutant *SCN5A*- R34fs/60, accentuated the late sodium current in R1195H-containing Na_v1.5 channels while exerting no effect on the *SCN5A*-WT channels suggesting the possibility that *SCN5A*-R1195H may be creating a “receptive binding site” as the peptide only augmented its late current but not the late current of WT channels. This intriguing mechanism is speculative, however, as no direct physical interaction between this mutant peptide and *SCN5A*-R1195H has been demonstrated and further, we did not measure single channel recordings to demonstrate that the observed accentuation in late sodium current stems from re-openings of the R1195H-containing Na_v1.5 channels. Nevertheless, such interaction and regulation between these monomeric alpha subunits have been demonstrated before by the observation that phenotypic severity is modified by several *SCN5A* polymorphisms. For example, the common polymorphism *SCN5A*-H558R restores the trafficking defect of a BrS1 mutation in a different allele³³ and the polymorphism *SCN5A*-R1193Q mitigates the adverse effect conferred by the nonsense mutation *SCN5A*-W1421X³⁴. Interestingly, we observed in vitro that despite the premature Na_v1.5 truncation; this peptide still has the potential to regulate the cellular phenotype of an alpha subunit monomer, albeit one containing the missense mutation, R1195H.

In summary, we identified compound heterozygosity mutations involving *SCN5A*: R34fs/60 and R1195H in a toddler with an unusual mixed phenotype characterized by fever-induced monomorphic ventricular tachycardia and QT interval prolongation. The “fusion” peptide, of course, yielded no sodium current. Unexpectedly, this 94-aminoacid “fusion” peptide accentuated the late or persistent sodium current of R1195H-containing Na_v1.5 channels. These in vitro results suggest an important regulation between Na_v1.5 subunits even in the presence of premature protein truncation and revealed a potential role of Na_v1.5's N-terminal region on its channel inactivation that requires further examination.

In the clinical setting, we observed a fever-induced monomorphic ventricular tachycardia, as the only clinical manifestation of sodium channel loss of function mutation; this has important clinical implications in the management of ventricular arrhythmias in pediatric population, because these cases might severely deteriorate after the administration of sodium channel blockers.

Acknowledgments

Dr. Medeiros received financial support through CONACyT and FUNSALUD fellowship. Dr. Tan was supported by American Heart Association, Greater Midwest Affiliate Postdoctoral fellowship and NIH grant HL71092 to J.C.M. Dr. Ackerman's research program is supported by the Mayo Clinic Windland Smith Rice Comprehensive Sudden Cardiac Death Program, the Dr. Scholl Foundation, the CJ Foundation for SIDS, the American Heart Association, and the National Institutes of Health (HD42569). We are particularly indebted to the index case and family members for their participation in this study.

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Figure 1. Clinical characterization of mixed channelopathy phenotype

A. Pedigree tree of the family. Circles indicate females, squares, males. Filled symbol, affected. The age of each family member is displayed above the symbol. The genotype is shown below the symbol. Arrow indicates the proband.

B. Lead II ECG (10 mm/mV, 25 mm/s) from the proband in sinus rhythm showing a prolonged QT interval (QTc, 519 ms)

C. Lead II ECG (10 mm/mV, 25 mm/s) from the proband showing monomorphic ventricular tachycardia at 250 bpm

Figure 2. Post-mortem genetic testing revealing compound heterozygosity in *SCN5A*

A. Proband sequence chromatogram showing a “G” base deletion at position 105, after codon 34 of *SCN5A* resulting in a frame shift mutation followed by 60 “scrambled” amino acids and premature truncation of the protein.

B. Proband sequence chromatogram showing a missense mutation in codon 1195 of *SCN5A* resulting in the replacement of an arginine “R” by a histidine “H”.

C. Topological diagram of sodium channel showing the maternally inherited *SCN5A*-R34fs/60 “fusion” peptide and the paternally inherited *SCN5A*-R1195H missense mutation.

D. Conservation across species of R1195-*SCN5A*.

Figure 3. Cellular electrophysiological phenotype of compound mutations in SCN5A

A. Whole-cell current traces from representative experiments of SCN5A-WT, SCN5A-WT + SCN5A-R34fs/60, SCN5A-R34fs/60, SCN5A-R1195H, and SCN5A-R1195H + SCN5A-R34fs/60.

B. Summary of I_{Na} density in SCN5A-WT, SCN5A-R34fs/60, SCN5A-R1195H, SCN5A-R1195H + SCN5A-R34fs/60, and SCN5A-WT + SCN5A-R34fs/60. The number of experiments is indicated near or in the bar.

C. Examples of late I_{Na} for SCN5A-WT, SCN5A-R1195H, SCN5A-R1195H + SCN5A-R34fs/60, and SCN5A-WT + SCN5A-R34fs/60 elicited by a depolarization pulse from 120 mV to 20 mV for 700 ms (here only 300 ms was shown). Late I_{Na} was normalized to cell capacitance and presented in pA/pF.

D. Summary of late I_{Na} normalized to peak I_{Na} . After the leak subtraction, the late I_{Na} was measured as the peak between 600 and 700ms after the initiation of the depolarization. The number of experiments is indicated above the bar.

*Statistically significant differences vs R1195H + R34fs/60 ($p < 0.05$).

Figure 4. Activation and inactivation properties of SCN5A mutations

A. Voltage-dependence of activation for SCN5A-WT, SCN5A-R1195H, SCN5A-R1195H + SCN5A-R34fs/60, and SCN5A-WT + SCN5A-R34fs/60. The voltage clamp protocol included a 24 ms step depolarization to different potentials in increments of 10 mV from a holding potential of -140 mV (see insert). The midpoints of activation were obtained by fitting to a Boltzmann function and negative shift activation midpoints were obtained with mutant channels containing SCN5A-R1195H.

B. Steady state inactivation for SCN5A-WT, R1195H, R1195H + R34fs/60, and SCN5A-WT + SCN5A-R34fs/60 mutants using the standard protocol indicated in the diagram and fitted using a Boltzmann function. The inactivation midpoint in mutant channels of SCN5A-R1195H were significantly negative shifted compared to WT channels.

C. Recovery from inactivation for SCN5A-WT, R1195H, R1195H + R34fs/60, and SCN5A-WT + SCN5A-R34fs/60 deletion mutants with time in a log scales to better show the early time course of recovery. Mutant channels of SCN5A-R1195H exhibit slower recovery from inactivation than WT channels.

D. Intermediate inactivation for SCN5A-WT, R1195H, R1195H + R34fs/60, and SCN5A-WT + SCN5A-R34fs/60 deletion mutants with a variant time of the prepulse duration. All data points are shown as the mean value and the bars represent the standard error of the mean. Intermediate inactivation was enhanced for mutants of SCN5A-R1195H compared to SCN5A-WT channels.

Table 1

Voltage-dependent gating parameters of WT and mutant channels

Samples	Activation		Inactivation		Recovery	
	$V_{1/2}$ (mV)	K	$V_{1/2}$ (mV)	τ_f (ms)	τ_s (ms)	A_s (%)
SCN5A WT	-38.6 ± 0.9	4 ± 0.2 (15)	-76 ± 1.2 (19)	1.3 ± 0.2	30 ± 7.3	23 ± 3.0 (15)
R1195H	$-47.8 \pm 2.8^*$	4 ± 0.2 (15)	$-86 \pm 1.9^*$ (14)	$2.8 \pm 0.4^*$	$53 \pm 7.0^*$	19 ± 1.2 (17)
R1195H+R34fs/60	$-46.1 \pm 2.0^*$	4 ± 0.2 (18)	$-84 \pm 1.9^*$ (20)	$2.5 \pm 0.3^*$	$51 \pm 6.0^*$	19 ± 1.4 (19)
SCN5A-WT+R34fs/60	-37.0 ± 2.2	4 ± 0.3 (9)	-76 ± 2.0 (9)	1.7 ± 0.5	31 ± 7.3	19 ± 2.3 (8)

The fitted values of voltage-dependent gating parameters represent the mean $\pm$ SEM for number of experiments in the parentheses. These parameters were obtained from fitting the individual experiments as in Figure 4 (A, B, C) to the appropriate model equations. For the Boltzmann fits the parameters of $V_{1/2}$ are the midpoint of activation and inactivation. For the double exponential fits the parameters of recovery are: τ_f , the fast time constant; τ_s , the slow time constant; and A_s , the fractional amplitude of slow component. All parameters were analyzed by one-way ANOVA across the WT and mutant channel.

* Statistically significant values compared with WT.