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A nonsense *SCN5A* mutation associated with Brugada type ECG and intraventricular conduction defects

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Abstract

Mutations of *SCN5A*, gene encoding α -subunit of cardiac sodium channel, can cause mixed phenotypes of Brugada syndrome (BrS) and cardiac conduction diseases (CCD). We have identified a nucleotide change of *SCN5A* (4178T>G) which results in a nonsense mutation, L1393X, in a 36 year-old Caucasian man who presented with intraventricular conduction delays and BrS type ECG change. To study biophysical characteristics of L1393X-*SCN5A*, electrophysiological and immunostaining studies were performed using mammalian expression systems. While WT-*SCN5A* showed significant currents (93.3 ± 10.6 pA/pF; 1 μ g plasmid), L1393X (5 μ g) did not generate any significant currents in NIH-3T3 cells. The cells co-transfected with WT (0.5 μ g) and L1393X (0.5 μ g) showed approximately 50% current amplitudes compared the WT (1 μ g). Voltage-dependency of steady-state activation and inactivation was not affected by the co-transfection of L1393X. Immunohistochemical stainings demonstrated that L1393X proteins were expressed in the plasma membranes. Our study demonstrated that L1393X-*SCN5A* does not form functional channel proteins, which might account for the patient's mixed phenotypes of BrS and CCD.

Keywords

Electrophysiology - Clinical; Electrophysiology - Basic

Introduction

Brugada syndrome (BrS) is an inherited disease that is characterized by ST-segment elevation in the right pericardial leads and lethal ventricular arrhythmias. Approximately one hundred *SCN5A* mutations were identified in 20-30% cases of Brugada syndrome patients. These mutations can cause loss-of-function in hNav1.5, which leads to the loss of action potential dome in the epicardial myocardium and exaggerates transmural voltage gradients.¹ On the other hand, approximately thirty *SCN5A* mutations were identified in cardiac conduction diseases (CCDs). Biophysical experiments demonstrated that these mutations, also, attenuate the sodium currents.^{2,3} In theory, these diseases might be overlapped since

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both share same pathophysiological mechanisms. However, to date, only a few *SCN5A* mutations were reported to account for the mixed phenotypes of BrS and CCD.²⁻⁶ We report a case of asymptomatic 36 year-old male who presented intraventricular conduction disturbance and BrS type phenotypes.

Methods

Case report

The patient is a 36-year-old Caucasian man with no apparent medical history and no family history of syncope or sudden cardiac death. Twelve lead-ECG showed premature ventricular contractions (PVCs) with a configuration of inferior axis and LBBB, intraventricular conduction disturbances (QRS: 160 ms in I; 140 ms in II; RBBB in chest leads) and mild coved-type ST-elevation in the V₁ and V₂ leads (Figure 1A). Complete cardiac work-up revealed no evidence of structural heart diseases. Since the provocation test with ajmaline caused further ST-elevation in precordial leads (data not shown), electrophysiological study (EPS) was performed according to standard protocols. VF was induced during a programmed stimulation at the right ventricle (R on T, Figure 1B). The patient was discharged after ICD (implantable cardioverter defibrillator) implantation.

Genetic screening and mutagenesis

The genetic analysis was performed according to the guideline approved by the Institutional Ethics Committee at Baylor College of Medicine. The blood sample was collected after the informed written consent was obtained. The genomic DNA was extracted from the peripheral blood lymphocytes as previously described.⁷ Polymerase chain reaction/denaturing high performance liquid chromatography (DHPLC) and direct DNA sequencing using a 3100 ABI DNA Analyzer (ABI, Foster City, CA) identified 4178T>G nucleotide change, resulting in a nonsense mutation at position 1393 (L1393X).

Site-directed mutagenesis was performed with the QuikChange Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA) using the vector containing WT-*SCN5A* as a template. The mutated L1393X-*SCN5A* clones were sequenced to ensure the presence of the mutation and the absence of other substitutions introduced by the DNA polymerase.

Cell culture and transient expression of L1393X

NIH-3T3 or HEK-293 cells were transfected with the plasmid pcDNA3.1-*SCN5A* containing L1393X cDNA and pEGFP-C3 (Clontech, Mountain View, CA) encoding green fluorescent protein using Lipofectamine 2000 transfection reagent (Qiagen, Valencia, CA). The cells were incubated at 37°C for 2-3 days before use.

Patch-clamp experiment

Patch-clamp experiments were performed as previously described.⁸ Pulse protocols were generated with Axopatch 200B and Pclamp 9 software (Axon Instruments, Sunnyvale, CA). The pipette solution contained 10 NaF, 110 CsF, 20 CsCl, 10 EGTA, and 10 HEPES (in mM, pH 7.35 with CsOH). Bath solution contained 145 NaCl, 4 KCl, 1 MgCl₂, 1.8 CaCl₂, 10 HPES, and 10 glucose (in mM, pH 7.35 with NaOH).

Data analysis

Data were analyzed using Clampfit (Axon Instruments, Sunnyvale, CA) and Igor software (Wavemetrics, Lake Oswego, OR). Data were presented as mean ± SE. Comparisons among data were made using unpaired Student's t-test with $p < 0.05$ considered statistically significant.

Results

To study the biophysical characteristics of WT and L1393X-SCN5A, mammalian cell lines (NIH-3T3 or HEK-293 cells) were transfected in three different conditions: WT-SCN5A (1 μ g), WT-SCN5A (0.5 μ g) and L1393X-SCN5A (0.5 μ g), or L1393X-SCN5A (5 μ g). GFP (0.5 μ g) was co-transfected as a reporter gene. Macroscopic sodium currents were induced using step-pulse protocols (20 ms; between -80 and 80 mV) from a holding potential of -140 mV. Figure 3A shows superimposed current traces obtained from cells in various conditions. While WT-SCN5A (1 μ g) generated significant currents, L1393X did not generate any significant currents. The cells transfected with WT-SCN5A (0.5 μ g) and L1393X-SCN5A (0.5 μ g) generated 54% of the WT-SCN5A currents (1 μ g), which might mimic a heterozygous condition. Figure 3B shows current-voltage relationships. Co-transfection of L1393X with WT did not affect the voltage dependency of onset and peak currents. The normalized conductance was plotted as a function of membrane potential (Figure 3C). The data were fitted with the Boltzman's equation, yielding half maximal voltage (V_h) of -42.6 mV for WT (1 μ g) and -40.0 mV for co-transfection of WT (0.5 μ g) and L1393X (0.5 μ g). Steady-state inactivation was studied with a pre-pulse protocol. The normalized currents were plotted as a function of membrane potential (Figure 1C). The data were fitted with the Boltzman's equation, yielding half maximal voltage (V_h) of -97.9 mV for WT and -92.7 mV for the mixture. Table I summarizes the basal gating kinetics in each condition.

It is reasonable to speculate that L1393X-SCN5A did not generate any currents because it lacks more than a quarter of the channel structure (from Domain III S6 till C-terminal). However, the lack of currents might be due to trafficking problems. To study cellular localization of L1393X, HEK-293 cells were transfected with FLAG-tagged L1393X. Forty-eight hours after transfection, the cells were fixed and stained with anti-FLAG antibody. Figure 3 shows that L1393X proteins were expressed in the plasma membrane (red staining). Thus, we conclude that L1393X proteins do not form functional sodium channels.

Discussion

We report a *SCN5A* mutation, L1393X, identified in the patient who represented with mixed phenotypes of CCD and BrS. As previously reported eight *SCN5A* nonsense mutations identified in BrS patients, L1393X did not form functional channels, which may severely affect the electrophysiological properties of patient's heart. Interestingly, none of those eight nonsense mutations have been reported to cause CCDs. Also, our patient did not show severe BrS phenotypes (*i.e.*, no syncope or aborted SCD) despite of the severe functional defects of sodium channels. However, this might be due to the timing of diagnosis. Probst reported that conduction defects can be developed over time in the patients who carry the *SCN5A* mutations.⁹ In addition, Some patients with typical Brugada ECG pattern at rest or during drug-challenge tests experience lethal ventricular arrhythmias, whereas other patients or family members carrying same mutations sometimes remain free of symptoms.¹⁰ Since this is a single case report, there is a limitation in linking our experimental data to the clinical significance. Further case studies are warranted to elucidate the underlying mechanism(s) that can compensate the functional defects by L1393X.

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References

1. Antzelevitch C, Brugada P, Brugada J, Brugada R, Towbin JA, Nademanee K. Brugada syndrome: 1992-2002: a historical perspective. *J Am Coll Cardiol.* 2003; 41:1665–71. [PubMed: 12767644]
2. Kyndt F, Probst V, Potet F, Demolombe S, Chevallier JC, Baro I, Moisan JP, et al. Novel SCN5A mutation leading either to isolated cardiac conduction defect or Brugada syndrome in a large French family. *Circulation.* 2001; 104:3081–6. [PubMed: 11748104]
3. Shirai N, Makita N, Sasaki K, Yokoi H, Sakuma I, Sakurada H, Akai J, et al. A mutant cardiac sodium channel with multiple biophysical defects associated with overlapping clinical features of Brugada syndrome and cardiac conduction disease. *Cardiovasc Res.* 2002; 53:348–54. [PubMed: 11827685]
4. Smits JP, Eckardt L, Probst V, Bezzina CR, Schott JJ, Remme CA, Haverkamp W, et al. Genotype-phenotype relationship in Brugada syndrome: electrocardiographic features differentiate SCN5A-related patients from non-SCN5A-related patients. *J Am Coll Cardiol.* 2002; 40:350–6. [PubMed: 12106943]
5. Schulze-Bahr E, Eckardt L, Breithardt G, Seidl K, Wichter T, Wolpert C, Borggrefe M, et al. Sodium channel gene (SCN5A) mutations in 44 index patients with Brugada syndrome: different incidences in familial and sporadic disease. *Hum Mutat.* 2003; 21:651–2. [PubMed: 14961552]
6. Makiyama T, Akao M, Tsuji K, Doi T, Ohno S, Takenaka K, Kobori A, et al. High risk for bradyarrhythmic complications in patients with Brugada syndrome caused by SCN5A gene mutations. *Journal of Am Coll Cardiol.* 2005; 46:2100–6. [PubMed: 16325048]
7. Vatta M, Dumaine R, Varghese G, Richard TA, Shimizu W, Aihara N, Nademanee K, et al. Genetic and biophysical basis of sudden unexplained nocturnal death syndrome (SUNDS), a disease allelic to Brugada syndrome. *Hum Mol Genet.* 2002; 11:337–45. [PubMed: 11823453]
8. Shuraih M, Ai T, Vatta M, Sohma Y, Merkle EM, Taylor E, Li Z, et al. A common SCN5A variant alters the responsiveness of human sodium channels to class I antiarrhythmic agents. *J Cardiovasc Electrophysiol.* 2007; 18:434–40. [PubMed: 17331104]
9. Probst V, Allouis M, Sacher F, Pattier S, Babuty D, Mabo P, Mansourati J, et al. Progressive cardiac conduction defect is the prevailing phenotype in carriers of a Brugada syndrome SCN5A mutation. *J Cardiovasc Electrophysiol.* 2006; 17:270–5. [PubMed: 16643399]
10. Keller DI, Barrane FZ, Gouas L, Martin J, Pilote S, Suarez V, Osswald S, et al. A novel nonsense mutation in the SCN5A gene leads to Brugada syndrome and a silent gene mutation carrier state. *Can J Cardiol.* 2005; 21:925–31. [PubMed: 16239976]

Figure1. ECG phenotypes

(A) Baseline 12 lead ECG. (B) Polymorphic ventricular tachycardia induced in the electrophysiological study.

Figure 2. Genetic analysis

DNA sequence analysis of genomic DNA identifies the 4178T>A substitution in exon 23 of *SCN5A*, in both forward and reverse orientation, leading to an amino acid substitution of a Lysine to a Stop codon at position 1393 (L1393X).

Figure 3. Basal characteristics of WT- and L1393X-SCN5A

(A) Superimposed whole-cell current traces obtained from the cells transfected with WT (1 μ g), WT (0.5 μ g) and L1393X (0.5 μ g), and L1393X (5 μ g). (B) I-V relationships. (C) Voltage-dependence of peak conductance and steady-state fast inactivation. Conductance $G(V)$ was calculated by the equation: $G(V) = I / (V_m - E_{rev})$, where I is the peak currents, E_{rev} is the measured reversal potential, V_m is the membrane potential. The normalized peak conductance was plotted against membrane potentials. Steady-state inactivation was estimated by a pre-pulse protocol (shown in the inset). The normalized peak currents were plotted as a function of membrane potentials. Steady-state activation and inactivation were fitted with the Boltzmann equation: $y = [1 + \exp((V_h - V_m)/k)]^{-1}$, where y represents variables; V_h , midpoint; k , slope factor; V_m , membrane potential. The data was represented as mean $\pm$ SD.

Figure 4. Immunohistochemical staining

(A) Fluorescopic images of the cells stably transfected with L1393X. (B) Corresponding microscopic images of the cells with visible light. Note that the L1393X proteins are expressed in the plasma membrane.

Table1

Biophysical characteristics

	WT	WT + L1393X	L1393X
Plasmid amount (μ g)	1	0.5 + 0.5	5
Current density (pA/pF)	(10)	(10)	(20)
	93.3 $\pm$ 10.6	50.8 $\pm$ 7.8**	0
Activation	(10)	(10)	
V_h (mV)	-42.6 $\pm$ 1.1	-40.0 $\pm$ 0.9	
k	8.9 $\pm$ 0.3	8.6 $\pm$ 0.5	
Fast-inactivation	(10)	(10)	
V_h (mV)	-97.7 $\pm$ 2.1	-92.7 $\pm$ 1.1	
k	7.0 $\pm$ 0.5	6.4 $\pm$ 0.2	

V_h , represents midpoint voltage of maximal activation/inactivation; k , slope factor

**
 $p < 0.001$ vs. WT; numbers in parenthesis, the number of patches.