SCN5A(K817E), a novel Brugada syndrome–associated mutation that alters the activation gating of $Na_V1.5$ channel

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BACKGROUND Brugada syndrome (BrS) is an inherited lethal arrhythmic disorder characterized by syncope and sudden cardiac death from ventricular tachyarrhythmias. Here we identified a novel K817E mutation of *SCN5A* gene in a man with type 1 BrS electrocardiogram pattern using next-generation sequencing targeted for 73 cardiac disorder-related genes. *SCN5A* encodes the α-subunit of $Na_V1.5$ voltage-gated Na^+ channel, and some of its mutations are linked to BrS. The proband had no mutation in any of the other arrhythmia-related genes sequenced.

OBJECTIVE We investigated whether the K817E mutation causes a functional change of $Na_V1.5$ channel responsible for the BrS phenotype.

METHODS We compared the electrophysiological properties of the whole-cell currents mediated by wild-type and mutant channels heterologously expressed in human embryonic kidney 293 cells by using a voltage-clamp technique.

RESULTS The K817E mutation reduced the Na^+ current density by 39.0%–91.4% at membrane potentials from −55 to −5 mV. This reduction resulted from a ∼24-mV positive shift in the voltage dependence of activation. The mutation also decelerated recovery from both fast and intermediate inactivation, whereas it had little effect on the cell surface expression, single-channel conductance, voltage-dependence of fast inactivation, entry into intermediate inactivation, use-dependent loss of channel availability, or closed-state inactivation.

CONCLUSION The K817E mutation of *SCN5A* gene leads to loss of function of $Na_V1.5$ channel and may underlie the BrS phenotype of the proband.

KEYWORDS Brugada syndrome; Sodium channel; Voltage sensor; Missense mutation; Genetics

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Introduction

The *SCN5A* gene encodes the pore-forming α-subunit of the human cardiac voltage-gated Na^+ channel $Na_V1.5$, which mediates the fast inward Na^+ current (I_{Na}) that contributes to the rapid depolarization of the cardiac action potential.[1,2] Mutations in *SCN5A* gene may cause cardiac Na^+ channelopathies, sometimes leading to sudden cardiac death. Such channelopathies include Brugada syndrome (BrS), long QT syndrome type 3, and dilated cardiomyopathy.[3–6] BrS is an inherited cardiac arrhythmia disorder possibly underlying the development of ventricular fibrillation and leading to sudden cardiac death in patients with structurally normal hearts.[7] Patients with BrS have a characteristic electrocardiogram (ECG) pattern with ST-segment elevation at the right precordial leads.[8,9] Some *SCN5A* mutations previously identified in patients with BrS cause the loss of function of $Na_V1.5$ channel by reducing the surface expression level of

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the channel or changing the voltage and time dependences of channel activation and/or inactivation.[4,5,10–12]

Here we report a functional study of a $Na_V1.5$ channel involving a novel SCN5A(K817E) mutant that was identified in a 38-year-old man. $Na_V1.5$ channel is composed of 4 domains, each of which has 6 membrane-spanning hydrophobic helical segments. K817 is located in the fourth segment of domain 2 (D2S4). D2S4 serves as a voltage sensor and its positively charged amino acids including K817 are considered important for voltage sensing of the channel.[13] The BrS-related mutations identified in the positively charged amino acids in the D2S4 region reportedly affect the voltage dependence of fast inactivation.[14,15] Similarly, a substitution of K817 with the negatively charged glutamate may affect channel gating. To assess this possibility, we performed functional analyses of the mutant $Na_V1.5$ channel in a heterologous expression system of human embryonic kidney 293 cells.

Methods

Plasmid construction

To express the α-subunit of $Na_V1.5$ channel, wild-type (WT) human *SCN5A* complementary DNA (GenBank: NM_198056) was subcloned into pReceiver-M12 vector containing the N-terminal 3× FLAG epitope (GeneCopoeia, Rockville, MD) [FLAG-SCN5A(WT)]. FLAG-SCN5A(K817E) was generated on the basis of FLAG-SCN5A(WT) using a site-directed mutagenesis kit (Toyobo, Osaka, Japan). To express the β-subunit of $Na_V1.5$ channel, WT *SCN1B* complementary DNA (GenBank: NM_001037) was subcloned into pReceiver-M12 vector containing the N-terminal 2× Myc epitope [Myc-SCN1B(WT)].

Cell culture and transfection

Human embryonic kidney 293 cells were cultured in Dulbecco's modified Eagle's medium (Life Technologies) supplemented with 10% fetal bovine serum at 37°C in 5% CO_2. The cells were transiently transfected with a 500 ng mixture of pCAGGS-EGFP, Myc-SCN1B, and either FLAG-SCN5A(WT) or FLAG-SCN5A(K817E) at a ratio of 1:4.5:4.5 using PolyMag (OZ Biosciences, Marseille, France).

Electrophysiological analysis

Rupture-patch whole-cell voltage-clamp recordings were made from EGFP-positive cells at room temperature (25°C) as previously described.[16] Briefly, the recording chamber was perfused with an extracellular bath solution containing (in mM) 147 NaCl, 4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 2-[4-(2-Hydroxyethyl)-l-piperazinyl]ethanesulfonic acid (HEPES), and 10 D-glucose (pH 7.4 adjusted with NaOH). Current signals were acquired with an EPC 9/2 amplifier controlled by PATCHMASTER software (HEKA, Lambrecht, Germany). A glass recording pipette was filled with (in mM) 105 cesium fluoride, 35 NaCl, 10 HEPES, and 10 Ethylene glycol-bis(β-aminoethyl ether)-N,N, N',N' -tetraacetic acid tetrasodium salt (EGTA) (pH 7.4 adjusted with CsOH) and had a tip resistance of 1–3 MΩ. Data analyses and statistical evaluation are described in Online Supplemental Methods.

Results

Clinical and genetic background

In a school physical examination, a 10-year-old girl (Figure 1A, IV-1) reported that she has a couple of relatives who suffered from a cardiac defect or died of sudden cardiac death. Based on this report, the hospital asked her family members, including the proband (her father, Figure 1A, III-1), for further investigation. The proband's ECG showed a type 1 BrS pattern consisting of a spontaneous coved-type ST-segment elevation and T-wave inversion in leads V_1-V_2 (Figure 1B). Although the proband has shown abnormal ECG patterns since childhood, he has not yet experienced any cardiac events without being prescribed any antiarrhythmic drugs. The proband's brother suffered from sick sinus syndrome and had a pacemaker implanted at the age of 30 (Figure 1A, III-3). In addition, his grandmother and uncle died of sudden cardiac death before the age of 40 (Figure 1A, I-2 and II-2). The proband's mother (Figure 1A, II-5), sister (Figure 1A, III-4), and 2 daughters (Figure 1A, IV-1 and -2) have never shown any cardiac symptoms. The ECG obtained from the mother did not show ST-segment elevation (Online Supplemental Figure 1). The detailed histories of the proband and his family are summarized in Online Supplemental Tables 1 and 2, respectively.

The proband's genomic DNA was analyzed by next-generation sequencing of 73 major cardiac disorder-related genes (Online Supplemental Table 3); this showed a heterozygous a2449g substitution in exon 16 of *SCN5A*, resulting in a K817E amino acid substitution (Figure 1C). There was no missense mutation in the other BrS-related genes including *CACNA1C*, *CACNB2*, *GPD1L*, *HCN4*, *KCNE3*, *SCN1B*, *SCN3B*, and *ABCC9*. In addition, there was no missense mutation in the other channelopathy- or cardiomyopathy-related genes except for a N478S (a1433g) mutation in *VCL* (NM_014000). K817 is the fourth of the 5 positively charged amino acids in D2S4 of *SCN5A* and conserved among mammalian homologues (Figure 1D and Online Supplemental Figure 2).

Electrophysiological characterization of the SCN5A (K817E) mutant channel

In this and the following sections, we used a voltage-clamp technique to examine the effect of the K817E mutation on I_{Na} in cells transfected with SCN1B(WT) and either of SCN5A(WT) or SCN5A(K817E) (WT or KE cells, respectively). The I_{Na} was activated by a voltage step from a holding potential of −120 mV to various test potentials (Figure 2A). The current-voltage plots showed that the I_{Na} density of KE cells were always lower than that of WT cells at test potentials ranging from −55 to −5 mV (Figure 2B). The reductions in the mean I_{Na} density were 91.4% and 39.0% at test potentials of −40 and −5 mV, respectively.