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Brief Communication

Diplotype analysis of the human cardiac sodium channel regulatory region in Japanese cases of sudden death by unknown causes

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ABSTRACT

Inherited mutations in the human cardiac sodium channel (SCN5A) gene cause arrhythmogenic diseases such as tachyarrhythmia and bradyarrhythmia. Moreover, mutation subsets in the coding region impair SCN5A function, potentially leading to sudden cardiac death (SCD). In the present study, we performed diplotype analysis of the regulatory region of the SCN5A gene in Japanese people who died suddenly because of an unknown cause (sudden death group; $n = 70$) and controls ($n = 112$). There were no significant differences at six polymorphic loci between the groups. However, 38 diplotypes of 6-nucleotide polymorphism variants were identified. One of these diplotypes—Dip.D (CTG–TC/CCG–TC)—occurred significantly more frequently in the sudden death group than in the controls ($p < 0.01$, OR = 5.18, 95% CI: 1.38–19.45). Dip.D has two variants (T-1062C and T-847G), and while it is unclear whether these directly affect mRNA expression, a common polymorphism in this region modulates SCN5A expression in vitro. Our results thus suggest that the transcription of the SCN5A Dip.D variant may be associated with arrhythmogenic diseases that can induce sudden death.

1. Introduction

The SCN5A gene—located on chromosome 3p21—encodes the voltage-gated pore-forming (α) subunit of the predominant sodium channel [1,2]. It has become increasingly clear that several mutations within the SCN5A coding region induce disturbances of the cardiac rhythm. Sudden death sometimes occurs in people with SCN5A gene mutations, which are implicated in congenital long QT syndrome (LQTS), Brugada syndrome (BrS), sudden infant death syndrome (SIDS), and progressive cardiac conduction system disease (PCCD, or Lev-Lenegre disease). In such cases, the clinical and physiopathological characteristics are poorly defined [3].

LQTS is a genetic disease characterized by a prolonged QT interval, and is a major cause of malignant ventricular tachyarrhythmia. A mutation of the SCN5A gene is responsible for LQTS [4–6]. BrS is an inherited cardiac arrhythmia syndrome that produces ventricular fibrillation and, potentially, SCD in patients with structurally normal hearts. Approximately 20–30% of patients with BrS have coding region variants of the SCN5A gene, and the transcription of these variants can enhance the risk of arrhythmia. Functional studies of cardiac sodium channels transcribed from BrS-associated gene mutations have shown lower than normal sodium currents, resulting from a defect in either the gating function or surface membrane expression [7–12]. SIDS is a multifactorial condition in which an interaction of environmental, genetic, and developmental factors is thought to be involved. Mutations in the SCN5A gene are reported to account for 2–10% of SIDS cases [13–15]. Effects associated with PCCD include a reduction in channel availability at the normal resting membrane potential or an increased level of depolarization for channel activation. Mutations in the SCN5A gene that segregate and cosegregate with PCCD in an autosomal dominant manner have been reported [16–18]. Such disease-associated mutations could disrupt sodium channel function via multiple molecular mechanisms, such as synthesis of defective sodium channels because of a reduced expression of sodium channel membranes [14]. However, the role of DNA variants within SCN5A transcriptional control has not been determined thus far [19–22].

The SCN5A gene core promoter includes multiple positive and negative *cis*-acting elements that extending into intron 1 and DNA polymorphisms [23]. It has been suggested that polymorphisms in the promoter region may be associated with interindividual variability in the transcription patterns of the SCN5A gene. Moreover, these polymorphisms could be attributed to a six-change haplotype variant in the promoter region, which is common in East Asian populations, and reduces the transcriptional activity of the gene [24].

Recent studies have shown that mutations of the sodium channel gene, particularly those occurring within the promoter region,

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can also lead to enhanced fibrosis in myocardial tissue [2]. This suggests that the detection of diplotype variants of the sodium channel regulatory region could aid the forensic diagnosis of sudden death cases.

In the present study, we report the frequency in a Japanese sample of diplotype variants consisting of six polymorphisms in the regulatory region of the SCN5A gene.

2. Materials and methods

2.1. Study population

We investigated 70 cases of sudden death between 2000 and 2008, the causes of which remained unknown after forensic autopsies performed at Osaka University Graduate School of Medicine (LQTS and BrS were diagnosed in 1 case each antemortem; SIDS was diagnosed in 20 cases postmortem). The autopsies involved an evaluation of the circumstances of death, familial and medical history, and microscopic examinations. The 20 cases of SIDS involved 10 males and 10 females, aged 2–12 months (mean = 8 ± 2 months). The remaining cases of sudden death involved adults: 35 males and 15 females, aged 20–60 years (mean = 41 ± 13 years). There were 112 Japanese control subjects: 70 males and 42 females, aged 0–60 years (mean = 40 ± 15 years). All control patients were free of cardiac, neurologic, and psychiatric illness, and no patient was related to the sudden death subjects. The protocol for the present study was approved by the local ethics committee, and informed consent was obtained from all the volunteers.

2.2. DNA extraction

The phenol/chloroform method was used to extract genomic DNA from whole blood. After incubation with proteinase K (125 mg/ml) in lysis buffer (3 ml containing 50 mM Tris–HCl, 100 mM NaCl, 0.5% SDS, and 1 mM EDTA) at 55 °C for 3 h, protein contaminants were removed by phenol/chloroform extraction, precipitated with ethanol, and then dried and stored at −20 °C in 10 mM Tris–HCl (pH 7.6). Optical density at 260 nm was used to quantify the amount of extracted DNA, with a value of 1.0 corresponding to 50 mg/ml DNA.

2.3. Identification of polymorphisms

Because the transcription start site for the human SCN5A gene is in exon 2, we detected six major DNA polymorphisms in the regulatory region (T-1418C, C-1062T, T-847G, -835insGC, and T-834C) and the proximal intron 1 region (C287T). The genomic organization of the regulatory region and the relative positions of the polymorphisms are shown in Fig. 1. The detected polymorphisms are known to be very common in Asian populations and are in complete linkage disequilibrium.

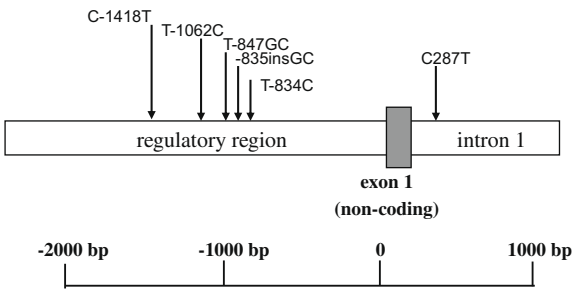

Fig. 1. The SCN5A regulatory region including 2.2 kb upstream of exon 1, exon 1 (which is 173 bp and non-coding), and the proximal 439 bp of intron 1. Six major nucleotide variations are indicated.

2.4. Polymorphism genotyping

Genotyping of the T-1418C and C-1062T single nucleotide polymorphisms (SNPs) was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis using restriction enzymes EarI (New England Biolabs, USA) and HaeIII (SibEnzyme, Russia), respectively, as described previously [24]. PCR was performed using a DNA thermal cycler (GeneAmp PCR System 9700, Applied Biosystems, USA) with a 50-μl reaction mixture containing 50 ng genomic DNA, 2.5 mM dNTP mixture, 10 pmol primers, and 0.5 μl PrimeSTAR™ HS DNA Polymerase (Takara Bio, Japan). The primers used to amplify the 123-bp fragment, including the T-1418C SNP, were F1 and R1. The PCR conditions were as follows: 98 °C for 1 min, followed by 30 cycles of 98 °C for 10 s, 66 °C for 15 s, and 72 °C for 30 s, with a final extension at 72 °C for 10 min. The primers used to amplify the 161-bp fragment, including the C-1062T SNP, were F2 and R2. The PCR conditions were as follows: 98 °C for 1 min, followed by 30 cycles of 98 °C for 10 s, 68 °C for 15 s, and 72 °C for 30 s, with a final extension at 72 °C for 10 min. The PCR products were digested with 2.5 U of restriction endonuclease at 37 °C for 3 h, after adding an appropriate incubation buffer. Both the digested and undigested products were electrophoresed in 2.5% agarose gel for 3 h at 150 V, before being directly visualized with ethidium bromide under UV light. A 100-bp ladder was used as a standard against which fragment sizes were estimated.

Genotyping of the T-847G, -835insGC, T-834C, and C287T polymorphisms was done by direct sequencing. The primers for PCR used to amplify the 638-bp fragment, including the T-847G, -835insGC, and T-834C polymorphisms, were F3 and R3. The PCR conditions were as follows: 98 °C for 1 min, followed by 35 cycles of 98 °C for 10 s, 65 °C for 15 s, and 72 °C for 30 s, with a final extension at 72 °C for 10 min. The primers used to amplify the 599-bp fragment, including the C287T SNP, were F4 and R4. The PCR conditions were as follows: 96 °C for 1 min, followed by 25 cycles of 96 °C for 10 s, 50 °C for 15 s, and 70 °C for 30 s, with a final extension at 70 °C for 10 min. Genotypes were confirmed using an ABI 310 Genetic Analyzer and BigDye™ Terminator Cycle Sequencing Kit version 3.1 (Applied Biosystems, USA), as per the manufacturer's instructions. The primer sequences are shown as Supplementary data on the journal web site.

2.5. Statistical analysis

Significant deviations from Hardy–Weinberg equilibrium (HWE) were revealed for all six of the selected polymorphisms. Diplotype frequencies were estimated from the genotypes observed for the polymorphisms. We designated the most common diplotype block in our sample as Dip.A, and the second, third, fourth, and fifth most common diplotypes as Dip.B, Dip.C, Dip.D, and Dip.E, respectively. Other diplotype blocks were designated as Dip.Others. Allele frequencies were derived from gene counts, and differences between the sudden death and control groups evaluated by χ^2-tests. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to evaluate the effects of the different genotypes.

3. Results

The sample was screened for SCN5A gene coding region mutations; however, no mutations were found.