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The global distribution of the p.R1193Q polymorphism in the *SCN5A* gene

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ABSTRACT

The *SCN5A* (sodium channel, voltage-gated, type V, alpha subunit) gene encodes the cardiac sodium channel, a member of the voltage-gated sodium channel family. The p.R1193Q (c.3578G>A) polymorphism in *SCN5A* is known to accelerate inactivation of the sodium channel current, and has been identified in patients with Brugada and long QT syndromes. In the present study, we investigated the frequency of the p.R1193Q substitution in more than 4000 genomic DNA samples from 34 Asian, European, and African populations using TaqMan and/or APLP (amplified product length polymorphism) assays. Allele A (p.1193Q) was detected in most Asian populations, but was sporadically observed or absent in European and African populations. These results demonstrated that the p.R1193Q substitution is characteristic of Asian populations.

1. Introduction

Sudden cardiac death is one of the most common causes of sudden natural unexpected death in forensic autopsy diagnoses. Many cases of sudden cardiac death exhibit organic changes, such as myocardial infarction and cardiomyopathy. However, difficulties have been associated with diagnosing the cause of death in cases of arrhythmia without sufficient information on the antemortem medical history because autopsy including pathological, toxicological, and biochemical examinations cannot reveal the cause of death.

A number of genes are known to be associated with arrhythmia [1,2], and represent good candidates for the definition of a genetic pro-arrhythmic profile. The *SCN5A* (sodium channel, voltage-gated, type V, alpha subunit) gene encodes specific voltage-dependent Na (+) channels that are abundant in cardiac muscle. *SCN5A* mutations are frequently detected in cases of arrhythmia, and are associated with cardiac rhythm syndromes including Brugada and long QT syndromes [3]. The p.R1193Q polymorphism (CGG>CAG, refSNP ID rs41261344) in the *SCN5A* gene is located at the link between domains II and III, several residues upstream of the proposed boundary of the S1 transmembrane segment of domain III. The arginine at amino acid position 1193 is conserved among the mammalian sodium channel isoforms [4]. The p.R1193Q substitution was previously shown to accelerate inactivation of the sodium channel current [5]. This substitution has been identified in patients with Brugada syndrome [5,6] and long QT syndrome [4,7]. The p.R1193Q substitution has been characterized functionally as either a "loss-of-function" variant consistent with Brugada syndrome [5], or as a "gain-of-function" variant consistent with previously defined long QT syndrome type 3 [4]. These findings suggest that the p.R1193Q polymorphism is a functional substitution that has the ability to increase susceptibility to these syndromes. We previously examined mutations in the *SCN5A* gene in cases of sudden cardiac death, and identified a heterozygous mutation causing the p.R1193Q substitution [8].

The allelic frequency of the p.R1193Q polymorphism has been investigated in several populations, suggesting that the p.R1193Q polymorphism is common in Asian populations [9,10]. In order to elucidate the geographical distribution of the p.R1193Q polymorphism, we herein investigated more than 4000 individuals from Asian, European, and African populations.

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2. Materials and methods

2.1. DNA samples

A total of 4044 unrelated individuals from 34 populations were examined (Table S1). The geographical locations of the Asian populations are shown in Fig. 1. Sub-Saharan African (Nigerian and Ghanaian) and Turkish samples were collected from people residing in Germany. This study was approved by the Fukuoka University School of Medicine Ethical Review Board.

2.2. Genotyping

A mutational analysis was performed using Custom TaqMan® SNP Genotyping Assays and APLP (amplified product length polymorphism) assay. Locus-specific PCR primers and allele-specific TaqMan® probes were designed, as shown in Table 1, and supplied by Applied Biosystems (Foster City, CA, USA). TaqMan® probes, specific to alleles A (p.1193Q) and G (p.1193R), had FAM and VIC, respectively, as the fluorescent reporter dyes at the 5′ end. Each of these had a non-fluorescent quencher (NFQ) with a minor groove binder (MGB) at the 3′ end. PCR was performed on a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA) in a 25-μL reaction volume. In each PCR, 11.25 μL of genomic DNA (1–20 ng) was mixed with 12.5 μL of 2× TaqMan® genotyping master mix, and 1.25 μL of 20× TaqMan® SNP genotyping assay mix. All reactions consisted of one cycle at 95 °C for 10 min, and 40 cycles at 92 °C for 15 s, 60 °C for 1 min. After real-time PCR was finished, an allelic discrimination analysis was performed using SDS 1.4 software (Applied Biosystems, Foster City, CA) to genotype the samples.

The APLP assay was carried out as follows: 100 μL of the PCR cocktail consisted of 50 μL of the KAPA2G Fast Multiplex PCR Kit (Kapa Biosystems, Wilmington, MA), 2 μL of each primer (Table 1) at a concentration of 10 pmol/μL, and 44 μL of water. PCR was performed in a volume of 8 μL containing 7.5 μL of the PCR cocktail and 0.5 μL of a solution containing approximately 10–20 ng of genomic DNA. The cycle conditions used were 95 °C for 3 min, then 32 cycles of 94 °C for 15 s, 65 °C for 15 s, and 72 °C for 15 s, and a final extension step of 3 min at 72 °C. The products were separated using a polyacrylamide gel (9%T, 5%C) together with positive and negative controls, and then visualized by staining with ethidium bromide.

The results of several samples by the Custom TaqMan® SNP Genotyping Assay and APLP assay were confirmed by direct sequencing. Allele frequency was estimated and Hardy–Weinberg equilibrium was tested using SNPAlyze Ver. 8 (Dynacom, Chiba, Japan). A contour map of the frequencies for allele A was generated from 34 present and 14 previously reported populations, of which a location was identified (Table S1) using the Surfer 12.0 program (HULINKS, Tokyo, Japan).

3. Results

The amplification plot of Custom TaqMan® SNP Genotyping Assays showed a clear separation of the p.R1193Q polymorphism (Fig. 2A). In the APLP assay, each genotype was clearly and unambiguously distinguished (Fig. 2B).

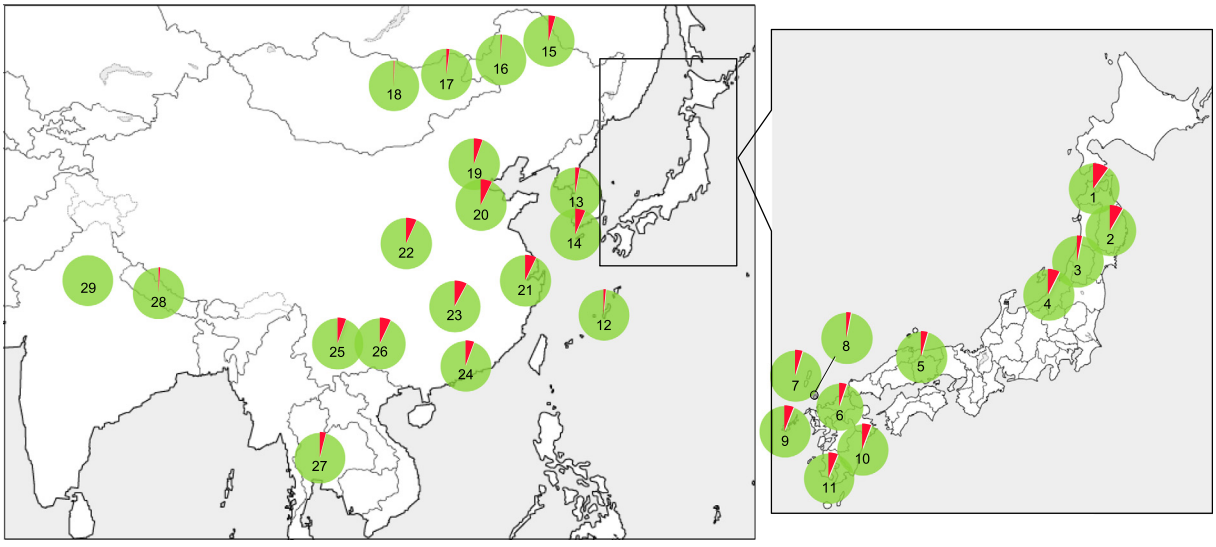

Fig. 1. The distribution of allele A (p.1193Q) frequencies in East and South Asian populations investigated in this study, as indicated by the red sector in each pie chart. (1) Aomori, (2) Iwate, (3) Yamagata, (4) Niigata, (5) Tottori, (6) Fukuoka, (7) Tsushima island, (8) Iki island, (9) Goto island, (10) Miyazaki, (11) Kagoshima, (12) Okinawa, (13) Seoul, (14) Kwangju, (15) Heihe, (16) Hailar, (17) Dashbalbar, (18) Ulaan Baator, (19) Beijing, (20) Gu'an, (21) Wuxi, (22) Xi'an, (23) Changsha, (24) Huizhou, (25) Kunming, (26) Yunnan, (27) Bangkok, (28) Kathmandu, (29) New Delhi.

Table 1
TaqMan® primers, probes, and APLP primers used for *SCN5A* R1193Q genotyping.

Method	Primer (5′–3′)	Probe
TaqMan®	Forward: ACTCTCTCCCATAGGCTGTGT Reverse: AGCTGTGCTCCACGATGTG	VIC-CTGGTGGCGGTTGC (Allele G) FAM-CTGGTGGCAGTTGC (Allele A)
APLP	Forward (Allele G): TAGCAGGTCTTGCGCtACC Forward (Allele A): ataGTAGCAGGTCTaGCGCAACT Reverse: GTGCaGTGGACACCACACA	

Non-complementary nucleotides are written in lower case letters.