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Genotyping of the c.1423C>T (p.P475S) polymorphism in the *ADAMTS13* gene by APLP and HRM assays: Northeastern Asian origin of the mutant

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ABSTRACT

ADAMTS13 is a von Willebrand factor-cleaving protease. The mutant types of p.P475S (c.1423C>T) polymorphism in ADAMTS13 have a reduced activity in comparison with the wild type. In the present study, we investigated the frequency of the C-to-T substitution in 2584 genomic DNA samples from 25 Asian, European, and African populations using APLP (amplified product length polymorphism) and/or HRM (high-resolution melting) assays. Allele T (*ADAMTS13*T*) was detected only in Asian populations and its frequency was observed to decrease gradually from north to south in 24 East Asian populations. Almost all *ADAMTS13*T* were associated with *ABO*O*. These results suggested that *ADAMTS13*T* had occurred on a chromosome with *ABO*O* in a northern part of East Asia. This SNP is useful as an ancestry-informative marker, and the present genotyping techniques are applicable to the investigation of an association between this SNP and aortic dissection (Kobayashi et al., 2012).

1. Introduction

ADAMTS13 (A disintegrin-like and metallopeptidase with thrombospondin type 1 motif 13) functions as a protease for the specific cleavage of von Willebrand factor (vWF), which is secreted into the plasma as unusually large multimers. ADAMTS13 quickly degrades these multimers into smaller forms to prevent microvascular thrombosis, platelet consumption, and hemolysis. An inherited deficiency of this protease is associated with congenital thrombotic thrombocytopenic purpura (TTP), or Upshaw-Schulman syndrome [1,2].

The human *ADAMTS13* gene (Genbank Gene ID 11093), neighboring the *ABO* gene, spans approximately 37 kb on chromosome 9q34.2 and consists of 29 exons, encoding a 1427-amino acid polypeptide comprising 14 domains [3–5]. To date, many common and rare variants have been identified in various populations [1,2,6]. A C-to-T transition (refSNP ID rs11575933) located at nucleotide position c.1423 in exon 12 of the *ADAMTS13* gene results in the p.P475S polymorphism in the Cys-rich domain. The frequency of allele T (*ADAMTS13*T*) was estimated to be 0.0508 in 364 Japanese individuals from Osaka. The mutant types, PS and SS, show a reduced activity in comparison with the wild type, but are unlikely to be responsible for TTP [7–9].

The p.P475S polymorphism has been investigated in patients suffering from acute ischemic stroke, acute myocardial infarction, and cerebral malaria [10,11]. Kobayashi et al. [12] investigated this polymorphism in autopsy cases with unexpected sudden death, and observed a significantly higher frequency of *ADAMTS13*T* in autopsy cases with aortic dissection (Table S1). Population studies of the p.P475S polymorphism have been performed only in four populations, consisting of Japanese, Koreans, Chinese, and Europeans, suggesting that the p.P475S polymorphism is restricted to Asian populations [7,10,13,14]. For a better understanding of the geographical distribution of the p.P475S polymorphism, we herein investigated more than 2500 individuals from Asian, European, and African populations.

2. Materials and methods

2.1. DNA samples

A total of 2584 unrelated individuals from 25 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

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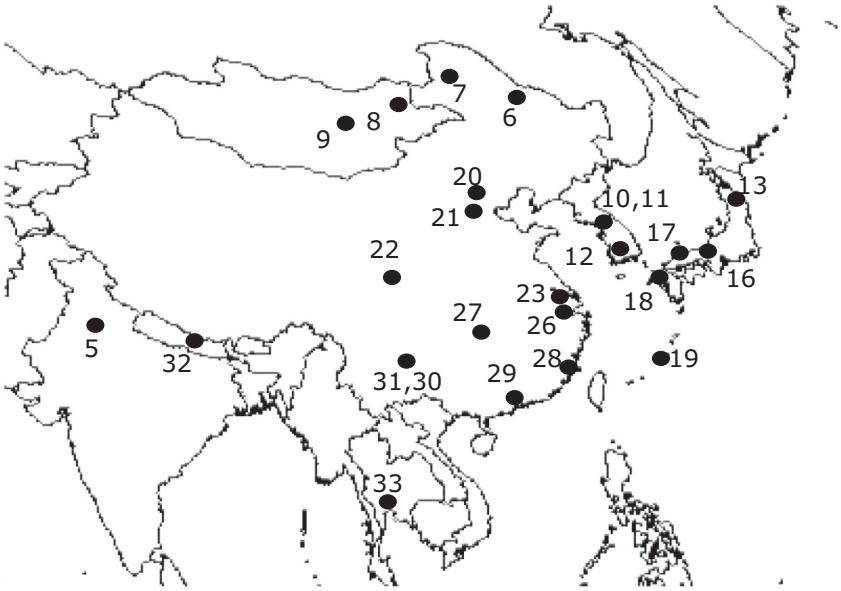

Fig. 1. Locations of Asian populations in the present study. Numbers correspond to those in Table S1.

residing in Germany. This study was approved by the Ethics Committee of the Faculty of Medicine, Tottori University.

2.2. Genotyping

Genotyping of the C-to-T mutation was performed using amplified product length polymorphism (APLP) and high-resolution melting (HRM) assays. The primers are shown in Table 1. 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, USA), 2 μL of each primer (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 were as follows: 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.

For the HRM assay, 150 μL of the PCR cocktail was prepared to contain 100 μL of LightCycler 480 High Resolution Melting Master (Roche, Mannheim, Germany), 6 μL of each primer (10 μM), 16 μL of 25 mM $MgCl_2$, and 22 μL of water. A mixture of 9 μL of HRM cocktail and 3 μL of template DNA containing about 15 ng was subjected to PCR, of which the conditions were as follows: 95 °C for 10 min, then 50 cycles of 95 °C for 10 s, 55 °C for 15 s, and 72 °C for 15 s. The samples were kept at 95 °C for 60 s and 40 °C for 60 s, and then were melted at a ramp rate of 2.2 °C/s from 60 °C to 97 °C. All these reactions were performed using a LightCycler 96 system (Roche, Mannheim, Germany).

Table 1
APLP and HRM primers used for ADAMTS13 c.1423C>T genotyping.

Method	Primer	Sequence (5′ → 3′)
APLP	ADAMTS13-F2	AACAGTGCaCCAGGACCGA
	ADAMTS13-TR2	tatatCCtCACCTTGGCTGTGTGA
	ADAMTS13-CR2	CtCCACCTTGGCTGTGTGG
HRM	ADAMTS13-F4	TTCTACCACTGGGGTGCTG
	ADAMTS13-R4	CTGTCCGAGGGTCTCCAG

Non-complementary nucleotides are written in lower case letters.

To confirm the results obtained by APLP and HRM assays, several samples were analyzed by direct sequencing and restriction fragment length polymorphism (RFLP) analysis using *Rsa*I [7,10,13,14]. ABO genotyping was carried out in three populations including Japanese in Tottori (Japanese-Tottori), Oroqen, and Evenki according to the APLP technique [15].

2.3. Data analysis

The allele frequency, Hardy-Weinberg equilibrium, pairwise F_{ST}, and linkage disequilibrium were examined using Arlequin program ver.3.5.1.2 [16]. A contour map of the frequencies for *ADAMTS13*T* was generated using the Surfer 12.0 program (HULINKS, Tokyo, Japan).

3. Results

Fig. 2A shows the electrophoretic results of the APLP assay, where each genotype was clearly and unambiguously distinguished. The PCR products of *ADAMTS13*C* and *ADAMTS13*T* were identified as two different bands with 99 and 104 bp, respectively, because the APLP assay used two allele-specific primers differing in size. Fig. 2B depicts the normalized melting curves after the HRM analysis, where three distinct curves were observed. Similarly, normalized melting peaks and difference plots showed a clear separation of the p.P475S polymorphism (Fig. S1). The genotyping results of several samples obtained by the APLP and HRM assays were confirmed by direct sequencing and RFLP analysis.

Table S1 summarizes the distribution of *ADAMTS13*T* in all 34 populations including nine previously reported frequency data. Our 25 populations passed the exact test for the Hardy–Weinberg equilibrium. Among 29 populations excluding five populations studied regarding the association with disease, the highest frequency of *ADAMTS13*T* was observed in Japanese-Tottori (0.0825). The frequency in the three other Japanese populations on the main islands of Japan (Honshu and Kyushu) was homogeneous, ranging from 0.0542 to 0.0508, and a Japanese population in Okinawa showed a low frequency of 0.0284. Calculation of the pairwise F_{ST} revealed a significant difference only between Tottori and Okinawa ($p = 0.0090$). The high frequency observed in Japanese-Tottori may be incidental. The Japanese population on