

Frequency of Pro475Ser Polymorphism of ADAMTS13 Gene and Its Association with ADAMTS-13 Activity in the Korean Population

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Purpose: The *in vitro* study suggested that proline to serine polymorphism in codon 475 (C1423T) of the A Disintegrin and Metalloprotease with ThromboSpondin type 1 repeats-13 (ADAMTS-13) gene is related to reduced activity of ADAMTS-13. In this study, the frequency of the Pro475Ser polymorphism in Koreans was studied and plasma ADAMTS-13 activity was measured to find out whether this polymorphism contributes to decreased ADAMTS-13 activity in Koreans. **Patients and Methods:** The frequency of the C1423T allele of the ADAMTS13 gene was studied along with measuring plasma ADAMTS-13 activity in 250 healthy Korean individuals. **Results:** The allele frequency of C1423T polymorphism was 4%, and the median activity of CT type was 107 (69 - 143)%, which was lower than in controls with the CC genotype [118 (48 - 197)%, ($p = 0.021$)]. **Conclusion:** Therefore, the Pro475Ser polymorphism seems to be popular in the Korean population, and attenuates ADAMTS-13 plasma activity.

Key Words: ADAMTS-13, polymorphism, thrombotic thrombocytopenic purpura

INTRODUCTION

ADAMTS-13 is a metalloprotease that specifically cleaves von Willebrand (VWF) multimer.^{1,2} The enzyme degrades unusually large VWF (UL-VWF) multimers by cleaving 842Tyr-843Met peptide bonds in susceptible A2 domains of VWF

monomeric subunits.³ A deficiency of ADAMTS-13 activity results in an accumulation of UL-VWF and increased platelet adhesion to vascular walls, and leads to thrombotic thrombocytopenic purpura (TTP) either by congenital deficiency or the presence of inhibitory antibodies of ADAMTS-13, which is a syndrome characterized by microangiopathic hemolytic anemia, thrombocytopenia, neurological disorder, renal failure, and fever.⁴⁻⁶

ADAMTS-13 was purified and its genetic structure was recently identified.^{1,7,8} Human ADAMTS13 gene is composed of 29 exons with a size of 37 kb on chromosome 9q34.⁵ ADAMTS13 is the gene responsible for familial TTP. Discrimination between congenital and acquired TTP is important for the therapeutic plan of acute crisis, and study on genetic mutation is increasingly required for accurate diagnosis. Moreover, growing evidence suggests that ADAMTS-13 is a key enzyme in a new pathway for the regulation of VWF and platelet function and a new candidate for a genetic modifier potentially affecting overall hemostatic balance and the risk of bleeding and/or thrombosis.⁹

Since the landmark study⁵ on genetic mutation of ADAMTS13 as a congenital cause was carried out by Levy et al. in 2002, more than 50 mutations and at least 8 common single-nucleotide polymorphisms have been identified to test this hypothesis.¹⁰⁻¹² Among them, the proline (Pro) to serine (Ser) polymorphism in codon 475 of the ADAMTS13 gene (Pro475Ser), caused by a base substitution of C1423 to T in exon 12 (C1423T) which was found to impair ADAMTS-13 activity

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in vitro, has been reported to be common in the Japanese population, and suggested that the importance of TTP or thrombosis-related gene in Japanese.¹⁰ However, subsequent studies carried out in the Chinese population¹³ or Caucasians¹⁴ did not show positive correlation of C1423T with thrombotic disorders. The allele frequency of C1423T was only 1.5% in the Chinese population, and the heterozygous genotype was not associated with an increased risk of acute ischemic stroke and acute myocardial infarction.¹³ No Pro475Ser polymorphism was identified in Caucasians and Afro-Americans, including ischemic stroke patients.¹⁴ In summary, the allele frequencies of Pro475Ser polymorphism are rare except in the Japanese population, and evidence to support the role of Pro475Ser polymorphism as a new candidate for a genetic modifier potentially affecting overall hemostatic balance and the risk of thrombosis is still lacking.

PATIENTS AND METHODS

Subjects

The study population was enrolled from January to February 2004 from patients who visited Bundang CHA Hospital's health promotion center for a periodic health examination. Two hundred-fifty healthy subjects were selected to represent a healthy Korean population of individuals with no abnormal findings on examination. They comprised of 135 males and 115 females. The Institutional Review Board of Bundang CHA hospital approved the research protocol and informed consent was obtained from all participating individuals and/or their guardians.

Genotyping of C1423T polymorphism

DNA was extracted from leukocytes with a DNA extraction kit (QIAmp blood kit, Qiagen) according to the manufacturer's protocol. Two hundred nanograms of DNA was amplified with PCR by using 100 pmol each of forward primer (F-5'-TGA GGC CAC ACC CAC ATC TTG) and reverse primer (R-5'-ATG CCA GAG CCT GAA CCA CTT), 1.5 mM MgCl₂, 0.2 M each of

deoxynucleotide triphosphates and 1 unit of Taq polymerase (Takara, WI, USA) in a total volume of 100 μ L. PCR conditions were as follows: denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 30s, and 72°C for 30s. The terminal elongation was performed at 72°C for 5 min, by which 365 bp product was obtained. The PCR products were digested with Rsa I restriction enzyme for 2 hrs at 37°C and subjected to electrophoresis. Two bands of 198 and 137 bp indicated a homozygous wild CC type, 3 fragments of 335, 198, and 137 bp indicated heterozygous CT type, and a 335 bp fragment indicated homozygous variant TT type.

Assay of ADAMTS-13 activity

The activity of plasma ADAMTS-13 was examined by fluorescence resonance energy transfer assay.¹⁵ Each 0, 1, 2, 3, 4, 5 and 6 μ L of normal plasma specimen was added to 100 μ L of substrate solution (FRET-S-VWF73, Peptides International, Louisville, KY, USA), and then each fluorescent degree of specimens was measured in 5-min intervals per hr by using a fluorescence spectrophotometer (VICTOR3, PerkinElmer, Yokohama, Japan). The slope of each specimen was measured and the standard curve was obtained from these measurements.

Since substrate solution was reacted with 4 μ L of plasma specimen of the object, the slope of each fluorescent degree detected at different times was compared with the standard curve, and the activation degree of ADAMTS-13 was expressed in percentages. The intra-assay coefficient of variation (CV) was 5.3%.

Statistical analysis

The distribution of allele frequencies for the Pro475Ser polymorphism was examined by χ^2 test to determine whether the observed genotype distributions confirm to the Hardy-Weinberg equilibrium expectation. Differences in ADAMTS-13 activity according to different genotypes were calculated using Mann-Whitney test. A 2-tailed *p* value of less than 0.05 was regarded as statistically significant. Results were expressed as median and range.

RESULTS

Frequency of Pro475Ser polymorphism

The median ages for the CC and CT groups were similar (44 and 41 yrs old, respectively). Male to female ratio was 125:105 for the CC group and 10:10 for the CT group without gender difference. The genotype distributions of C1423T polymorphic loci did not significantly deviate from the Hardy-Weinberg equilibrium. The genotype of the C1423T polymorphism was the CC type of 230 people (92%), CT type of 20 (8%), and TT type (none). The allele and heterozygote frequencies of the C1423T polymorphism were 4% and 8% (Table 1).

Plasma ADAMTS-13 activity according to C1423T genotypes

We measured ADAMTS-13 activity with 219 healthy individuals chosen from 230 people of CC type and 19 healthy individuals of CT type

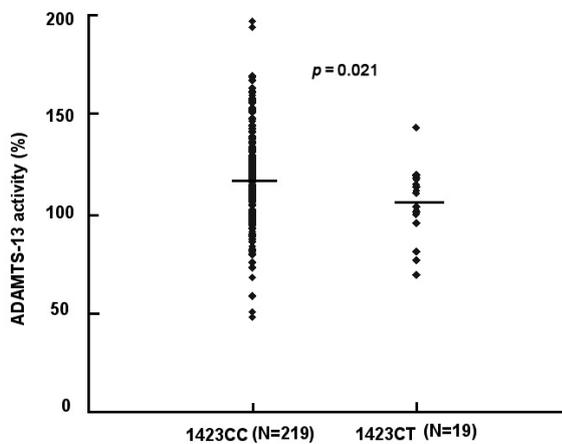


Fig. 1. Plasma ADAMTS-13 activity according to the genotype of C1423T polymorphism in a healthy Korean population.

because 11 plasma samples of 230 CC type and 1 plasma sample of 20 CT type were exhausted during repeated experiments. The median (range) ADAMTS-13 activity of the CT type was 107 (69-143)%, which was slightly lower than that of the CC type, 118 [48 - 197)% ($p = 0.021$)] (Fig. 1). This difference was not affected by the presence of 1.5 M urea (data not shown). There was no severe ADAMTS-13 activity deficient case in CT heterozygote individuals.

DISCUSSION

The allele frequency of Pro475Ser polymorphism in Koreans was 4%, which is between the range of the Japanese (5.1%)¹¹ and Chinese Han race (1.5%).¹² Since Pro475Ser polymorphism has been shown to be absent in Caucasians or Afro-Americans,¹³ our results suggest that the frequency of the C1423T allele is different among races (Table 2) and higher in Koreans and Japanese than other races.

In the present study, we confirmed that Pro475Ser polymorphism contributes to decreased ADAMTS-13 activity and demonstrated mild decrease of plasma ADAMTS-13 activity in individuals with Pro475Ser polymorphism. This is the first demonstration of decreased activity of ADAMTS-1 Pro475Ser polymorphism in human samples, and the result is consistent with that by Kokame et al.,¹⁰ who demonstrated decreased ADAMTS-13 activity in the media of C1423T mutant transfected HeLa cells.

Severe deficiency of ADAMTS-13 activity less than 5% is specific for TTP.¹⁶ Although we confirmed that Pro475Ser polymorphism contributed to decreased ADAMTS-13 activity, the clinical implication of only 11% difference seemed to be questionable because there was no severe

Table 1. Genotypes and Allele Frequencies of C1423T Polymorphism in a Healthy Korean Population

	Genotypes			Allele frequencies
	CC	CT	TT	T
Observed number (%)	230 (92.0)	20 (8.0)	0 (0.0)	0.04
Expected number	230.4	19.2	0.4	0.04

$\chi^2 = 0.433$, $df = 1$, $0.5 < p < 0.8$.

Table 2. Genotype and Allele Frequencies of C1423T Polymorphism in Different Populations

Population	Tested subjects (n)	Genotypes			Allele frequencies		References
		CC	CT	TT	C	T	
Japanese	364	328	35	1	0.949	0.051	Kokame et al. ¹⁴
Chinese	400	388	12	0	0.985	0.015	Gao et al. ¹⁵
Caucasian	250	250	0	0	1.000	0.000	Boners et al. ¹⁶
Korean	250	230	20	0	0.960	0.040	Present study

ADAMTS-13 deficient case in all heterozygotes of the C1423T allele examined. Therefore, this substitution is not likely to be clinically relevant to develop TTP or thrombotic disorders because the decrease in the activity is minimal. Further studies are required to clarify whether the C1423T substitution may act synergistically with other ADAMTS13 substitution or modifiers to develop TTP or other thrombotic disorders in the Korean population.

In conclusion, the allele frequency of C1423T polymorphism in Koreans was 4%. The ADAMTS-13 Pro475Ser polymorphism is not a major determinant to develop thrombotic thrombocytopenic purpura or thrombotic disease in the Korean population because its contribution to decreased ADAMTS-13 activity is minimal.

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