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BRIEF REPORT

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Racial differences in protein S Tokushima and two protein C variants as genetic risk factors for venous thromboembolism

Hiroko Tsuda MD, PhD[1] | Kenta Noguchi PhD[1] | Doyeun Oh MD, PhD[2] | Zsuzsanna Bereczky MD, PhD[3] | Lai H. Lee MD, PhD[4] | Dongchon Kang MD, PhD[5] | Luci M. S. Dusse PhD[6] | Maria das G. Carvalho PhD[6] | Eriko Morishita MD, PhD[7] | The SSC Subcommittee on Plasma Coagulation Inhibitors of the ISTH

[1]Department of Nutritional Sciences, Nakamura Gakuen University, Fukuoka, Japan

[2]Division of Hemato-oncology, School of Medicine, CHA University, Seongnam, South Korea

[3]Division of Clinical Laboratory Science, Department of Laboratory Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

[4]Department of Haematology, Singapore General Hospital, Singapore City, Singapore

[5]Department of Clinical Chemistry and Laboratory Medicine, Kyushu University Hospital, Fukuoka, Japan

[6]Faculty of Pharmacy, Federal University of Minas Gerais, Minas Gerais, Brazil

[7]Department of Laboratory Medicine, Kanazawa University Medical School, Kanazawa, Japan

Correspondence
Hiroko Tsuda, Department of Nutritional Sciences, Nakamura Gakuen University, 5-7-1, Befu, Johnan-ku, Fukuoka 814-0198, Japan.
Email: tsuda@nakamura-u.ac.jp

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Abstract

Background: Racial differences in genetic risk factors for venous thromboembolism (VTE) are elucidated, with factor V Leiden and prothrombin G20210A being prevalent among the Caucasian population but rare among non-Caucasians.

Objectives: To assess the worldwide distribution of three gene polymorphisms previously identified as genetic risk factors among East Asian subpopulations: protein S (PS) Tokushima (p.Lys196Glu), protein C (PC) p.Arg189Trp, and PC p.Lys193del.

Methods: An international collaborative study group of seven centers in five countries—Japan, South Korea, Singapore, Hungary, and Brazil—was created, and genotype analyses were performed. A total of 2850 unrelated individuals (1061 patients with VTE and 1789 controls) were included.

Results: PS Tokushima was confined to Japanese patients with VTE (allele frequency, 2.35%) and controls (1.12%), with an odds ratio (OR) of 2.15 (95% confidence interval, 1.16-3.99). PC p.Arg189Trp carriers were prevalent among Chinese and Malay patients with VTE in Singapore, with allele frequencies of 10.53% and 22.73%, respectively. Carriers of PC p.Lys193del were identified among Japanese and Korean patients with VTE (0.87% and 2.35%, respectively) and controls (0.36% and 1.07%, respectively), with the OR for VTE not being significant, and Chinese patients with VTE in Singapore (5.26%). In contrast, no carriers of PS Tokushima and two PC gene variants were found among patients with VTE or controls from Hungary, Brazil, or Indians in Singapore.

Conclusion: The three variants were prevalent among East and Southeast Asians, having some differences in geographic distribution, but were absent among Caucasian subpopulations and Brazilians.

KEYWORDS

genetic risk factors, protein C, protein S, racial difference, venous thromboembolism

Essentials

- Racial differences in genetic risk factors for venous thromboembolism are elucidated.
- The worldwide distribution of PS Tokushima, PC p.Arg189Trp, and PC p.Lys193del was assessed.
- PS Tokushima was confined to Japanese; PC variants were prevalent in East and Southeast Asia.
- Three variants were absent among Caucasian subpopulations and Brazilians.

1 | INTRODUCTION

Venous thromboembolism (VTE), consisting mainly of deep vein thrombosis (DVT) and pulmonary embolism (PE), is a typical multifactorial disease caused by both genetic and acquired risk factors. Genetic risk factors for VTE include loss of function in anticoagulant factors, including antithrombin, protein C (PC), and protein S (PS), or gain of function in procoagulants such as factor V Leiden and prothrombin G20210A.[1] The latter two gene variants are prevalent among Caucasian populations but rare among non-Caucasians. In contrast, inherited deficiencies in PS and PC are prevalent among Japanese and Chinese patients with VTE but are rare among Caucasian patients.[2,3] A PS gene (*PROS1*) variant, PS Tokushima (p.Lys196Glu, K155E in the mature protein numbering; rs121918474) with a plasma phenotype of type II PS deficiency, is a genetic risk factor for DVT with an allele frequency of 0.6%-0.9% in the Japanese population and a 3.7- to 8.6-fold higher risk of DVT among heterozygotes.[4-6] For Chinese populations, two PC gene (*PROC*) variants, p.Arg189Trp (R147W; rs146922325)[7,8] and p.Lys193del (K150del; rs199469469)[9], were identified as genetic risk factors for VTE with allele frequencies of 0.4% and 1.2% in the general population, and related 5.1- to 7.3-fold and 2.7-fold higher risks of VTE among heterozygotes, respectively. However, systematic analyses of the worldwide distribution of these three variants have not yet been performed.

From this perspective, we conducted an international collaborative study in an attempt to assess the racial differences in the allele frequency of these three variants in the general population and in patients with VTE, and their significance as genetic risk factors for VTE.

2 | METHODS

This study was performed as a project of the Plasma Coagulation Inhibitors Subcommittee of Scientific and Standardization Committee of the ISTH between June 2013 and April 2019. A total of seven centers in five countries in four geographic regions participated: Eastern Asia (Japan and South Korea), Southeastern Asia (Singapore), Eastern Europe (Hungary), and South America (Brazil). The geographic regions were determined according to the definition by the United Nations[10]. All centers enrolled patients with VTE and healthy controls except one, Nakamura Gakuen University (Fukuoka, Japan), which enrolled only healthy controls. The study protocol was submitted to the institutional review board of each center according to local requirements, and signed informed consent was received from all subjects. Data were collected using a standardized registry sheet on the general demographics, including age, sex, and racial group classified as White (Caucasian), Hispanic, Black (African), Japanese, Korean, Chinese, Malay, Indian, or others. As most of the Brazilian population is the result of the miscegenation of three racial groups, namely European colonizers, especially Portuguese, Africans, and autochthonous Amerindians,[11] we categorized them as Brazilian. Medical histories were obtained from patients with VTE, including type of thrombosis, date of events, diagnostic methods, and environmental risk factors. The inclusion criteria for patients with VTE were symptomatic DVT, PE, or venous thrombosis at other locations (other venous thrombosis), which was confirmed objectively, regardless of age and period after the onset. DVT was diagnosed mainly by Doppler ultrasound and D-dimer investigation, PE by spiral computed tomography (CT) of pulmonary arteries or ventilation-perfusion lung scan, and other venous thrombosis by spiral CT. Healthy unrelated individuals, including university students and workers; nonrelative accompanying persons of patients with or without VTE; examinees of medical checkups; or residents from the same geographic area as patients with VTE were enrolled as controls; the controls varied among centers. Control subjects were excluded if they had a history of thrombosis, including VTE; were receiving anticoagulants or oral contraceptives; or were pregnant or in puerperium. In total, 1112 patients with VTE and 1802 controls were enrolled. Eight patients were excluded because they were family members of patients with VTE, 29 patients and 11 controls were excluded because of blood samples insufficient to perform gene analyses, and 14 patients and 2 controls were excluded due to incomplete information about the general demographics.

2.1 | Genotype analyses

The frozen buffy coat of citrated blood samples obtained from participants of all centers except two in Japan was sent to the coordinating center, Nakamura Gakuen University. Genomic DNA was purified from the buffy coat, and the genotype was determined by the quantitative PCR method using cycling probe technology for the PS p.Lys196Glu and PC p.Arg189Trp variants, and TaqMan probe technology for the PC p.Lys193del variant, as reported previously.[12] At two other centers in Japan, the genotypes were determined by the quantitative PCR method using TaqMan probe technology and/or direct-sequencing methods of *PROS1* and *PROC* genes.

2.2 | Statistical analysis

To investigate the associations of gene variants with VTE and the subtypes of VTE, we calculated the odds ratio (OR) with 95% confidence interval (CI) and *P* value using the chi-square test. Statistical analyses were performed using PASW Statistics version 23 (SPSS Inc, Chicago, IL, USA), and a *P* value of $< .05$ indicated significance.

3 | RESULTS AND DISCUSSION

3.1 | Characteristics of participants

After applying the exclusion criteria, 2850 unrelated individuals, 1061 patients with VTE (female, 52.7%) and 1789 controls (female, 61.9%), were included in this study (Table 1). Racial backgrounds of the participants from Japan, South Korea, and Hungary were homogeneous, and solely comprised Japanese, Korean, and Caucasian, respectively. In contrast, Singapore is a multiracial nation, and approximately 70% of patients with VTE were Chinese, 13% Malay, 10% Indian, and 7% others; the racial composition of patients with VTE was almost the same as that of controls. All participants from Brazil were classified as Brazilians. In Japan, 44.2% of patients with VTE had DVT only, 20.4% PE only, 20.6% DVT + PE, and 14.8% other venous thrombosis; in South Korea, 44.7% had DVT only, 23.5% PE only, 31.8% DVT + PE; in Singapore, 61.5% had DVT only, 2.4% PE only, 33.7% DVT + PE, and 2.4% other venous thrombosis; in Hungary, 75.1% had DVT only, 7.9% PE only, and 17.1% DVT + PE; and in Brazil, 87.0% had DVT only, 0.7% PE only, 3.2% DVT + PE, and 9.1% other venous thrombosis.

TABLE 1 Characteristics of patients with VTE and controls in five countries

Country	Geographic region[a]		VTE	Controls
Japan	Eastern Asia	Total, n	446	1031
		Age, y, mean (SD)	44.7 (17.4)	47.1 (19.0)
		Female, n (%)	235 (52.7)	559 (54.2)
		Racial group, n (%)		
		Japanese	446 (100)	1031 (100)
South Korea	Eastern Asia	Total, n	85	140
		Age, y, mean (SD)	58.2 (17.6)	57.6 (9.3)
		Female, n (%)	38 (44.7)	58 (41.4)
		Racial group, n (%)		
		Korean	85 (100)	140 (100)
Singapore	Southeastern Asia	Total, n	83	74
		Age, y, mean (SD)	58.4 (13.9)	43.4 (15.4)
		Female, n (%)	37 (44.6)	48 (64.9)
		Racial group, n (%)		
		Chinese	57 (68.7)	59 (79.7)
		Malay	11 (13.3)	8 (10.8)
		Indian	9 (10.8)	4 (5.4)
		Others	6 (7.2)	3 (4.1)
Hungary	Eastern Europe	Total, n	293	243
		Age, y, mean (SD)	55.5 (16.4)	33.4 (11.4)
		Female, n (%)	144 (49.1)	155 (63.8)
		Racial group, n (%)		
		Caucasian	293 (100)	243 (100)
Brazil	South America	Total, n	154	301
		Age, y, mean (SD)	40.7 (14.5)	28.3 (11.9)
		Female, n (%)	105 (68.2)	287 (95.3)
		Racial group, n (%)		
		Brazilian	154 (100)	301 (100.0)

Abbreviation: VTE, venous thromboembolism.

[a]The geographic regions were determined according to the definitions by the United Nations.[10]

3.2 | PS Tokushima (p.Lys196Glu) variant

Genotype analyses revealed that the carriers of PS Tokushima (p.Lys196Glu) variant were only Japanese patients with VTE and controls (Table 2). Absence of this variant was reported in Chinese and Korean populations[13], and in patients with VTE and controls mainly of Caucasian ethnicity.[14] However, the present study is the first to demonstrate that the PS p.Lys196Glu variant is confined within Japan among four geographic regions. The allele frequencies of PS p.Lys196Glu variant in Japanese patients with VTE and controls were 2.35% and 1.12%, respectively. Although the age and sex of controls were not exactly matched with those of patients with VTE, we evaluated the risk of this variant for VTE: The OR (95% CI) was 2.15 (1.16-3.99; $P = .01$). The OR for VTE in this study was relatively lower than the previously reported values for DVT of 3.74-8.56 in not-matched case-control studies.[4-6] To assess whether the PS p.Lys196Glu variant has a similar differential effect on DVT and PE as factor V Leiden, the so-called factor V Leiden paradox,[15,16] we calculated the allele frequencies and OR (95% CI) based on the subtypes of VTE: for DVT only (n = 197), 3.30% (11 heterozygotes and one homozygote) and 2.98 (1.45-6.12, $P = .002$); for PE only (n = 91), 2.20% (4 heterozygotes) and 2.11 (0.71-6.26; $P = .17$); for DVT + PE (n = 92), 1.09% (2 heterozygotes) and 1.02 (0.24-4.40; $P = .98$), respectively (Table S1). Accordingly, the PS p.Lys196Glu variant was likely to be a risk factor for DVT but not for PE with or without DVT. As with the factor V Leiden paradox,[16] this finding needs to be confirmed using prospective cohort studies.

3.3 | PC p.Arg189Trp variant

As shown in Table 2, the allele frequencies of the PC p.Arg189Trp variant were markedly high in Singapore patients with VTE and controls (10.84% and 1.35%, respectively), and the OR (95% CI) for VTE was 9.27 (2.06-41.67; $P = .001$). However, no carrier was found in other countries except for a heterozygous patient with VTE in Japan. As Singapore is a multiracial nation, we then examined the genotypes of each racial group in Singapore (Table 3). Among Chinese, the allele frequencies of this variant in patients with VTE and controls were 10.53% and 0.85%, respectively, and the OR (95% CI) was 13.87 (1.73-111.39; $P = .002$). These values are higher than the previously reported allele frequency in the general population (0.4%) and the OR for VTE (5.1-7.3) of Chinese in Taiwan[7] and in China[8]. Among Malay, the allele frequency in patients with VTE was also high, 22.73%, but no carrier was found among controls. One Batak patient with VTE and a control of Myanmar were heterozygotes; however, no carrier was identified among the Indian participants. This suggests that the PC p.Arg189Trp variant is a highly frequent thrombophilia among Chinese and Malay, and possibly among Batak and Myanmar. Recently, this variant was reported to be prevalent in

TABLE 2 Genotypes of PS Tokushima (p.Lys196Glu), PC p.Arg189Trp, and PC p.Lys193del variants of patients with VTE and controls in five countries

	PS p.Lys196Glu			PC p. Arg189Trp			PC p.Lys193del		
		VTE	Controls		VTE	Controls		VTE	Controls
Country		n (%)	n (%)		n (%)	n (%)		n (%)	n (%)
Japan	Lys/Lys	426 (95.5)	1009 (97.9)	Arg/Arg	282 (99.6)	868 (100)	Lys/Lys	440 (98.7)	961 (99.3)
	Lys/Glu	19 (4.3)	21 (2.0)	Arg/Trp	1 (0.4)	0	Lys/del	4 (0.9)	7 (0.7)
	Glu/Glu	1 (0.2)	1 (0.1)	Trp/Trp	0	0	del/del	2 (0.4)	0
South Korea	Lys/Lys	85 (100)	140 (100)	Arg/Arg	85 (100)	140 (100)	Lys/Lys	82 (96.5)	138 (98.6)
	Lys/Glu	0	0	Arg/Trp	0	0	Lys/del	2 (2.4)	1 (0.7)
	Glu/Glu	0	0	Trp/Trp	0	0	del/del	1 (1.2)	1 (0.7)
Singapore	Lys/Lys	83 (100)	74 (100)	Arg/Arg	66 (79.5)	72 (97.3)	Lys/Lys	77 (92.8)	74 (100)
	Lys/Glu	0	0	Arg/Trp	16 (19.3)	2 (2.7)	Lys/del	6 (7.2)	0
	Glu/Glu	0	0	Trp/Trp	1 (1.2)	0	del/del	0	0
Hungary	Lys/Lys	293 (100)	243 (100)	Arg/Arg	293 (100)	243 (100)	Lys/Lys	293 (100)	243 (100)
	Lys/Glu	0	0	Arg/Trp	0	0	Lys/del	0	0
	Glu/Glu	0	0	Trp/Trp	0	0	del/del	0	0
Brazil	Lys/Lys	154 (100)	301 (100)	Arg/Arg	154 (100)	301 (100)	Lys/Lys	154 (100)	301 (100)
	Lys/Glu	0	0	Arg/Trp	0	0	Lys/del	0	0
	Glu/Glu	0	0	Trp/Trp	0	0	del/del	0	0

Abbreviations: PC, protein C; PS, protein S; VTE, venous thromboembolism.

TABLE 3 Genotypes of PC p.Arg189Trp and PC p.Lys193del variants of patients with VTE and controls of each racial group in Singapore

	PC p. Arg189Trp			PC p.Lys193del		
		VTE	Controls		VTE	Controls
Racial group		n (%)	n (%)		n (%)	n (%)
Chinese	Arg/Arg	46 (80.7)	58 (98.3)	Lys/Lys	51 (89.5)	59 (100)
	Arg/Trp	10 (17.5)	1 (1.7)	Lys/del	6 (10.5)	0
	Trp/Trp	1 (1.8)	0	del/del	0	0
Malay	Arg/Arg	6 (54.5)	8 (100)	Lys/Lys	11 (100)	8 (100)
	Arg/Trp	5 (45.5)	0	Lys/del	0	0
	Trp/Trp	0	0	del/del	0	0
Indian	Arg/Arg	9 (100)	4 (100)	Lys/Lys	9 (100)	4 (100)
	Arg/Trp	0	0	Lys/del	0	0
	Trp/Trp	0	0	del/del	0	0
Others	Arg/Arg	5 (83.3)	2 (66.7)	Lys/Lys	6 (100)	3 (100)
	Arg/Trp[a]	1 (16.7)	1 (33.3)	Lys/del	0	0
	Trp/Trp	0	0	del/del	0	0

Abbreviations: PC, protein C; VTE, venous thromboembolism.

[a]The racial group of a patient with VTE and a control was Batak and Myanmar, respectively.

the general Thai population (allele frequency, 3.0%) and was identified as a risk factor for thromboembolism in subjects <18 years old.[17]

3.4 | PC p.Lys193del variant

Carriers of the PC p.Lys193del variant were identified in Japan, South Korea, and Singapore, but not in Hungary or Brazil (Table 2). The allele frequencies of this variant in Japanese patients with VTE and controls were 0.90% and 0.36%, respectively, and those in Korean patients with VTE and controls were 2.35% and 1.07%, respectively; however, neither OR (95% CI) for VTE was significant (1.87 [0.63-5.60] and 2.52 [0.41-15.42], respectively). The subtype analyses of Japanese patients with VTE revealed that this variant was not present among patients with PE and not a risk factor for either DVT or DVT + PE (2.85 [0.83-9.81] and 1.51 [0.18-12.40], respectively; Table S1). Subtype analysis was not performed on Korean patients with VTE because of the small sample size of each subtype of VTE. In Singapore, six heterozygotes were found among 83 patients with VTE (allele frequency, 3.61%), but there was no carrier among the controls (Table 2). As shown in Table 3, the heterozygous carriers of Singapore patients with VTE were all Chinese, with an allele frequency of 5.26%; this value was slightly higher than that in the previous report on patients with VTE in China (3.44%).[9] However, the PC p.Lys193del variant was not identified as a genetic risk factor for VTE in the present study, in contrast to the previous report on Chinese patients with VTE.[9] Recently, we found that the PC anticoagulant activities of heterozygotes of the PC p.Lys193del variant were not significantly different from those of wild-type individuals, but their PC antigen levels were high, resulting in a markedly lower activity/antigen ratio.[12] This suggests that the thrombophilic effects of this variant are mild and additional underlying risk factors may induce thrombosis.

A Chinese patient with unprovoked DVT whose first onset was at the age of 44 years old was a compound heterozygote for PC p.Lys193del and PC p.Arg189Trp variants. Thus, 16 (28.1%) of 57 Chinese patients with VTE in Singapore were carriers of either the PC p.Lys193del or PC p.Arg189Trp variant.

3.5 | Limitations

This study has some limitations as a case-control study. First, the age and sex of healthy controls were not matched with those of the patients with VTE. Second, neither the environmental risk factors of VTE, such as advanced age, history of cancer, pregnancy, obesity, and long travel, nor additional genetic risk factors of VTE were evaluated. Third is the lack of detailed information regarding VTE such as proximal or distal DVT, or provoked or unprovoked VTE. Therefore, matched case-control studies and logistic regression analyses adjusted for variables are needed to clarify the true risk of each variant for VTE.

3.6 | Conclusion

This study demonstrated that racial differences exist in the allele frequency of PS Tokushima (p.Lys196Glu), PC p.Arg189Trp, and PC p.Lys193del variants. PS Tokushima was confined to Japanese patients with VTE and controls: PC p.Arg189Trp carriers were prevalent among Chinese and Malay patients with VTE in Singapore: PC p.Lys193del carriers were identified among Japanese, Korean, and

Chinese patients with VTE. In contrast, no carriers of these three gene variants were found among Caucasian subpopulations and Brazilians. Thus, the distributions of the three variants, being prevalent among East and Southeast Asians but absent among Caucasians are opposite to those of factor V Leiden and prothrombin G20210A[2]. Tsay et al suggested a single origin of PC p.Arg189Trp based on *PROC* haplotype analyses for nine heterozygous carriers.[7] Accordingly, PC p.Arg189Trp and PC p.Lys193del mutations may have developed after the divergence of Asians from Caucasian subpopulations, and PS Tokushima may have developed after settlement of the Japan islands by Japanese ancestors. Detailed haplotype analyses of *PROC* and *PROS1* are necessary to clarify the genetic origins of these variants.

From the findings of this study, we propose that the racial differences in genetic predisposition be considered to develop strategies to minimize VTE; however, further prospective studies evaluating this as a risk factor are needed.

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RELATIONSHIP DISCLOSURE

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

Study conception and design: HT. Data acquisition: HT, KN, DO, ZB, LHL, DK, LMSD, MGC, and EM. Statistical analysis: HT, KN. Interpretation of the data: all authors. Drafting of the manuscript: HT. Critical revision of the manuscript for important intellectual content: all authors. Final approval of the manuscript: all authors.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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