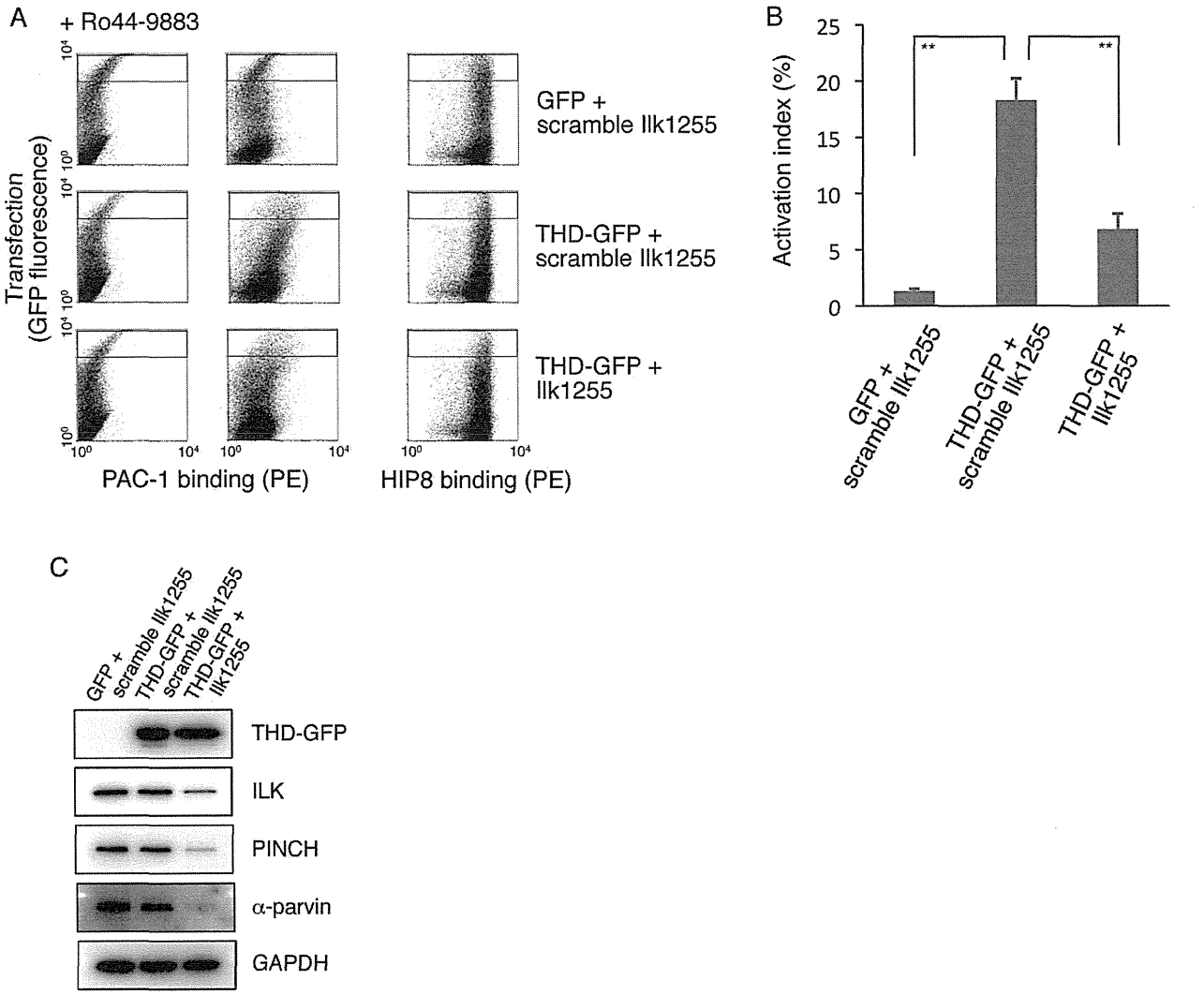

Fig. 5

Figure 5. Knockdown effects of ILK on THD-mediated αIIbβ3 activation. (A) Dot plot detecting PAC-1 binding to cotransfected cells. Inactive αIIbβ3-expressing CHO cells were transiently cotransfected with GFP cDNA plus scrambled ILK siRNA (scramble Ilk1255), with THD-GFP cDNA plus scrambled ILK siRNA (scramble Ilk1255), or with THD-GFP cDNA plus ILK siRNA (Ilk1255). Highly transfected cells (cells in gated regions) were analyzed for PAC-1 binding or HIP8 (an αIIbβ3-specific mAb) binding. (B) The activation indexes of transfected cells. The activation index was determined by the formula shown in Materials and Methods. A value of 100% implies the median fluorescence intensity of HIP8 binding to the cells in gated regions. Data represent means ± SD of three independent experiments. ** indicates $P < 0.01$. (C) Immunoblotting to evaluate expression levels of IPP and THD-GFP. Cell lysates were electrophoresed and immunoblotted with indicated Abs.

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mutually exclusive [34,42]. PINCH-1 is expressed in hematopoietic systems, and strong expression of it has been observed in megakaryocytes during fetal liver hematopoiesis [27]. PINCH-2 also joins in the IPP complex and contributes to the stabilization of individual proteins. We examined only PINCH-1 expression in CHO cells since we were unable to find PINCH-2 mRNA in parental CHO cells. Our knockdown experiment using PINCH-1-specific siRNA revealed the reduction of both ILK and α-parvin expression levels. In addition, published amino acid sequences of hamster PINCH-2

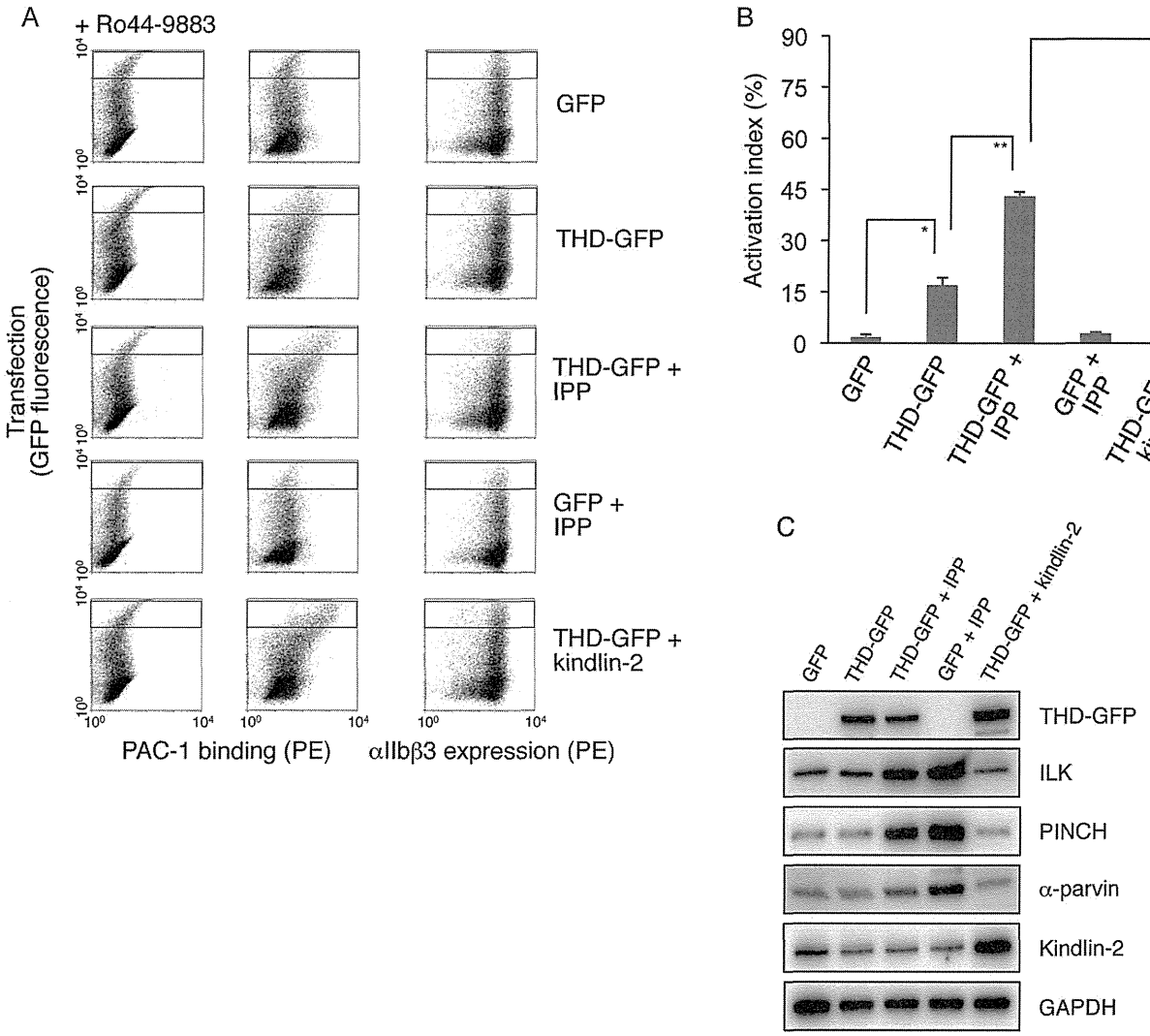

Fig. 6

Figure 6. Effects of IPP overexpression on THD-mediated integrin activation in inactive αIIbβ3-expressing CHO cells. (A) Dot plot detecting PAC-1 binding to transfected cells. Inactive αIIbβ3-expressing CHO cells were transiently transfected with GFP cDNA, with THD-GFP cDNA, with THD-GFP cDNA plus IPP (ILK, PINCH, and α-parvin) cDNAs, with GFP cDNA plus IPP cDNAs, or with THD-GFP cDNA plus kindling-2 cDNA. Highly transfected cells in the gated regions were analyzed for PAC-1 binding or HIP8 (an αIIbβ3-specific mAb) binding. (B) The activation indexes of transfected cells. The index was determined by the formula shown in Materials and Methods. A value of 100% implies the median fluorescence intensity of HIP8 binding to the cells in gated regions. Data represent means ± SD of three independent experiments. * and ** indicate $P < 0.05$ and $P < 0.01$, respectively. (C) Immunoblotting to evaluate expression levels of IPP, THD-GFP, and kindlin-2. Cell lysates obtained from transfected cells were electrophoresed and immunoblotted with indicated Abs.

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(GenBank accession number EGW10997) showed an amino acid length composed of 144 residues, shorter than that of mouse PINCH-2 (accession number NP659111) composed of 341 residues. This suggested that proper PINCH-2 may not be expressed in CHO cells. Unlike PINCH, α- and β-parvins were expressed in CHO cells and the knockdown of both parvins but

not either α- or β-parvin decreased ILK and PINCH expression to a similar extent as the parvins. Thus, the parvins are complemented with each other in the formation of the IPP complex, and either one seems to support integrin activation by maintaining the IPP complex.

Platelets are likely to have α- and β-parvins, and both parvins contribute to the formation of the IPP complex [43,44]. The functional importance of the IPP complex for platelet integrin regulation has not been fully elucidated. There are only a few reports in which the IPP complex stably exists to a similar extent between resting and stimulated platelets [43,44]. It has been shown in human platelets that ILK is activated and binds to the β subunit of αIIbβ3 and the integrin collagen receptor α2β1 after stimulation with thrombin, phorbol 12-myristrate 13-acetate, and collagen [45,46]. These processes seem to aggregation-dependently occur in αIIbβ3 or aggregation-independently arise in α2β1. In a recent study using an ILK-conditional knockout mouse, ILK-deficient platelets exhibited reduced abilities of aggregation, fibrinogen binding, and α-granule secretion [33]. The ILK-deficient platelets also showed decreased expression levels of PINCH and α-parvin, suggesting that the IPP complex is involved in the regulation of integrin affinity. In platelets, the IPP complex may be translocated from the cytoplasm to the integrin β cytoplasmic domain in response to agonist stimulation and may participate in the control of integrin affinity.

The important role of ILK as a signal regulatory protein has been well investigated. Most works demonstrated that ILK functions in outside-in signaling through integrins [20,25,26,47-50]. In the present study, we showed that ILK supports integrin activation by assembling the IPP complex in the CHO cells. This effect may occur through a process in which kindlin-2 recruits ILK to integrins [37,51]. The IPP complex may participate in the cooperation of talin and kindlins in a final step of integrin activation and stabilize the active conformation of integrin.

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Author Contributions

Conceived and designed the experiments: SH ST YT TM. Performed the experiments: SH HSI. Analyzed the data: SH HSI ST YT TM. Wrote the manuscript: SH YT TM.

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Dysfunction of protein C anticoagulant system, main genetic risk factor for venous thromboembolism in Northeast Asians

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Abstract Venous thromboembolism (VTE) is a life-threatening medical disorder worldwide. A great deal of evidence suggests that prevalence of VTE varies significantly among ethnic populations, with consistently lower incidence found in Asians. While the distribution of genetic risk factors may vary among races, genetic risk factors can play a major role among individuals with different genetic backgrounds. Two clinically evaluated low-frequency genetic mutations that predispose to VTE—the factor V Leiden mutation and prothrombin G20210A mutation—are found predominantly in Caucasians, and virtually never in Asians. The findings of a recent genetic study of VTE in northeast Asians, which greatly advanced our knowledge in this area, indicate that the most frequent genetic risk factors for VTE in northeast Asians can be attributed to a dysfunction of the protein C anticoagulant system. Several low-frequency genetic mutations, *PROS1* p.Lys196Glu in Japanese and *PROC* p.Arg189Trp and p.Lys193del in Chinese, are significantly associated with increased risk for VTE, with odds ratio more than 2 through the reduced protein C anticoagulant activity. Construction of a multifactorial model based on the genetic risk factors in the protein C anticoagulant system could facilitate genetic counseling for VTE risk in these populations. The influence of prevalent genetic mutations on the risk of VTE should be further investigated in Asian countries.

Keywords Asian thrombophilia · Genetic risk factor · Protein C anticoagulant system · Venous thromboembolism

Introduction

Venous thromboembolism (VTE), a multifactorial disorder consisting of deep venous thrombosis (DVT) and pulmonary embolism (PE), represents a major thrombotic medical disorder worldwide. Despite acknowledged problems with different criteria and misclassification in determining VTE, there is strong evidence that the prevalence of VTE varies significantly among different ethnic/racial populations. Among the few studies with sufficiently diverse ethnic population samples to make direct comparisons [1–4] (Table 1), the most notable findings were from epidemiological studies based on ethnically diverse populations in California [1.3.4]. They suggested that the annual incidence of idiopathic DVT in persons over 18 years is higher among African Americans (29 per 100,000 individuals per year) than among Caucasians (23 per 100,000 individuals per year), is significantly lower among Hispanic populations (14 per 100,000 individuals per year), and is strikingly lower among Asian-Pacific Islanders (6 per 100,000 individuals per year) [1]. Population-based epidemiological studies of VTE are relatively rare in Asians. Recently, Sakuma et al. [5] reported the annual estimated incidence of PE and DVT in Japanese to be 6.19 and 11.55 patients per 100,000 individuals per year, respectively. Lee et al. [6] analyzed the incidence of symptomatic VTE in almost the entire population of

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Table 1 Prevalence of VTE in diverse ethnic populations

Population origin	Diagnosis	Incidence rate per 100,000 individuals/year					Year	Reference
		African	Caucasian	Hispanics	Asian	Others		
Hospital patients	Idiopathic DVT	29	23	14	6	–	1991–1994	White et al. [1]
General population	PE or DVT	22	21	9	2	15[a]	1978–1985	Klatsky et al. [2]
Hospital patients	Total VTE	141	104	55	21	64	1996	White et al. [3]
Hospital patients	Idiopathic VTE	32	28	15	6	–	1996	White et al. [3]
General population	First-time VTE	141	103	62	29	23	1996	White and Keenan [4]

VTE venous thromboembolism; *DVT* deep vein thrombosis; *PE* pulmonary embolism

[a] In mixed or other ethnic populations

Taiwan. The incidence of PE and DVT among Taiwanese adults was 4.8 and 16.5 per 100,000, respectively, which is lower than among Caucasians and African-Americans, and similar to that of other Asians. Nevertheless, prevalence of PE and DVT in Asia may be increasing with improvements in diagnosis and access to healthcare [7].

VTE is recognized to be a multifactorial, complex disorder, which results from an interaction between environmental, clinical, and genetic risk factors. While racial differences in the incidence of VTE have been well established, interactive risk factors also vary by race [8]. Generally accepted environmental and clinical risk factors for VTE—such as obesity and access to complex surgery and cancer treatments—as well as prevalence of VTE risk factors such as human immunodeficiency virus and the sickle cell trait, are likely to emerge as important mediators of the racial difference in VTE [7]. Data from studies in Asian patients indicate a lower incidence of symptomatic VTE complicating trauma, immobilization, surgery, and/or use of thalidomide [7]. In addition to these well-established risk factors for VTE, it is conceivable that genetic risk factors may vary and play a major role in the different distribution of VTE among people from different genetic backgrounds [8].

The involvement of genetic factors in increased risk for VTE was confirmed in family-based studies in Caucasians, where genetics were thought to account for up to 60 % of risk [9]. Well-established genetic risks for VTE include increased pro-coagulant activities and hereditary deficiencies of natural anticoagulants. Two well-known and clinically evaluated genetic mutations associated with VTE are factor V Leiden mutation (factor V p.Arg506Gln mutation) and prothrombin G20210A mutation, which are found predominantly in populations with European ancestry, and are virtually non-existent in Asians without European admixture [8]. The relatively lower incidence of VTE in Asians compared to Caucasians may partly be due to the lower prevalence of these predisposing genetic factors.

Recently, in northeast Asian populations, where VTE incidence appears low, the most represented genetic risk factors are congenital deficiency of natural anticoagulants, especially genetic deficiency concerning the dysfunction of the protein C anticoagulant system [10]. The purpose of this review is to discuss the prevalence of this deficiency in Asians, and to evaluate the influence of genetic mutations in the protein C anticoagulant system on the risk of VTE in these populations. In this review, the A of the ATG initiator Met codon is denoted as nucleotide +1, and the initial Met residue is denoted as amino acid +1 [11].

Protein C anticoagulant system

Natural anticoagulation in healthy individuals is primarily achieved through the actions of the anticoagulant systems, which include antithrombin, tissue factor pathway inhibitor, protein C, and protein S. Antithrombin plays a major role through the inhibition of thrombin and factor Xa. Tissue factor pathway inhibitor performs its physiological and pathological roles through the inhibition of factor Xa and factor VIIa-tissue factor complex. Unlike these protease inhibitors, the inactive serine-protease zymogen, protein C, must first be activated by thombomodulin (TM)-bound thrombin on the endothelial surface, with the help of the endothelial cell protein C receptor (EPCR); this produces activated protein C (APC) that then proteolytically inactivates factors Va and VIIIa in the presence of protein S. Protein S also stimulates factor Xa inhibition by tissue factor pathway inhibitor, resulting in the down-regulation of the extrinsic coagulation pathway. Thus, the protein C anticoagulant system, consisting of protein C, protein S, TM, and EPCR, regulates the balance between procoagulant and anticoagulant activities. Thrombus formation occurs when this balance is disturbed.

Deficiency of natural anticoagulants and prevalence in Asians

Deficiencies of proteins C and S result in the dysfunction of the protein C anticoagulant system. Hereditary protein C deficiency is usually inherited as an autosomal dominant trait. It is associated with an increased risk of VTE, and is thus considered hereditary thrombophilia. Protein C deficiency is classified into type I (low plasma concentration of both functional and immunological protein C) and type II (low concentration of functional protein C with normal antigen concentration). The inheritance pattern of protein S deficiency is usually autosomal-dominant. Protein S deficiency is classified as type I (quantitative deficiency of both activity and antigen concentration), type II (qualitative deficiency characterized by decreased activity with normal antigen concentration), or type III (normal concentration of total protein S and low concentration of free protein S).

The frequency of deficiencies of protein C, protein S, and antithrombin in VTE patients of Western ethnicity was reported to be 1.4–8.6, 1.4–7.5, and 0.5–4.9 %, respectively [12]. Compared with Caucasians, deficiencies of protein C and protein S in Asians were higher in both the general population and in VTE patients [13–23]. As shown in Table 2, the most prevalent deficiencies in Asian VTE patients were protein S deficiency, followed by protein C deficiency. A report from Hong Kong claimed that as many as 42 % of Chinese VTE patients have reduced activity of the protein C anticoagulant system [13]. In the Taiwanese population, about 50 % of VTE patients showed reduced activity of protein C and protein S [24]. In the Japanese population, the frequency of mutations of the protein C gene was almost three times higher than in Caucasian patients, and protein S deficiency was approximately 5–10 times more prevalent in Japanese VTE patients [20]. These studies suggested that Asian individuals have thrombophilias that differ from those of Caucasians, with a high likelihood of thrombophilia being due to an abnormality of protein C or protein S. They also indicate that there may be an overall higher prevalence of abnormality in protein C or protein S in Asian populations in general, and that a higher occurrence of this class of genetic risk factors may be expected in patients with VTE from the same genetic background [8].

Genetic mutations in the protein C anticoagulant system with VTE in Asians

Recently, several genetic mutations that are associated with a reduction of protein C anticoagulant activity and increased risk for VTE have been confirmed in the protein C anticoagulant system in Japanese and Chinese populations. While differences in VTE by race due to genetic predisposition will probably always be present, understanding the racially specific genetic risk factors for VTE can provide important information about etiological mechanisms, as well as novel therapeutic targets.

PROS1 p.Lys196Glu as a genetic risk of VTE in Japanese

Approximately 8–47 % of Japanese and Chinese individuals who develop VTE have reduced activities of protein S (Table 2). At present, more than 200 mutations have been described in the protein S gene (*PROS1*), and large deletions/duplications can also be identified as causes of protein S deficiency [25, 26]. The most common *PROS1* mutation is a p.Lys196Glu mutation (rs121918474, c.586A>G, protein S Tokushima, p.Lys155Glu in the mature protein numbering), which accounts for 9–30 % of protein S molecule abnormalities in people of Japanese decent [10, 20, 27–29].

An abnormal protein S molecule with the p.Lys196Glu mutation was identified in thrombophilic Japanese patients almost simultaneously by two independent groups in 1993 [30, 31]. It is a missense mutation that causes Lys196 to be replaced by Glu, formerly known as protein S Tokushima. This mutation is present in the second EGF-like domain of the protein S molecule. The allele frequency is approximately 0.9 % in the Japanese population, which means that 1 out of 55 Japanese carries the mutation as a heterozygote [29, 32, 33]. The frequency is much higher, approximately 6–10 % among DVT patients (Table 3) [20, 28, 32]. While homozygotes for this mutation have been identified in VTE patients, with a prevalence of one homozygote out of approximately 85 patients [20, 32], they have thus far not been identified in the general population [29]. The protein S p.Lys196Glu mutation can also be found in VTE patients with congenital protein C deficiency, thereby facilitating the development of VTE [34], and is frequently seen in VTE patients who are pregnant [35]. So far, 3 independent case–control studies, all performed in Japan, have reached the conclusion that the protein S p.Lys196Glu mutation is a risk factor for VTE, with odds ratio between 3.74 and 8.56 [20, 28, 32] (Table 3).

A genotype–phenotype study of the general Japanese population showed that individuals heterozygous for the mutant Glu-allele had a 16 % mean reduction in plasma protein S anticoagulant activity compared to wild-type individuals [27]. A patient with DVT who is a homozygote of protein S p.Lys196Glu mutation showed 35 % protein S anticoagulant activity and 37 % specific anticoagulant activity (activity/amount of protein S) [20]. In vitro studies using the recombinant proteins have shown that mutant protein S with Glu196 had impaired APC cofactor function

Table 2 Prevalence of protein S, protein C, and antithrombin deficiency in Asians

Population	Number of deficiency/total (%)						Reference
	Protein S deficiency		Protein C deficiency		Antithrombin deficiency		
	VTE patients	General population	VTE patients	General population	VTE patients	General population	
Japanese	20/113 (17.70 %)	8/392 (2.02 %)	9/113 (7.96 %)	2/392 (0.51 %)	2/113 (1.7 %)	0/392 (0 %)	Suehisa et al. [16]
Japanese	–	–	7/108 (6.48 %)	6/4,517 (0.13 %)	6/108 (5.56 %)	7/4,517 (0.15 %)	Sakata et al. [19]
Japanese	–	Male: 14/1,252 (1.12 %) Female: 23/1,438 (1.60 %)	–	–	–	–	Sakata et al. [18]
Japanese	40/85 (47.06 %)	1/126 (0.79 %)	27/85 (31.76 %)	1/95 (1.05 %)	6/85 (7.06 %)	0/95 (0 %)	Kinoshita et al. [20]
Chinese	10/52 (19.23 %)	–	9/52 (17.31 %)	–	5/52 (9.62 %)	–	Liu et al. [13]
Chinese	28/85 (32.94 %)	–	16/85 (18.82 %)	–	3/85 (3.53 %)	–	Shen et al. [24]
Chinese	39/116 (33.62 %)	8/125 (6.40 %)	20/116 (17.24 %)	5/125 (40.00 %)	6/116 (5.17 %)	8/125 (6.40 %)	Shen et al. [14]
Chinese	4/50 (8.00 %)	–	2/50 (4.00 %)	–	2/50 (4.00 %)	–	Ho et al. [15]
Chinese	6/56 (10.71 %)	–	6/56 (10.71 %)	–	4/56 (7.14 %)	–	Chen et al. [17]
Chinese	–	2/3,493 (0.06 %)	–	10/3,493 (0.29 %)	–	3/3,493 (0.09 %)	Zhu et al. [22]
Chinese	11/32 (34.40 %) (protein S or protein C deficiency)						Tang et al. [23]
Thai	10/85 (11.76 %)	–	8/85 (9.41 %)	–	4/85 (4.71 %)	–	Angchaisuksiri et al. [21]

– Data unavailable

Table 3 Influence of genetic variants in the protein C anticoagulant system on VTE in Asians

Gene	Nucleotide change	Amino acid change(in mature protein)	rs number	Risk allele	No. of deficiency/total (%)		Odds ratio (95 % CI)	*p* value	Population	Reference
					Cases	Controls				
PROS1	c.586A>G	p.Lys196Glu (p.Lys155Glu)	rs121918474	G	5/85 (5.88)	5/304 (1.64)	3.74 (1.06–13.2)	–	Japanese	Kinoshita et al. [20]
PROS1	c.586A>G	p.Lys196Glu (p.Lys155Glu)	rs121918474	G	15/161 (9.32)	66/3,651 (1.81)	5.58 (3.11–10.01) adjusted: 4.72 (2.39–9.31)	<0.001	Japanese	Kimura et al. [32]
PROS1	c.586A>G	p.Lys196Glu (p.Lys155Glu)	rs121918474	G	6/60 (10.00)	3/234 (1.28)	8.56 (2.07–35.30)	<0.05	Japanese	Ikejiri et al. [28]
PROC	c.565C>T	p.Arg189Trp (p.Arg147Trp)	rs146922325	T	5/116 (4.31)	11/1,292 (0.85)	5.10 (1.7–14.8)	–	Chinese	Tsay et al. [38]
PROC	c.565C>T	p.Arg189Trp (p.Arg147Trp)	rs146922325	T	59/1,003 (5.88)	9/1,031 (0.87)	7.10 (3.50–14.39) adjusted: 7.34 (3.61–14.94)[a] or 7.13 (3.49–14.56)[b]	3.31×10^{-10} adjusted: 3.88×10^{-8} [a] or 6.88×10^{-8} [b]	Chinese	Tang et al. [23], [39]
PROC	c.565C>T	p.Arg189Trp (p.Arg147Trp)	rs146922325	T	68/1,304 (5.21)	12/1,334 (0.90)	6.06 (3.26–11.25)	1.03×10^{-10}	Chinese	Tang et al. [48]
PROC	c.574_576del	p.Lys193del (p.Lys151del)	rs199469469	Del	68/1,003 (6.78)	25/1,031 (2.42)	2.93 (1.84–4.67) adjusted: 2.71 (1.68–4.36)	2.59×10^{-6} adjusted: 4.59×10^{-5}	Chinese	Tang et al. [23]
PROC	c.574_576del	p.Lys193del (p.Lys151del)	rs199469469	Del	85/1,304 (6.52)	32/1,334 (2.40)	2.84 (1.88–4. 29)	2.77×10^{-7}	Chinese	Tang et al. [48]
THBD	c.2729A>C in tight LD with c.1418C>T	In tight LD with p.Ala473Val	rs3176123	C	33/55[c] (60.00)	462/1,032[c] (44.77)	2.76[c] (1.14–6.67)	0.02[c]	Japanese	Sugiyama et al. [47]
THBD	c.−151G>T	–	rs16984852	T	35/1,304 (2.68)	13/1,334 (0.97)	2.80 (1.48–5.32)	1.02×10^{-3}	Chinese	Tang et al. [48]
PROCR	c.4600A>G	p.Ser219Gly	rs867186	G	15/65[d] (23.07)	7/71[d] (9.86)	2.75 (1.04–7.30)	<0.05	Chinese	Chen et al. [52]
PROCR	c.4600A>G	p.Ser219Gly	rs867186	G	41/112 (36.61)	23/112 (20.54)	1.78 (1.11–2.89)	<0.05	Chinese	Yin et al. [53]

CI confidence interval, *LD* linkage disequilibrium, – Data unavailable

[a] Data were analyzed by logistic regression adjusted for age, gender, smoking status, alcohol abuse, malignant tumor, type 2 diabetes, sedentariness/immobilization, and pregnancy/puerperium

[b] Data were calculated by unconditional logistic regression adjusted for age, gender, smoking status, malignant tumor, sedentariness/immobilization, and pregnancy/puerperium

[c] Male patients

[d] Patients with one G allele

[36]. Plasma protein S activities in carriers of the p.Lys196Glu mutation showed reduced activity as described, but antigen levels were within normal limits [27, 30, 31].

The protein S p.Lys196Glu mutation is race-specific; so far this mutation has not been identified in any population other than Japanese. Chinese and Koreans populations, despite being geographically and genetically close to Japanese, did not carry this mutation [37]. Thus, the protein S p.Lys196Glu mutation must be a recent occurrence and fixed within the Japanese population.

PROC p.Arg189Trp and p.Lys193del as genetic risks of VTE in Chinese

At least 161 different protein C gene (*PROC*) mutations have been reported, and most of them are missense mutations. The predominant genetic defects in the *PROC* gene may be different for different races. Recently, the p.Arg189Trp mutation of protein C (rs146922325, c.565C>T, p.Arg147Trp in the mature protein numbering) was reported by two independent studies to be not only the most frequent variant for protein C deficiency but also a significant risk factor for VTE in Chinese populations [38, 39]. This missense mutation was initially described in an American patient with symptomatic protein C deficiency [40], and was later reported in an asymptomatic individual [41]. Although a rare mutation in Western populations, the p.Arg189Trp mutation was present in approximately 0.9 % of the general Chinese population (Table 3) [38, 39]. The heterozygous state of the p.Arg189Trp mutation is associated with decreased plasma functional activity and a relatively normal protein C antigen level, indicating type II protein C deficiency. This mutation was identified in almost half of the probands with hereditary protein C deficiency [38, 39]. First-degree relatives bearing this variant had an 8.8-fold increased risk of VTE [39]. Two independent population-based case–control studies showed the odds ratio of VTE in carriers of the variant ranged from 5 to 7 (Table 3) [38, 39]. The p.Arg189Trp mutation is located at the C-terminal region of the light chain adjacent to the EGF-2 like domain, and may impair the interaction of protein C with other molecules suggesting that Arg189 may constitute an exosite for the binding of factor Va and/or the thrombin–thrombomodulin complex. Further functional studies are needed to elucidate the deleterious effect of this mutation on the activation of protein C and the inactivation of factor Va by APC. Data on the prevalence of this mutation and the thrombotic risk associated with it in other populations (especially other Asian populations) are still quite limited, and should be further evaluated.

Recently, using coagulation screening tests, resequencing, and a case–control study, Tang et al. revealed that the *PROC* p.Lys193del mutation (rs199469469, c.574_576del, p.Lys151del in the mature protein numbering) was associated with both decreased protein C anticoagulant activity and an increased risk of VTE in Chinese, with an odds ratio of 2.7 (Table 3) [23]. The nomenclature of one amino acid deletion in this case is somewhat complicated, as positions 192 and 193 of protein C are both Lys and one of the Lys residues is deleted in this case. We call the mutation the "p.Lys193del" according to the recommendation of the Human Genome Variation Society [11]. This variant was first described in three Japanese patients who suffered from protein C deficiency [42]. In other studies on protein C and protein S deficiencies in Japanese individuals, this mutation was identified in 2 of 85 VTE patients, as well as in 1 of 30 healthy individuals in one study [20], and in 4 of 173 VTE patients in another [34]. Despite being identified as a rare genetic mutation in Japanese, the contribution of the variant to the risk for VTE was not further evaluated in the general Japanese population. Another recent study found that the prevalence of p.Lys193del mutation was 2.36 % in the general Chinese population [23]. It was identified in 68 of 1,003 VTE patients (6.78 %) and in 25 of 1,031 healthy individuals (2.42 %), therefore, it conferred an increased risk of VTE with an adjusted odds ratio of 2.7 (Table 3) [23]. Patients with the p.Lys193del mutation showed lower anticoagulant activity of protein C, but relatively normal amidolytic activity compared to the wild-type carriers [23, 34, 42]. The anticoagulant activity of the recombinant mutant protein C showed about 40 % of the wild-type, consistent with the value of plasma from the homozygous patient [23]. Although this mutation has been reported previously in Japanese populations, further studies are needed to evaluate its prevalence in other Asians, and to determine whether this polymorphism is a risk factor for VTE in other Asian populations.

Some other *PROC* mutations were also reported in VTE patients from Asia [39, 40, 43–45]. Both protein C p.Arg211Trp and p.Met406Ile (p.Arg169Trp and p.Met364Ile in the mature protein numbering), which are related to type I protein C deficiency, were first reported in Japanese patients with VTE [43, 44]. Protein C p.Arg211Trp is a recurrent mutation occurring at a CpG mutation hotspot at the thrombin cleavage site in the heavy chain; it has also been described in Caucasian patients with VTE. It was reported to account for about 10 % of *PROC* mutations in Japanese [45]. In contrast, p.Met406Ile, which occurs at a non-CpG site of the serine protease domain, has been described exclusively in Japan, accounting for ~8 % of *PROC* mutations in Japanese VTE patients [45]. In resequencing the *PROC* gene in probands of protein C deficiency, 8 novel coding sequence mutations contributed to 7 amino acid exchanges; 3 evidently detrimental novel null mutations were also supposed to contribute to the development of VTE in Chinese [39].

THBD mutations as VTE risk in Asians

Thrombomodulin (TM encoded by *THBD*), another critical component of the protein C anticoagulant system, is a transmembrane glycoprotein of 557 amino acids, and is expressed mainly on the endothelial cells. TM binds thrombin and alters its substrate specificity. The resulting TM–thrombin complex efficiently catalyzes protein C activation. The intron-less human *THBD* gene is 3.6 kb in length. Based on the important anticoagulant role of TM, mutations within *THBD* could predispose individuals to VTE. In addition, *THBD* mutations may affect the plasma-soluble TM level. Several studies have focused on the influence of genetic polymorphisms in *THBD* on soluble TM level and VTE. One study conducted in the USA found mutations—including c.127G>A (p.Ala43Thr), c.1418C>T (p.Ala473Val), c.1752C deletion, and c.3645A>G—were not associated with VTE [46]. An association study of the Japanese population that included 2,247 individuals showed that c.2729A>C in tight linkage disequilibrium with c.1418C>T (p.Ala473Val) was associated with the soluble TM level [47]. This mutation also showed a marginal association with VTE, but only in males (Table 3).

A recent large study of the Chinese population showed an association of the soluble TM levels with c.−151G>T in *THBD* (Table 3) [48]. Furthermore, this genetic mutation increased risk of VTE. The study enrolled 1,304 individuals with VTE and 1,334 age- and sex-matched controls. By resequencing and genotyping of the *THBD* gene, the study showed that c.−151G>T in *THBD* could cause a predisposition to VTE, with a 2.8-fold increased risk of developing VTE in the population and a 3.42-fold increased risk of VTE in the family [48]. The prevalence of this variant in the Chinese population was 0.97 %, indicating an allele frequency of 0.49 %. Compared with the wild-type allele, the c.−151G>T mutation significantly reduced the reporter gene-expression level in cultured cells [48].

In addition, rare nonsynonymous mutations, p.Ser190Trp, p.Ser212Ter, p.Leu220Ter, and p.Asp126Tyr in *THBD* were also identified in 108 thrombophilic individuals with VTE [48]. The prevalence and relative risk of VTE with these mutations in other populations, especially in Asians, will require further evaluation.

The extensive resequencing studies on *THBD* in VTE patients revealed yet another aspect of the *THBD* mutations, that is, a possible link between the nonsynonymous mutations and atypical hemolytic uremic syndrome (aHUS), a type of microangiopathy characterized by uncontrolled complement activation. One of the causative genes for aHUS is *THBD* [49]. In vitro, TM binds to C3b and complement factor H and negatively regulates the complement by accelerating complement factor I-mediated inactivation of C3b. The TM mutations were less effective than wild-type TM in enhancing factor I-mediated inactivation of C3b. Thus, some missense mutations of TM are characterized as causative for the development of aHUS [49]. A missense mutation, p.Asp486Tyr, in the Ser/Thr rich domain of TM, which has been identified in both VTE patients and controls [46–48], was characterized as a causative mutation for aHUS. Missense mutations in the lectin-like domain of TM are also reportedly causative for aHUS. Thus, nonsynonymous mutations in *THBD* would affect not only VTE but also aHUS to a certain degree.

PROCR mutations as VTE risk in Asians

On the pathway of the protein C anticoagulant system, protein C is activated on the endothelial surface by the membrane-bound TM–thrombin complex. Protein C activation is enhanced approximately 20-fold when protein C binds to the endothelial protein C receptor (EPCR) encoded by *PROCR*. EPCR also serves as a cellular binding site for factor VII and factor VIIa. A soluble form of this receptor (sEPCR) in plasma inhibits both APC activity and protein C activation by competing for protein C with membrane-bound EPCR. These findings suggest an important role for EPCR in VTE.

Several studies have reported that the *PROCR* p.Ser219Gly mutation (rs867186, c.655A>G) present within the membrane-spanning region reduced plasma sEPCR levels to 56–87 % [50]. Significantly higher levels of factor VII, factor VIIa, and downstream markers of activated coagulation in the extrinsic pathway (factor IX activation peptide, factor X activation peptide), and prothrombin F1 + 2 were also identified in Gly carriers, compared to Ser/Ser [51]. Evidence for the association between the p.Ser219Gly mutation and VTE is conflicting in ethnically diverse populations. A recent meta-analysis in 4,821 VTE patients and 6,070 controls found a significant association of this mutation with VTE [50]. Under an additive genetic model, the odds of VTE increased by a factor of 1.22 for every additional copy of the G allele in all ethnic populations, suggesting a moderate effect for VTE. The reported frequency of the G allele in northeast Asians is approximately 10 % [52, 53]. Thus far, only two independent, small-scale studies of Chinese populations have reported a significant association between the p.Ser219Gly mutation and VTE in Asian populations (Table 3) [52, 53]. Further studies restricted to idiopathic VTE patients in Asian might facilitate the positive association of this variant.

Perspectives

The genetic mutations in the protein C anticoagulant system (*PROS1* p.Lys196Glu, *PROC* p.Arg189Trp, *PROC*

p.Lys193del, and *THBD* c.−151G>T) associated with risk of VTE in Asians are all classified into low-frequency variations with allele frequencies of less than 5 %. Three genetic mutations in the protein C anticoagulant system (*PROC* p.Arg189Trp, *PROC* p.Lys193del, and *THBD* c.−151G>T) were detected concurrently in the Chinese population, with a respective frequency of 0.90, 2.40, and 0.97 %, and a respective odds ratio for VTE of 6.06, 2.84, and 2.80. Their estimated population-attributable risks were therefore calculated to be 4.67, 4.14, and 1.48 %, respectively [48]. Taken together, about 10 % of VTE events in the general Chinese population could be explained by these mild to moderate thrombophilic risk factors. Hence, as we have described [29], these low-frequency genetic variations could play an important role in the development of VTE. The risk loci may act in concert with each mutation adding or detracting a small amount from the phenotype; the environment also interacts with the genotype to produce the final phenotype [8].

Recent genome-wide association studies have found additional genetic polymorphisms that are potentially related to VTE risk, but most have been detected predominately in European-ancestry populations [54, 55]. Genome-wide association studies do serve an important role in identifying new loci of interest, as well as confirming previously suggested loci for VTE. However, their main potential is for identifying common mutations (>5 %) with relatively lower risk (odds ratio <1.5). The candidate gene resequencing in the protein C anticoagulant system or the exome sequencing would facilitate the discovering of low-frequency variations with high risk for VTE in Asians. An accumulating body of evidence strongly suggests that genetic studies should be carried out in ethnically diverse populations, and that studies of common variations, as well as low-frequency variations, are warranted [29].

As VTE is a complex disease with genetic factors accounting for part of the risk, a multifactorial non-Mendelian inheritance model that includes the influence of genetic and environmental factors should be proposed for genetic counseling of VTE risk. Recently, a multiple single-nucleotide polymorphism test based on 31 VTE-associated polymorphisms or the 5 most strongly associated polymorphisms was found to improve risk prediction of first venous thrombosis in Caucasians [56]. Future studies should consider the construction of a multifactorial model based on the genetic risk factors in the protein C anticoagulant system, which is specific for Asian populations.

In summary, the genetic mutations leading to dysfunction of the protein C anticoagulant system could be a major risk factor for VTE in northeast Asian populations, especially in Japanese and Chinese. Conditions where the procoagulant activity surpasses the anticoagulant activity, including the protein C anticoagulant system, could trigger the development of thrombosis in individuals with risk genetic variants. Genetic analysis for VTE is highly restricted in Japanese and Chinese populations, and other Asian populations are not yet well studied. Even in geographically close populations, such as Japanese and Chinese, low-frequency mutations are not evenly distributed. The *PROS1* p.Lys196Glu mutation, for example, is exclusively identified in Japanese populations. Whether dysfunction of the protein C anticoagulant system occurs in other Asian countries is an important unresolved issue of the thrombophilia study among Asians, and an international survey is warranted to disclose it.

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非典型溶血性尿毒症症候群（aHUS）

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Key words : Hemolytic uremic syndrome, Complement, Complement Regulatory factors, Eculizumab

緒 言

溶血性尿毒症症候群 hemolytic uremic syndrome（HUS）は細血管障害性溶血性貧血，血小板減少，急性腎障害を3主徴（Triad）とする全身性重篤疾患で1955年にドイツのGasserらによって最初に報告された[1]。その後，1982年の米国でのハンバーガー食中毒事件をきっかけに，志賀毒素（Shigatoxin, Stx）を産生する腸管出血性病原大腸菌（Enterohemorrhagic *Escherichia coli*, EHEC）O157による腸炎に合併して高頻度にHUSが発生することが示された。最近では，本邦及びドイツにおいて，O111やO104などO157以外のStx産生EHECによって脳症併発などの重篤HUS例が多数報告されている。

一方，このような下痢に伴う［diarrhea, D(+)］HUS以外に，下痢を伴わないD(−)HUSが散発性あるいは家族性に発症することが早期から知られていた。しかし，これら患者群の詳細なnatural history解析では非出血性下痢を伴うことは屢々あり，D(−)HUSという表現は適切ではなく，最近は「非典型溶血性尿毒症症候群［atypical (a)］HUS」[2]と統一して呼ばれるようになった。これより，本稿ではaHUS研究の歴史的背景，次に補体と補体調節因子の基本的役割を紹介し，その後，本邦aHUSの診断と治療の現状を欧米のそれと対比しながら解説する。

歴史的背景

aHUSの原因には諸説あったが，同種腎移植後にHUS再発が見られること，また限られてはいたが血漿交換療法に良く反応する症例もあったことから，患者のHUS発症には何らかの血漿因子が関与するものと推定されていた。実際，aHUS患者数名においては補体調節因子であるcomplement Factor H（CFH）蛋白量の著減（5〜10%）と，この疾患が劣性遺伝を示すことは1981年にThompson & Winterborn[3]によって，また1994年にはPichetteら[4]によって報告された。しかしながら1990年，Roodhooftら[5]は，ある一家系において，aHUS患者はCFH蛋白量が48%と略半減していたが，母親は正常で，父親は34%と低下していたが無症状であったことより，CFHの量的低下は優性遺伝と考えられるが，症状は必ずしもこれに一致しないことを報告した。この後，1998年にWarwickerら[6]は患者DNAの多点連鎖解析にてCFHの遺伝子異常と疾患関連性を証明するというブレークスルーをなし得た。これ以降，補体や補体関連因子に注目が集まり，complement (C) 3やCFHを含む様々な補体調節因子であるcomplement factor B（CFB）やcomplement factor I（CFI），またその関連膜糖蛋白であるmembrane cofactor protein（MCP）やthrombomodulin（THBD）がaHUSの原因となることが示された。より最近にはdiacylglycerol kinase ε（DGKE）という血小板活性化に必須のアラキドン酸代謝経路シグナルを遮断する蛋白の遺伝子異常もaHUSの原因となることが報告されている[7]。

本邦でのaHUS解析状況

欧米でのaHUS研究は上記のごとく，1998年以降，遺伝子解析を中心に大きな進展があり，その病態概念として「補体活性化の制御不能」が本疾患の根底をなし，それ故に治療には補体活性化の最終点に位置するC5の活性化を阻害する分子標的療法が奏功することが示された。これに対し，本邦でのaHUS研究は欧米からは大きく立ち遅れていたが，2011年に信州大学の天野・日高ら[8]によりCFH missense変異をヘテロ接合体で持つ

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Table 1　本邦の非典型溶血性尿毒症症候群の診断基準[2)]

非典型溶血性尿毒症症候群　診断基準

非典型溶血性尿毒症症候群診断基準作成委員会
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外部委員
藤村吉博　奈良県立医科大学輸血部
吉田瑤子　奈良県立医科大学輸血部

疾患の定義

非典型溶血性尿毒症症候群（atypical hemolytic uremic syndrome, aHUS）は、志賀毒素による HUS と ADAMTS13（a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs, member 13）活性著減による血栓性血小板減少性紫斑病（thrombotic thrombocytopenic purpura, TTP）以外の血栓性微小血管障害（thrombotic microangiopathy, TMA）で、微小血管症性溶血性貧血・血小板減少・急性腎障害（acute kidney injury, AKI）を三主徴とする疾患である。

診断基準

Definite:

三主徴がそろい、志賀毒素に関連するものでないこと。血栓性血小板減少性紫斑病でないこと。

微小血管症性溶血性貧血；Hb 10g/dl 未満

血中 Hb 値のみで判断するのではなく、血清 LDH の上昇、血清ハプトグロビンの著減、末梢血スメアでの破砕赤血球の存在をもとに微小血管症性溶血の有無を確認する

血小板減少；PLT 15 万/μl 未満

急性腎障害（AKI）；

小児例：年齢・性別による血清クレアチニン基準値の 1.5 倍

（血清クレアチニンは、小児腎臓病学会の基準値を用いる。）

成人例：AKI の診断基準を用いる

Probable:

急性腎障害（AKI）、微小血管症性溶血性貧血、血小板減少の 3 項目のうち 2 項目を呈し、かつ志賀毒素に関連するものでも、血栓性血小板減少性紫斑病でもないこと。

付則事項

① 志賀毒素産生性大腸菌感染症の除外診断：

大腸菌の関与を確認する方法：培養検査・志賀毒素直接検出法（EIA）・抗 LPS-IgM 抗体など

② 血栓性血小板減少性紫斑病（TTP）の除外診断：

従来、TTP は古典的 5 徴候で診断されてきた。しかし ADAMTS13 の発見により、TTP 症例は人種にかかわらず、その 60〜90%は ADAMTS13 活性が<5%と著減している事が判明した。従って aHUS の診断において ADAMTS13 活性著減例（<5%）は TTP と診断し、これを除外する必要がある。しかしながら、TTP の古典的 5 徴候は今も臨床現場で用いられており、この中には ADAMTS13 活性が正常ないし軽度低下に留まるものもある。従って、ADAMTS13 活性 5%以上を示す患者についてはその他の臨床症状も加味して aHUS であるか TTP であるかを判断する。

③ 明確な他の原因による TMA の除外診断：

DIC、強皮症腎、悪性高血圧、抗リン脂質抗体症候群など、TMA の病態を生じることが明らかな疾患を除外する。

④ Probable に該当すれば、aHUS の可能性を念頭に置き、各種鑑別診断に必要な検査検体の採取に努める。

aHUS の診療に精通した施設にコンサルトし治療方針を決定する。

⑤ HUS の病態を呈し、以下の状況にある場合には、下痢の有無にとらわれず aHUS を考慮する。

- 生後 6 か月未満の症例
- 発症時期が明確でない症例（潜在性発症例）
- HUS の既往がある症例（再発症例）
- 原因不明の貧血の既往
- 腎移植後 HUS の再発
- HUS の家族歴（食中毒事例は除外する）
- 下痢や血便を伴わない症例

aHUS（ADAMTS13*1 欠損による TTP を除外）の病因分類

（1）補体制御異常：
　（ア）先天性
　　補体蛋白の遺伝子変異：H 因子、I 因子、membrane cofactor protein（MCP, CD46）、C3、B 因子、トロンボモジュリン*2
　（イ）後天性
　　抗 H 因子抗体などの自己抗体産生*3
（2）コバラミン代謝異常症*4
（3）感染症*5
　（ア）肺炎球菌
　（イ）HIV
　（ウ）百日咳
　（エ）インフルエンザ
　（オ）水痘
（4）薬剤性*6
　（ア）抗悪性腫瘍薬
　（イ）免疫抑制薬
　（ウ）抗血小板薬
（5）妊娠関連
　（ア）HELLP 症候群
　（イ）子癇
（6）自己免疫疾患・膠原病*7
　（ア）SLE
　（イ）抗リン脂質抗体症候群
（7）骨髄移植・臓器移植関連
（8）その他

*1 ADAMTS13、フォンビルブランド因子（von Willebrand factor, VWF）の特異的切断酵素
*2 溶血試験、補体蛋白・制御因子の蛋白量定量、遺伝子解析。ただし、補体蛋白や補体制御因子の蛋白量が正常範囲内であっても、補体関連の aHUS を否定する根拠にはならない。
*3 ELISA、ウェスタンブロット法による抗 H 因子抗体などの検出
*4 発症年齢で考慮：生後 6 か月未満、血漿アミノ酸分析で高ホモシステイン血症、低メチオニン血症
*5 病原微生物の同定、血清学的検査による確定診断
*6 原因薬剤の同定
*7 自己抗体検査、抗リン脂質抗体検査、血清学的検査による確定診断

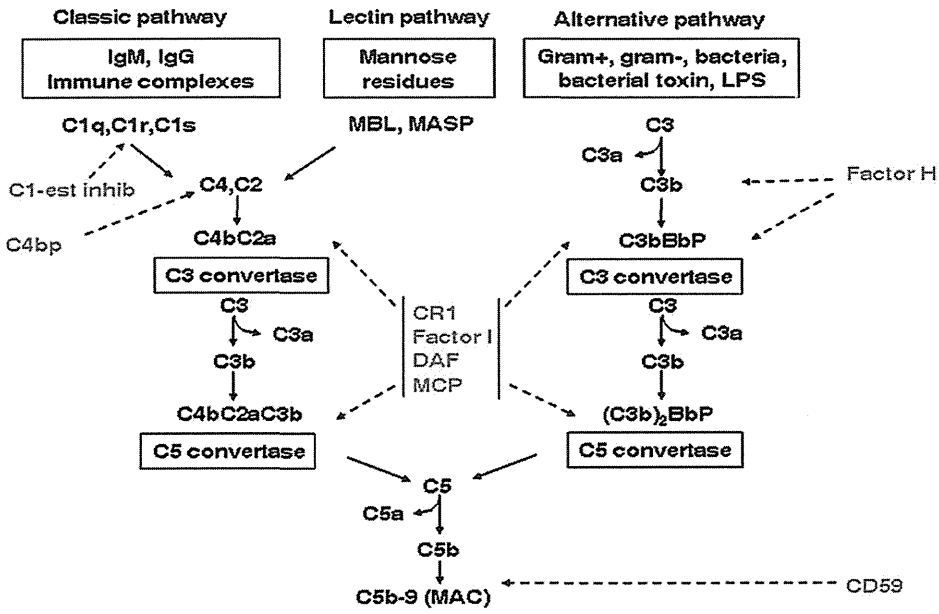

Fig. 1 Activation pathways of the complement system and their regulators (in red) (Noris & Remuzzi, 2005[9])

aHUS 症例が発見され，本疾患に対する本邦研究者の認知度も急激に高まった。

奈良医大輸血部は 1998 年以降，国立循環器病研究センターと共同で，ADAMTS13 解析を通じて本邦の血栓性微小血管障害症（thrombotic microangiopathy, TMA）の患者診断と登録のコホート研究を行ってきた。この結果，2012 年末の時点で 1149 名の TMA 患者数を登録することになった（論文未発表）。また，2013 年 2 月には「本邦における aHUS 診断ガイドライン」が，日本腎臓学会と日本小児科学会のホームページにて公表された。本診断基準は，徳島大学小児科の香美祥二委員長と東京大学腎臓・内分泌内科の南学正臣先生を中心とした aHUS 診断基準作成ワーキング・グループの尽力のもとで作成された（Table 1）[2]。この診断基準に照らすと，我々のコホート研究の中で，2011 年迄は原因不詳の先天性 HUS というカテゴリーに属していた症例の殆どが aHUS に該当することが判明し，最終的に前記 TMA 患者中，55 名が先天性 aHUS と分類された。また，ADAMTS13 活性を遺伝性に欠く先天性血栓性血小板減少性紫斑病（thrombotic thrombocytopenic purpura, TTP）—別名，Upshaw-Schulman 症候群（USS）—も 49 名同定された。これより，前記コホート研究では本邦 TMA 患者中で 9.1%（104/1149）は先天性 TMA と分類された。

補体活性化とその調節機構

補体系は個体の免疫機構構築に必須で，3 つの基本的経路を介して活性化される。これらは古典経路（classical pathway），レクチン経路（lectin pathway），第二経路（alternative pathway）である（Fig. 1）[9]。古典経路では抗原抗体反応によって，またレクチン経路は血清中のマンノース結合レクチンが細菌膜表面のマンノースに結合することにより活性化が開始される。さらに第二経路では，病原微生物上に C3 が結合することにより活性化される。一方で，自然界では C3 内のチオエステル結合の持続的加水分解が絶えず生じており，C3 が自動的に活性化されている状態にある。即ち，これらいずれの経路を介しても，最終的には C3 分解活性化反応が進行し，細胞膜侵襲複合体である C5b-9（membrane attack complex, MAC）が形成される。

1974 年以降，aHUS 患者では C3 低下が見られるが，C4 低下は見られないとの報告がなされた[10]。また，第二経路では活性化反応に C3 分解を伴うが他の経路とは異なり C4 分解は伴わない。これより aHUS には第二経路の活性化が特異的に関与していることが予想された[11]。補体活性化第二経路（Fig. 1）では，C3 が加水分解反応により C3a と C3b に分解され反応が開始する。C3 の分解によって生成した C3b は CFB と結合し，続いて CFD により分解されることで C3bBb（C3 convertase）を形成する。C3 convertase は C3 の分解を促進させ，生じた C3b とさらに結合して C5 convertase（C3bBbC3b）を形成する。C5 convertase は C5 を C5a と C5b に分解し，生じた C5b が C6-C9 と複合体（C5b-9）を形成，膜侵襲複合体として病原体膜に結合し，溶

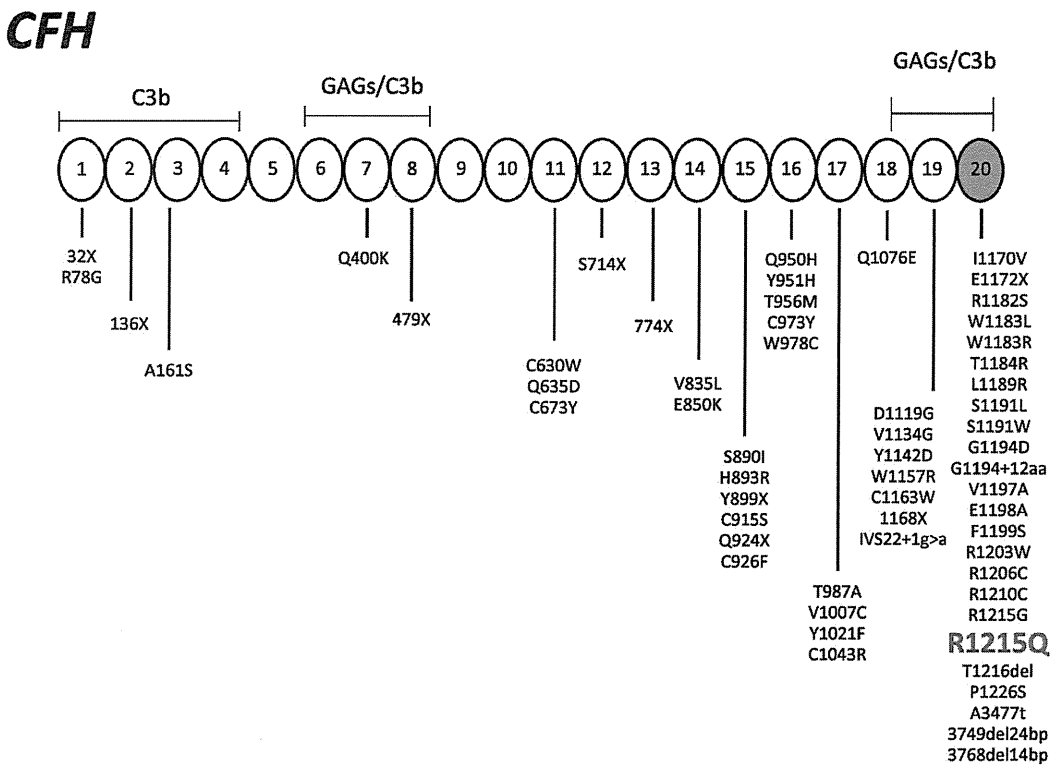

Fig. 2 Structure of complement factor H (CFH) and its gene mutations identified with aHUS patients (modified from Kavanagh & Goodship, 2010[14])
The figure demonstrates the 20 CCP modules of CFH. The glycosaminoglycan (GAG) and C3b binding sites of CFH are indicated on the diagram. Mutations in CFH reported in aHUS are listed below the figure.

菌，細胞膜融解を引き起こす。また，第二経路で活性化されたC3bはオプソニン効果を持ち，抗体によるウイルス中和反応を増強する作用を持つ。特に病原体に結合したC3bは貪食細胞による病原体の貪食と破壊を促進する。またC3a，C5aは好塩基球や肥満細胞からヒスタミンなどを放出させるアナフィラトキシンとして働く。

補体調節因子は補体活性化作用において，活性化と非活性化（制御）のいずれか一方の機能を持つものに大別される。活性化因子として代表的なものはCFB，CFD，properdinであり，制御因子としてはCFIとCFHがある。さらに細胞膜上の制御因子としてMCP（CD46），decay accelerating factor（DAF），THBDなどが知られている。aHUSでは，これら補体調節因子の中でも特に第二経路の補体制御因子の異常が数多く報告されている。これら補体制御因子はregulators of complement activation（RCA）proteinと呼ばれ，ヒトでは染色体1q32上にgene clusterを形成している。また，これら因子は共通してcomplement control protein（CCP）と呼ばれる約60のアミノ酸からなる相同性の高いドメイン構造を持つ。C3ステップでの制御因子にはCFH，C4結合蛋白，補体レセプター1（CR1），MCP，DAFが含まれる。aHUSにおけるこれら補体制御因子の異常は，過剰な補体の活性化や補体による自身の細胞障害を引き起こすと考えられる。

CFH

CFHは分子量150kDの血漿糖蛋白質であり，主に肝臓で産生される。この分子は20個のCCPから構成され，分子内に3ヶ所のC3b結合ドメインと2ヶ所のグリコサミングリカン（GAG）結合ドメインを持つ（Fig. 2）。CFHは主に第二経路における制御因子として働き，1）CFIによるC3b分解の補助，2）C3bへのCFBの接着阻害，3）C3 convertase（C3bBb）の解離促進などの機能を持つ[12]。これらの働きはN末端側のCCP1-4で行われ，この領域は制御ドメインと呼ばれる。CFHはヘパラン硫酸プロテオグリカンなどを介して血管内皮細胞などに結合し，補体による攻撃から自身の細胞を保護するといった極めて重要な役割を持つ。細胞膜表面への結合はC末端側のCCP19, 20を介して行われることからこの領域は認識ドメインと呼ばれる。最近の詳細な研究により，CCP19はC3bに結合し，CCP20はヘパラン硫酸プロテオグリカンに結合することが示された。欧米で

Table 2 Complement genetic abnormalities and frequency in patients with aHUS (modified from Noris et al. 2005)

Gene	Protein Affected	Frequency (%)		Response to short-term plasma therapy
		Overseas	Japan (n=30 ※)	
No identified mutation	*	30–50%	26.7% (8/30) (2 patients had anti-FH autoantibody)	No data
CFH	Factor H	20–30%	10.0% (3*/30)	Rate of remission 60%
CFHR1/3	CFHR1/3	6%	6.7% (2/30)	Rate of remission 70–80% (plasma exchange combined with immunosuppression)
MCP	MCP	10–15%	13.3% (4**/30)	No definitive indication for therapy
CFI	Factor I	4–10%	0% (0/30)	Rate of remission 30–40%
CFB	Factor B	1–2%	3.3% (1/30)	Rate of remission 30%
C3	C3	5–10%	43.3% (13**/30)	Rate of remission 40–50%
THBD	Thrombomodulin	5%	3.3% (1*/30)	Rate of remission 60%

※ 24 patients were analyzed in National Cerebral and Cardiovascular Center.
* One patient had the mutation in CFH+THBD. **One patient had the mutation in C3+MCP.

はaHUSの20～30%（本邦では10.0%）でCFH遺伝子異常が存在することが示され[13,14]，aHUSにおける遺伝子異常の中で最も頻度が高いことが示された（Table 2）。報告された遺伝子異常は分子全体に認められるが，その約60%が認識ドメインであるCCP19, 20に集中しており（Fig. 2），この領域における遺伝子変異は細胞表面における補体の攻撃からの保護機構の破綻を引き起こすと考えられている。本邦ではCCP20にR1215Q変異のみが観察されている。

aHUS患者の6～10%（本邦では13.3%）でCFHに対する自己抗体の存在が確認されている[15]。CFHに対する自己抗体はIgG型で，遺伝子異常の好発部位と同じC末領域を認識し，C3bへの結合を阻害することで，補体の過剰な活性化を引き起こす。近年，CFHに対する自己抗体生成の機序にはCFH related（CFHR）蛋白質1～5の遺伝子異常が深く関わっていることが明らかとなってきた。自己抗体陽性患者では*CFHR1*と*CFHR3*の遺伝子が欠損していることが報告されている[16]。これらCFHR蛋白質の遺伝子はCFHと同様，染色体1q32上に存在し，CFHと非常に類似した遺伝子配列を持つ。このようにCFH抗体陽性で，かつ*CFHR*遺伝子の欠損が見られるaHUS症例はDEAP（Deficiency of CFHR plasma protein and autoantibody-positive form）-HUSとして近年注目されている[16]。

MCP

MCPは膜結合型糖蛋白で，CFIの補助因子として同一細胞上のC3bやC4bを分解するが，CFHとは異なりC3 convertaseの崩壊促進には関与しない。*MCP*遺伝子異常は欧米ではaHUS患者の約10～15%（本邦では13.3%）と報告されている[17]。*MCP*遺伝子異常の患者では，CFH異常など他の因子の異常に比べ，比較的予後が良いことが知られている。また，血漿治療の有無による予後の差はなく，90%以上の症例で寛解が得られている[18]。腎移植後の再発率も低く，これは移植腎に十分なMCPが含まれているためと考えられる。

CFI

CFIは分子量88kDの血漿糖蛋白であり，主に肝臓で合成される。CFIはCFHやMCP，C4結合蛋白などを補助因子としてC3bやC4bを分解するセリンプロテアーゼである。*CFI*遺伝子異常は，欧米ではaHUS患者の4～10%（本邦では未発見）と報告されている[17,19]。Table 2に示すように，血漿交換などの治療に対する反応は悪く予後は不良である。

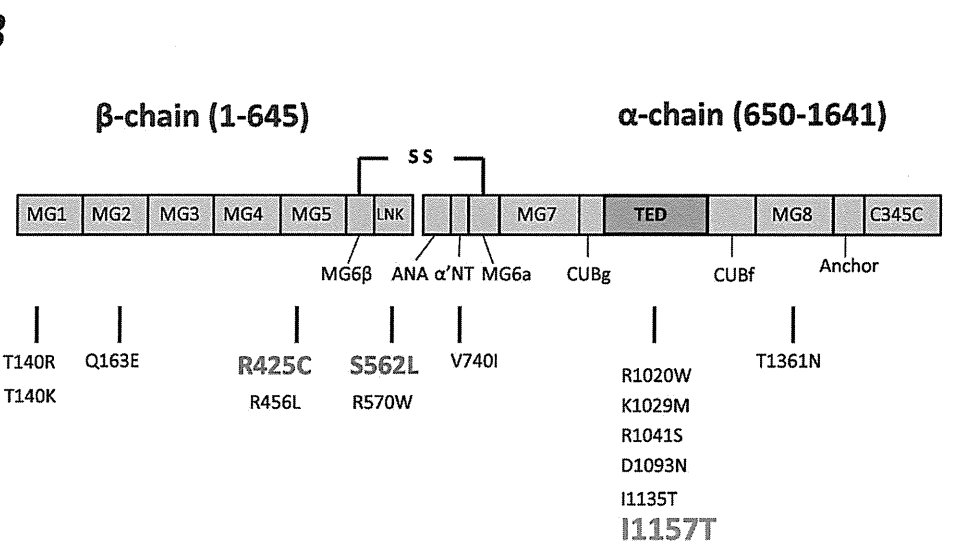

Fig. 3 Structure of complement (C) 3 and its gene mutations identified with aHUS patients (from Noris et al. 2010[18])
The C3 mutations are spread all over the gene; however, a hot spot is evidenced in the thioester-containing domain (TED domain) with six independent mutations.

THBD

THBD は血管内皮細胞上に存在する膜蛋白で，抗血栓，抗炎症，細胞保護作用を有する。THBD は C3b と CFH に結合し，CFI を介した C3b 不活化を促進する。また，THBD に結合したトロンビンは血漿 thrombin-activatable fibrinolysis inhibitor (TAFI) を活性化し，生じた TAFIa は C3a，C5a を不活化する。2009 年に Delvaeye ら[20]により，THBD 遺伝子異常が aHUS に関与していることが示された。彼らは 152 例の aHUS 患者の中で 7 例の患者において，6 種類の *THBD* 遺伝子変異を発見した。欧米での頻度は約 5%（本邦では 3.3%）である。THBD に遺伝子変異があると C3b 不活化能と TAFIa 形成能が減弱し，結果として補体活性化を制御できず aHUS が発症すると考えられている。

CFB と C3

aHUS では機能獲得型 (gain-of-function) 異常として補体機能が過剰に活性化する症例も報告されている。これに相当するのが CFB と C3 の遺伝子異常である[21, 22]。CFB 遺伝子異常は欧米では aHUS の約 1～2%（本邦 3.3%）に認められる稀変異である。変異 CFB は C3b へ過剰に結合し，C3 convertase を活性化することで C3b を過剰に産生させる。

C3 遺伝子変異は欧米では aHUS 患者の 5～10% と比較的少ないと報告されている。しかし本邦では C3 遺伝子変異は aHUS 全体の 43.3% を占め，欧米とはその頻度において大きな差異が確認されている。しかもその種類は I1157T 変異が圧倒的に多い（後述）。C3 変異患者（例えば C3-I1157T）では変異 C3b の CFH や MCP への結合能が低下し，C3b 分解が減じるために aHUS が発症すると説明されている（Fig. 3）。

DGKE

DGKE は血管内皮細胞，血小板，腎 podocyte などの細胞質と膜の両方に存在する分子量 64kD の蛋白で，その作用は血小板活性化に必須であるアラキドン酸代謝経路のシグナル伝達を遮断する機能を持つ。最近の米国の研究グループでの報告[7]では，この DGKE 遺伝子異常による aHUS 患者は常染色体性劣性遺伝形式を示し，いずれも aHUS の初発は 1 才以下と early-onset の特徴を持つ。症状は持続性高血圧，血尿，蛋白尿（屢々ネフローゼ様）で，その後加齢と共に慢性腎不全に移行する。本邦では未発見である。

本邦での aHUS 診断

aHUS 診断については，2011 年にフランスの Loirat & Fremeaux-Bacchi[23]により，Fig. 4 のようなアルゴリズムが提唱されたが，本邦ではルーチン検査として行える C3 や C4 の定量を除いて，CFH，CFI，そして CFB を日常臨床の中で測定することは殆ど不可能である。また MCP 発現量測定には生細胞とフローサイトメーター解析が必要で，この実施も容易ではない。さらに，これら蛋白の定量が出来ても，それらが missense 変異であ