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ORIGINAL ARTICLE

Genetic analysis of patients with deep vein thrombosis during pregnancy and postpartum

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Abstract Deep vein thrombosis (DVT) is a serious pregnancy-related complication. Recent studies indicate that the genetic background for DVT differs with ethnicity. In our study, we enrolled 18 consecutive Japanese patients who had developed DVT during pregnancy and postpartum. We performed a genetic analysis of three candidate genes for DVT, protein S, protein C and antithrombin, in these patients. We found that four patients had missense mutations in the protein S gene, including the K196E mutation in two patients, the L446P mutation in one patient, and the D79Y and T630I mutations in one patient, as well as one patient with the C147Y mutation in the protein C gene. All five patients with genetic mutations had DVT in their first two trimesters. Nine of the patients without genetic mutations developed DVT in the first two trimesters, and four in the postpartum period. Thus, genetic mutations in the protein S gene were predominant in pregnant Japanese DVT women, and DVT in pregnant women with genetic mutations occurred more frequently at the early stage of pregnancy than postpartum. Considering the rapid decrease in protein S activity during pregnancy, we may need to assess thrombophilia in women before pregnancy.

Keywords Deep vein thrombosis · Protein S · Thrombophilia · Pregnancy

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1 Introduction

Venous thromboembolism is the leading cause of maternal deaths in Western countries [1]. The incidence of pregnancy-related venous thromboembolism was 13 per 10,000 deliveries [2]. A 30-year population-based study reported that the unadjusted incidence of deep vein thrombosis (DVT) was 151.8 per 100,000 woman-years [3]. Most studies have found that the risk for thrombosis were 3–12 times higher in postpartum than during pregnancy [3, 4]. One study, however, reported twice as many events antenatally as postpartum [5]. Most of these studies involved patients in Western countries. A study in Japan showed that pulmonary thromboembolism occurred in 0.02% of total births, and the mortality rate was 2.5 per 100,000 deliveries [6]. Women with pregnancy-related thrombosis tend to have inherited thrombophilia, thus the prevention of DVT during pregnancy and postpartum is important for pregnant women. Therefore, the identification of inherited or acquired thrombophilia in pregnant women is urgently needed for the prevention of pregnancy-related thrombosis.

In Caucasian populations, two thrombotic mutations, the factor V Leiden mutation and the prothrombin G20210A mutation, for venous thromboembolism are widely distributed, with 30–60% of women with pregnancy-related thrombosis having these mutations [7, 8]. Both mutations are well-established risk factors for venous thromboembolism during pregnancy and postpartum in Caucasian populations. Several prophylactic therapies for pregnant women, such as heparin administration in the perinatal period, are recommended based on the type of thrombophilia and history of thrombosis [9]. However, these two genetic mutations are not found in the Japanese population [10, 11]. Thus, Caucasians and Japanese have clear genetic differences for thrombosis [12].

Deficiencies in protein S, protein C, and antithrombin are well-known risk factors for DVT [13]. The frequency of protein C deficiency and antithrombin deficiency in the general Japanese population was estimated to be 0.13 and 0.15%, respectively, and was comparable to the Caucasian population [12, 14–16]. The frequency of protein S deficiency in Japanese, however, seemed to be higher than that in Caucasians [17, 18], although the assays for plasma protein S levels differed among the studies. Actually, the frequency of protein S deficiency in 2,690 individuals randomly selected from the general Japanese population was estimated to be 1.12%, higher than reported in Caucasian populations (0.03–0.13%) [17, 18]. In a study of Japanese patients with venous thromboembolism, the frequency of inherited protein S deficiency was higher than that in Caucasian patients [19, 20]. It was recently reported that 17% of Japanese patients with venous thromboembolism had genetic mutations in the protein S gene [20]; this was much higher than in selected Caucasian patients with thromboembolism (1.4–8.6%) [13]. Furthermore, we and others reported the significant association with a missense mutation, K196E, in the protein S gene and venous thromboembolism in Japanese populations [19, 21, 22]. The carriers of this mutation showed low protein S activity [19, 23]. The prevalence of this mutant allele in the general Japanese population was about 0.009, suggesting that a substantial proportion of the Japanese population carried the protein S E-allele and was at risk of developing DVT [12, 19, 21, 22, 24]. This mutation seems to be ethnically specific, because it has not so far been identified in Caucasians.

It is well recognized that plasma levels of protein S activity and antigen are significantly reduced during pregnancy [25] and in oral contraceptive users [26]. The activities of protein S, protein C, and antithrombin can be affected at the acute stage of thrombotic events or after antithrombotic therapies. Therefore, the plasma assay may have an intrinsic limitation for the diagnosis of thrombophilia, and alternative ways to diagnose thrombophilia are expected. Genetic analysis might fulfill this requirement if it is applicable.

In this study, we performed DNA analysis for the genes of protein S, protein C, and antithrombin in patients with DVT during pregnancy and postpartum. We measured their plasma activities of protein S, protein C, and antithrombin. Based on these analyses, we described the clinical characteristics of the DVT events in patients with genetic mutation.

2 Materials and methods

2.1 Study patients

In this study, 18 consecutive patients with DVT during pregnancy and postpartum were enrolled from two tertiary perinatal centers: the National Cerebral and Cardiovascular Center and the Osaka Medical Center and Research Institute for Maternal and Child Health. Both centers are located in the Osaka Prefecture, which has the third-largest population in Japan. Postpartum was defined as the first 3 months after delivery. DVT was diagnosed by ultrasonography, venography, or magnetic resonance imaging angiography. We enrolled only patients with symptomatic DVT. Each patient's age, body mass index, gestational weeks of DVT onset, complications of pregnancy, delivery mode, and other information were reviewed.

The protocol of this study was approved by the Ethics Review Committee of the National Cerebral and Cardiovascular Center and by that of the Osaka Medical Center and Research Institute for Maternal and Child Health. Only those who had given written informed consent for genetic analyses were included.

2.2 Activity measurements of protein S, protein C, antithrombin, and antiphospholipid syndrome screening

The plasma samples were obtained after at least 3 months' postpartum and at least 3 months without the use of warfarin. Samples were subjected to a thrombophilia screening, including prothrombin time, activated partial prothrombin time, and activities of protein S, protein C, and antithrombin. Protein S activity was measured as cofactor activity for activated protein C on the basis of the activated partial thromboplastin time assay using Staclot protein S (Diagnostica Stago, Asnieres, France) [18]. Protein C amidolytic activity was measured using S-2366 as a chromogenic substrate and Protac derived from *Agkistrodon contortrix* venom as the activator [16]. Antithrombin activity was measured as a heparin cofactor activity using chromogenic substrate S-2238 (Chromogenix AB, Stockholm, Sweden) [16, 27]. Samples were also subjected to an antiphospholipid syndrome screening of

lupus anticoagulant, anticardiolipin antibody, and anti-β2-glycoprotein-I antibody [28].

2.3 DNA sequencing of protein S, protein C, and antithrombin genes

We sequenced the entire coding region of protein S, protein C, and antithrombin genes in 18 patients with DVT. The method of direct sequencing using the 96-capillary 3730xl DNA Analyzer (Applied Biosystems Japan, Tokyo, Japan) has been described previously [20, 29]. We have adopted the numbering standards of the Nomenclature Working Group, wherein the A of the ATG of the initiator Met codon is denoted as nucleotide +1, and the initial Met residue is denoted as amino acid +1 [30].

3 Results

3.1 DVT history of enrolled patients

We enrolled 18 Japanese symptomatic DVT patients in this study, and only one patient had previous DVT event. All patients were negative for the antiphospholipid syndrome. Thirteen patients were primiparous and five were multiparous. One patient without genetic mutation had a history of miscarriage. One patient without genetic mutation had a history of first trimester artificial abortion that was also complicated with DVT at the time. As an additional risk factor, two out of 13 DVT patients without genetic mutation showed hyperemesis, but all five patients with genetic mutation did not show hyperemesis. Other risk factors such as bed rest, preeclampsia, multiple pregnancy, and preterm labor were not observed in all 18 patients. One patient without genetic mutation had the travelers' thrombosis in the first trimester. One patient without genetic mutation showed paradoxical embolism after DVT postpartum.

3.2 Identification of genetic mutation in DVT patients

We sequenced the coding regions of the protein S, protein C, and antithrombin genes in the 18 DVT patients and identified missense mutations in the protein S gene in four cases, and in the protein C gene in one case, but not in the antithrombin gene (Table 1). Two patients, cases 1 and 2, had the K196E mutation in the protein S gene; this is the most popular thrombophilic mutation in the Japanese population [19, 21, 24]. These two patients had protein S anticoagulant activity above 50% (Table 1). Case 3 had a missense mutation, L446P, in the protein S gene. Case 4 had two missense mutations, D79Y and T630I, in the protein S gene with very low anticoagulant activity of 4%, with family history of DVT in her father. The protein S anticoagulant activities during pregnancy in cases 2, 3, and 4 were decreased to 25, <20, and <1%, respectively. Case 5 had the C147Y mutation in the protein C gene with 45% amidolytic activity. Her protein C activity did not change during pregnancy (Table 1). None of the 18 patients with DVT had nonsynonymous mutations in the antithrombin gene. All patients were not obese with body mass index between 18 and 24. Case 1, 2, and 3 had term vaginal delivery; however, case 4 and 5 had cesarean section due to other obstetric indication.

3.3 Onset of DVT in patients with genetic mutation

Table 2 shows the onset of the DVT events in patients with or without genetic mutation. DVT was found in all five patients with genetic mutations in their first and second trimesters, but not in postpartum. In 13 patients without genetic mutations, DVT events occurred in postpartum for four patients and in the first and second trimesters for nine patients. Two out of four patients without genetic mutation underwent cesarean section. Thus, DVT in pregnant patients with genetic mutation tended to occur in the first and second trimesters and not postpartum.

4 Discussion

Although the relationship between DVT and genetic mutations in protein S, protein C, and antithrombin genes is well established, the clinical courses of DVT patients with genetic mutation among Japanese women during pregnancy and postpartum have not been well characterized. Recent genetic analysis of inherited thrombophilia revealed ethnic differences in DVT between Caucasians and Asians [19, 21], suggesting that the study of venous thromboembolism within individual ethnic populations is highly valuable [12]. It has been established that Caucasians have factor V Leiden mutation and prothrombin G20210A mutation as genetic risk factors for DVT, whereas Japanese do not carry them [10, 11]. However, Japanese have the K196E mutation in the protein S gene as a genetic risk for DVT [19, 21, 22]. The study of DVT in a Japanese population without factor V Leiden mutation or prothrombin G20210A mutation may reveal different clinical characteristics and give rise to hitherto unrecognized issues. In particular, sub-group analyses, such as DVT during pregnancy and postpartum, would be valuable. In the present study, we enrolled 18 pregnant Japanese women with DVT and found that five out of 18 patients (28% patients) had genetic mutations in the protein S or protein C gene. None carried mutations in the antithrombin gene.

The question of when DVT events occur in pregnant women with genetic mutations has been debated. Studies of

Table 1 Nonsynonymous mutations identified in protein S and protein C genes in patients ($n = 18$) with DVT during pregnancy and postpartum

Patient	cDNA[a]	Region	Amino acid change	Protein S[b] or protein C[c] activity (%)	Protein S[b] or protein C[c] activity, during pregnancy (%)	Age	Gravida	Parity	Body mass index	Family history	Other complications of pregnancy	Onset of DVT (weeks of gestation)	Delivery mode	Recurrence of DVT	Complication of PTE (weeks of gestation)
Protein S gene															
Case 1	c.586	Exon 6	K196E	57[b]	n.d.	30	1	1	18.6	None	None	27	TVD	None	27
Case 2	c.586	Exon 6	K196E	68[b]	25[b]	27	0	0	20.3	None	None	10	TVD	None	None
Case 3	c.1337	Exon 12	L446P	13[b,d]	<20[b]	30	0	0	18.8	None	None	27	TVD	None	None
Case 4	c.235	Exon 3	D79Y	4[b]	<1[b]	35	0	0	22.5	Father	None	6	C/S	None	None
	c.1889	Exon 15	T630I												
Protein C gene															
Case 5	c.440	Exon 6	C147Y	45[c]	57[c]	28	0	0	24.2	None	None	20	C/S	None	None

TVD term vaginal delivery, *C/S* cesarean section, *PTE* pulmonary thromboembolism

[a] Position from A of initial ATG in cDNA

[b] Protein S anticoagulant activity

[c] Protein C amidolytic activity

[d] Protein S activity was obtained under warfarin treatment

pregnant Caucasian women have reported a 3- to 12-times higher risk of thrombosis postpartum than during pregnancy [3, 4]. On the other hand, a large retrospective study found that events were twice as likely during pregnancy as postpartum [5]. In our new study, we found that Japanese patients with genetic mutations manifested DVT events in their first two trimesters (Table 2). In particular, pregnant Japanese patients with genetic mutation had no DVT events postpartum. Although this trend went against previous findings [3, 4], it was consistent with the results that there were twice as many DVT events during pregnancy as postpartum [5]. DVT onset at the early stage of pregnancy in patients with genetic mutation might be reasonable, since genetic mutation accelerates DVT onset, and patients with mutation might have DVT events in their early stage of pregnancy.

In the present study, we enrolled 18 pregnant Japanese women with DVT and found that four out of 18 patients (22% patients) had genetic mutations in the protein S gene. A previous study on thrombophilia activity screening in Japanese patients with DVT reported a high prevalence of protein S deficiency [31], and this was later confirmed by genetic analysis [19]. Taken together with these previous findings, our study reinforced the theory that protein S deficiency is an important risk factor for DVT in Japanese. This observation was in stark contrast to the case in Caucasians, in whom factor V Leiden and prothrombin G20210A mutations are involved in almost 50% of all DVT cases in pregnant women [8]. It is well known that the level of protein S activity was decreased immediately after pregnancy [25]. Therefore, predisposed thrombophilia should be considered in the care of patients with pregnancy-related complications, and antithrombotic prophylactic therapy might be applicable for those patients. Also, it might be good for women of child-bearing years to know their own thrombophilic nature.

A previous study reported on DNA sequence analyses of the protein S, protein C, and antithrombin genes in 173 Japanese DVT patients [20]. In this study, 55 patients (accounting for 32% of total patients) had nonsynonymous mutations in one of three genes. Among the three genes, mutations in the protein S gene were predominant, being found in 29 patients (17% of the total). Among various nonsynonymous mutations in the protein S gene, the K196E mutation was most prevalent. It was found in one out of 55–70 Japanese individuals, from analyses of general Japanese populations [19, 21, 22, 24]. In our study, we sequenced three genes in 18 patients with pregnancy-related thrombosis and identified missense mutations in five patients (accounting for 28% of the patients). Among five patients, four (22% of the total) had missense mutations in the protein S gene, which reconfirmed the predominance of inherited protein S deficiency in Japanese patients with

Table 2 Onset of DVT according to trimester of pregnancy and postpartum, and according to delivery mode

	Onset of DVT				Delivery mode			Complication of PTE
	First trimester	Second trimester	Third trimester	Postpartum period	Artificial abortion	Term vaginal delivery	Term cesarean section	
Patients with genetic mutation								
Protein S ($n = 4$)	2	2	0	0	0	3	1	1
Protein C ($n = 1$)	0	1	0	0	0	0	1	0
Total ($n = 5$)	2	3	0	0	0	3	2	1[a]
Patients without genetic mutation								
Total ($n = 13$)	4	5	0	4[c]	1[d]	8	4	1[b]

PTE pulmonary thromboembolism

[a] PTE events with genetic mutation occurred during the second trimester

[b] PTE events in the patients without genetic mutation occurred postpartum after cesarean section

[c] Two out of 4 patients without genetic mutation underwent cesarean section

[d] First trimester

DVT. Two of these patients had K196E mutation. Thus, K196E mutation in the protein S gene would be a genetic risk for not only DVT in general, but also for pregnancy-related DVT.

There are limitations to the present study. This was a small-scale retrospective study with 18 patients. We performed genetic analysis in those patients and identified five patients with genetic mutation. To understand the DVT risk in pregnant Japanese patients with inherited or acquired thrombophilia, we will have to recruit patients consecutively and perform thrombophilic screening, including genetic analysis, in the future evaluation.

In conclusion, we identified inherited thrombophilia in pregnant Japanese women with DVT and found protein S deficiency to be a predominant cause of thrombophilia. By DNA sequence analysis, we found two patients with a K196E mutation in the protein S gene that is prevalent in the Japanese population. Since pregnant women showed reduced protein S levels, a diagnosis of protein S deficiency based on its activity has an intrinsic limitation. Since the onset of DVT tends to occur at an early stage during pregnancy, the genetic analysis might be an alternative diagnostic tool.

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