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Table 1 Relative risk of clinical manifestations of APS for each aPL test

Test	cutoff	sensitivity (%)	specificity (%)	OR (95%CI)	aPL score
APTT mixing	>49 s	39.1	89.3	5.36 (2.53–11.4)	5
Confirmation test, ratio	>1.3	19.6	95.2	4.81 (1.79–12.9)	2
	>1.1	30.4	90.9	4.38 (1.96–9.76)	1
KCT mixing	>29 s	45.6	88.8	6.64 (3.17–13.9)	8
dRVVT mixing	>45 s	28.2	90.9	3.93 (1.74–8.88)	4
Confirmation test, ratio	>1.3	17.4	94.7	3.72 (1.38–10.1)	2
	>1.1	28.3	90.4	3.7 (1.65–8.27)	1
IgG aCL, GPL					
high titers	>30	15.2	98.4	11 (2.72–44.5)	20
medium/low titers	>18.5	19.5	94.6	4.31 (1.63–11.3)	4
IgM aCL,MPL	>7	6.5	96.3	1.79 (0.45–7.22)	2
IgG ab2GPI, units					
high titers	>15	23.9	98.4	19.3 (5.11–72.7)	20
medium/low titers	>2.2	30.4	92.5	5.4 (2.35–12.4)	6
IgM ab2GPI, units	>6	8.7	91.4	1.02 (0.32–3.20)	1
IgG aPS/PT, units					
high titers	>10	19.6	97.8	11.1 (3.25–38.1)	20
medium/low titers	>2	28.3	95.7	8.81 (3.39–22.9)	13
IgM aPS/PT, units	>9.2	6.5	98.9	6.45 (1.05–39.8)	8

APS: antiphospholipid syndrome; aPL: antiphospholipid antibodies; APTT: activated partial thromboplastin time; OR: odds ratio; CI: confidence interval; KCT: kaolin clotting time; dRVVT: dilute Russell's viper venom time; Ig: immunoglobulin; aCL: anticardiolipin antibodies; MPL: IgM phospholipid units; aβ2GPI: anti-beta2glycoprotein I antibodies; aPS/PT: phosphatidyl-dependent antiprothrombin antibodies.

having APS manifestations (thrombosis and/or pregnancy morbidity) for each of the 11 aPL tests using the first cohort of patients (Table 1). For LA the results of the three mixing procedures and the two confirming tests for LA were included. In the aCL, aβ2GPI and aPS/PT ELISAs, a second cut-off level was defined to separate patients with high antibody levels from those with lower levels (Table 1).

We devised an original formula in which aPL-S was determined by OR: aPL−S = 5 × exp ([OR] −5)/4 (Table 1). The aPL-S for each patient was calculated as total scores for positive tests. Further, we defined the partial aPL-S using only aPL tests included in the updated classification criteria for APS[1] IgG/IgM aCL, IgG/IgM aβ2GPI and LA (only APTT and dRVVT).

Results

In our first cohort of patients, the aPL-S ranged from 0 to 86 and the scores for patients with APS manifestations were higher than the scores for patients who did not have such manifestations. The prevalence of APS manifestations increased in accordance with increasing aPL-S. Patients were subdivided into five groups according to aPL-S: scores of 0, 1–9, 10–29 and >30.

The prevalence of APS manifestations in the groups was 10%, 26%, 29% and 56%, respectively. Similar results were observed with the partial aPL-S ranging from 0 to 56. The prevalence of APS manifestations was 13%, 23%, 36% and 44% in patients with scores of 0, 1–9, 10–19 and 20 <, respectively. The receiver operating characteristic (ROC) curves for aPL-S, partial aPL-S and revised Sapporo criteria showed a hyperbolic pattern, implying that aPL-S is a potential quantitative marker for APS diagnosis. The area under the curve (AUC) values were 0.752 for aPL-S, 0.692 for partial aPL-S, and 0.686 for revised Sapporo criteria. When the cut-off levels for aPL-S and partial aPL-S were defined as 30 and 20, respectively, the OR for aPL-S (13.6 (95% confidence interval (95% CI) 4.81–38.7)) was higher than that for the revised Sapporo criteria (4.91 (2.36–10.2)) and partial aPL-S (7.85 (2.99–20.7)).

To evaluate the relationship between aPL-S and the risk of thrombosis, we retrospectively analyzed all the thrombotic events developed in the second cohort of patients during the follow-up period. There were 36 new thrombotic events in 32 patients (22 arterial and 14 venous). The aPL-S and partial aPL-S among patients in whom thrombosis developed were significantly higher than that among those without thrombotic events (median score 5.5 vs. 0 and 5.5 vs. 0; $p < 0.012$ and $p < 0.001$, respectively).
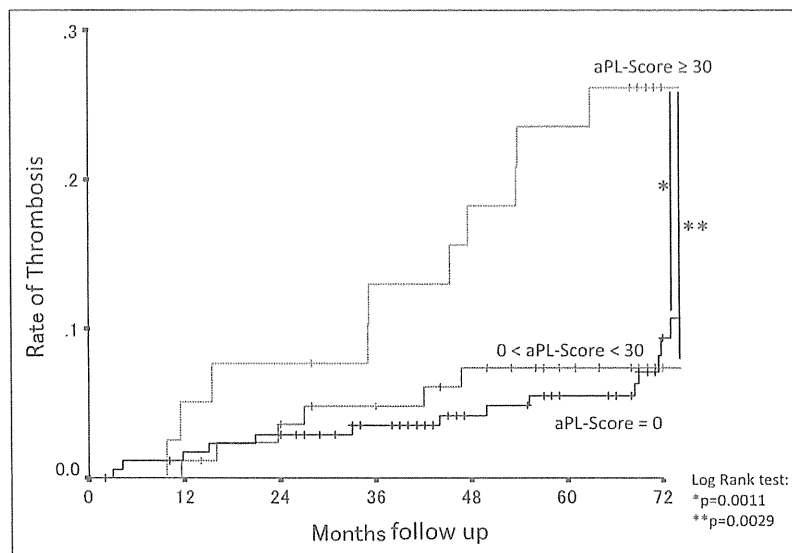
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Figure 1 Kaplan-Meier analysis of the rate of thrombosis in patients with different aPL scores.
aPL-Score: antiphospholipid score.

Patients with higher aPL-S had a stronger risk of thrombosis compared with patients with lower scores. The ORs for newly developed thrombosis in patients with aPL-S of >10 and >30 were 2.86 ((1.33–6.6), $p < 0.006$) and 5.27 ((2.32–11.95), $p < 0.0001$). The positive predictive values of aPL-S >10 and >30 were 20% and 31%, respectively, whereas the negative predictive values were 92% and 92%, respectively. For the partial aPL-S, the positive predictive values of scores >10 and >20 were 21% and 16%, respectively, and the negative predictive values were 92% and 91%, respectively.

The effect of antithrombotic treatment on aPL-S was evaluated in patients with aPL-S $\geq$ 30. This group included 39 patients (14 primary APS, 15 APS and systemic lupus erythematosus (SLE), 10 others), and 34 (87%) received some antithrombotic medications. The prevalence of thromboses among patients with aPL-S $\geq$ 30 was higher than that among those with a lower aPL-S (OR 5.40 (2.38–12.23) $p < 0.005$) (Figure 1).

A multivariate Cox regression test was conducted to analyze the risk of thrombosis using the following data: aPL-S > 30, age, gender, glucocorticoids treatment, and the presence of hypertension, hyperlipidemia, diabetes, SLE, or rheumatoid arthritis at the time the aPL assays were performed. An aPL-S of > 30 appeared to be an independent risk factor for thrombosis (hazard ratio 3.144 (1.383–7.150), $p < 0.006$).

Conclusion

aPL profile can be quantitated using the aPL-S. The aPL-S correlated with APS manifestations, suggesting that aPL-S is a potential quantitative marker of APS. Furthermore, aPL-S showed its predictive value for the onset of thrombosis, suggesting that treatment of APS can be modified considering aPL-S.

Although aPL have a strong link to thrombosis/pregnancy morbidity, the value of each aPL determination as an APS marker is still not yet elucidated.[6,7] One of the major issues on APS classification has been avoiding overdiagnosis of APS by not accepting positive results of nonspecific aPL tests. Also, standardization of each aPL assay has been extremely difficult as numbers of variables in the assay, such as techniques, reagents and standards, have hampered achievement of consensus.[2] Considering the history of standardization, the establishment of a single aPL to define APS is unlikely. In contrast, the premise that aPL represent the risk of thrombosis both in the past and in the future would not be disputed. Accordingly, it would be more sensible to use the aPL profile as a marker of thromboses rather than diagnosis. Furthermore, combining multiple aPL tests would compensate for or reduce the disadvantage of each single test. From this point of view, our definition of aPL-S has been proven to represent the "probability" or "likelihood" of having APS.

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We demonstrated a positive correlation between aPL-S with the risk of thrombosis. Despite receiving standard prophylaxis, many patients developed thromboses during the follow-up periods. Those data would lead to a potential therapeutic strategy in which the intensity of antithrombotic treatment could be determined according to aPL-S.

In clinical practice, all aPL tests are not available to all physicians. Therefore, we also defined a partial aPL-S that corresponds to aPL tests included in the revised Sapporo criteria[1] that seems to be useful for evaluating the risk of thrombosis in patients with aPL.

We proved the efficacy of aPL-S as a marker of the "probability" of APS and its value for predicting thrombosis. aPL-S is the first to attempt scoring the aPL profile, and successfully correlated with risk of thrombotic events. However, the score could have other definitions, according to the population, and obviously the "true" predictive value should be validated in prospective studies. Higher accuracy of aPL-S is obtained when all aPL tests are included. In case all of the tests are not accessible, a partial aPL-S will provide important information regarding the thrombosis risk for each patient and consequently will help clinicians in making decisions about the therapeutic approach.

Funding

Conflict of interest statement

The authors have no conflicts of interest to declare.

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GYNECOLOGICAL ENDOCRINOLOGY

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ORIGINAL ARTICLE

History of biochemical pregnancy was associated with the subsequent reproductive failure among women with recurrent spontaneous abortion

Yoko Maesawa, Hideto Yamada, Masashi Deguchi, and Yasuhiko Ebina

Department of Obstetrics and Gynecology, Kobe University Graduate School of Medicine, Kobe, Japan

Abstract

The aim of this study was to evaluate whether the presence of history of biochemical pregnancy (BP) was associated with clinical characteristics and the subsequent pregnancy outcome among women with recurrent spontaneous abortion (RSA). One-hundred and seventy-five RSA women with two or more clinical pregnancy losses were enrolled. The clinical characteristics were compared between 164 women with history of 0–1 BP (Group A) and 11 women with two or more BP (Group B). The frequency of previous pregnancy loss and history of in vitro fertilization and embryo transfer in Group B was higher than that in Group A; while frequency of secondary RSA in Group B was lower than Group A. The subsequent pregnancy outcome was assessed prospectively; and live-birth rate in Group A (72.9%) was higher ($p < 0.05$) than that in Group B (41.7%). The incidence of reproductive failure (58.3%, $p < 0.05$) and spontaneous abortion with normal chromosome (25.0%, $p = 0.050$) in Group B was higher than those (27.1 and 5.9%, respectively) in Group A. RSA women with two or more BP had higher risk of reproductive failure and spontaneous abortion with normal chromosome together with lower chance of live-birth. The results of the present study involve important information and are helpful for clinical practitioners.

Introduction

The definition of clinical pregnancy is based on the existence of gestational sac in the uterus confirmed by ultrasonography. The International Committee for Monitoring Assisted Reproductive Technology (ICMART) and World Health Organization (WHO) have defined biochemical pregnancy (BP) as a condition that human chorionic gonadotropin (hCG) is detected in maternal urine or blood, but clinical pregnancy is not achieved [1]. BP is not rare; and it has been reported that the incidence of BP ranges from 8 to 33% of a total of pregnancies [2–5]. The presence of BP does not significantly affect the menstrual cycle, and therefore the pathophysiology of BP has been still uncertain.

Recurrent spontaneous abortion (RSA) is defined as two or more consecutive losses of clinical pregnancies during early gestation and affects 1–1.8% of women [6]. A wide variety of causes participate in the pathogenesis of RSA, including uterine anomalies, cervical incompetence, autoimmune diseases, antiphospholipid antibody, chromosomal abnormalities of couples, thrombophilic disorders, endocrinological abnormalities and microbial infections.

Women with RSA often have a history of repeated BP. The aim of this study was to evaluate whether the presence of a history of

BP was associated with clinical characteristics, etiologies of RSA or the subsequent pregnancy outcome among RSA women.

Patients and methods

This study was approved by the institutional ethical boards of the Kobe University Hospital; and was conducted with informed consent from all of the subjects. During the period between June 2009 and September 2012, consecutive 175 RSA women who had a history of two or more clinical pregnancy losses visited the university hospital.

All the RSA women underwent examinations of ultrasound, hysterosalpingography, endometrial biopsy, basal body temperature (BBT) and conventional blood analyses for screening of the etiologies. The blood analyses included chromosome karyotypes of couple; measurements of progesterone in mid-luteal phase, prolactin, testosterone, follicle-stimulating hormone, luteinizing hormone, thyroid, liver, kidney functions, natural killer (NK) cell activity, hemostatic coagulation factors including D-dimer, coagulation factor XII, protein C and protein S; and autoimmune factors including antinuclear antibody (ANA), lupus anticoagulant (LA), anticardiolipin (aCL), β2-glycoprotein-I dependent anticardiolipin (aCLβ2GPI) and antiphosphatidylethanolamine antibodies (aPE).

Findings associated with risks or etiologies of RSA in the present study were defined as the following: the presence of uterine abnormality, thyroid dysfunction, chromosome abnormality of the couples, antiphospholipid antibody (aPL) including LA, aCL and aCLβ2GPI, aPE, luteal dysfunction, polycystic ovary (PCO), hyperprolactinemia, protein S deficiency, protein C deficiency, low level of factor XII, ANA and high NK cell

Address for correspondence: Prof. Hideto Yamada, MD, PhD, Department of Obstetrics and Gynecology, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho Chuo-ku, Kobe 650-0017, Japan. Tel: +81-78-382-6000. Fax: +81-78-382-6019. E-mail: yhideto@med.kobe-u.ac.jp

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activity. Luteal dysfunction in the present study was defined from low level of progesterone, <12 days of luteal phase on BBT, or delay of two or more days on endometrial dating biopsy. When multiple findings were noted in one woman, each finding was counted for categorization.

Information about pregnancy history was obtained from referral letters and detailed inquiry to patients by the same doctor (H.Y.). BP was diagnosed according to criteria of the ICMART and WHO. Clinical information including maternal age at the initial visit, pregnancy history, history of *in vitro* fertilization and embryo transfer (IVF-ET), number of previous BP, findings associated with risks or etiologies of RSA were collected from inquiries and medical records. The subsequent pregnancy outcome was assessed prospectively. When the subsequent pregnancy ended in spontaneous abortion, the patient was suggested undergoing chromosome karyotype analysis of the villi. The Mann–Whitney *U*-test and Fisher's exact test were used for the comparison of two groups. Statistical significance was determined by $p < 0.05$.

Results

Of the 175 RSA women, 14 had experienced BP once, 7 had twice, 1 had three times, 1 had four times and 2 had five times of BP, while the remaining 150 women had no history of BP. Of a total of 598 previous pregnancies in the 175 RSA women, 45 (7.5%) pregnancies were found to be BP.

Table 1 shows comparison of maternal age and reproductive history according to number of previous BP. When the 175 RSA women were divided into 164 women with history of 0–1 BP (Group A) and 11 women with two or more BP (Group B), number of previous pregnancy loss including BP/excluding BP in Group B was significantly higher than that in Group A. The frequency of women with secondary RSA who had experienced RSA after live-birth in Group A was significantly higher than that in Group B. The frequency of women who had experienced pregnancy losses of IVF-ET in Group B was significantly higher than that in Group A. There was no statistical difference in maternal age between Group A and Group B.

Table 2 shows the incidence of findings associated with risks or etiologies of RSA according to number of previous BP. There was no statistical difference in the incidence of findings among women without history of BP, women with one BP, and women with two or more BP; or between Group A and Group B.

Until March 2014, 109 of the 175 RSA women experienced a total of 130 pregnancies undergoing appropriate therapies for individual etiologies. The 130 pregnancies consisted of 109 pregnancies from 92 women without history of BP, 9 pregnancies from eight women with one BP, and 12 pregnancies from nine women with two or more BP. The pregnancy outcome was classified into live-birth and reproductive failure that included spontaneous abortion with normal chromosome karyotype

(SANK), spontaneous abortion with abnormal chromosome karyotype (SAAK) of the conception, unknown karyotype due to insufficient specimen or no consent, and BP.

Table 3 shows the outcome of the subsequent pregnancy according to number of previous BP. The live-birth rate in Group A was significantly ($p < 0.05$) higher than that in Group B. The incidence of reproductive failure ($p < 0.05$) and SANK ($p = 0.050$) in Group B was higher than those in Group A.

Discussion

Several prospective studies of hCG measurements using serum and urine samples have demonstrated that the incidence of BP for a total of human pregnancy ranges from 8 to 33% [2–5]. A wide variety of the BP incidence might be due to differences in study design, hCG cutoff values, measurement methods or subject characteristics. The incidence of BP in 175 RSA women was found to be 7.5% of their previous pregnancies in the present study. The BP incidence of 7.5% was lower than the abovementioned incidence. The frequencies of previous pregnancy loss and IVF-ET history in women with two or more BP were higher than those in women with 0–1 BP. The frequency of secondary RSA in women with two or more BP was lower than women with 0–1 BP. Therefore, women with two or more BP seemed to have more severe condition of RSA than women with 0–1 BP in the present study. The outcome of the subsequent pregnancy was assessed prospectively. It was for the first time found that RSA women with two or more BP had higher risk of

Table 2. The incidence (percentage) of findings associated with risks or etiologies of RSA according to number of previous BP.

		Number of previous BP			
	Total ($n = 175$)	0 ($n = 150$)	1 ($n = 14$)	0–1 ($n = 164$)	≥2 ($n = 11$)
Uterine abnormality	2.9	2.7	7.1	3.1	0
Thyroid dysfunction	10.9	11.3	14.3	11.6	0
Chromosome abnormality	4.6	4.67	7.1	4.9	0
aPL	13.7	14	21.4	14.6	0
aPE	19.4	19.3	21.4	19.5	18.2
Luteal dysfunction	29.7	31.3	14.3	29.8	27.3
PCO	2.9	3.3	0	3	0
Hyperprolactinemia	4	4.67	0	4.3	0
Protein S deficiency	13.7	14.7	7.1	14.1	9.1
Protein C deficiency	1.7	2	0	1.8	0
Low level of factor XII	7.4	8	7.1	7.9	0
Antinuclear antibody	13.1	14	14.3	14	0
High NK cell activity	20.6	22	7.1	20.7	18.2
Not detected	14.3	13.3	14.3	13.4	17.3

No statistical differences between women with 0–1 BP (Group A) and women with two or more BP (Group B). aPL including LA, aCL and aCLβ2GPI.

Table 1. Comparison of maternal age and reproductive history according to number of previous BP.

		Number of previous BP				
	Total ($n = 175$)	0 ($n = 150$)	1 ($n = 14$)	0–1 ($n = 164$)	≥2 ($n = 11$)	*p* Value
Maternal age* (years)	35 (24–46)	35 (24–46)	39 (30–39)	35 (24–46)	35 (29–44)	0.730
Number of previous pregnancy loss*						
Excluding BP	2 (2–13)	2 (2–13)	2 (2–7)	2 (2–13)	4 (2–12)	<0.05
Including BP	3 (2–14)	2 (2–13)	3 (3–8)	3 (2–13)	8 (4–14)	<0.01
Secondary RSA	57	54	3	57	0	<0.05
History of IVF-ET	16	10	2	12	4	<0.05

*Median (range). *p* Values indicate comparison between women with 0–1 BP (Group A) and women with two or more BP (Group B).

Table 3. The outcome of the subsequent pregnancy according to number of previous BP.

| | Total ($n = 130$) | Number of previous BP | | | | p Value |
		0 ($n = 109$)	1 ($n = 9$)	0–1 ($n = 118$)	≥2 ($n = 12$)	
Live-birth	91 (70.0)	78 (71.6)	8 (88.9)	86 (72.9)	5 (41.7)	<0.05
Reproductive failure	39 (30.0)	31 (28.4)	1 (11.1)	32 (27.1)	7 (58.3)	<0.05
Spontaneous abortion	29 (26.7)	23 (21.1)	1 (11.1)	24 (20.3)	5 (41.7)	0.138
Normal chromosome karyotype	10 (7.7)	7 (6.4)	0	7 (5.9)	3 (25.0)	0.050
Abnormal chromosome karyotype	10 (7.7)	8 (7.3)	1 (11.1)	9 (7.6)	1 (8.3)	0.940
Unknown karyotype	9 (6.9)	8 (7.3)	0	8 (6.8)	1 (8.3)	0.594
Biochemical pregnancy	10 (7.7)	8 (7.3)	0	8 (6.8)	2 (16.7)	0.231

Parentheses indicate percentages. p Values indicate comparison between women with 0–1 BP (Group A) and women with two or more BP (Group B).

reproductive failure and SANK together with lower chance of live-birth in the subsequent pregnancy than women with 0–1 BP. The risks of SAAK and BP were not statistically different between two groups. These findings suggested that there might be a clinical entity with certain pathophysiology on the reproduction by which women had reduced fecundity and increased risks for repeated BP or SANK. The results of the present study involve important information and are helpful for clinical practitioners.

One study has demonstrated that women with BP history have an increased rate of positive tests for aPL, but not for ANA or high NK cell activity [7]. In the present study, there was no statistical difference in the incidence of each finding associated with risks or etiologies of RSA between women with 0–1 BP and women with two or more BP. No women with two or more BP had a positive test for aPL. Therefore, the etiology predisposing to BP is still obscure, and further studies are necessary.

The present study enrolling not a large number of RSA women with two or more previous BP included several uncertainties. The presence of previous BP among RSA women was determined by information about pregnancy history obtained from referral letters, inquiry and medical record. However, all the 175 women equally underwent etiological assessments for RSA, and their subsequent pregnancy outcome was assessed prospectively under the same condition including chromosome analysis for abortion. It is too difficult to confirm prospectively whether each pregnancy is BP or clinical pregnancy for nulliparous woman until she experiences two or more RSA. Further studies are necessary to confirm conclusions of the present study.

Declaration of interest

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ORIGINAL ARTICLE

Management of thromboembolism in pregnant women with inherited antithrombin deficiency: genotype-phenotype analysis

Yasuhiko Ebina[1], Yuji Nakamachi[2], Kenji Tanimura[1], Mayumi Morizane[1], Satoko Morikami[1], Masashi Deguchi[1], Seiji Kawano[2], Hideto Yamada[1]

1 Kobe University Graduate School of Medicine, Department of Obstetrics and Gynecology
2 Kobe University Hospital, Department of Clinical Laboratory

Abstract

Objective   Women with inherited antithrombin (AT) deficiency have an increased risk of thromboembolisms (TEs) occurring during pregnancy and puerperium. Although thromboprophylaxis during pregnancy is recommended, the drug selection and dosage remain controversial, and little is known about the correlation between mutations and clinical manifestations. We therefore analysed the molecular defects of the AT gene (*SERPINC1*) and clinical courses in pregnant women with inherited AT deficiency to provide general guidance for optimal management.

Methods   Pregnant Japanese women genetically diagnosed with inherited AT deficiency and being treated at Kobe University Hospital were included in the study, and their detailed clinical information and family histories of TE were examined. Genetic analysis was performed of *SERPINC1* in patients and family members.

Results   Out of a total of 16 pregnancies in 15 women affected by inherited AT deficiency, 10 were complicated by TEs, nine had deep venous thrombosis (DVT), and one had DVT and pulmonary embolism. Of the 15 cases, 14 had causative mutations (five novel mutations) in *SERPINC1*, including six missense mutations, two nonsense mutations, and one splice site mutation. The use of AT concentrate and unfractionated heparin (UFH) in pregnancy and puerperium was associated with a successful pregnancy outcome, with venous thromboembolism (VTE) rates being lower than previously reported.

Conclusion   Despite the heterogeneous background of AT deficiency, we concluded that molecular

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analysis of *SERPIN1* might help to further elucidate the pathogenesis of AT deficiency and to establish genotype-phenotype associations.

Key words: Antithrombin concentrate, antithrombin deficiency, pregnancy

Correspondence to: Yasuhiko Ebina, MD, PhD, Associate professor
Department of Obstetrics and Gynecology, Kobe University Graduate School of Medicine,
7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan
Tel: +81-78-382-6005, Fax: +81-78-382-6019
E-mail: ebiyas@med.kobe-u.ac.jp

Introduction

Antithrombin (AT) plays an important role in the regulation of haemostasis by inactivating thrombin and other activated coagulation factors. AT deficiency is a rare, autosomal dominant thrombotic disorder associated with a 1.7–4.0% overall annual incidence of venous thromboembolism (VTE) and affecting 1/500 to 1/5,000 individuals worldwide. Women with inherited AT deficiency have an increased risk of thromboembolism (TE) during pregnancy and puerperium; this has been found to occur in more than 70% of cases in the absence of anticoagulant therapy. Several clinicians have described the management of pregnant women with AT deficiency, which has included the administration of heparin and AT concentrate. Although thromboprophylaxis during pregnancy is recommended, the drug selection and dosage remain controversial.

The AT gene (*SERPINC1*) is located on chromosome 1q23.1-23.9, and comprises seven exons and six introns. Many different *SERPINC1* mutations have been reported, but little is known about the correlation between mutations and clinical manifestations. Moreover, because of the disease rarity, no large trials have been conducted. We speculated that the severity of maternal thromboembolic risks and effective management might vary according to the mutation. Therefore, the objectives of the present study were to clarify *SERPINC1* molecular defects and clinical courses in pregnant women with inherited AT deficiency, and to provide general guidance for optimal disease management.

Patients and Methods

Study population

The institutional ethical boards of Kobe University Hospital approved this study, which was conducted according to the Declaration of Helsinki. We obtained written informed consent from all subjects. Between January 2006 and August 2013, pregnant Japanese women genetically diagnosed with

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inherited AT deficiency and treated at our institution were included in the study. Plasma AT activity (normal range, 79–121%), and the concentration of plasma AT protein (15–31 mg/dl) were determined by commercial kits. The AT activity/antigen ratio was calculated to classify the AT deficiency into type I and type II. We also measured levels of protein C, protein S, anticardiolipin antibodies, and lupus anticoagulant upon patient referral, but not Factor V Leiden since this disease is not present in the Japanese population (1). We took pharmacologic agents (especially heparin) and other causes of acquired antithrombin deficiency into consideration when interpreting the AT results, and did not test for AT deficiency during an acute thrombotic event. Testing was also carried out at least five days after stopping heparin therapy. To differentiate between inherited and acquired AT deficiency, we carried out *SERPINC1* genetic analysis. We used the reported typing result for cases with previously known mutations.

Thromboembolic treatment and prophylaxis

The treatment and prophylactic protocol for patients with AT deficiency are shown in Figure 1. Most cases with deep vein thrombosis (DVT) during pregnancy were first suspected because of the presence of a swollen and painful lower extremity. The DVT diagnosis was based on continuous-wave Doppler ultrasonography, while that of pulmonary embolism (PE) was confirmed by computed tomography.

After the diagnosis of VTE, unfractionated heparin (UFH) and AT concentrate therapy was commenced. Although there have been many investigations in Europe describing low molecular-weight heparin (LMWH) therapy as a form of thromboprophylaxis during pregnancy, this is not permitted in Japan. Therefore, continuous intravenous injection of unfractionated heparin was administrated to maintain an activated partial thromboplastin time (APTT) that was 1.5–2.0 times that of the control value. AT concentrate (Antithrombin® P 1500 for injection, CSL Behring, Japan or Neuart® LV, 1,500 units, Japan Blood Products Organization, Japan) was also injected to maintain plasma AT levels above 70%. AT concentrates manufactured from donated blood are currently available and costs are covered by Japanese government　national health insurance.

To avoid PE, a temporary inferior vena cava (IVC) filter was inserted in severe cases. The indications for the placement of temporally IVC filters are documented DVT, and temporary contraindications for anticoagulation and PE despite prophylaxis. Doppler ultrasound examinations of lower extremities were performed repeatedly to check for thrombus formation. After the succession of thrombus, women with prior VTE received subcutaneous heparin twice daily. Patients were admitted for labour induction at 37 gestational weeks. On the day before delivery, intravenous heparin infusion was substituted for subcutaneous heparin, but discontinued at least 4–6 h before delivery. To maintain plasma AT activity above 70%, a sufficient dose of AT concentrate was important. Six hours after delivery, intravenous heparin infusion was recommenced. Warfarin administration was started on the first postpartum day.

All women in whom inherited AT deficiency was already diagnosed began anticoagulant treatment with heparin when pregnancy was confirmed. AT supplementation was also commenced and continued to maintain plasma AT levels above 70%. Peripartum management was the same as for the treatment

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group. Thromboembolic events were considered to be puerperal-associated if they occurred within the first 40 days after delivery.

Genetic analysis of SERPINC 1

Venous blood samples were collected from patients and their family members. Genomic DNA from blood leukocytes was extracted using the QIAamp DNA Mini Kit (Qiagen, Tokyo, Japan) according to the manufacturer's instructions. All exons were amplified using the seven sets of primers as described by Vidaud et al. with some modifications (2). All reactions were performed in a final volume of 50 μl containing 15 mM Tris-HCl buffer, 50 mM KCl, 200 μmol/l each dNTP, 2 mmol/l MgCl$_2$, 200 nmol/l each primer, 1.25 U Taq polymerase (Applied Biosystems, Foster City, CA), and 2 μl genomic DNA.

Samples were amplified as follows: denaturation at 95°C for 10 min, followed by 30 cycles of 95°C denaturation for 30 s, annealing at 57°C for 30 s, and extension at 72°C for 30 s; the last cycle was extended for 10 min. PCR products were electrophoresed on 3% TAE agarose gels, stained with ethidium bromide, and purified with Ultrafree-DA (Millipore, Bedford, MA) according to the manufacturer's instructions with slight modifications. Sequencing analysis of purified PCR products was performed with the corresponding PCR primers using the dye terminator method with the DNA Sequencing Kit, BigDye Terminator Cycle Sequencing Ready Reaction (Applied Biosystems) according to the manufacturer's instructions. Reaction solutions were purified and sequenced with Genetic Analyzer (Applied Biosystems) according to the manufacturer's instructions.

Data analysis

Continuous variables were expressed as median and min-max values. To evaluate the efficacy of anticoagulation treatment, thrombolysis, placement of IVC filter, time and mode of delivery, the weight of the neonate and interpartum bleeding volumes were analysed. Spontaneous abortion was defined as the loss of pregnancy before 20 weeks of gestation. Stillbirth was defined as the intrauterine death of the foetus after 22 weeks of gestational age. Premature newborns were live births before 37 weeks of gestation. Foetal growth restriction (FGR) was defined as a newborn with a weight at birth under the 10th percentile for its gestational age, according to Japanese standards. Pregnancy-induced hypertension was defined as new onset hypertension after the 20th week of gestation plus proteinuria. Placental abruption was diagnosed clinically.

Results

We treated 16 pregnancies in 15 unrelated women affected by inherited AT deficiency (Table 1). The median age during pregnancy was 32.5 years (range, 22–41 years). Thirteen women were primipara and two were porous women. The diagnosis of inherited AT deficiency was made prior to the current pregnancy because of a familial study in two asymptomatic women (patients 12 and 13). Patient 14 was diagnosed when she developed ovarian hyperstimulation syndrome. Patient 15 was tested at a former

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clinic during early pregnancy. The remaining 11 women were diagnosed after a thromboembolic event: in patient 11 before pregnancy (at 28 years of age), while the other 10 women (patients 1–10) suffered thromboembolic complications during the current pregnancy. Eight of the 15 women had no family history of TE (53.3%).

Maternal thrombotic manifestations and treatment

Ten pregnancies (patients 1–10) complicated by TE were classified as the Treatment group. Eight patients (80%) carried type I AT deficiency, and the other two (20%) had type II AT deficiency. The median age during pregnancy of these 10 patients was 32.5 years (range, 22–38 years). The median time of TE onset was the 12.5th gestational week (range, 6–33 weeks). The onset of TE showed a bimodal distribution, with one at 6–14 weeks of gestation (*n*=5) and another at 21–33 weeks (*n*=5). Both groups had the same frequency of type I deficiency (20%). Nine cases had DVT, and one had DVT and PE. DVT was present on the left side (*n*=8) or right side (*n*=2) of the lower extremity. Primary symptoms were leg oedema (*n*=3), leg oedema with leg pain (*n*=3), leg pain (*n*=1), leg oedema and pain with discoloration (*n*=1), and no symptoms (*n*=1).

Asymptomatic thrombosis was detected by ultrasound screening during the treatment of threatened premature labour. Intravenous injection of heparin was administrated to maintain an APTT that was 1.5–2.0 times that of the control value. AT concentrate was administered with a median dose of 4,875 U per week (range, 3,000–10,500 U per week) to maintain an AT level >70%. To administrate anticoagulation therapy, temporary IVC filters were inserted to prevent fatal PE in eight women. The length of filter placement ranged from two to four weeks. The median length of hospital stay for anticoagulant treatment was 38 days. After regression of thrombus, subcutaneous heparin was administrated twice daily. Supplementation of AT concentrate was continued at an in-patient clinic. There were no cases of arterial thrombosis, cerebral infarction, or pregnancy-induced hypertension.

No TE occurred in the six women in the Prophylaxis group. AT concentrate was administered with a median dose of 4,500 U per week (range, 1,500–6,000 U per week) to maintain an AT level >70%. Patient 3(2) had a history of TE in her first pregnancy (3(1)) at 22 years of age. The prophylactic protocol was therefore commenced early in the present pregnancy. She experienced no TE during her entire second pregnancy and delivered a healthy baby. Patient 15 was referred in the 32nd week of gestation. Administration of AT concentrate was commenced at week 33. No heparin injection was used, with the exception of patient 15.

There were no adverse effects of AT concentrate administration. However, heparin-induced thrombocytopenia occurred in patient 2 as previously reported (3). Anticoagulation therapies together with AT supplementation were commenced with the use of argatroban and fondaparinux. There were no cases of thromboembolic events or heavy bleeding during the puerperal period. This treatment regimen allowed neuraxial anaesthesia with no complications in all cases.

Pregnancy outcome

Pregnancy outcomes are summarised in Table 1. The mean birth-weight was 2,771 g (range,

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2,122–3,710 g). The median length of gestation was 38 weeks (range, 30–39 weeks). There were three cases (18.8%) of preterm births. Patient 1 underwent cervical cerclage at 20 weeks of gestation, and required a caesarean section at the 35th week of gestation because of a nonreassuring foetal status (NRFS). Another caesarean section was performed because of NRFS during delivery at the 38th week of gestation in patient 6. Patient 7 had a twin pregnancy and placenta previa. Caesarean section was required in this patient at the 30th week of gestation because of failure of tocolysis and genital bleeding. The median estimated blood loss at delivery was 559 ml (range, 265–2,180 ml). There were no cases of miscarriage, stillbirth, FGR, or placental abruption.

Genetic analysis of SERPINC1

Table 2 shows genetic variants of the SERPINC1 gene according to exon order. Patients 4, 9, 13, and 15 carried a type II AT deficiency, while the other 11 patients had type I AT deficiency. Of the 15 cases, 14 had causative heterozygous mutations (93.3%) in SERPINC1, including six missense mutations, two nonsense mutations, and one splice site mutation. There were four previously reported mutations (4-7). The other five mutations are novel (Figure 1); all of these were proved to segregate in the families, and the corresponding mutations were identified in a total of 11 family members (Table 2).

The details of the novel mutations are as follows: patient 10 carried a T to C mutation at nucleotide 72 of SERPINC1, which changed the codon for Met-32 to Thr (start codon). Her level of plasma AT activity was 40%. Her son and daughter also carried this mutation. Although we did not measure this patient's AT antigen level, we diagnosed type I AT deficiency because of the mutation in the start codon. Patient 8 carried a G to A mutation at nucleotide 2624 (exon 1) of SERPINC1, which changed the codon for Ala54 to Thr. Her level of plasma AT activity and antigens were 48% and 18 mg/dl, respectively. Her father also carried this mutation. Patient 11 carried a T to G mutation at nucleotide 2637 (exon 2), which changed the codon 58 for Phe to Cyc. Her level of plasma AT activity and antigens were 45% and 32 mg/dl, respectively. Patient 1 carried a C to T mutation at nucleotide 2762 (exon 2), which changed the codon for Gln100 to Stop. Her level of plasma AT activity and antigens were 61% and 13 mg/dl, respectively. Her mother and daughter also carried this mutation. Patient 14 carried a C to A mutation at nucleotide 7457 of SERPINC1, which changed the codon for Ala249 to Glu. Her level of plasma AT activity and antigens were 30% and 19 mg/dl, respectively. Her father also carried this mutation. The AT deficiency remained unexplained after sequencing analysis in patient 5. However, we suspected the presence of a large SERPINC1 deletion.

It is noteworthy that three patients (patients 9, 13, and 15) carried the SERPINC1 variant AT III Nagasaki (6). They carried a T to C mutation at nucleotide 5342 (exon 3a), which changed the codon for Ser116 to Pro. This variation is related to a heparin-binding defect. Also of interest is that four patients (patients 3, 6, 7, and 12) carry a previously reported splice site mutation: a G to A mutation 14 bp upstream from the beginning of the coding sequence at nucleotide 9788 in intron 4. This mutation creates a *de novo* exon 5 splice site, with a subsequent modification of the coding sequence. It leads to the insertion of four additional amino acids (Val, Phe, Leu, and Pro) between Proline at position 352 and

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Glycine at position 353, and results in an AT deficiency (5, 7, 8).

Discussion

This is the first report of molecular analysis and genotype-phenotype correlation in pregnant women with AT deficiency. The prevalence of AT deficiency in Japanese patients with DVT was 5.56%, which is much higher than the previously reported prevalence of AT deficiency in 4,517 Japanese healthy blood donors of 0.15% (9). In the present study, we describe both the genetic basis and clinical thrombotic profile of AT deficiency in 15 pregnant Japanese women. A total of 14 mutations were identified, five of which were novel.

SERPINC1 gene mutations are highly heterogeneous in corresponding populations (10), and few ethnic-specific mutations have been identified. Miyata previously identified 14 nonsynonymous mutations, including eight missense, three frameshift, two nonsense, and one splice-site mutation, in 173 Japanese patients with AT deficiency and DVT (mean age, 46.6 years)(11). There were no *SERPINC1* mutations common to both our patients and those of Miyata's report. Picard reported that 63 of 86 probands (73%) with suspected type I AT deficiency carried a causative heterozygous point mutation, and multiplex ligation-dependent probe amplification identified a large *SERPINC1* deletion in 10 of these subjects (12).

The aim of comparing phenotypic characteristics with genetic alterations is to identify a correlation between the two. A previous 51-year-old patient with a splice site mutation at position 9788 in intron 4 of *SERPINC1* developed TE at the age of 19. Because of a splicing abnormality, mutant mRNA with a 12 bp insertion was generated which was less stable and more fragile (18%) than the normal allele (50%) (13). A second patient with this mutation developed DVT at 44 years of age, which was complicated with PE. His daughter presented with her first thrombotic event at the age of 18 years after taking oral contraceptives. Six other affected family members suffered recurrent thromboembolic events. An episode of PE in a 20-year-old woman carrying the same mutation resulted in her death (7), while a report of a French female patient with this mutation showed that she suffered her first DVT at the age of 22, after giving birth. She then had an iliac DVT at the age of 36 (8).

In the present study, four patients carried the same splice-site mutation. Of these, three (patients 6, 7, and 12) had a family history of severe thromboembolic events, PE ($n=3$), and cerebral infarction ($n=1$). In the treatment group ($n=10$), 30% of patients including the only PE case carried this mutation. Consequently, its presence might be associated with an increased risk of developing TE during pregnancy. Patient 12 was diagnosed with AT deficiency at 35 years of age because of her familial history, which led to her taking warfarin orally. In her present pregnancy, she commenced anticoagulant therapy with AT concentrate and heparin injections. There was no thromboembolic event and she successfully delivered a healthy baby.

Mitsuguro reported that the risk for VTE in non-pregnant patients with type I AT deficiency was much higher than in patients with type II deficiency (hazard ratio: 7.3; 95% confidence interval

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1.9–12.2; p=0.0009) (14). However, 20% of the patients in this study who had TE complications had type II AT deficiency. Thus, it might be necessary for pregnant patients to undertake a sufficient prophylaxis policy regardless of the type of AT deficiency.

Yamada et al. reported two cases with AT deficiency who were successfully treated with infusion of AT concentrates (15). This previous study also reviewed the 45 case reports of pregnancy with inherited AT deficiencies published between 1976 and 1995. Of the different prophylactic therapies, heparin (including LMWH) infusion was the most popular, being performed in 41 of 45 cases. Warfarin was administrated in 33 cases but was restricted to the second trimester, early third trimester, and puerperal period. AT concentrate infusion was only reported during and after the 1980s, and occurred in 25 patients including those whose treatment was combined with heparin. Some patients were controlled by AT concentrate infusion alone and most experienced a favourable outcome. Twenty-four of 25 patients treated with AT were given AT concentrate for prophylactic therapy during delivery, while 12 received only AT concentrate during delivery (15).

Another case report from Japan described the use of AT concentrate for antepartum thromboprophylaxis (16). Since then, the only anticoagulants used alone for antepartum prophylaxis have been LMWH (17-20) and, more recently, enoxaparin in 18 pregnancies with AT deficiency (21) in which thromboprophylaxis was stopped at the initiation of labour or 12 h prior to caesarean section. Four of the latter pregnancies (22.2%) were complicated by TE. Similarly, Rogenhofer reported that TE occurred in 22% of pregnancies despite the use of LMWH prophylaxis (22). In the present study, no TE events occurred in the prophylactic group. Plasma-derived AT concentrates therefore appear to be well tolerated, with few observed adverse reactions, so pose an extremely low risk for the transmission of infection agents. Indeed, there have been no confirmed reports of infectious transmission with Anthrobin P® and Neuart® LV.

In conclusion, the use of AT concentrate and UFH in pregnancy and puerperium has been associated with a successful pregnancy outcome, with VTE rates lower than previously reported. Despite the heterogeneous background of AT deficiency, we conclude that molecular analysis of *SERPIN1* would help to further elucidate the pathogenesis of AT deficiency and to establish genotype-phenotype associations.

Conflicts of interest
None

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Table 1: Maternal thrombotic manifestations, treatment, and pregnancy outcomes.

Patient number	Age	Family history of TE	Pregnancy history	ATD diagnosis before present pregnancy	Type of deficiency	TE in present pregnancy	Time of TE (weeks+days)	DVT	PE	Location of TE	Inferior vena cava filter placemant	Gestational age at delivery (weeks+days)	Delivery	Body weight of neonate (g)	Bleeding at delivery (g)
1	38	No	None	-	I	+	6 + 4	+	-	Rt popliteal, femoral vein	-	35 + 3	CS	2122	265
2	33	No	None	-	I	+	7 + 2	+	-	Lt posterior tibial, external iliac vein	+	38 + 2	VD	2834	838
3 (1)	22	No	None	-	I	+	9 + 3	+	-	Lt popliteal, external iliac vein	+	38 + 1	VD	2666	1343
4	33	No	None	-	II RS	+	11 + 2	+	-	Lt femoral, common iliac vein	+	39 + 1	VD	3078	331
5	23	No	None	-	I	+	14 + 0	+	-	Lt popliteal, external iliac vein	+	38 + 1	VD	2542	1100
6	29	Father: PE Aunt:Cerebral infarction	1) VD (26 y/o) 2) SA (26 y/o) no previous history of TE	-	I	+	21 + 4	+	+	Rt popliteal, femoral vein, PE	+	38 + 1	CS	2888	470
7	36	Cousins: PE	None	-	I	+	24 + 1	+	-	Lt femoral vein	+	30 + 0	CS	1590/1655	2180
8	24	No	None	-	I	+	24 + 6	+	-	Lt popliteal vein	+	38 + 0	VD	2730	480
9	36	No	None	-	II HBS	+	25 + 2	+	-	Lt popliteal, external iliac vein	-	36 + 6	VD	2872	740
10	32	Mother: TE Cousins on mother side: DVT	None	-	I	+	33 + 1	+	-	Lt fibular, external iliac vein	+	37 + 0	VD	2730	546
3 (2)	26	-	1) VD (22 y/o) Warfarin	DVT (22 y/o)	I	-	-	-	-	-	-	37 + 5	VD	2546	358
11	32	Father: ATD (cerebral infarction)	None	DVT (28 y/o) Warfarin	I	-	-	-	-	-	-	38 +1	VD	2570	302
12	41	Brother: ATD (PE) Mother: ATD (asymptomatic)	1) VD (27 y/o) 2) AA (30 y/o) 3) VD (31 y/o) 4) AA (38 y/o) no previous history of TE	Tested due to FH (35 y/o) Warfarin	I	-	-	-	-	-	-	38 + 6	VD	3710	572
13	34	Sister: ATD	None	Tested owing to FH (34 y/o)	II HBS	-	-	-	-	-	-	39 + 1	VD	2808	893
14	34	Cousins: Stillbirth	None	Tested in OHSS treatment (34 y/o)	I	-	-	-	-	-	-	38 + 5	VD	2800	1710
15	30	No	None	Tested in early pregnancy	II HBS	-	-	-	-	-	-	40 + 2	VD	2974	488

TE, thromboembolism; ATD, antithrombin deficiency; DVT, deep venous thrombus; PE, pulmonary embolism; RS, reaction site; HBS, heparin binding site; CS, Caesarean section; VD, vaginal delivery;

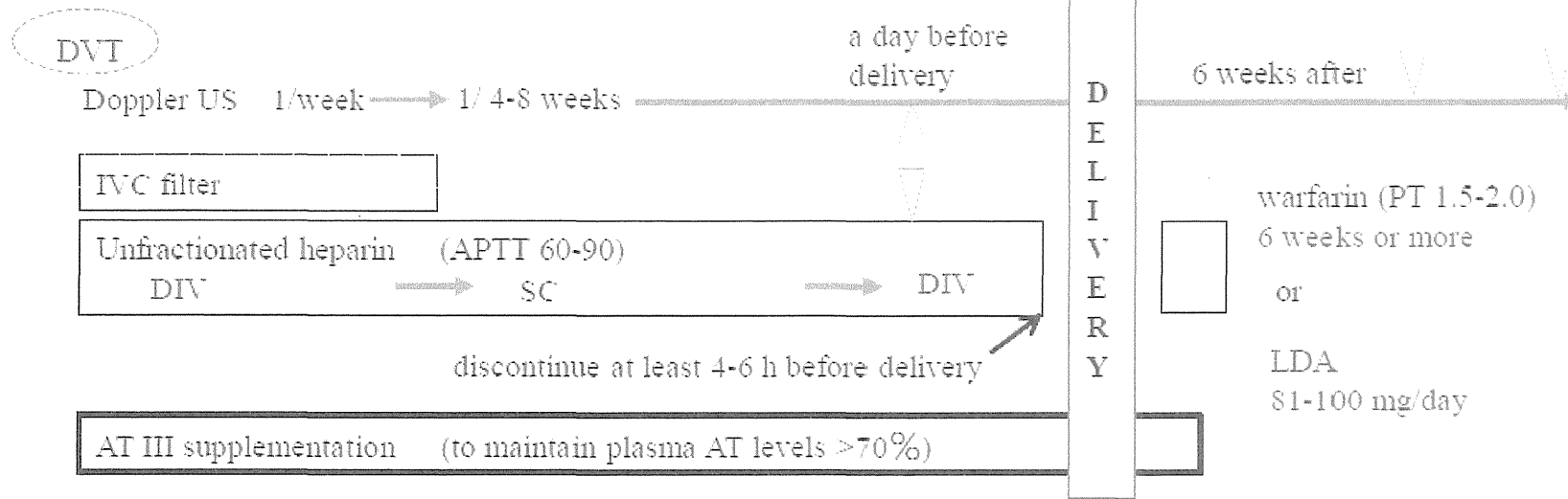FH, family history; OHSS, ovarian hyperstimulation syndrome.

Table 2   Genetic variants of the *SERPINC 1* gene and familial study

	Nucleotide variation (transcription start site +1)	Codon (mature AT start site: 1) and amino acid variation	Genetic alteration profile	Patient number	AT activity in pregnancy (%)	AT antigen (mg/dl)	Type of AT deficiency	Family member carrying the same mutation (AT activity, antigen)
Exon 1	72 T>C	-32 (start codon) Met→Thr	missense mutation (novel mutation)	10	40	N/A	I	Son, Daughter
Exon 2	2624 G>A	54 Ala→Thr	missense mutation (novel mutation)	8	47	18	I	Father (73%, 20 mg/dl)
	2637 T>G	58 Phe→Cyc	missense mutation (novel mutation)	11	45	32	I	Father
	2762 C>T	100 Gln→Stop	nonsense mutation (novel mutation)	1	61	13	I	Mother (60%, 51 mg/dl) Daughter
Exon 3a	5342 T>C	116 Ser→Pro	missense mutation (AT-III Nagasaki)(6)	9	37	N/A	II HBS	N/A
	5342 T>C	116 Ser→Pro	(same as above)	13	46	53	II HBS	N/A
	5342 T>C	116 Ser→Pro	(same as above)	15	49	N/A	II HBS	N/A
Exon 4	7457 C>A	249 Ala→Glu	missense mutation (novel mutation)	14	30	19	I	Father (61%, 24 mg/dl)
Intron 4	9788 G>A	None	splice site mutation (7-8)	3	36	18	I	N/A
	9788 G>A	None	(same as above)	6	41	20	I	N/A
	9788 G>A	None	(same as above)	7	37	N/A	I	N/A
	9788 G>A	None	(same as above)	12	53	N/A	I	N/A
Exon 5	9819 C>T	359 Arg→Stop	nonsense mutation (5)	2	38	14	I	Mother (35%)
Intron 5	9893 G>C	None	suspicion of large deletion	5	36	16	I	Father (37%) Sister (50%)
Exon 6	13299 C>T	394 Sel→Leu	missense mutation (AT-3 Denver)(4)	4	27	25	II RS	Mother

AT, antithrombin;   RS, reaction site;   HBS, heparin binding site;   N/A,   not available.

Figure 1 Treatment and prophylactic protocol for pregnant women with AT deficiency.

1) Treatment group

DVT

Doppler US   1/week ——▶ 1/ 4-8 weeks ———————————— a day before delivery ———————————— 6 weeks after

IVC filter

Unfractionated heparin   (APTT 60-90)
    DIV ——▶ SC ——▶ DIV

discontinue at least 4-6 h before delivery

AT III supplementation   (to maintain plasma AT levels >70%)

DELIVERY

warfarin (PT 1.5-2.0)
6 weeks or more

or

LDA
81-100 mg/day

2) Prophylaxis group

Doppler US 1/ 4-8 weeks ———————————— a day before delivery ———————————— 6 weeks after

Unfractionated heparin   (APTT40-50)
    SC ——▶ DIV

discontinue at least 4-6 h before delivery

and/or

LDA 81-100 mg/day

AT III supplementation   (to maintain plasma AT levels >70%)

DELIVERY

warfarin (PT 1.5-2.0)
6 weeks