

## PROTHROMBIN AND FACTOR V MUTATIONS IN WOMEN WITH A HISTORY OF THROMBOSIS DURING PREGNANCY AND THE PUERPERIUM

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### ABSTRACT

**Background** Venous thromboembolism is a leading cause of morbidity and mortality during pregnancy and the puerperium. However, the role of mutations in the prothrombin and factor V genes and other thrombophilic abnormalities as risk factors for thromboembolism in women during pregnancy and the puerperium is not known.

**Methods** In a study of 119 women with a history of venous thromboembolism during pregnancy and the puerperium and 233 age-matched normal women, we measured the activity of antithrombin, protein C, protein S, and lupus anticoagulant. We also performed genetic analyses to detect the G1691A mutation in the factor V gene (factor V Leiden), the G20210A mutation in the prothrombin gene, and the C677T mutation in the methylenetetrahydrofolate reductase gene. Blood samples were obtained at least three months post partum or after the cessation of lactation.

**Results** Among the women with a history of venous thromboembolism, the prevalence of factor V Leiden was 43.7 percent, as compared with 7.7 percent among the normal women (relative risk of venous thromboembolism, 9.3; 95 percent confidence interval, 5.1 to 16.9); that of the G20210A prothrombin-gene mutation, 16.9 percent as compared with 1.3 percent (relative risk, 15.2; 95 percent confidence interval, 4.2 to 52.6); and that of both factor V Leiden and the G20210A prothrombin-gene mutation, 9.3 percent as compared with 0 (estimated odds ratio, 107). Assuming an overall risk of 1 in 1500 pregnancies, the risk of thrombosis among carriers of factor V Leiden was 0.2 percent, among carriers of the G20210A prothrombin-gene mutation, 0.5 percent, and among carriers of both defects, 4.6 percent, as calculated in a multivariate analysis.

**Conclusions** The G20210A prothrombin-gene mutation and factor V Leiden individually are associated with an increased risk of venous thromboembolism during pregnancy and the puerperium, and the risk among women with both mutations is disproportionately higher than that among women with only one mutation. (N Engl J Med 2000;342:374-80.)

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**T**HE risk of venous thromboembolism is five times as high among pregnant women as among nonpregnant women of similar age.<sup>1-3</sup> Estimates of the incidence of pregnancy-associated venous thromboembolism vary from 1 in 1000 to 1 in 2000 deliveries.<sup>4</sup> Thromboembolic complications, including pulmonary embolism, are major causes of death among women during pregnan-

cy and the puerperium.<sup>5</sup> Thus, venous thromboembolism is an uncommon but leading cause of morbidity and mortality among women during this period.

During normal pregnancy, the plasma concentrations and activities of several proteins involved in blood coagulation and fibrinolysis change. These changes may promote coagulation, decrease anticoagulation, and inhibit fibrinolysis<sup>5-7</sup> and thus may increase the risk of thromboembolic events, especially among pregnant women who have acquired or genetic risk factors for thrombosis.<sup>5,8-10</sup>

Among the genetically determined changes, the mutation in the gene encoding factor V at nucleotide 1691 (G1691A, referred to as factor V Leiden) is the most common inherited abnormality in patients with thromboembolic disease, occurring in 20 percent of patients with a first episode of venous thrombosis and up to 50 percent of those with recurrent venous thrombosis.<sup>9</sup> A guanine-to-adenine mutation in the prothrombin gene, G20210A, associated with elevated plasma prothrombin concentrations and an increased risk of venous thrombosis, has been identified.<sup>11</sup> In addition, homozygosity for a common cytosine-to-thymidine mutation in the gene encoding 5,10-methylenetetrahydrofolate reductase (MTHFR), C677T, is associated with high plasma homocysteine concentrations and venous thrombosis,<sup>12-14</sup> although this association has been questioned by some investigators.<sup>15</sup>

To date, there are limited data on the role of these genetic abnormalities in causing the venous thromboembolism associated with pregnancy and the puerperium.<sup>5,8,10,16</sup> We studied the relative risk of venous thromboembolism associated with these genetic markers and other thrombophilic abnormalities in pregnant and puerperal women.

### METHODS

#### Subjects

We studied 119 consecutive women with a history of venous thromboembolism during pregnancy and the puerperium (up to six weeks post partum) and 233 normal women. The women with a history of thromboembolism were referred from local hospitals to the Düsseldorf University Medical Center (Düsseldorf, Ger-

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many) for treatment of venous thromboembolism between January 1990 and December 1998 and were seen again during 1999 for determination of the presence or absence of genetic risk factors and other thrombophilic abnormalities. In both the women with a history of thromboembolism and the normal women, blood samples were obtained at least three months post partum or three months after the cessation of lactation to rule out any pregnancy-related alterations of coagulation and fibrinolysis. Eight of the 119 women with a history of thromboembolism were receiving long-term oral anticoagulant therapy at the time of blood sampling; 30 of the women with a history of thromboembolism and 92 of the normal women were taking an oral contraceptive at this time.

None of the women had overt evidence of autoimmune or neoplastic disease. Thirteen women with a history of thromboembolism had one or more pregnancy-associated complications: gestational hypertension (3 women), immobilization due to preterm labor (13 women), and twin gestation (2 women). The other 106 women with a history of thromboembolism had normal singleton pregnancies except for the occurrence of the thrombotic event. In four women, none of whom had pregnancy-associated complications, the pregnancy resulted from in vitro fertilization and embryo transfer. Of the 119 women with a history of thromboembolism, 81 had vaginal deliveries (68 percent) and 38 delivered by cesarean section (32 percent).

All the women with a history of thromboembolism had an objectively diagnosed episode of deep venous thrombosis or pulmonary embolism. Deep venous thrombosis was diagnosed by Doppler ultrasonography or computed tomography during pregnancy and by venography or Doppler ultrasonography after delivery. In 77 of these women (65 percent), thrombectomy was performed with complete removal of the thrombus for the prevention of pulmonary embolism, thrombus extension, and the postphlebotic syndrome. In particular, thrombectomy was performed if the thrombus extended into the iliac veins and the inferior caval vein or if a floating thrombus was documented. The procedure was completed with the creation of a temporary arteriovenous fistula, which was removed three to six months later. The details and results of this procedure have been reported elsewhere.<sup>17-20</sup> Among these 77 women, thrombectomy was performed in 39 (51 percent) during pregnancy and after delivery in 38 (49 percent).

The 233 normal women were recruited by the Heinrich Heine University Blood Donation Center and matched with the women with a history of thromboembolism according to age. They were from the same geographic region as the women with a history of thromboembolism, but were unrelated to them. One hundred fifty-seven had previous pregnancies with no thromboembolic complications; 148 of these had a vaginal delivery (94 percent) and 9 delivered by cesarean section (6 percent).

Personal histories to document the presence or absence of thromboembolic disease were obtained from all the women with the use of a standardized questionnaire. This procedure has been evaluated previously.<sup>21</sup> The study was performed in accordance with the Helsinki Declaration, and written informed consent was given by all the women.

#### Laboratory Tests

All the women were screened for inherited and acquired defects in blood coagulation. We determined the activities of antithrombin, protein C, protein S, and lupus anticoagulant in plasma and performed a genetic analysis to determine the presence or absence of factor V Leiden, the G20210A prothrombin-gene mutation, and the C677T MTHFR mutation. The activities of plasma protein C and protein S were measured by a functional clotting assay (Instrumentation Laboratory, Milan, Italy), and total protein S antigen and free protein S antigen were measured by immunoelectrophoresis (Boehringer Mannheim, Mannheim, Germany). Plasma antithrombin activity was measured with the use of Berichrom (Dade Behring, Liederbach, Germany). Resistance to activated protein C was determined with a kit (Coatest APC Resistance, Chromogenix, Mölndal, Sweden). Lupus anticoagulant was measured

with the DVV test and DVV confirm test (American Diagnostica, Greenwich, Conn.).

The criteria for the diagnosis of deficiencies of protein C, protein S, and antithrombin were activity levels below the lower limit of normal (cutoff, 95th percentile), as determined in blood samples obtained from the 233 normal women. The lower limits of normal were 70 percent for protein C activity, 75 percent for protein C activity after the exclusion of carriers of factor V Leiden, 55 percent for protein S activity, 65 percent for protein S activity after the exclusion of normal women taking an oral contraceptive and carriers of factor V Leiden, and 80 percent for antithrombin activity (with and without oral-contraceptive therapy).

#### Genetic Analysis

DNA was extracted from peripheral-blood leukocytes according to standard protocols with the use of the Chelex system (Bio-Rad, Munich, Germany). The presence or absence of factor V Leiden was determined by allele-specific restriction-enzyme analysis.<sup>22</sup> For confirmation of the genotypes, an oligonucleotide-ligation assay was performed.<sup>23</sup> The results of the oligonucleotide-ligation assay were in 100 percent concordance with the results of genotypic analysis, as determined by an allele-specific restriction-enzyme assay. The G20210A prothrombin-gene mutation was identified by allele-specific restriction-enzyme analysis.<sup>11</sup> Screening for the presence of the C677T MTHFR polymorphism was performed by the method described by Frosst et al.<sup>12</sup>

#### Statistical Analysis

The SAS statistical package (version 6.12, SAS Institute, Cary, N.C.) was used for all statistical analyses. Depending on the type of data, the Wilcoxon rank-sum test, chi-square analysis, or Fisher's exact test (two-tailed) was used to assess the differences between the groups. Multivariate analyses, including the calculation of predictive values, were performed with the use of a stepwise logistic-regression procedure. For the calculation of predictive values, an incidence of 1 thromboembolic event in 1500 pregnancies was assumed. The relative risk was estimated from the odds ratio. Complete data were not available for all the women (e.g., the activities of protein C and protein S were not measured in women with thromboembolism who were taking an oral anticoagulant).

### RESULTS

The characteristics of all the women are presented in Table 1. Among the 119 women with a history of thromboembolism, 62 (52 percent) had deep venous thromboembolism during pregnancy (first trimester, 14; second trimester, 13; third trimester, 35) and 57 (48 percent) had thromboembolism in the postpartum period (vaginal delivery, 38; cesarean section, 19). In the 35 women with a history of thromboembolism during the third trimester, 11 (31 percent) had a cesarean section followed immediately by thrombectomy. In 96 women, the venous thrombosis occurring during pregnancy or the puerperium was their first thrombotic event, whereas in 23 women the venous thrombosis was a recurrent thrombotic event. The prevalence of genetic risk factors did not differ significantly between the women with a history of thromboembolism who underwent thrombectomy and those who did not or between the women with a history of thromboembolism who had no pregnancy-associated complications and those with thromboembolism who had pregnancy-associated complications. We therefore do not present separate analyses of the results.

**TABLE 1. CHARACTERISTICS OF THE WOMEN WITH A HISTORY OF VENOUS THROMBOEMBOLISM DURING PREGNANCY AND THE PUERPERIUM AND THE NORMAL WOMEN.**

CHARACTERISTIC	WOMEN WITH HISTORY OF THROMBOEMBOLISM (N=119)	NORMAL WOMEN (N=233)
Age at the time of thrombosis — yr		NA*
Median	27	
Range	13–45	
Age at the time of blood sampling — yr		
Median	34	34
Range	14–67	19–65
Use of oral contraceptives (current or previous) — no. (%)†	69 (58.0)	137 (58.8)
Cigarette smoking (current or previous) — no. (%)†	69 (58.0)	156 (67.0)
Body-mass index >30 — no. (%)‡	17 (14.3)	15 (6.4)

\*NA denotes not applicable.

†The difference between the two groups was not significant.

‡The relative risk of venous thromboembolism associated with a body-mass index (the weight in kilograms divided by the square of the height in meters) of more than 30 was 2.3 (95 percent confidence interval, 1.0 to 5.2; P=0.04).

**Prevalence of Inherited Risk Factors**

The prevalence of factor V Leiden was significantly higher among the women with a history of thromboembolism than among the normal women (Tables 2 and 3). Similarly, the prevalence of the G20210A prothrombin-gene mutation was significantly higher among the women with a history of thromboembolism than among the normal women (Tables 2 and 3). Of the women with a history of venous thromboembolism who had factor V Leiden, 5 were homozygous and 47 were heterozygous for the mutation; all of the women with the G20210A prothrombin-gene mutation were heterozygous for the mutation.

Eleven of 118 women with a history of thromboembolism (9.3 percent) had both factor V Leiden and the G20210A prothrombin-gene mutation (estimated odds ratio for thromboembolism during pregnancy and the puerperium, 107). Since no normal women had this combined defect, the estimated odds ratio was calculated on the basis of the probability of a combined defect in the normal women (7.7 percent × 1.3 percent = 0.10 percent).

The prevalence of the other risk factors for thrombophilia (deficiencies of antithrombin, protein C, and protein S) was 24.8 percent in the women with a history of thromboembolism and 11.4 percent in the normal women. A total of 19.3 percent of the women with a history of thromboembolism (23 of 119) had an antithrombin deficiency (<80 percent of normal

**TABLE 2. PREVALENCE OF HEREDITARY COAGULATION DEFECTS.\***

COAGULATION DEFECT	WOMEN WITH HISTORY OF THROMBOEMBOLISM (N=119)	NORMAL WOMEN (N=233)	P VALUE
	% (no. with defect/total no.)		
Factor V Leiden†	43.7 (52/119)	7.7 (18/233)	<0.001
G20210A prothrombin-gene mutation‡	16.9 (20/118)	1.3 (3/226)	<0.001
677TT MTHFR genotype	9.6 (11/114)	9.4 (20/212)	0.95
Antithrombin deficiency (<80 percent of normal activity)	19.3 (23/119)	2.6 (6/231)	<0.001
Antithrombin deficiency (<80 percent of normal activity) after exclusion of women taking oral contraceptives	6.7 (6/89)	1.5 (2/131)	0.06
Protein C deficiency (<70 percent of normal activity)	14.2 (15/106)	3.9 (9/230)	<0.001
Protein C deficiency (<75 percent of normal activity)§	10.3 (6/58)	3.3 (7/212)	0.03
Protein S deficiency (<55 percent of normal activity)	12.4 (13/105)	4.8 (11/228)	0.01
Protein S deficiency (<65 percent of normal activity)¶	7.8 (4/51)	6.6 (8/121)	0.77

\*Complete data were not available for all the women. Tests for antiphospholipid antibodies were negative in all the women.

†Among the women with factor V Leiden who had thromboembolism, five were homozygous for the mutation; none of the normal women were homozygous for the mutation.

‡None of the women with the prothrombin G20210A mutation were homozygous for the mutation.

§Carriers of factor V Leiden were excluded from the analysis.

¶Women who were taking an oral contraceptive and carriers of factor V Leiden were excluded from the analysis.

**TABLE 3.** RELATIVE RISK OF VENOUS THROMBOEMBOLISM ASSOCIATED WITH HEREDITARY COAGULATION DEFECTS.\*

RISK FACTOR	UNIVARIATE ANALYSIS		MULTIVARIATE ANALYSIS†	
	RELATIVE RISK (95% CI)	P VALUE	RELATIVE RISK (95% CI)	P VALUE
Factor V Leiden	9.3 (5.1–16.9)	<0.001	6.9 (3.3–15.2)	<0.001
G20210A prothrombin-gene mutation	15.2 (4.2–52.6)	<0.001	9.5 (2.1–66.7)	0.007
Antithrombin deficiency (<80 percent of normal activity)‡	4.7 (0.9–21.8)	0.06	10.4 (2.2–62.5)	<0.001
Protein C deficiency (<75 percent of normal activity)§	3.4 (1.1–10.5)	0.03	2.2 (0.8–6.1)	0.13

\*Univariate analyses were performed with the use of the chi-square test. Multivariate analyses were performed with the use of the stepwise logistic-regression procedure. CI denotes confidence interval.

†All stepwise logistic-regression analyses were performed with the following variables: presence of factor V Leiden; presence of prothrombin G20210A mutation; presence of the MTHFR 677TT genotype; low protein C, protein S, and antithrombin activity; body-mass index of more than 30; and use of oral contraceptives. The P values indicate the significance of each risk factor independently.

‡Women who were taking an oral contraceptive were excluded from the univariate analysis.

§Carriers of factor V Leiden were excluded from the univariate analysis.

activity), as compared with 2.6 percent of the normal women (6 of 231) (relative risk, 9.0; 95 percent confidence interval, 3.5 to 22.7;  $P < 0.001$ ); a difference, although not significant, persisted after the exclusion of the women taking an oral contraceptive (Table 2). A total of 12.4 percent of the women with a history of thromboembolism (13 of 105) and 4.8 percent of the normal women (11 of 228) had reduced protein S activity (<55 percent of normal activity; relative risk, 2.8; 95 percent confidence interval, 1.2 to 6.5;  $P = 0.01$ ); this difference did not persist after the exclusion of the women taking an oral contraceptive and the carriers of factor V Leiden (Table 2). A total of 14.2 percent of the women with a history of thromboembolism (15 of 106) had protein C deficiency, as compared with 3.9 percent of the normal women (9 of 230) (relative risk, 4.0; 95 percent confidence interval, 1.7 to 9.6;  $P < 0.001$ ). The difference persisted after the exclusion of the carriers of factor V Leiden (Table 2). Among the women with a history of thromboembolism who had deficiencies of antithrombin, protein C, and protein S, most had activity levels that ranged between 50 percent and 65 percent of normal activity. One woman with a history of thromboembolism had an antithrombin activity of <50 percent, and two had a protein C activity of <50 percent, whereas none of the normal women had levels this low.

In a logistic-regression analysis in which adjustments were made for age, body-mass index, and oral contraceptive use or nonuse (Table 3) and in which protein C deficiency, protein S deficiency, antithrombin deficiency, and presence of factor V Leiden, the G20210A prothrombin-gene mutation, and the 677TT MTHFR genotype were included in the same model, only antithrombin deficiency (<80 percent

of normal activity) and the presence of factor V Leiden and the G20210A prothrombin-gene mutation could be identified as independent risk factors that were predictive of thrombosis in the women with thromboembolism.

**Predictors of Thrombosis in Pregnancy as Compared with Those in the Puerperium**

There was no significant difference in the prevalence of factor V Leiden, the G20210A prothrombin-gene mutation, protein C deficiency, and protein S deficiency between the 62 women with a history of thromboembolism during pregnancy and the 57 women with a history of thromboembolism during the puerperium. There was a trend toward a higher prevalence of antithrombin deficiency among the women with a history of venous thromboembolism in pregnancy than among the women with a history of thromboembolism during the puerperium (prevalence, 11.1 percent vs. 2.3 percent;  $P = 0.10$ ). By contrast, the MTHFR 677TT genotype was predominantly associated with thrombosis in the puerperium (prevalence, 30.0 percent among the women with thromboembolism during the puerperium vs. 8.8 percent among the women with thromboembolism during pregnancy;  $P = 0.04$ ).

There was no statistical difference with regard to the prevalence or relative risk of inherited risk factors between the women with a history of thromboembolism who had vaginal deliveries and those who had cesarean deliveries.

**Recurrent Venous Thromboembolism**

The prevalence and relative risk of genetic risk factors among the women with a first episode of throm-

**TABLE 4.** PREVALENCE AND RELATIVE RISK OF HEREDITARY COAGULATION DEFECTS IN WOMEN WITH FIRST OR RECURRENT VENOUS THROMBOEMBOLISM AS COMPARED WITH NORMAL WOMEN.\*

CHARACTERISTIC	PREVALENCE AMONG NORMAL WOMEN (N=233)	PREVALENCE AMONG WOMEN WITH FIRST EPISODE (N=79)	RELATIVE RISK (95% CI)	PREVALENCE AMONG WOMEN WITH RECURRENCE (N=40)	RELATIVE RISK (95% CI)	P VALUE†
	percent			percent		
Factor V Leiden	7.7	43.0	9.0 (4.7–17.4)	45.0	9.8 (4.4–21.5)	0.78
G20210A prothrombin-gene mutation	1.3	12.7	10.8 (2.9–40.3)	25.6	26.0 (6.7–98.6)	0.08
Factor V Leiden and G20210A prothrombin-gene mutation	0	7.6	69.0‡	12.8	152.0‡	0.37
Antithrombin deficiency (<80 percent of normal activity)	2.6	14.1	6.2 (2.2–17.3)	30.0	16.1 (5.6–46.2)	0.04
Antithrombin deficiency (<80 percent of normal activity) after exclusion of women taking oral contraceptives	1.5	5.0	3.4 (0.6–20.9)	10.3	7.4 (1.2–46.8)	0.40
Protein C deficiency (<70 percent of normal activity)	3.9	8.5	2.3 (0.8–6.6)	26.5	8.8 (3.2–24.3)	0.01
Protein C deficiency (<75 percent of normal activity)§	3.3	5.0	1.5 (0.3–7.7)	22.2	8.4 (2.2–32.0)	0.05
Protein S deficiency (<55 percent of normal activity)	4.8	14.1	3.2 (1.3–8.0)	9.1	2.0 (0.5–7.5)	0.50
Protein S deficiency (<65 percent of normal activity)¶	6.6	9.1	1.4 (0.4–5.7)	5.6	0.8 (0.1–7.1)	0.70

\*CI denotes confidence interval.

†P values are for the univariate comparison between women with a first episode of venous thromboembolism and those with a recurrent episode. In the multivariate analysis after adjustments were made for a body-mass index of more than 30 and use of oral contraceptives, P=0.01 for the effect of antithrombin deficiency (<80 percent of normal activity), P<0.001 for the effect of protein C deficiency (<65 percent of normal activity), and P=0.10 for the effect of the G20210A prothrombin-gene mutation.

‡Because no normal women had this combined defect, the estimated relative risk was calculated on the basis of the probability of a combined defect in these women. For this reason, no confidence interval is given.

§Carriers of factor V Leiden were excluded from the analysis.

¶Women who were taking an oral contraceptive and carriers of factor V Leiden were excluded from the analysis.

boembolism and among those with recurrent thromboembolism, as compared with normal women, are shown in Table 4. We also compared the prevalence and relative risk of these risk factors among 79 women with a first episode of thromboembolism with those among the 40 women who had recurrent thromboembolism. The presence of the G20210A prothrombin-gene mutation, the presence of antithrombin deficiency, and the presence of protein C deficiency were associated with recurrent venous thrombotic events. Women with recurrent thromboembolism had significantly more combined defects than did women with a first episode (prevalence of factor V Leiden plus protein C deficiency, 14.7 percent vs. 1.4 percent, P=0.006; prevalence of factor V Leiden plus antithrombin deficiency, 20 percent vs. 7.7 percent, P=0.05; prevalence of the G20210A prothrombin-gene mutation plus antithrombin deficiency, 12.8 percent vs. 0 percent, P=0.004).

#### Positive Predictive Value of Hereditary Risk Factors

For the carriers of hereditary risk factors, the positive predictive values indicating the probability of thrombosis per pregnancy and puerperium were cal-

culated in a multivariate analysis. An incidence of 1 thromboembolic event in 1500 pregnancies was assumed. The probability of thrombosis among women without any risk factor was 0.03 percent (95 percent confidence interval, 0.02 to 0.04 percent); among those with factor V Leiden, 0.25 percent (95 percent confidence interval, 0.2 to 0.3 percent); among those with the G20210A prothrombin-gene mutation, 0.5 percent (95 percent confidence interval, 0.3 to 0.8 percent); among those with antithrombin deficiency (<80 percent of normal activity), 0.4 percent (95 percent confidence interval, 0.3 to 0.6 percent); and among those with protein C deficiency (<75 percent of normal activity), 0.1 percent (95 percent confidence interval, 0.04 to 0.13 percent). The combination of factor V Leiden plus the G20210A prothrombin-gene mutation was associated with a probability of thrombosis of 4.6 percent (95 percent confidence interval, 2.6 to 8.2 percent).

#### DISCUSSION

Thromboembolic complications, including pulmonary embolism, that occur during pregnancy and the puerperium are a major cause of maternal morbidity

and mortality. Our results indicate that the relative risk of venous thromboembolism among women with the G20210A prothrombin-gene mutation is at least as high as that among those with factor V Leiden. Previous studies demonstrated a relative risk of venous thromboembolism of between 3.0 and 5.0 associated with the G20210A prothrombin-gene mutation<sup>11,24-26</sup>; in contrast, we found a much higher relative risk of venous thromboembolism during pregnancy and the puerperium among women with this defect. There appears to be a relation between the presence of the G20210A prothrombin-gene mutation and pregnancy-related alterations in coagulation or fibrinolysis that lead to thrombosis in women who are carriers of the G20210A prothrombin-gene mutation. A similar observation was recently reported for cerebral-vein thrombosis in women taking an oral contraceptive.<sup>27</sup>

We confirmed the importance of factor V Leiden as a risk factor for venous thromboembolism during pregnancy and the puerperium.<sup>10,16</sup> In addition, we found that factor V Leiden is a risk factor that is independent of other known determinants of the risk of thrombosis; specifically, it is independent of the G20210A prothrombin-gene mutation. It is remarkable that as many as 9.3 percent of the women had both the G20210A prothrombin-gene mutation and factor V Leiden, leading to a disproportionately higher risk than with either defect alone.

There are conflicting results regarding the role of a homozygous 677TT MTHFR genotype as a risk factor for venous thromboembolism. Homozygosity for this polymorphism was associated with high plasma homocysteine concentrations and venous thrombosis in some studies<sup>12-14</sup> but not in others.<sup>15</sup> In our study, the 677TT MTHFR genotype had no influence on the risk of venous thromboembolism, although the prevalence of the 677TT MTHFR genotype in the subgroup of women with venous thromboembolism during the puerperium was higher than in the subgroup of women with venous thromboembolism during pregnancy.

In the analysis of the risk associated with deficiencies of antithrombin, protein C, and protein S, only antithrombin deficiency was an independent risk factor for thromboembolism after adjustments were made for the presence of factor V Leiden and oral-contraceptive use. However, the number of women with deficiencies of protein C and protein S may be too low for it to be possible to identify an influence of each of these risk factors independently in a multivariate analysis. It is well known that resistance to activated protein C is associated with decreased protein S activity, as determined by routine assays. We found that resistance to activated protein C was also associated with decreased protein C activity in a group of 500 normal men and women (data not shown). In addition to antithrombin deficiency and the

G20210A prothrombin-gene mutation, the presence of protein C deficiency was associated with recurrent venous thromboembolism during pregnancy and the puerperium, particularly when it was present with other defects.

The predictive value of factor V Leiden and other inherited markers for the purpose of screening for thrombotic risk is low among pregnant women. A significant increase in the probability of thrombosis can be demonstrated only in cases in which a woman has a combined defect (as evidenced by a probability of 4.6 percent for women with both factor V Leiden and the G20210A prothrombin-gene mutation).

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