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THROMBOSIS AND HEMOSTASIS

Hereditary risk factors for thrombophilia and probability of venous thromboembolism during pregnancy and the puerperium

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Key Points

- Women with severe thrombophilia have a high absolute risk of pregnancy-associated VTE independent of a positive family history of VTE.
- These women should be considered for routine antenatal thromboprophylaxis regardless of family history of VTE.

Venous thromboembolism (VTE) is a leading cause of maternal mortality. Few studies have evaluated the individual risk of gestational VTE associated with heritable thrombophilia, and current recommendations for antenatal thromboprophylaxis in women with severe thrombophilia such as homozygous factor V Leiden mutation (*FVL*) depend on a positive family history of VTE. To better stratify thromboprophylaxis in pregnancy, we aimed to estimate the individual probability (absolute risk) of gestational VTE associated with thrombophilia and to see whether these risk factors are independent of a family history of VTE in first-degree relatives. We studied 243 women with the first VTE during pregnancy and the puerperium and 243 age-matched normal women. Baseline incidence of VTE of 1:483 pregnancies in women ≥35 years and 1:741 deliveries in women <35 years was assumed, according to a recent population-based study. In women ≥35 years (<35 years), the individual probability of gestational VTE was as follows: 0.7% (0.5%) for heterozygous *FVL*; 3.4% (2.2%) for homozygous *FVL*; 0.6% (0.4%) for heterozygous prothrombin G20210A; 8.2% (5.5%) for compound heterozygotes for *FVL* and prothrombin G20210A; 9.0% (6.1%) for antithrombin deficiency; 1.1% (0.7%) for protein C deficiency; and 1.0% (0.7%) for protein S deficiency. These results were independent of a positive family history of VTE. We provide evidence that unselected women with these thrombophilias have an increased risk of gestational VTE independent of a positive family history of VTE. In contrast to current guidelines, these data suggest that women with high-risk thrombophilia should be considered for antenatal thromboprophylaxis regardless of family history of VTE. (*Blood*. 2016;128(19):2343-2349)

Introduction

Venous thromboembolism (VTE) is an important cause of maternal morbidity and mortality in the Western world.[1-4] Although the relative risk of VTE is 5 times higher in pregnant women than in nonpregnant women of similar age,[5] the absolute risk remains low. Estimates of the incidence of pregnancy-associated VTE vary from 1:500 to 1:1500 pregnancies, with higher estimates in more recent reports (1:500 to 1:1000).[5-10]

VTE is a multicausal disorder, in which acquired and hereditary risk factors interact.[11] Pregnancy is an acquired and independent risk factor for the development of VTE. Acquired risk determinants can significantly increase the thrombotic risk further during pregnancy and the puerperium. These include maternal age (≥35 years), caesarean section, obesity, high parity (≥4), infection, and a personal or family history of VTE.[8,12-18]

Knowledge of the etiology of VTE advanced with the discovery of several genetic polymorphisms that contribute to venous thrombosis. As acquired and innate risk factors interact, it is important to quantify the influence of heritable thrombophilias on risk of thrombosis. In women with pregnancy-associated VTE, there are data for the risk associated with the heterozygous genotype of the G1691A polymorphism in the gene encoding factor V (factor V Leiden [*FVL*]) and the heterozygous genotype of the G20210A polymorphism in the gene encoding prothrombin (prothrombin G20210A).[19-21] However, few data exist in pregnant women with a single homozygous polymorphism or a compound heterozygous polymorphisms.[19,22,23] Moreover, previous reports on the risk calculation for deficiencies of antithrombin, protein C, and protein S may have led to an overestimation of the risk of thrombosis due to selection bias in family studies.[24-27]

Limited data on the magnitude of the thrombosis risk associated with heritable thrombophilias and the interaction of risk factors have impeded the development of evidence-based risk stratification to guide thromboprophylaxis. Consequently, there is a lack of consensus on thromboprophylaxis in national and international guidelines.[21,28-32] Therefore, assessment of the individual thrombotic risk during pregnancy and the puerperium is important to provide evidence-based thromboprophylaxis management and the disease burden. Current recommendations regarding prophylactic anticoagulation during this period, particularly in women with severe thrombophilia such as homozygous *FVL*, are based on the absence or presence of a positive family history of VTE.[21]

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The aims of our study were (1) to estimate the individual probability (absolute risk) of gestational VTE due to thrombophilic risk factors, (2) to examine whether these risk factors are independent of a positive family history of VTE in first-degree relatives, and (3) to consider whether or not this approach would be suitable to improve thromboprophylaxis management.

We performed an analysis of the risk factors associated with venous thrombosis in women during pregnancy and the puerperium to quantify the contribution of hereditary thrombophilias to the risk of VTE and to examine whether these risk factors are independent of a positive family history of VTE in first-degree relatives. As age >35 years is a known risk factor, and because of demographic changes with increasing rates of older childbearing women, the absolute risk of thrombosis was stratified for age (<35 and ≥35 years).

Methods

Subjects

We retrospectively studied 243 consecutive women with a history of first VTE (patient group) during pregnancy or the puerperium (6 weeks postpartum) and 243 age-matched control women with ≥1 prior pregnancy and no history of venous thromboembolism. Three patients and 1 control woman were pregnant with twins.

The women with a history of thromboembolism were referred for treatment of venous thromboembolism or were sent for consultation for prophylaxis by local hospitals to the Düsseldorf University Medical Center between January 1990 and December 2008.

None showed overt evidence of autoimmune or neoplastic disease. At the time of blood sampling, only a small number of women had comorbidities (6 patients and 6 control women had diabetes, 17 patients and 15 control women had hypertension, and 20 patients and 26 control women had elevated lipid levels; in addition, 3 patients and no control women had had a myocardial infarction, and 4 patients and no control women had had an ischemic stroke). All participants underwent assessment of their coagulation parameters, with blood sampling ≥3 months postpartum or 3 months after the cessation of lactation to exclude any pregnancy-related alterations in coagulation and fibrinolysis. In addition, in the patient group, blood samples were taken ≥6 months after VTE to exclude any thrombosis- and/or inflammation-related effects on laboratory test results.

All women in the patient group had an objectively diagnosed episode of deep venous thrombosis (DVT) or pulmonary embolism (PE). DVT was diagnosed by Doppler ultrasonography, impedance plethysmography, computerized tomography, or nuclear magnetic resonance tomography during pregnancy and Doppler ultrasonography or X-ray venography after delivery. PE was diagnosed by computerized tomography, ventilation-perfusion scanning, or nuclear magnetic resonance tomography during pregnancy, and by computerized tomography, ventilation-perfusion scanning, or pulmonary angiography in the postpartum period.

The 243 control women with ≥1 previous pregnancy were recruited by the Heinrich Heine University Blood Donation Centre and matched for age with the 243 women with a history of VTE. Matching for age was performed at the time of blood sampling (maximum age difference allowed: 5 years), because plasma levels of coagulation factor activities change with age. However, we assumed that functional assay values were the same as they had been before VTE. Because values were divided according to levels of deficiency, one would not expect a more severely deficient patient to normalize with age. After matching, the mean age was 37.8 years in patients and 37.7 years ($P = .97$) in control women. To avoid a referral bias, all women with prior evaluation of genetic risk factors were excluded. Women with antiphospholipid syndrome were also excluded. The control women were from the same geographic region as the women with a history of VTE but were unrelated to them. The controls were chosen from blood donors because the presence of thrombophilic risk factors is not a selection criterion for blood donors and therefore a selection bias is not to be expected. Personal histories were obtained from all women using a standardized questionnaire, documenting the presence or absence of thromboembolic disease. The study was approved by the Ethics Committee of the Faculty of Medicine, Heinrich Heine University, Düsseldorf, and written informed consent was given by all participants in this study.

Laboratory tests

Samples of whole blood were collected in vacuum tubes containing 3.8% (w/v) sodium citrate in a 1:9 ratio (v/v) of anticoagulant to blood. Platelet-poor plasma was prepared by centrifugation at 2000*g* for 10 minutes. Antithrombin activity was analyzed immediately. Plasma for determination of other parameters was stored at −80°C until analysis.

The activities of plasma proteins C and S were measured using a functional clotting assay (Instrumentation Laboratory, Milano, Italy), and free protein S antigen was determined by means of an enzyme-linked immunosorbent assay kit (Diagnostic International, Schriesheim, Germany). Antithrombin activity was measured using Berichrom (Siemens Healthcare, Eschborn, Germany). For screening of lupus anticoagulant, a lupus-sensitive activated partial thromboplastin time (Siemens Healthcare, Eschborn, Germany) and a dilute Russell's viper venom time test (American Diagnostica, Greenwich, CT) were used. For confirmation of positive results, a dilute Russell's viper venom time confirm test (American Diagnostica) was performed. Cardiolipin and β_2-glycoprotein I antibodies were quantified using an enzyme-linked immunosorbent assay (Orgentec Diagnostika, Mainz, Germany).

For diagnosis of protein C, protein S, and antithrombin deficiencies, percentiles of activity or antigen levels were determined in the age-matched control women. The 5th percentiles were as follows: antithrombin activity, 90%; protein C activity, 76%; protein S activity, 56%; free protein S, antigen 57%. To identify severe deficiencies reliably, we chose the cutoff for severe deficiencies to be 2/3 (67%) of the 5th percentile cutoff and rounded the results (see Tables 2 and 3). Reduced levels of antithrombin, protein C, protein S, and free protein S antigen were routinely confirmed using another blood sample drawn on a different occasion. Measurements of antithrombin, protein C, and protein S activity were performed in women without hormone intake (see tables for exact numbers). DNA was extracted from peripheral blood leukocytes according to standard protocols using the Chelex system (BIO-RAD, München, Germany) or the Qiagen system (Qiagen, Hilden, Germany). The presence or absence of *FVL* and prothrombin G20210A was identified using an allele-specific restriction enzyme analysis.[33,34]

Statistical analysis

The SAS statistical package (version 9.4; SAS Institute, Cary, NC) was used for all statistical analyses. Depending on the type of data, a Wilcoxon rank-sum test or a Fisher exact test (2-tailed) was used to compare the different groups. Relative risk was estimated using odds ratios (ORs). The calculation of relative risks of thrombophilic risk factors was performed with regard to the patient and control population as a whole, without stratification for age (<35/≥35 years). We used a multivariate logistic regression analysis to adjust for a positive family history of venous thrombosis in first- and second-degree relatives.

Absolute risks (incidence) of VTE per delivery were determined by the option of calculating a predicted probability as integrated part of the SAS logistic regression procedure (SCORE statement). Details of the mathematics are given in the SAS/STAT(R) 9.2 User's Guide, Second Edition Online documentation.[35] This procedure takes into account that the baseline risk includes thrombophilic and nonthrombophilic women to avoid an overestimation of the absolute risk (see also footnote to Table 3). The data set was modified using the PRIOREVENT option in the SCORE statement, specifying the prior event probability for a binary response model (0.207% = 1:483 for women ≥35 years, 0.135% = 1:741 for women <35 years). After specifying the correct prior probabilities, the posterior probabilities are calculated based on the ORs of the single risk determinants.

The combined confidence intervals (Cis) of the predicted probabilities given depend on the CI associated with thrombophilic risk factors and on that of the population-based incidence rates.[7] Thus, we have 2 parameters, p_1 and p_2, and 2 CIs. The variances of p_1 and p_2 can be calculated from their respective CIs in the following way (this is valid for a 95% CI): $\mathrm{SE}(p_1) = (\text{upper limit} - \text{lower limit})/3.92$. Then $\mathrm{Var}(p_1) = \mathrm{SE}(p_1)^2$. The combined variance is $\mathrm{Var}(p_1 p_2) = \mathrm{Var}(p_1) + \mathrm{Var}(p_2) + 2\mathrm{C}(p_1, p_2)$, where the covariance between p_1 and p_2 is denoted by

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$C(p_1, p_2)$.[36] Because the variables are independently distributed, the variance of their sum is simply the sum of their variances, $\mathrm{Var}(p_1 p_2) = \mathrm{Var}(p_1) + \mathrm{Var}(p_2)$, and the covariance may be neglected. The corresponding standard error is the square root of the above expression. The 95% CI is calculated by $p_1 p_2 \pm 1.96 \times$ SE.[36,37]

Complete data for genetic analyses were obtained for all women in the patient group; however, some data on coagulation assays were incomplete due to logistical issues associated with their management (eg, results of coagulation assays may have been obtained earlier than the required ≥3 months postpartum or after the cessation of lactation). Exact numbers of women included in each of the analyses are indicated in the tables. For calculation of ORs of homozygous carriers of *FVL*, individuals with heterozygous defects were excluded and vice versa (see Table 2).

Based on the Hardy-Weinberg equilibrium, the frequency of homozygous *FVL* carriers in the normal population was calculated as 153/100 000 and the frequency of compound heterozygous *FVL* and prothrombin G20210A as 183/100 000. The frequency of antithrombin and protein C deficiency in the normal population was assumed to be 20/100 000[38] and 300/100 000,[39] respectively.

Results

Patient characteristics

Patient characteristics are presented in Table 1.

Table 1. Patient characteristics

Characteristics	Women with history of venous thromboembolism during pregnancy and puerperium (n = 243)
Age at the time of thrombosis, years	
Mean	27.9
Range	16.2-45.2
Age at the time of blood sampling, years*	
Mean	37.8
Range	16.9-78.2
Body mass index >30 kg/m^2 (%)†	17.2
Cigarette smoking (current or previous)‡	50
Venous thromboembolism, no. (%)	
During pregnancy	132 (54.3)
First trimester	28
Second trimester	30
Third trimester	75
Postpartum	111 (45.7)
Vaginal delivery	65
Caesarean section	46
Venous thromboembolism during, no. (%)	
First pregnancy	153 (62.97)
Second pregnancy	43 (17.7)
Third pregnancy	27 (11.11)
Fourth pregnancy	14 (5.76)
Fifth pregnancy	3 (1.23)
Sixth pregnancy	3 (1.23)
Pulmonary embolism, no. (%)§	
During pregnancy	11 (8.3)
First trimester	2
Second trimester	3
Third trimester	6
Postpartum	24 (21.6)
Vaginal delivery	13
Caesarean section	11

*The mean age at time of blood sampling among normal women was 37.7 years (range, 18.1-75.2 years; $P = .97$).

†BMI at age of blood sampling: 12.8% of control women had a BMI >30 ($P = .23$; OR, 1.42).

‡Forty-five percent of control women smoked at the time of blood sampling or previously ($P = .392$; OR, 1.22).

§Already counted in the VTE group.

ORs and probability of venous thromboembolism associated with genetic defects

Patients with heterozygous and homozygous *FVL* and heterozygous prothrombin G20210A had significantly increased ORs for VTE (Table 2).

Thirteen of 243 women with a history of VTE had a compound heterozygosity for both *FVL* and prothrombin G20210A (estimated OR, 47). Because no control woman had this combined defect, the estimated OR was calculated based on the probability of a combined defect in the population using the prevalence of heterozygous defects according to the Hardy-Weinberg equilibrium (Table 2).

The corresponding age-dependent absolute risks of VTE per pregnancy for these heritable thrombophilias are higher in women aged ≥35 years than in women aged <35 years (Table 3).

OR and probability of venous thromboembolism associated with endogenous coagulation inhibitor deficiencies

ORs and age-dependent absolute risks of VTE were calculated for both mild and severe deficiencies (Table 2). Mild antithrombin deficiency (<90% activity) was associated with no significant increase in OR for VTE, whereas antithrombin activity of 60% or lower carried a considerably higher risk (OR, 49; 95% CI, 11.5-204; 2 patients with antithrombin deficiency type I). Decreased activities of protein C and protein S or free protein S antigen concentration were associated with an increased OR for VTE (Table 2). Patient numbers were low for individual thrombophilias; consequently, the 95% CIs for the OR were wide.

Incidence of thrombophilic risk factors in women with venous thromboembolism during pregnancy compared with those in the puerperium

Because there was no statistical difference in the prevalence of thrombophilic risk factors in pregnancy compared with the puerperium, the absolute risks result from the distribution of VTE events in pregnancy compared with the puerperium (eg, a total risk of 0.73% for heterozygous *FVL* and age above 35 years leads to an absolute risk of 0.45% in pregnancy vs 0.28% in the puerperium [distribution 61.3% in pregnancy vs 38.7% in puerperium[7]]).

Positive family history of venous thrombosis in association with thrombophilic risk factors

A positive family history of VTE in first-degree relatives (numbers for second-degree relatives in parentheses) were as follows: heterozygous *FVL* 21/60, (13/60); homozygous *FVL*, 3/4 (2/4); heterozygous prothrombin G20210A, 5/13 (3/13); compound heterozygous *FVL* and prothrombin G20210A, 7/13 (5/13); deficiencies of antithrombin (activity <90%), 8/19 (7/19); antithrombin (activity <60%), 2/2 (1/2); protein C (activity <76%), 6/19 (7/19); protein C (activity <50%), 3/3 (2/3); protein S (activity <56%), 11/19 (6/19); protein S (activity <40%), 6/7 (3/7); free protein S antigen (activity <57%), 10/19 (6/19); and free protein S antigen (activity <40%) 6/10 (2/10).

The prevalence of a positive family history of thrombosis in first-degree relatives was significantly higher among women with a history of VTE than among control women (Table 2). For further evaluation, the risk of thrombosis in patients with genetic defects was adjusted for a positive family history of VTE in first-degree relatives. This analysis

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Table 2. Prevalence of thrombophilic risk factors and associated OR of venous thromboembolism

	Women with history of thromboembolism during pregnancy and puerperium (n = 243)		Control women (n = 243)		Univariate analysis		
Coagulation defect	%	No. with defect/ total no.	%	No. with defect/ total no.	*P* value	OR	95% CI
Genetic defects*,†							
FVL heterozygous	28.44	60/211	8.02	19/237	<.0001	4.6	2.65-7.95
FVL homozygous	2.58	4/155	0.15‡		<.0001	17.2	6.3-47
Prothrombin G20210A heterozygous	7.93	13/164	2.68	6/224	.029	3.1	1.16-8.41
FVL and prothrombin G20210A (compound heterozygous)	7.93	13/164	0.18‡		<.0001	47	26-84
Antithrombin deficiency (activity)							
Mild deficiency (cutoff <90%)	9.18	19/207	4.83	10/207	.083	2.0	0.9-4.93
Severe deficiency (cutoff <60%)	0.97	2/207	0.02‡		<.0001	49	11.5-204
Protein C deficiency (activity)							
Mild deficiency (cutoff <76%)	10.67	19/178	4.98	10/201	.037	2.3	1.03-5.1
Severe deficiency (cutoff <50%)	1.69	3/178	0.31‡		.019	5.5	1.8-17.3
Protein S deficiency (activity)							
Mild deficiency (cutoff <56%)	10.73	19/177	4.50	9/200	.021	2.6	1.12-5.8
Severe deficiency (cutoff <40%)	3.95	7/177	1.0	2/200	.089	4.1	0.84-19.9
Free protein S deficiency (concentration)							
Mild deficiency (cutoff <57%)	12.1	19/157	4.17	6/144	.02	3.2	1.23-8.17
Severe deficiency (cutoff <40%)	6.37	10/157	0.69	1/144	.011	9.7	1.2-76.9
Family history of VTE in first-degree relatives§	39.1	95/243	16.5	40/243	<.0001	3.3	2.2-5.0

There were no patients and no control women with a positive result for lupus anticoagulant or cardiolipin antibodies.

*These categories are exclusive, ie, to be included in one of the groups, subjects must display the exact genetic constellation. This leads to, for example, individuals with compound heterozygous *FVL* and prothrombin G20210A to be excluded from both the heterozygous *FVL* and the heterozygous prothrombin G20210A groups and vice versa.

†After adjustment for a positive family history, the OR was 4.3 (95% CI, 3.15-5.76) for heterozygous *FVL*, 23 (95% CI, 8.3-63.5) for homozygous *FVL*, 4.5 (95% CI, 2.4-7.8) for heterozygous prothrombin G20210A, and 61 (95% CI, 33.8-113) for compound heterozygous *FVL* and prothrombin G20210A.

‡Because no normal woman had a homozygous defect or a compound defect, the estimated ORs were calculated on the basis of the probability of a homozygous defect in this group using the Hardy-Weinberg equilibrium. Because no normal woman had an antithrombin activity <60% or a protein C activity <50%, the ORs for deficiencies of antithrombin activity <60% and of protein C activity <50% were calculated on the basis of the prevalence of antithrombin deficiency type I of 0.02% in the general population[38] and on the basis of the prevalence of protein C deficiency of 0.3% in the general population,[39] respectively.

§The OR for a positive family history of VTE was 2.8 (95% CI, 2.1-3.7) after adjustment for heterozygous *FVL*, 3.5 (95% CI, 2.5-4.8) after adjustment for homozygous *FVL*, 3.71 (95% CI, 2.7-5.1) after adjustment for heterozygous prothrombin G20210A, and 3.7 (95% CI, 2.7-5.1) after adjustment for compound heterozygous *FVL* and prothrombin G20210A.

did not yield a relevant decrease in OR: 4.3 (95% CI, 3.15-5.76) for heterozygous *FVL*, 23 (95% CI, 8.3-63.5) for homozygous *FVL*, 4.5 (95% CI, 2.4-7.8) for heterozygous prothrombin G20210A, and 61 (95% CI, 33.8-113) for compound heterozygous *FVL* and prothrombin G20210A. In addition, we performed a multivariate analysis to assess interactions between genetic risk factors and a family history of

Table 3. Incidence (absolute risk) of venous thromboembolism in pregnancy and puerperium associated with thrombophilic risk factors

	Probability of thrombosis during pregnancy and puerperium* <35 years (basic risk 1:741)		Probability of thrombosis during pregnancy and puerperium* ≥35 years (basic risk 1:483)	
Coagulation defect	%	95% CI	%	95% CI
Genetic defects†				
FVL heterozygous	0.5	0.23-0.72	0.7	0.35-1.11
FVL homozygous	2.2	0.0-9.9	3.4	0.0-14.87
Prothrombin G20210A heterozygous	0.4	0.01-0.78	0.6	0.02-1.20
FVL and prothrombin G20210A (compound heterozygous)	5.5	0-21.92	8.2	0.0-31.97
Antithrombin deficiency (activity)				
Mild deficiency (cutoff <90%)	0.2	0.05-0.32	0.3	0.07-0.50
Severe deficiency (cutoff <60%)	6.1	0.0-68.16	9.0	0.0-98.4
Protein C deficiency (activity)				
Mild deficiency (cutoff <76%)	0.3	0.05-0.53	0.4	0.077-0.80
Severe deficiency (cutoff <50%)	0.7	0.0-2.90	1.1	0.0-4.43
Protein S deficiency (activity)				
Mild deficiency (cutoff <56%)	0.3	0.0-0.66	0.5	0.0-1.01
Severe deficiency (cutoff <40%)	0.7	0.0-2.14	1.0	0.0-3.26
Free protein S deficiency (concentration)				
Mild deficiency (cutoff <57%)	0.3	0.03-0.61	0.5	0.04-0.93
Severe deficiency (cutoff <40%)	1.0	0.0-3.03	1.5	0.0-4.61

*The results for noncarriers in each separate analysis were between 0.105% for analyses with high prevalence (heterozygous *FVL*) and 0.20% for analyses with low prevalence (homozygous *FVL*).

†These categories are exclusive; ie, to be included in one of the groups, subjects must display the exact genetic constellation. This leads to, for example, individuals with compound heterozygous *FVL* and prothrombin G20210A to be excluded from both the heterozygous *FVL* and the heterozygous prothrombin G20210A groups and vice versa.

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thrombosis in first-degree relatives. No interaction was detectable for heterozygous *FVL* and for compound heterozygous *FVL* and prothrombin G20210A. Due to the low numbers of patients, no interaction analysis could be performed for homozygous *FVL* and for heterozygous prothrombin G20210A.

Further, the prevalence of a positive family history of thrombosis in second-degree relatives was significantly higher among women with a history of VTE (58/243, 23.97%) than among control women (25/243, 10.29%, $P < .0001$; OR, 2.73; 95% CI, 1.64-4.5). In addition, an adjustment for VTE in second-degree relatives was performed, showing a significant independent influence of the thrombophilic risk factors comparable to the OR found after adjustment for VTE in first-degree relatives (data not shown).

Discussion

There is marked inconsistency between national and international clinical guidelines for antenatal thromboprophylaxis in pregnant women with heritable thrombophilias.[21,28-32] This reflects varying estimates of thrombosis risk associated with these thrombophilias, particularly in contemporary studies, lack of information on the interaction with a family history of VTE, and differing perceptions of the threshold of risk above which pharmacologic prophylaxis is considered appropriate. In particular, for some thrombophilias, especially antithrombin deficiency, there are concerns that older studies overestimate the risk[24,25] due to methodologic limitations including referral bias and objective diagnosis.

This study now addresses many of these issues and can inform management decision for the need of thromboprophylaxis by quantifying the influence of heritable thrombophilias on VTE risk during pregnancy and the puerperium, stratifying for age (<35 and ≥35 years), and assessing the influence independent of a family history of VTE. Risk factors of clinical relevance include the G1691A polymorphism in the gene encoding factor V (*FVL*), prothrombin G20210A, and deficiencies in antithrombin or proteins C and S. Women with homozygous *FVL*, those who are compound heterozygous for *FVL* and prothrombin G20210A, or those with antithrombin deficiency have a particularly high risk for pregnancy-associated thrombosis, especially if ≥35 years.

To date, there are limited data on the risk of pregnancy-associated VTE among unselected homozygous carriers of *FVL* G1691A. In this study, the OR of 17.2 (95% CI, 6.3-47; probability of thrombosis 3.4% in women aged ≥35 years) for VTE associated with homozygosity for *FVL* is in agreement with a systematic review of 9 studies by Robertson et al,[40] which assessed the risk of VTE in pregnant women with inherited thrombophilia, but not necessarily with a family history of VTE, and reported an OR of 34 (probability of thrombosis, 4.8%).

In contrast to these results obtained from unselected patients, a much higher risk was reported in women with previous VTE and in women with symptomatic relatives. Several family-based cohort studies found that women with homozygous *FVL* and a positive family history, but without a previous episode of VTE, have a risk of up to 14.0% of developing a first VTE episode during pregnancy or the postpartum period.[22,23,41]

Our data show that a compound defect of heterozygous *FVL* and prothrombin G20210A is associated with a disproportionate increase in risk compared with the risk of either alone. The results confirm our previous data[19,20] but are in contrast to 2 previous small studies that showed that the risk of first VTE during pregnancy and the puerperium in compound heterozygous carriers of *FVL* and prothrombin G20210A is low and similar to that of single heterozygous carriers.[23,42] In view of these conflicting results, a multiplicative risk increase in combined defects may be a plausible estimate (eg, OR, 20).

Regardless of the presence or the kind of thrombophilia, a positive family history of VTE increases the risk of VTE two- to fourfold.[21,43] In our study, only 10 of 17 women with VTE and homozygous *FVL* or compound heterozygous *FVL* and prothrombin G20210A had first-degree relatives with a history of venous thrombosis. Using a multivariate analysis, the relative risk of thrombosis associated with homozygous defects or compound heterozygous *FVL* and prothrombin G20210A was independent of a positive family history of VTE in first-degree relatives. Because of the high risk of VTE in women with homozygous *FVL* or with compound heterozygous *FVL* and prothrombin G20210A, our data are the first to provide evidence to support the indication for routine antepartum thromboprophylaxis (with heparin) in unselected pregnant women with no prior history of VTE in the presence of these defects, even if the family history of VTE is negative.

The relevance and, more specifically, the predictive value of deficiencies of antithrombin or proteins C and S are controversial. The clinical relevance of quantitatively different deficiencies in particular is still an unsolved problem. We now present ORs and individual probabilities of pregnancy-associated VTE for different degrees of deficiencies. As anticipated, mild deficiencies (eg, antithrombin activity <90% or protein C activity <76%), which represent the majority of clinical cases, are associated with a less pronounced increase in risk compared with severe deficiencies (Table 3).

Much of our knowledge about the risk of VTE in pregnant and postpartum women with inhibitor deficiencies has been derived from family studies, which are likely to overestimate the risk for unselected women with these defects, particularly for those with mild deficiencies.[24-27] In these studies, the risk of thromboembolism among antithrombin-deficient pregnant women not receiving anticoagulant therapy was estimated at up to 40% (3-40% in the antenatal period, 0-20% in the puerperium). For pregnant women with abnormalities in the protein C and S system not receiving anticoagulant therapy, the risk of thrombosis during pregnancy ranged from 3% to 10% for protein C deficiency and from 0% to 6% for protein S deficiency. In postpartum women, the risk was 7% to 19% for protein C deficiency and 7% to 22% for protein S deficiency.[24-27] In contrast to these results obtained from family studies, a much lower risk is found in unselected women.[19,20] Our data support these findings by confirming a lower risk of deficiencies of antithrombin, protein C and protein S in unselected women. The most likely explanation for the particularly high risk in women from thrombophilic families is that familial thrombophilia is caused by multigenic effects. Thus, in each of the families, several known and unknown risk factors of thrombosis may be present, leading to an overestimation of the single risk factor under study.[11]

A strength of our study is that we, for the first time, provide estimates of the absolute risk of thrombosis according to age and thrombophilic defects, allowing for an individualized risk assessment as a basis for prophylactic treatment decisions. The age stratification takes into account the demographic changes in the society with increasing rates of childbearing women ≥35 years of age.

A possible limitation of our study is its case-control design (as opposed to cohort studies) because risk estimates obtained from case-control studies may be biased (referral/selection bias for patients, recall bias for family history) leading to an overestimation of the risk. However, the OR of 4.6 for heterozygous *FVL*, found in our analysis, is in agreement with previously published cohort studies with the pooled OR of 4.5 found in a meta-analysis,[44] which specifically aimed at providing an estimate of the association of

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FVL with pregnancy-associated VTE. This result may support the value of our data. Although a recall bias might increase the risk estimates for a positive family history of VTE, the multivariate adjustment of thrombophilic risk factors for a positive family history of VTE is likely not influenced. Due to the limitations of the case-control design and to the low numbers of patients with severe thrombophilias, all results found in our study should be considered estimations, which is evident by the wide CIs in the risk calculation of deficiencies of antithrombin, protein C, and protein S.

In summary, we demonstrate that unselected women with homozygous *FVL*, compound heterozygous *FVL* and prothrombin G20210A, or severe antithrombin deficiency have a high risk of pregnancy-associated thrombosis, particularly in women aged ≥35 years. This risk is similar to or higher than the level that would usually trigger prophylactic anticoagulation in the nonpregnant populations (ie, 3%).[21] Whereas carrier status of isolated heterozygous *FVL* or prothrombin G20210A does not require routine thromboprophylaxis in pregnant women, women with the aforementioned types of high-risk thrombophilia may be considered for routine antepartal prophylactic anticoagulation even with a negative family history of VTE. However, a clinical trial demonstrating the efficacy and safety of antepartal prophylactic anticoagulation in this population is required before a formal recommendation can be made.

The question remains whether these findings should lead to a general screening for thrombophilic risk factors in all pregnancies, regardless of family history. Current practical considerations (the cost of general screening vs the low absolute number of pregnant women who are carriers of the aforementioned mutations and who will develop a venous thromboembolism) lead us to suggest that women with additional, previously well-established thrombophilic risk factors (eg, obesity) may particularly benefit from a screening.[14] Although a family history is not the sole decisive factor for an indication for heparin prophylaxis in women with the aforementioned mutations, it nevertheless represents such an independent, additional risk factor (see above). In conclusion, we suggest that even though general screening of thrombophilic risk factors in all pregnant women may be considered ideal to estimate individual risk, this is unlikely to be cost effective; therefore, women with additional risk factors may constitute the main focus for counseling and selected thrombophilia screening in pregnancy.[45,46] However, in case a cost calculation is desired, the prevalences given in Tables 2 and 3 may be used as a basis.

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Authorship

Contribution: A.G. and R.B.Z. provided the hypothesis, were involved in study conception and design, provided data analysis and interpretation, and wrote the paper; and I.A.G. and R.E.S. were involved in study conception, wrote the paper, and provided critical revisions.

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