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Summary

Background A successful outcome of pregnancy requires an efficient uteroplacental vascular system Since this system may be compromised by disorders of haemostasis associated with a prothrombotic state we postulated that maternal thrombophilia might be a risk factor for fetal loss We studied the relation between heritable thrombophilic defects and fetal loss in a cohort of women with factor V Leiden or deficiency of antithrombin protein C or protein S

Methods We studied 1384 women enrolled in the European Prospective Cohort on Thrombophilia (EPCOT) Of 843 women with thrombophilia 571 had 1524 pregnancies of 541 control women 395 had 1019 pregnancies The controls were partners of male members of the EPCOT cohort or acquaintances of cases We analysed the frequencies of miscarriage (fetal loss at or before 28 weeks of gestation) and stillbirth (fetal loss after 28 weeks of gestation) jointly and separately

Findings The risk of fetal loss was increased in women with thrombophilia (168/571 *vs* 93/395 odds ratio 1 35 [95% CI 1 01–1 82]) The odds ratio was higher for stillbirth than for miscarriage (3 6 [1 4–9 4] *vs* 1 27 [0 94–1 71]) The highest odds ratio for stillbirth was in women with combined defects (14 3 [2 4–86 0]) compared with 5 2 (1 5–18 1) in antithrombin deficiency 2 3 (0 6–8 3) in protein C deficiency 3 3 (1 0–11 3) in protein S deficiency and 2 0 (0 5–7 7) with factor V Leiden The corresponding odds ratios for miscarriage in these subgroups were 0 8 (0 2–3 6) 1 7 (1 0–2 8) 1 4 (0 9–2 2) 1 2 (0 7–1 9) and 0 9 (0 5–1 5) Significantly more pregnancy terminations had been done in women with thrombophilia than in controls (odds ratio 2 9 [1 8–4 8]) this discrepancy was apparent in nine of 11 participating centres and for all thrombophilia subgroups

Interpretation Women with familial thrombophilia especially those with combined defects or antithrombin deficiency have an increased risk of fetal loss particularly stillbirth Our findings have important implications for therapy and provide a rationale for clinical trials of thromboprophylaxis for affected women with recurrent fetal loss

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Introduction

Until lately, a clear relation with a genetic thrombophilic defect was evident in only 5–10% of patients presenting with venous thromboembolism and was confined to those with deficiencies of antithrombin, protein C, and protein S The importance of heritable defects as risk factors for venous thromboembolism has increased with the discovery of a genetic thrombophilic disorder that manifests as resistance to activated protein C (APC)[1] and the observation that this disorder is present in about 20% of individuals presenting with venous thrombosis Bertina and colleagues[2] have shown that in most individuals APC resistance is the result of a single point mutation in the factor V gene at nucleotide 1691, which codes for the APC cleavage site (factor V Leiden mutation) In much of Europe and in the USA the prevalence of the factor V Leiden defect is 3–5% However, in other parts of the world, such as southeast Asia and Africa, the prevalence of the defect is less than 1% [3]

In pregnancy, a successful outcome is highly dependent on satisfactory placental development and sustained placental function These processes, in turn, require the establishment of an adequate fetomaternal circulatory system Since this system may be compromised by disturbances of haemostasis leading to a prothrombotic state, we postulated that maternal thrombophilia might be a risk factor for fetal loss We have therefore studied the relation between heritable thrombophilic defects and fetal loss in a cohort of women who have factor V Leiden or deficiencies of antithrombin, protein C, or protein S (the European Prospective Cohort on Thrombophilia [EPCOT]) and in a control group Although EPCOT is a prospective follow-up study, the data here were collected at baseline and give information on lifetime occurrence of miscarriage and stillbirth

Patients and methods

The women described here were enrolled in EPCOT between January, 1994, and November 1995 The primary aim of the study is to establish the risk of thrombosis in this group of disorders A secondary aim is to investigate the possible relation between these disorders and the risk of fetal loss

The index group consists of male and female individuals of all ages with or without symptomatic disease Each participating centre enrolled all registered patients who had hereditary thrombophilia caused by deficiencies of protein C protein S or

	Deficiencies of			Factor V Leiden	Combined defects*	All patients	Controls
	Antithrombin	Protein C	Protein S				
Women ever pregnant	108	162	145	141	15	571	395
Number of pregnancies	260	430	378	410	46	1524	1019
Number of pregnancies ending in miscarriage	44 (16 9%)	68 (15 8%)	55 (14 6%)	43 (10 5%)	6 (13%)	216 (14 2%)	118 (11 6%)
Number of pregnancies ending in stillbirth	6 (2 3%)	5 (1 2%)	7 (1 9%)	5 (1 2%)	7 (15%)	30 (2 0%)	6 (0 6%)
Number of women with fetal loss†	34 (31 5%)	48 (29 6%)	42 (29 0%)	38 (26 9%)	6 (40%)	168 (29 4%)	93 (23 5%)
Number of pregnancies resulting in fetal loss†	50 (19 2%)	73 (17 0%)	62 (16 4%)	48 (11 7%)	13 (28%)	246 (16 1%)	124 (12 2%)

*4 protein C deficiency+factor V Leiden 7 protein S deficiency+factor V Leiden 2 antithrombin deficiency+factor V Leiden and 2 protein C and protein S deficiencies †Excludes terminations of pregnancy

Table 1 **Number of pregnancies and fetal loss**

antithrombin, or by factor V Leiden There were no exclusion criteria Healthy controls are enrolled in the study for reasons of comparison The patients were asked whether their spouses (or partners) would be willing to serve as controls Single patients were asked to find an acquaintance who would be willing to serve as a control We left the patient to decide whether the control was of the same sex or not Since the controls are partners (65%) or acquaintances (35%) of the index individuals, the control group is of about the same age as the index group We excluded from the control group blood relatives of the index patient and individuals known to have familial thrombophilia All data collection, at baseline, and at follow-up was the same for the patients and controls We report here the data collected at baseline, referring to events before entry to EPCOT

Before study enrolment, informed consent was obtained from all individuals, including controls Data were collected at entry to the study and annually thereafter, by questionnaire or by telephone or personal interview Data recorded include general demographic information, thrombosis history, medication, risk factors for thrombosis, family history, obstetric history, and details on type and subtype of thrombophilia Standard data-collection forms are used at all participating centres For each patient, the diagnosis of heritable thrombophilia is confirmed by the diagnostic criteria of the study protocol All contributing centres participate in an external quality-assessment scheme for thrombophilia testing

We calculated the number of women in each group who had been pregnant and analysed the outcome of all pregnancies—miscarriage, pregnancy termination, stillbirth, or livebirth Miscarriage was defined as fetal loss during the first or second trimester (ie, up to and including 28 weeks' gestation) Stillbirth was defined as intrauterine death during the third trimester (ie, after 28 weeks' gestation) We could obtain information only on known or confirmed pregnancies We assessed differences in the risk of fetal loss by comparing the number of women who had ever experienced miscarriage or stillbirth by cross-tabulation and χ test We also compared the frequencies of pregnancy termination between the groups

The structure of the control group made study of the effect of paternal thrombophilia on fetal loss possible For this report only the women from EPCOT are included as patients, and the controls are female partners of thrombophilic men, or unrelated acquaintances of thrombophilic patients By comparing the frequency of fetal loss in control women who are partners of thrombophilic men and those who are not, we investigated the effect of paternal thrombophilia on miscarriage and stillbirth

The mean number of pregnancies differed among the various study groups, this difference had to be taken into account since the risk of fetal loss obviously increases with the number of pregnancies We therefore carried out logistic regression analysis with ever-experience of fetal loss as the outcome (dependent) variable and the total number of pregnancies as one of the covariates (independent variable) When a variable of interest is included in the model (for example, whether a woman is a thrombophilic patient or a control), this regression analysis will produce an odds ratio for that variable This odds ratio is a measure of relative risk, and is the ratio of the odds of fetal loss in an index woman over the odds in a control woman, adjusted for the number of pregnancies An odds ratio of more than one indicates a risk exceeding that of controls As a variable of interest we also looked at the type of thrombophilia, a dummy variable model gave separate odds ratios, each compared with the controls, for each subtype of thrombophilia Since the participating centres are spread widely across Europe, and since we investigated lifetime risk of fetal loss and our study period encompassed several decades, we also assessed the effect of adjustment for possible regional and time effects, by adding centre (as a dummy variable) and current age (as a continuous term) into the models used Since adjustment for age did not lead to any change, this variable was omitted from the models presented We then investigated the effect of thrombophilia on miscarriage and stillbirth separately To allow for the possibility that heterogeneity among women in other, unknown, factors might affect the risk of fetal loss, we also analysed the risk of pregnancies ending in fetal loss by a random effects model

Results

Patients and controls were enrolled by 11 centres from nine countries (Leiden, Barcelona, Bologna, Frankfurt, Glasgow, Malmo, Paris, Rome, Sheffield, Tel-Hashomer, Vienna) On Nov 1, 1995, 1384 women had been enrolled (843 patients, 541 controls) Most of the controls (354 [65%]) were partners of index patients, this proportion was 79% (311/395) among the controls who had been pregnant at least once The patients were 242 women with protein-C deficiency, 214 with protein-S deficiency, 159 with antithrombin deficiency, 203 with the factor V Leiden defect, and 25 with combined defects 571 patients had had 1524 pregnancies compared with 1019 pregnancies in 395 controls

Here we report on fetal loss among women who had been pregnant at least once (571 patients—162 with *protein-C deficiency, 145 with protein-S deficiency,* 108 with antithrombin deficiency, 141 with the factor V Leiden defect, and 15 with combined defects) The mean age of the 966 women who had been pregnant at least once (571 patients, 395 controls) at enrolment was 46 2 years (range 20–92), the mean age was similar for patients and controls (mean 45 7 [SD 13 8] *vs* 47 0 [12 2] years) The number of pregnancies ranged from 1 to 14 (patients 1–14, controls 1–11) and the number of fetal losses per

	Patients (n=571)	Controls (n=395)	Odds ratio (95% CI)
Any fetal loss	168	93	1 35 (1 01–1 82)
Miscarriage	154	89	1 27 (0 94–1 71)
Stillbirth	25	5	3 6 (1 4–9 4)

Numbers of miscarriages and stillbirths do not add up to the numbers under any fetal loss or overall figures in table 1 since some women have experienced miscarriages and stillbirths

Table 2 **Odds ratios of fetal loss in women with thrombophilia**

Type	All spontaneous fetal losses	Miscarriage	Stillbirth
Antithrombin deficiency	2 1 (1 2–3 6)	1 7 (1 0 2 8)	5 2 (1 5–18 1)
Protein C deficiency	1 4 (0 9–2 2)	1 4 (0 9–2 2)	2 3 (0 6–8 3)
Protein S deficiency	1 3 (0 8–2 1)	1 2 (0 7–1 9)	3 3 (1 0 11 3)
Factor V Leiden	1 0 (0 6–1 7)	0 9 (0 5 1 5)	2 0 (0 5–7 7)
Combined defects	2 0 (0 5–8 1)	0 8 (0 2–3 6)	14 3 (2 4–86 0)

Table 3 **Odds ratios (95% CI) for fetal loss and type of thrombophilia, with control group as reference, adjusted for number of pregnancies and centre**

woman ranged from 0 to 6 in both groups The mean number of pregnancies was similar in patients and controls (2 7 [1 7] *vs* 2 6 [1 4])

Fetal loss

Significantly more women with thrombophilia than controls had experienced fetal loss (miscarriage or stillbirth, table 1, p=0 04) The odds ratio for fetal loss associated with thrombophilia was 1 35 (95% CI 1 01–1 82) Random effects modelling led to a very similar result (1 40 [1 04–1 90]) The percentage of pregnancies ending in fetal loss (with terminations of pregnancy excluded) was significantly greater in each of the subgroups of patients with deficiencies of antithrombin, protein C, or protein S and in the subgroup with combined defects than in the control group The percentage of pregnancies ending in fetal loss in women with factor V Leiden did not differ significantly from that in the control group

For all women with thrombophilia compared with controls the relative risk of stillbirth was greater than that for miscarriage (table 2)

We carried out logistic regression to adjust the risk of fetal loss in thrombophilia for differences in the number of pregnancies Table 3 shows the odds ratios for both stillbirths and miscarriages for women with each type of thrombophilia and for those with combined defects, with the control group as reference, adjusted for the number of pregnancies and centre Further adjustment for age did not affect the estimates The random effects model gave essentially the same coefficients as the fixed model The odds of ever having had a spontaneous fetal loss were 30–36% greater in women with deficiencies of protein C or protein S than in controls without thrombophilia and 200% higher in women with antithrombin deficiency or with combined defects than in controls After we had adjusted for the higher number of pregnancies in carriers of factor V Leiden, we no longer found an excess risk of fetal loss associated with this abnormality (odds ratio 1 0 [0 6–1 7])

We repeated the multivariate analysis for miscarriages and stillbirths as separate outcomes, adjusted for the number of pregnancies This analysis confirmed the more pronounced odds ratio for stillbirths than for miscarriages

	% of women with pregnancies ending in fetal loss	
	Terminations included	Terminations excluded
Thrombophilic subgroup		
Antithrombin deficiency	49 1	31 5
Protein C deficiency	38 3	29 6
Protein S deficiency	40 7	29 0
Factor V Leiden	37 6	26 9
Combined defects	46 7	40 0
Controls	28 6	23 5

Table 4 **Percentage of women with pregnancies resulting in fetal loss with and without terminations of pregnancy**

but since there were fewer women with stillbirths than with miscarriages the confidence intervals were wider For all subtypes of thrombophilia the odds ratio for stillbirths exceeded that for miscarriages Again, we found no indication of an excess risk of miscarriage in carriers of factor V Leiden However, an increased risk of stillbirth in carriers of factor V Leiden does remain possible, although the confidence interval of the odds ratio was wide (2 0 [0 5–7 7]) The odds ratio for stillbirth was higher for all other subtypes of thrombophilia than for factor V Leiden and again highest in those with combined defects

Termination of pregnancy

15 8% of all the thrombophilic patients who had been pregnant had had at least one induced termination of pregnancy compared with 6 1% of the control group (p<0 001, χ^2 test, odds ratio 2 9 [1 8–4 6]) An increased frequency of pregnancy terminations compared with the control group was seen in all the thrombophilic subgroups (table 4) Since these observations could have been influenced by differences between participating centres, the data were reanalysed for each centre In nine of the 11 centres, terminations were more frequent among thrombophilic women than among controls

Influence of paternal thrombophilia

Many female partners of men with known thrombophilia have been enrolled in the EPCOT study control group Since these thrombophilic disorders are transmitted with an autosomal dominant pattern, we investigated the pregnancy outcome in the 311 control women who had been pregnant and were partners of men with thrombophilia We compared their results with those for 84 control women who had been pregnant and whose partners had no known thrombophilic defect There was no difference in the number of pregnancies or fetal losses between these two groups (data not shown)

Discussion

In this cross-sectional, multicentre study we found an increased risk of fetal loss in women with heritable deficiencies of antithrombin, protein C, or protein S The risks were greatest for women with antithrombin deficiency and for those with combined defects

Although the increased risk of fetal loss was found for both miscarriage and stillbirth, analysed jointly and separately, the effect of thrombophilia was especially pronounced for stillbirths This finding is not unexpected miscarriage is a common event with many possible causes, so positive identification of individual causative factors is difficult, whereas stillbirth is a much rarer event and strong risk factors are more readily identifiable Since our study dealt only with known or confirmed pregnancies, no conclusions can be drawn on the possibility of an effect of thrombophilia on very early fetal loss

No increased risk of fetal loss was evident in women with factor V Leiden For this group, although there was no excess of miscarriages, our results do suggest the possibility of an increased risk of stillbirth We can, however, conclude that the risk of total fetal loss is less than that of the other thrombophilic groups

An increased frequency of pregnancy terminations among women with all types of thrombophilia, including factor V Leiden, was found in nine of the 11 participating centres Overall, proportionately more women with factor

V Leiden than women in the other groups had had abortions This factor may have influenced the number of observed spontaneous fetal losses in these women We did not include these data in our analysis of fetal loss in thrombophilic women, since we believe that they relate to maternal rather than to fetal conditions We cannot identify the reasons for the increased number of pregnancy terminations but speculate that decisions to terminate pregnancy might have been influenced by previous episodes of venous thromboembolism, occurring either during pregnancy or in association with oral-contraceptive use Conception during treatment with coumarins might also have been a reason for termination

Since we report on miscarriage and fetal loss assessed retrospectively at the beginning of follow-up, we must consider the possibility of recall bias—ie, the tendency for patients to recall past events more readily than do controls A well-known example of recall bias is that mothers of newborn infants with birth defects are much better able to remember minor illnesses or drugs they took during pregnancy than are mothers of healthy babies We do not think that recall bias is likely to be important in our study, since in this setting thrombophilia is the minor event, and this diagnosis would be unlikely to make more women remember miscarriage The possibility that the diagnosis would affect the memory of a fetal loss occurring after 28 weeks of gestation is inconceivable

The control group consisted of partners of thrombophilic men and acquaintances of thrombophilic individuals The inclusion of the latter type of controls might theoretically lead to cases and controls being too much alike, which would lead to biased estimates However, since only a small proportion of controls were acquaintances of patients in this cohort, this bias can have had only a slight effect If present, this bias would lead to an underestimate of the difference between cases and controls—thus it cannot explain the higher risk of fetal loss observed in the patients Most of the controls were partners of thrombophilic men We do not think their inclusion introduced a bias, since the possibility that women who choose thrombophilic men as their partners have an intrinsically lower risk of fetal loss is extremely unlikely The frequency of fetal loss in the control group was similar to that in the general population [7] Because of the composition of the control group, we were able to investigate the effect of paternal thrombophilia on fetal loss, no effect was found Since this study deals with genetic abnormalities, confounding bias is unlikely to be present

In view of the well-established relation between the thrombophilic disorders and venous thromboembolism, and the increased thrombotic risk in people with combined defects, the causal mechanism seems likely to involve impaired placental development and function due to a compromised vascular support system A similar mechanism has been proposed for the increased risk of fetal loss associated with the presence of antibodies directed against phospholipids Similar mechanisms for increased fetal loss to those reported here may operate in women with those antibodies, the thrombotic risk in such people has been attributed, at least partly, to disturbances of the protein C protein S pathway [8] This view is strengthened by evidence from an animal model of the importance of that pathway for successful pregnancy outcome

The greatly increased risk of stillbirth in women with deficiencies of antithrombin, protein C, and protein S strongly suggests the possibility of uteroplacental insufficiency as a causative factor Another possibility is fetal thrombosis In the group of patients some of the women are likely to have partners with unidentified thrombophilia, and the fetus could then inherit genes for familial thrombophilia from both parents and be at increased risk of venous thromboembolism by virtue of having combined gene defects, or homozygosity for factor V Leiden [9]

The physiological mechanisms, including the contribution of paternal genes, for implantation and placental and fetal development are poorly understood Our results clearly indicate that the familial thrombotic disorders described here are not associated with female infertility We also provide evidence that in the context of genetic thrombophilic disorders, the increased fetal losses relate to maternal and not paternal abnormalities

The demonstration of increased fetal losses in women with familial thrombophilia, and especially in those with combined defects or isolated deficiencies of antithrombin, protein C, or protein S, has important therapeutic implications and provides a rationale for clinical trials of thromboprophylaxis for affected women with recurrent fetal loss We must emphasise, however, that although we have shown a significant difference in pregnancy outcome between women with coagulation-inhibitor deficiencies and controls, the probability of a favourable pregnancy outcome is good

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