Thrombophilic mutations are a main risk factor for placental abruption

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Background and Objectives. The aim of the present study was to evaluate inherited thrombophilic factor V Leiden and factor II A20210 mutations in women presenting with abruption of a normally implanted placenta.

Design and Methods. In a multi-center, case-control study, 50 consecutive women requiring immediate delivery because of abruption of the placenta were enrolled. Inclusion criteria were: *abruptio placentae* requiring immediate delivery, normally implanted placenta, Caucasian ethnic background, parity <3, delivery performed at Institutions. Exclusion criteria were: history of thromboembolism, history of 2 or more spontaneous abortions, uterine leiomyomas with a diameter >5 cm, illicit drug abuse, premature rupture of membranes, multiple pregnancy. One hundred Caucasian women with uneventful pregnancies carried to term, matched for parity and age, served as controls.

Results. Heterozygotes were found to be significantly more prevalent among women with *abruptio placentae* than among controls. The carriership of the FV Leiden mutation confers a OR of 9.12 (95% C.I.: 2.18-31.7; p=0.0005). Women carrying F II A20210 mutation have a OR of 12.25 (95% C.I.: 2.36-29.6; p=0.0004). No homozygotes or double heterozygotes were found. Twenty-three patients (46%) also met the criteria for a diagnosis of pre-eclampsia (PE). In such cases the prevalence of mutations (factor V: 6 cases, 26.1%; factor II: 5 cases, 21.7%) was similar to that in women without pre-eclampsia (factor V: 5 cases, 18.7%; factor II: 5 cases, 18.5%).

Interpretation and Conclusions. The presence of either of the above reported thrombophilic mutations represents a relevant risk factor for the occurrence of placental abruption in Caucasians. This risk is independent of the development of pre-eclampsia. Patients who have had dramatic abruption of a normally implanted placenta should undergo evaluation for the presence of genetic mutations of coagulation factors V and II.

Key words: *abruptio placentae*, factor V Leiden, factor II mutation A20210.

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Abruption of a normally implanted placenta is a life-threatening condition for both mother and fetus. Nowadays, abruption accounts for one fifth of maternal deaths due to hemorrhage.[1] Abruption is associated with variable accompanying symptoms such as abdominal pain and bleeding. Some risk factors have been identified and include prior abruption, great parity, any form of hypertension and premature rupture of membranes. Additional risks are smoking, cocaine use and leiomyomas.[1]

Recently, attention has been focused on thrombophilia, either genetic or acquired, a condition predisposing to deep venous thromboembolism and which has also been associated with several obstetric complications.[2-4] Placentas of thrombophilic women are also characterized by increased rate of vascular damage, multiple infarcts and fibrinoid necrosis.[5]

The prevalence of factor V (FV) Leiden mutation is 10-fold higher in placentas with infarction than in placentas carrying thrombophilia.[6] Moreover, it has been recently reported that this mutation is significantly more prevalent than in controls, the prevalence ranging from 25-29.6% in a total of 39 women with placental abruption.[7-8] In a further small series of women with *abruptio placentae*, mutation of factor II (F II) A20210 was found to be markedly higher than in controls.[9] However, the above studies were carried out in small series of cases sometimes in selected populations, and/or without strict clinical criteria for inclusion of patients.

Therefore, the aim of the present study was to evaluate the inherited thrombophilic FV Leiden and FII A20210 mutations in women presenting with abruption of a normally implanted placenta. A control group was formed of women who had carried at least one uneventful pregnancy to term.

Design and Methods

In a multicenter, case-control study, 50 consecutive women requiring immediate delivery because of *abruptio placentae* were enrolled. The diagnosis of abruption was based on clinical findings of abdominal pain and/or vaginal bleeding, with signs of fetal distress. The diagnosis was subsequently confirmed by histological examination of the placenta. Subjects were enrolled in the period January 2001-July 2002 at the Departments of Obstetrics and Gynaecology, University-Hospital S. Anna of Turin and University-Hospital Policlinico of Modena,

Italy. In this period, the incidence of *abruptio placentae* was 0.6% (61 cases/10516 deliveries). In eleven cases genetic evaluation was not possible. Eight more cases were excluded because they did not fulfil the criteria reported hereafter.

Inclusion criteria were: *abruptio placentae* requiring immediate delivery, normally implanted placenta confirmed at routine ultrasound screening in pregnancy, Caucasian ethnic background, parity <3, delivery performed at Institutions. Exclusion criteria were: history of thromboembolism, history of 2 or more spontaneous abortions, uterine leiomyomas with a diameter >5 cm, illicit drug abuse, premature rupture of membranes, multiple pregnancy. Such features are all confounders since they are associated with a possible increase in either the incidence of *abruptio placentae* or the prevalence of thrombophilic mutations.

One hundred Caucasian women with uneventful pregnancies carried to term, matched for parity and age (in the range of 2 years) from a random selection of the birth records, served as controls in a 2:1 ratio with respect to the cases.

After informed consent, 9 mL of blood were collected in sodium citrate (1 mL) at the moment of hospital discharge. Fresh samples were stored at -20°C for not more than 5 days. Leukocyte DNA was obtained from frozen blood by standard techniques. A 220-base-pair (bp) DNA fragment of the factor V gene including nucleotide 1691 was amplified by polymerase chain reaction, as previously described.[10] To identify the G20210A mutation of the prothrombin gene, a 345-bp fragment was obtained and digested using the Hind III endonuclease, according to Poort *et al.*[11]

Clinical and laboratory data were downloaded into a personal computer, and the results were analyzed using SPSS, the Statistical Package for Social Science (SPSS Inc., Chicago, IL, USA). Student's t-test and the χ^2 test were applied when appropriate for the detection of significant differences between groups. Odds ratios and 95 % confidence intervals were calculated.

Results

Age, parity, gestational age at delivery, birthweight, perinatal death as well disseminated intravascular coagulation are reported in Table 1.

The frequencies of heterozygous carriers (no homozygotes were found) of FV Leiden and FII A20210 are reported in Table 2. Heterozygotes for either mutation were found to be significantly more prevalent among women with *abruptio placentiae* than among controls. The carriership of the FV Leiden mutation confers a OR of 9.12 (95% C.I.: 2.18-31.7; p=0.0005). Women carrying F II A20210 mutation have a OR of 12.25 (95% C.I.: 2.36-29.6; p=0.0004). None of the subjects included was simultaneously a carrier of both mutations.

Among cases, 23 patients (46%) also met the criteria for a diagnosis of pre-eclampsia defined as the simultaneous occurrence of hypertension (at least two values higher than 140 mmHg systolic or 90 mmHg diastolic, evaluated 6 hours apart), and proteinuria (>300 mg/24-h or >30 mg on spot urine samples). Fifteen of them received antihypertensive treatment.

In patients with concomitant pre-eclampsia the prevalence of mutations (factor V: 6 cases, 26.1%; Factor II: 5 cases, 21.7%) was similar to that in patients without pre-eclampsia (factor V: 5 cases, 18.7%; factor II: 5 cases, 18.5%). At variance, a worse outcome was recorded in patients with pre-eclampsia (delivery at 33.3±4.3 weeks; birthweight: 1742±827g) than in patients without (delivery at 35.7.±4.1, p=0.046; birthweight: 2547±773, p=0.009).

Table 1. Clinical features and outcomes of pregnancy in subjects under study.

	Controls (100)	*Abruptio placentae (50)*
Age (years)	31.5±5.1* (18-42)	31.7±5.9 (17-43)
Primiparae	63 (63%)	34 (68%)
Gestational age at delivery (weeks)	39.3±1.4 (37-42)	34.6±4.4 (24-42)
Birthweight (g)	3178±367 (2500-4280)	2177±888 (500-3880)
Disseminated intravascular coagulation	0	14 (28%)

**M±SD; ranges in brackets.*

Table 2. Distribution of F V Leiden and F II A20210 in subjects under study.

	Controls		*Abruptio placentae*		*p*
	–/–	*+/–*	*–/–*	*+/–*	
F V Leiden	97 (97%)	3 (3%)	39 (78%)	11 (22%)	0.0005
F II A20210	98 (98%)	2 (2%)	40 (80%)	10 (20%)	0.0004

Discussion

These data demonstrate an impressively high rate of thrombophilic mutations in patients who have had *abruptio placentae* requiring immediate delivery. Almost half of such cases showed a congenital thrombophilic mutation. Until now only three case-control studies have been published.[7-9] Forty-seven cases with abruptio placentae have been tested for the presence of factor V Leiden (13 carriers) and 27 cases have been tested for the prothrombin gene mutation (5 carriers). Thus, including the data from our study, 97 subjects have been studied and the rate of mutations is quite homogeneous, ranging between 20-30% for factor V and 18-20% for factor II. In a prospective study, Lindqvist *et al.*[12] evaluated factor V in 2480 pregnant women. Their data on *abruptio placentae* confirm the higher prevalence of this mutation (15.3%). However, they were unable to find a significant increase of the incidence of abruption among activated protein C resistant subjects because, as in our experience, the prevalence of abruption is low. Interestingly, we did not find double heterozygotes among patients with *abruptio placentae*, as seems to be the case also in the previous series reported in the literature.

It is possible to conclude that, in a Caucasian population, the presence of either of the above reported thrombophilic mutations is the most relevant risk factor for the occurrence of placental abruption. This conclusion might not be applicable to black African women since polymorphisms of both these coagulation genes have been found to be absent.[13] The above observation clearly indicates that the presence of thrombophilia is not *per se* an absolute risk, and other facilitating factors are required for abruption.

Abruptio placentae is almost exclusively a diagnosis based on clinical reports. This could introduce a bias into any study of the condition. Thus, we decided to include only patients admitted to our institutions whose clinical condition necessitated immediate delivery because of acute fetal distress. Indeed, the prevalence of severe complications, such as perinatal death and disseminated intravascular coagulation, was very high in our series, as expected in this condition.[1] These criteria exclude any possibility of misdiagnosing patients and give more strength to the findings of the study.

Pre-eclampsia is a well known risk factor for placental infarcts and thrombi, and this was again confirmed in our series of women since hypertension and proteinuria were present in half of them. Moreover, pre-eclampsia has been considered among those pregnancy complications possibly linked to a thrombophilic trait.[2-3] Both factor V Leiden and prothrombin gene mutations have been found to be significantly more prevalent in pre-eclampsia and this could represent a confounding factor.[14,15] However, we report here for the first time that the high prevalence of factor V and factor II mutations in patients with *abruptio placentae* was independent of the absence or presence of clinical features of PE.

In conclusion, these data suggest that patients who have dramatic abruption of a normally implanted placenta should undergo evaluation for the presence of genetic mutations of coagulation factors V and II.

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Contributions

FF: principal author; LM: conception and design; EG: analysis of data; CP: analysis of data; AV: revising the article critically; CB: conception and design.

All the authors were part of a project funded by the Ministero dell'istruzione, Università e della Ricerca. The research team designed the study, analyzed/interpreted findings and concurred to produce a draft of the paper. Patients were collected by the Modena and Turin groups. Laboratory analyses were done in S. Giovanni Rotondo. Tables were created by CP.

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In the following paragraphs, Professor Vicente summarizes the peer-review process and its outcomes.

What is already known on this topic

Different inherited thrombophilic states have been associated with several obstetric complications.

What this study adds

This paper confirms that FV Leiden or FII A20210 polymorphisms represent a relevant risk factor for the occurrence of placental abruption.