

Familial thrombophilia and the occurrence of fetal growth restriction

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Background and Objectives. To evaluate the association between unexplained or gestational-hypertension-associated fetal growth restriction (FGR) and factor V Leiden, prothrombin A²⁰²¹⁰ mutations, and methylenetetrahydrofolate reductase (MTHFR) TT 677 genotype.

Design and Methods. Sixty-one women with a previous history of FGR and 93 parous women with uneventful pregnancies from the same ethnic background were investigated for the presence of factor V (FV) Leiden, prothrombin A²⁰²¹⁰ mutations, and MTHFR TT 677 genotype. Moreover, antiphospholipid antibodies, antithrombin, protein C, and total and free protein S antigen were determined in all patients.

Results. Among the controls, 2 (2.2%) carried the FV Leiden mutation, 19 (20.4%) were TT MTHFR homozygotes and 1 (1.6%) carried the prothrombin A²⁰²¹⁰ allele. The FV Leiden mutation was present in 8 women with FGR (13.1%, OR: 6.9, 95%CI 1.4-33.5), the TT MTHFR homozygosity in 17 (27.8%, OR: 1.5, 95%CI 0.7-3.2) and the A²⁰²¹⁰ prothrombin allele in 7 (11.5%, OR: 5.9, 95%CI 1.2-29.4). In six cases (9.8%) there was coexistence of more than one mutation (2 had the FV Leiden and the TT MTHFR genotype and 4 carried the A²⁰²¹⁰ prothrombin allele and TT MTHFR genotype). A logistic regression analysis showed that FV Leiden and A²⁰²¹⁰ prothrombin mutations were independently associated with the occurrence of FGR.

Interpretation and Conclusions. Present data indicate an association between prothrombotic genetic factors and FGR.

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Key words: fetal growth restriction, FV Leiden, A²⁰²¹⁰ prothrombin allele

Fetal growth restriction (FGR), defined as a birth-weight under the tenth percentile,¹ can be associated with a birth before 38 weeks of gestation and with respiratory and/or neurologic deficiencies in the newborn. One of the histologic features of the placenta from FGR fetuses is the presence of multi-infarctual areas.²

The identified causes of fetal growth restriction are of maternal origin (i.e. hypertensive disorders, prolonged pregnancy, maternal diseases) or fetal (malformations, transplacental infections, multiple pregnancy). In about 20% of FGR, there is an apparent absence of underlying pathologic conditions.

A series of prothrombotic genetic risk factors have recently been associated with some obstetric complications.³ Factor V (FV) Leiden, a gene variant of the FV responsible for activated protein C resistance, has been demonstrated to be associated with repeated and recurrent unexplained fetal losses.⁴ Moreover, it has been demonstrated that the FV Leiden mutation may predispose women to pre-eclampsia.⁵

Hyperhomocyst(e)inemia has been identified as an important risk factor for occlusive vascular disease in non-pregnant adults,⁶ but its effect on the uteroplacental vasculature is not known. A cause of moderate hyperhomocyst(e)inemia is a mutation (C→T at position 677) in the gene coding for 5,10 methylenetetrahydrofolate reductase (MTHFR).⁷ This mutation substitutes a valine for an alanine residue and the TT homozygous status is relatively frequent in the general population (about 18% in the Italian population).⁸ More recently, the G→A mutation at nucleotide 20210 within the prothrombin gene,⁹ causing elevated levels of prothrombin, has been associated with an increased incidence of venous thrombosis^{10,11} and gestational hypertension.¹²

We investigated the presence of FV Leiden mutation, TT 677 MTHFR genotype and prothrombin A²⁰²¹⁰ gene variant in a group (n=61) of women with intrauterine growth-restricted babies. As controls, 93 parous women with uneventful pregnancies were recruited.

Design and Methods

Patients

Between March 1997 and October 1998, 286 pregnant women were observed in the "At-risk pregnancies" Units of the Obstetrics and Gynecology Departments of IRCCS "Casa Sollievo della Sofferenza", S. Giovanni Rotondo and "Federico II" University Medical School in Naples. From among these women, 68 with a diagnosis of FGR were recruited. Three women refused the enrollment and 4 were lost during the follow-up. Thus, 61 women (median age 29 yrs, range 17-44) were enrolled. Of them, 19 had an unexplained FGR (i.e. absence of maternal diabetes, hypertension, kidney and liver disease) and 42 had FGR due to gestational hypertension (GH) with (n=18) or without (n=24) significant proteinuria (urine protein excretion > 300 mg/24h). GH was defined as the occurrence, after the 20th week of gestation, of a systolic blood pressure >140 mmHg and a diastolic blood pressure >90 mmHg occurring on two occasions > 4 hrs apart, in a previously normotensive woman.¹²

Fetal growth-restricted newborns with malformations, fetal infection or part of multiple pregnancies were not considered. FGR was defined as a birth weight under the tenth percentile.¹

All babies considered were born weighing less than the tenth percentile of the Neonatal Italian Standards¹³ and among them, 13 were under the fifth percentile and 5 under the third percentile. Gestational age was determined by last menstrual period and confirmed or corrected on the basis of an early ultrasound scan.

As controls, 93 healthy parous women (median age 29 yrs, range 18-44 yrs) with uneventful pregnancies and newborns with a birth weight > 10th percentile were enrolled. All cases and controls were Caucasian women from Southern Italy.

Information on socio-economic characteristics and a detailed obstetric and reproductive history were obtained. All individuals signed informed consent to the study which, after approval from the local Ethics Committees, was carried out according to the Principles of the Declaration of Helsinki.

Blood was collected in a 1:10 ratio in sodium citrate 0.1 M and the samples stored at -80°C until DNA extraction. Leukocyte DNA was obtained from frozen blood using standard techniques.

A 220-base-pair DNA fragment of the factor V gene that includes nucleotide 1691 was amplified by polymerase chain reaction (PCR), as previously described.⁴ To identify the G→A mutation of the prothrombin gene, a 345-bp fragment was obtained and then digested using the Hind III endonuclease, as described elsewhere.¹⁰

A fragment of 198 bp containing the C677T polymorphism of MTHFR gene was generated and investigated as elsewhere described.⁴

One control for FV Leiden and 1 case for both prothrombin and MTHFR polymorphism could not be typed. Antiphospholipid antibodies-lupus anticoagulant (LA) and IgG anticardiolipin antibodies (aCL) (ELISA, Byk Gulden,

Italy), antithrombin, protein C, amidolytic and immunologic (Behring, Marburg, Germany) and total and free protein S antigen (ELISA, Diagnostica Stago, Asnières, France) were determined in all patients, as reported elsewhere.¹¹

Clotting assays were performed on a KC4 Amelung coagulometer (Amelung, Germany). Inter and intra-assay coefficients of all the variables never exceeded 8.0% and 5.0%, respectively.

Statistical analysis

All the analyses were performed using the Statistical Package for Social Science (SPSS 6.1 for Macintosh). Difference in means were compared by a non-parametric test, whereas the significance of any difference in proportions was tested by the χ^2 statistic. Allele frequencies were estimated by gene counting in cases and controls; genotypes were scored for each subject. The significance of the difference of alleles and genotypes observed between the groups was tested using the χ^2 analysis, after grouping homozygous and heterozygous carriers of the FV Leiden.

Odds ratios (OR) and 95% confidence intervals (CI) were calculated.

Adjusted OR and 95% CI were calculated by logistic-regression models. Potential confounding variables were: age, parity, gravidity. A two-tailed statistical significance was taken as $p < 0.05$.

Results

Demographic and obstetric features of the cases and controls are shown in Table 1.

Among cases, mean gestational age at the time of delivery was 34.6±3.4 weeks. Mean birth weight (±SD) was 1,584±586 grams. Among all the cases, 58 (95.1%) were delivered by means of Cesarean section. No patient had antithrombin, protein C, protein S deficiencies or antiphospholipid positivity.

Overall, 26 pregnancy losses were recorded in the history of 21 (27.3%) patients.

As far as concerns smoking habits, 5 (8.2%) women in the patients' group smoked 1-10 cigarettes/day and one (1.6%) 10-20/day, while among controls 8 (8.6%) smoked 1-10 cigarettes/day. This difference was not statistically significant ($p=ns$). Twenty-nine (47.5%) newborns were admitted to a NICU and the perinatal mortality was 5.0% (3/61). One of these babies was from a mother with the TT MTHFR genotype.

Among the controls, 2 (2.2%) were FV Leiden carriers, 19 (20.4%) were homozygotes for the TT MTHFR genotype and 2 (2.2%) showed the A²⁰²¹⁰ allele of the prothrombin. The presence of two mutations was not observed in any control.

Among the cases, 8 (13.1%, OR: 6.9, 95%CI 1.4-33.5) carried the FV Leiden mutation (one was homozygous), 17 (28.3%, OR: 1.5, 95% CI 0.7-3.2) were homozygotes for the TT MTHFR genotype and 7 (11.7%, OR: 5.9, 95%CI 1.2-29.4) were heterozygotes for the prothrombin A²⁰²¹⁰ allele. Moreover, 6 women (9.8%) had co-existence of more than one mutation (2 had FV Leiden and

Table 1. Clinical features of cases and controls.

	Controls (n=93)	FGR (n=61)	χ^2	p
Age, median (range)	29 (18-44)	29 (17-44)		
Gravidity, median (range)	1 (1-4)	1 (1-4)		
Parity, median (range)	1 (1-3)	1 (1-3)		
Factor V Leiden carrier, % (n)				
No	97.8 (91)	86.9 (53)	7.29	0.007
Yes	2.2 (2)	13.1 (8)		
Odds Ratio (95% CI)	Reference	6.9 (1.4-33.5)		
Prothrombin A ²⁰²¹⁰ carrier, % (n)				
No	98.4 (92)	88.5 (54)	5.82	0.016
Yes	1.6 (1)	11.5 (7)		
Odds Ratio (95% CI)	Reference	5.9 (1.2-29.4)		
TT 677 MTHFR genotype, % (n)				
No	79.6 (74)	52.8 (44)	1.14	0.286
Yes	20.4 (19)	47.2 (17)		
Odds Ratio (95% CI)	Reference	1.5 (0.7-3.2)		

Table 2. Factors independently associated with FGR.

	B	FGR		95%CI
		SE	OR	
FV Leiden	2.05	0.81	7.7	1.6-37.9
Prothrombin A ²⁰²¹⁰	1.91	0.82	6.8	1.4-33.9

The multivariate logistic regression contained the following not significant variables: age, parity, gravidity and MTHFR TT genotype. B: estimated coefficient; SE: standard error; OR: odds ratio; 95%CI: 95% confidence interval.

Table 3. Prevalence of prothrombotic genetic risk factors in mothers, according to the birth weight percentile of their newborn infants.

Percentile of growth	Birth-weight >5 th < 10 th percentile (n=43)	Birth-weight <5 th percentile (n=13)	Birth-weight <3 rd percentile (n=5)
	FV Leiden	6	2*
Prothrombin A ²⁰²¹⁰	7	/	/
TT 677 MTHFR genotype	16	/	1
Combined defects	2	2	2

*One of these women was homozygous.

TT MTHFR genotype and 4 carried the A²⁰²¹⁰ prothrombin allele and TT MTHFR genotype).

Overall, in the subgroup of GH-proteinuric patients (n=18) 2 women had the FV Leiden mutation, 1 had the A²⁰²¹⁰ allele of the prothrombin gene and 3 the TT MTHFR genotype; 2 women showed the association of two molecular variants (1 FV Leiden and TT MTHFR genotype and 1 the A²⁰²¹⁰ prothrombin allele and the TT MTH-

FR genotype). In the subgroup of patients with GH but without proteinuria (n=24), 4 had the FV Leiden mutation, 4 the TT MTHFR genotype and 2 the coexistence of the A²⁰²¹⁰ prothrombin allele and the TT MTHFR genotype. The differences in the prevalence of the molecular variants described were not statistically significant.

Only 2 cases carrying the prothrombotic mutations (1 had FV Leiden and TT MTHFR and 1 had the A²⁰²¹⁰ prothrombin with the TT MTHFR genotype) had an obstetric history of 1 fetal loss.

To evaluate the impact of potential confounding variables on the associations previously described we performed a logistic regression analysis (Table 2). Variables entered into the model were: maternal age, parity, gravidity, FV Leiden carrier status, prothrombin G20210A mutation carrier status and 677T MTHFR homozygosity. The FV Leiden and prothrombin A²⁰²¹⁰ mutations were independently associated with the occurrence of FGR.

We also divided the FGR newborns according to their percentile weight (which corrects the birth-weight for gestational age) and analyzed the presence of these mutations in the mothers in each group (Table 3).

None of the women had arterial thrombosis, or deep venous thrombosis. Two superficial venous thromboses of the lower limbs were recorded in two controls (one during pregnancy and one in the post-partum period). Neither of them carried any of the molecular variants investigated.

Discussion

The successful outcome of a pregnancy is strongly dependent on satisfactory placental development and sustained placental function. These processes require an adequate fetomaternal circulatory system.

This system may be compromised by disturbances of hemostasis leading to a prothrombotic state. In view of this, congenital or acquired causes of thrombophilia may be involved in the pathogenesis of FGR. Nevertheless, studies addressing this issue are small and inconclusive in this regard.^{3,14}

We have already reported an association between the FV Leiden mutation and repeated or recurrent unexplained pregnancy loss, and there are data about favorable outcomes in women with recurrent fetal loss treated with aspirin or heparin.¹⁵ On the other hand, extensive necrotic areas have been demonstrated in women with FGR and in women with gestational hypertensive disorders.

This study shows an association between prothrombin A²⁰²¹⁰ and FV Leiden mutations and the occurrence of both forms of FGR, either associated with GH or occurring in absence of other predisposing conditions. This association was highlighted by univariate analysis and confirmed using a multivariate model, showing that these mutations are independent risk factors for the occurrence of FGR. No significant difference in the prevalence of these mutations was found between the unexplained FGR and those associated with GH.

A recent report from Rotmensch *et al.*¹⁴ suggests that

activated protein C resistance (APCR) is associated with a poor pregnancy outcome. Our data confirm these findings and suggest that both forms of FGR might have a vascular pathogenesis.

The role of the TT MTHFR genotype in arterial or venous thrombosis is still matter of investigation. The coexistence of more than one mutation has been demonstrated to increase the risk of venous thrombosis.¹⁶ It has been suggested that the TT MTHFR molecular variant may have a role only when associated with other risk factors, such as FV Leiden or antiphospholipid antibodies.^{17,18} On the other hand, the C677T polymorphism plays an important role in the regulation of homocysteinemia only in the presence of a low folate status and most of the women (n=54) considered here received folate supplementation during pregnancy.

FV Leiden and prothrombin mutations have been suggested to play a role in the occurrence of GH, regardless of proteinuria.¹² Furthermore, the same gene variants have been demonstrated to be associated with pregnancy-related venous thromboembolism.^{11,19}

Present data further strengthen the relevance of prothrombotic genetic factors in a series of obstetric complications, in which histologic² and epidemiological studies³ clearly show the relevance of disturbances leading to an imbalance of hemostasis.

Moreover, present findings provide the rationale for carrying out prospective studies, addressing the predictive power of these gene variants, in order to identify prophylactic regimens in women at risk of FGR.

Contributions and Acknowledgments

PM and EG made equivalent contributions to this work. DC was responsible for polymorphism evaluations. DP and NS selected cases and controls and performed clinical and ecographic evaluations. MM performed the statistical analysis and contributed to the writing of the paper. GDM critically revised the data and the draft.

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Disclosures

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Manuscript processing

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Potential implications for clinical practice

These data provide the rationale for anti-thrombotic prophylaxis in women with a family or personal history of unexplained or GH-induced FGR.

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