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Lower birth-weight in neonates of mothers carrying factor V G1691A and factor II A^{20210} mutations

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Background and Objectives. Inherited thrombophilia has been associated with fetal and maternal complications of pregnancy. It is reasonable to suppose that an imbalance of maternal hemostasis could lead to decreased fetal growth.

Design and Methods. We retrospectively investigated the birth-weight of neonates in relation to the presence of factor V G1691A and factor II A^{20210} mutations in the mothers. Overall, 755 women (194 with a history of unexplained recurrent pregnancy loss, 202 with gestational hypertension with or without proteinuria, 359 with at least one uneventful pregnancy) and 1100 alive neonates were considered.

Results. Among 980 neonates from mothers without mutations, 136 (13.9%) weighed <2500 grams, whereas 34 out 123 (27.6%) neonates from mothers carrying the factor V G1691A or factor II A^{20210} mutation were under this birth-weight (OR: 2.4, 95%CI: 1.5- 3.7). Adjusting for diagnosis, parity, and age, the risk of having a baby <2500 grams was 2.0 (95%CI: 1.1-3.6) in women carrying factor V G1691A or factor II A^{20210} mutation. When we analyzed all the neonates according to growth centiles and the presence of a thrombophilic mutation in the mothers, we found 142 (14.5%) and 28 (22.8%) neonates under the 10th centile from mothers without and with thrombophilic mutations, respectively (OR: 1.7, 95%-CI: 1.1-2.7). Adjusting for confounding variables (diagnosis, parity and age), the association between thrombophilic mutations and <10th growth centile did not change (OR: 1.7, 95% CI: 1.0-3.0).

Interpretation and Conclusions. Mothers carrying the factor V G1691A or factor II A^{20210} mutation have a significantly higher risk of delivering neonates with a lower birth-weight.

Key words: factor V G1691A, prothrombin A^{20210} mutation, birth-weight, growth centiles.

Current knowledge indicates that there is an association between obstetric complications and heritable causes of thrombophilia.[1] Broadly categorised, causes of growth deficiency common to any obstetric practice include maternal vascular disease. A large body of evidence has been accumulated on a high incidence of mutations predisposing to thrombosis in women with obstetric complications.[2-4] The factor V G1691A (FV Leiden) mutation, responsible for most cases of activated protein C resistance (APCR), has been recognized as the most frequent genetic predisposition to thrombosis.[5] It has been associated with pre-eclampsia, unexplained repeated or recurrent pregnancy loss, *abruptio placentae*, unexplained fetal growth restriction and unexplained stillbirths.[6,7] More recently, it has been reported that an acquired APCR is associated with restricted fetal growth.[8]

Like factor V G1691A, the G^{20210A} substitution in the factor II (FII) gene has been associated with the occurrence of venous thrombosis and obstetric complications.[1,2,4,9]

Moreover, several studies showed the presence of extensive necrotic areas in the placentae from pregnancies complicated by obstetric pathologies

(e.g. fetal losses or gestational hypertension), suggesting that an imbalance in maternal hemostasis could lead to abnormalities in placental perfusion and in turn to decreased intrauterine fetal growth.[10,11]

We reasoned that an imbalance of maternal hemostasis, due to factor V G1691A and FII A^{20210} mutations, could be associated with a low birthweight. To address this issue we investigated the birth-weight of neonates in relation to the presence of factor V G1691A and FII A^{20210} mutations in a cohort of Italian women with a known obstetric history.

Design and Methods

From August 1994-December 1998, 586 women with a history of recurrent pregnancy losses or gestational hypertension with or without significant proteinuria were referred to the Atherosclerosis and Thrombosis Unit of the I.R.C.C.S. *Casa Sollievo della Sofferenza* from the Obstetrics and Gynecology Departments of the I.R.C.C.S. *Casa Sollievo della Sofferenza* and *Federico II* University of Naples. Recurrent pregnancy loss was defined as the occurrence of three or more fetal losses during the first or second trimester 2 (*i.e.*, up to and including 24 weeks' gestation). Gestational hypertension[4] was defined as a systolic blood pressure >140 mmHg and a diastolic blood pressure > 90 mmHg occurring on two occasions > 4 hrs apart. Significant proteinuria was defined as urine protein excretion > 300 mg/24h.[4] All the women were studied for the presence of antiphospholipid antibodies. Anticardiolipin antibodies and lupus anticoagulant were investigated at least 6 weeks after a pregnancy, and when one or both tests were positive, they were confirmed after 6/8 weeks, as recommended by the SSC criteria.[12] Women with persistent anticardiolipin antibodies and/or lupus anticoagulant, uterine malformations, diabetes mellitus, autoimmune disease, abnormal karyotype (also for partners), infectious disease, pulmonary or renal chronic disease, or thyroid disease were excluded. Thus, 396 women with a history of recurrent pregnancy losses (n=194) or gestational hypertension with/without proteinuria (n=202) were included in the study.

In the same period we randomly selected 359 women with one or more uneventful pregnancies, from the same ethnic background, in order to form a comparison group. Relatives of patients and women with a history of repeated fetal loss or gestational hypertension were not included in this group. None had persistent anticardiolipin antibodies and/or lupus anticoagulant, diabetes mellitus, autoimmune disease, abnormal parental karyotype, infectious disease, pulmonary or renal chronic disease or thyroid disease. Thus, 755 women were analyzed. All the outcomes of these pregnant women were collected during a personal interview or from medical records. In each case the birth-weight was registered. Information on demographic characteristics, a detailed obstetric and reproductive history (previous full-term pregnancies, voluntary and spontaneous abortions, the occurrence of gestational hypertension with or without proteinuria in previous pregnancies) and a history of thrombotic events, were obtained from each woman. All individuals signed informed consent and, after approval of the local Ethics Committees, the study was carried out according to the Principles of the Declaration of Helsinki.

Blood collection and laboratory analysis

Blood was collected in a 1:10 ratio in sodium citrate 0.1 M and the samples were stored at -80°C until DNA extraction. Leukocyte DNA was obtained from frozen blood by standard techniques.[4] To screen the G20210A polymorphism of the FII gene, a 345-bp fragment was obtained and digested using the Hind III endonuclease, according to Poort *et al.*[9] A 220-bp DNA fragment of the FV gene that includes the nucleotide 1691 was amplified by a polymerase chain reaction (PCR), as previously described,[5] with some modifications.[4]

Statistical analysis

All the analyses were performed using the Statistical Package for Social Science (SPSS 6.1 for Macintosh). The significance of differences in means was evaluated by non-parametric tests, whereas the significance of any difference in proportions was tested by the χ^2 statistic. The significance of the difference of alleles and genotypes observed between the groups was tested using χ^2 analysis, after grouping homozygous and heterozygous carriers of the factor V G1691A mutation. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. A general factorial ANOVA model, adjusted for maternal diagnosis of pregnancy losses or gestational hypertension, sex and twin births, was used to investigate the birth-weight of infants in relation to the presence of factor V G1691A or FII A^{20210} mutation in the mothers. A logistic regression analysis with forward stepwise variable selection was used to evaluate the impact of each variable on the association with a history of low birth-weight or growth under the 10th centile. Variables entered into the model were:

Table 1. Clinical features of the whole sample.

	Total	*No mutation carriers*	*FV G1691A carriers**	*FII A^{20210}*
Women ever pregnant n (%)	755 (89.0%)	672 (5.4)	41* (5.8)	41*
Age (mean±SD)	32.0±6.5	32.0±6.6	32.0±6.8	31.9±6.0
Pregnancies n (%)	1790 (89.3%)	1598 (5.7%)	101 (5.3%)	94
Pregnancies ending in a live neonate (%)	1100 (61.5%)	980 (61.3%)	63 * (62.4%)	60 * (63.8%)
Gravidity (mean ± SD)	2.4±1.4	2.4±1.4	2.5±1.4	2.1±1.2
Parity (mean ± SD)	1.5 ±1.2	1.5±1.2	1.5±1.2	1.4±1.0
BMI (kg/m^2)	24.1±4.2	24.0±4.2	25.2±3.9	24.3±4.5
Smoke	48^	41^	3	4

**Factor V G1691A and FII A^{20210} mutations coexisted in two women, who delivered three live neonates; ^six women smoked >10 cigarettes/day.*

Table 2. Birth-weight of neonates delivered by women carrying factor V G1691A or factor II A^{20210} mutations.

	Total	*No mutation*	*Factor V G1691A*	*FII A^{20210}*
Birth-weight <1,000 g	20	14 (70%)	3 (15%)	3 (15%)
Birth-weight >1,000<2,499 g	150	122 (81.3%)	16 (10.7%)	12 (8.0%)
Birth-weight > 2,500* g	933	844 (90.5%)	44 (4.7%)	45 (4.8%)

**Factor V G1691A and FII A^{20210} mutations coexisted in two women, who delivered three live neonates.*

maternal age, parity, factor V G1691A or FII A^{20210} mutation carrier status, the presence of gestational hypertension or fetal loss, body mass index, twin births and smoking habits. Adjusted OR and 95% CI were calculated by logistic-regression models. A two-sided 5% level was considered significant for all statistical tests.

Results

Study Group. The clinical features of the entire group are reported in Table 1. The mean age (±SD) was 32.0±6.5 years. Overall, 1,790 pregnancies were registered and all were analyzed. Of them, 665 (37.1%) finished with a pregnancy loss; 9 (0.5%) were ectopic pregnancies; 16 (0.9%) were excluded because of fetal dysmorphology (n=9) or hydatiform mole (n=4) or gestational trophoblastic disease (n=3). Mean gravidity (±SD) was 2.4±4.4, while the mean parity (±SD) was 1.5±1.2. In the whole group (n=755), 41 (5.4%) women carried the factor V G1691A mutation (one was homozygous for the mutation) and 44 (5.8%) women were heterozygous for the FIIA^{20210} allele. Two women showed both mutations. The frequencies observed were not significantly different from those found in a general population from the same ethnic background.[13] In 1100 (61.4%) pregnancies there was delivery of one live neonate. Among the three groups, the rate of pregnancies ending in delivery of a live neonate was similar (Table 1). No woman reported a history of myocardial infarction, stroke, or cancer. As far as venous thromboembolism is concerned, 8 women showed superficial venous thrombosis (one with the factor V G1691A mutation) and 3 suffered from deep venous thrombosis (one woman had the factor V G1691A mutation).

Thrombophilic mutations and birth-weight. Birth-weights were divided into three groups (Table 2). Neonates delivered by mothers with thrombophilic mutations formed 30% (n=6) of babies weighing < 1,000 g, 18.7% (n=28) of those ranging from 1,001-2,499 g, and 9.5% (n=89) among babies weighing ≥ 2,500 g. Overall, 136 out of 980 (13.9%) neonates from mothers without mutations had a birth-weight < 2,500 g. In mothers carrying either the factor V G1691A or the FII A^{20210} mutation, 34/123 neonates (27.6%) weighed <2,500 g (OR: 2.4, 95% CI: 1.5-3.7).

The estimated risk of delivering a neonate weighing <2,500 g in the presence of one of the mutations described was 3.9 (95% CI: 1.0-15.4) among the 359 women with one or more uneventful pregnancies and 2.0 (95% CI: 1.0-4.0) in the 396 women with a previous obstetric pathology.

A logistic regression model with stepwise selection of variables was used to evaluate the impact of potential confounding variables (different diagnosis, parity, twin birth). This showed that carriership of prothrombotic mutations was significantly associated with a birth-weight < 2,500 g, (OR: 2.0, 95% CI: 1.1-3.6).

The possibility that factor V G1691A and FII A^{20210} mutations could influence birth-weight was further investigated by means of an analysis of variance (factorial ANOVA) that considered the birth-weight of each newborn, in addition to sex, to twin birth and to different clinical subgroups (*i.e.* personal history of fetal loss, gestational hypertension with or without proteinuria). The adjusted mean birth-weight was 3,145 g in the neonates from mothers without prothrombotic genetic risk factors, and 2,934 g (p= 0.013) in women carrying prothrombotic mutations.

Table 3. Growth centiles of neonates of mothers with or without prothrombotic mutations in relationship to maternal carriership of the factor V G1691A or the FIIA^{20210} allele.

	Centile <3		*Centile 4-10*		*Centile 11-25*		*Centile 26-50*		*Centile> 51*	
No mutation	61	83.6%	81	83.5%	149	88.2%	250	89.3%	439	90.7%
FVG1691A	3	4.1%	7	7.2%	11	6.5%	19	6.8%	23	4.8%
FII A^{20210}	9	12.3%	9	9.3%	9	5.3%	11	3.9%	22	4.5%

In this table growth centiles of newborn as related to the material carriership of the factor V G1691A or the FIIA^{20210} allele are showed.

Growth centiles and thrombophilic mutations. We then performed the same analyses considering the birth-weight corrected for gestational age (*i.e.* growth centiles) and evaluated the association between thrombophilic mutations and different growth centiles, using Italian neonatal standard curves (Table 3).[14]

Among neonates under the 10th centile in the group of uneventful pregnancies, 7.7 % (n=53) were born to mothers without prothrombotic mutations and 9.7% (n=7) to mothers with factor V G1691A or FII A^{20210} mutation (p = ns). Among neonates under the <10th centile born to women with gestational hypertension 38.0% (n=70) were from mothers without mutations and 45.3% (n=19) from women with these risk factors (p = ns). Finally, in the group of women with a history of unexplained fetal losses, 18.9% (n=14) of babies <10th centile were born to mothers without and 20% (n=2) to women with factor V G1691A or factor II A^{20210} mutation (p = ns). Overall, 142 (14.5%) and 28 (22.8%) neonates under the 10th centile were, respectively, from mothers without and with thrombophilic mutations (OR 1.7, 95% CI: 1.1-2.7).

A logistic regression model with stepwise selection of variables used to evaluate the impact of potential confounding variables (*i.e.* age, parity, the presence of pregnancy losses, of gestational hypertension, BMI, smoking habit) showed that thrombophilia carrier status was significantly associated with a birth-weight under the 10th centile (OR: 1.7 95%-CI: 1.0-3.0).

Discussion

A successful outcome of a pregnancy depends on satisfactory placental development and sustained placental function. These processes require an adequate feto-maternal circulatory system. The association between heritable thrombophilia and increased fetal losses[2] confirms and extends this concept. The factor V G1691A and the FII A^{20210} mutations have been associated with pregnancy complications,[1,3,4,15] in which a low birth-weight is known to occur. The main goal of this study was to analyze the birth-weight of neonates born to mothers with common causes of inherited thrombophilia, such as factor V G1691A and FII A^{20210} mutations. In most cases of intrauterine fetal growth restriction the time of delivery depends on medical advice. We reasoned that this aspect could have influenced the data analysis: for this reason we decided to use two different models, one using birth-weight regardless of gestational age, and the other one using centiles.

Clark *et al.*[8] recently showed that during pregnancy, an acquired APCR is associated with a low birth-weight. Moreover, prothrombotic mutations have been recognized as a risk factor for preterm birth.[16]

Our data extend the relevance of these findings. In this study, we found that neonates from mothers carrying inherited prothrombotic factors (i.e. factor V G1691A and the FII A^{20210} mutations) had a lower birth-weight than that of neonates from women without such mutations. Overall, neonates from women carrying the factor V G1691A mutation or the FII A^{20210} allele formed 30% of those weighing <1,000 g, 18.7% of those weighing 1,000-2,499 g and 9.5% of those weighing ≥2,500 g.

Our data demonstrated that mothers carrying the factor V G1691A or the FII A^{20210} allele had a significantly higher associated risk of delivering a neonate with a lower birth-weight. This association was independent of potential confounding factors, such as maternal age, parity, body mass index and the presence of obstetric complications such as pregnancy loss or gestational hypertension. When the growth centiles (that are a measure of the fetal growth corrected for gestational age) were considered, both the univariate and the multivariate analyses showed an association between the lowest growth centiles and the presence of prothrombotic mutations. Similarly to findings from a previous model of multivariate analysis, this association was not influenced by potential confounding variables such as maternal diagnosis of pregnancy loss or gestational hypertension, or age and parity.

Twins have lower birth weights than singleton deliveries. In multivariate analyses, adjusting for twin births, the significance obtained in univariate analyses did not change. In addition, exclusion of twins from analyses did not materially change the results obtained (data not shown). It could be hypothesized that the inheritance of one of these

molecular variants at the feto-placental interface may have a role in determining a lower birth-weight.

It is possible that carriers of inherited thrombophilia may more often have a placental disorder than non-carriers, perhaps caused by deficient trophoblast invasion of the maternal spiral arteries, leading to underperfusion of the uteroplacental circulation and placental ischemia.

Contributions and Acknowledgments

EG and MM designed and wrote the study. DC, GP, DP selected and studied the women. GDM and PM critically revised and gave important suggestions for the final version of the manuscript.

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Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

References

1. Kupferminc MJ, Eldor A, Steinman N, Many A, Bar-Am A, Jaffa A, et al. Increased frequency of genetic thrombophilia in women with complications of pregnancy. N Engl J Med 1999; 340:9-13.
2. Grandone E, Margaglione M, Colaizzo D, d'Addedda M, Cappucci G, Vecchione G, et al. Factor V G1691A is associated with repeated and recurrent fetal losses. Thromb Haemost 1997; 77:822-4.
3. Dizon-Townson DS, Nelson LM, Easton K, Ward K. The factor V G1691A mutation may predispose women to severe preeclampsia. Am J Obstet Gynecol 1996; 175: 902-5.
4. Grandone E, Margaglione M, Colaizzo D, Cappucci G, Sciannamé N, Montanaro S, et al. Prothrombotic genetic risk factors and the occurrence of gestational hypertension with or without proteinuria. Thromb Haemost 1999; 81:349-52.
5. Bertina RM, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994; 369:64-7.
6. Rotmensch S, Liberati M, Mittelmann M, Ben-Rafael Z. Activated protein C resistance and adverse pregnancy outcome. Am J Obstet Gynecol 1997; 177:170-3.
7. Dekker GA, de Vries JI, Doelitzsch PM, Huijgens PC, von Blomberg BM, Jakobs C, et al. Underlying disorders associated with severe early-onset preeclampsia. Am J Obstet Gynecol 1995; 173:1042-8.
8. Clark P, Walker ID, Greer I. Acquired activated protein-C resistance and association with increased thrombin generation and fetal weight. Lancet 1999; 353:292-3.
9. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 1996; 88:3698-703.
10. Rai RS, Regan L, Chitolie A, Donald JG, Cohen H. Placental thrombosis and second trimester miscarriage in association with activated protein C resistance. Br J Obstet Gynaecol 1996; 103:842-4.
11. Dizon-Townson DS, Meline L, Nelson LM, Varner M, Ward K. Fetal carriers of the factor V Leiden mutation are prone to miscarriage and placental infarction. Am J Obstet Gynecol 1997; 177:402-5.
12. Brandt JT, Triplett DA, Alving B, Scharrer I. Criteria for the diagnosis of lupus anticoagulants: an update. On behalf of the Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the ISTH. Thromb Haemost 1995; 74:1185-90.
13. Margaglione M, Brancaccio V, Giuliani N, D'Andrea G, Cappucci G, Iannaccone L, et al. Increased risk for venous thrombosis in carriers of the prothrombin G→A20210 gene variant. Ann Intern Med 1998; 129:89-93.
14. SMILA Neonatal Standards for North-East Italy. Montecatini: SEP; 1996. p. 26-31.
15. Pabinger I, Grafenhofer H, Kaider A, Ilic A, Eichinger S, Quehenberger P, et al. Preeclampsia and fetal loss in women with a history of venous thromboembolism. Arterioscler Thromb Vasc Biol 2001; 21:874-9.
16. Göpel W, Kim D, Gortner L. Prothrombotic mutations as a risk factor for preterm birth. Lancet 1999; 353:1411-2.

Peer Review Outcomes

What is already known on this topic

Inherited thrombophilia has been associated with fetal losses and obstetric complications.

What this study adds

This study demonstrates that mothers carrying factor V Leiden or prothrombin 20210A allele have a significantly higher risk of delivering lower birth-weight newborns.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Vicente Vicente, Deputy Editor. The final decision to accept this paper for publication was taken jointly by Professor Vicente and the Editors. Manuscript received October 24, 2001; accepted December 3, 2001.

Potential implications for clinical practice

This observation may suggest that carriers of FV Leiden or FII20210A might have placental disorders, leading to underperfusion of the uteroplacental circulation and placental ischemia.

Vicente Vicente, Deputy Editor