Factor V Leiden and prothrombin G20210A mutations, but not methylenetetrahydrofolate reductase C677T, are associated with recurrent miscarriages

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The aim of this study was to investigate the relationship between recurrent miscarriages and factor V Leiden, prothrombin G20210A and C677T methylenetetrahydrofolate reductase (MTHFR) mutations. In this case-control study the prevalence of factor V Leiden, prothrombin G20210A and C677T methylenetetrahydrofolate reductase mutations was determined in a consecutive series of 80 recurrent miscarriage patients and 100 controls. Fifteen of 80 recurrent miscarriage patients and four out of 100 controls carried the factor V Leiden mutation (19 versus 4%, \( P = 0.003 \), odds ratio 5.5, 95% confidence interval (CI): 1.7–17). Seven of 80 recurrent miscarriage patients and two of 100 controls were carriers of the prothrombin G20210A mutation (9 versus 2%, \( P = 0.038 \), odds ratio 4.6, 95% CI: 0.9–23.2). Six of 80 recurrent miscarriage women and 15 of 100 controls were homozygotes for the C677T MTHFR mutation (8 versus 15%, \( P = 0.134 \), odds ratio: 0.4, 95% CI: 0.1–1.2). Our results suggest that the presence of factor V Leiden and prothrombin G20210A polymorphism, but not MTHFR C677T homozygosity, could be additional risk factors for recurrent miscarriages. Furthermore, it was suggested that the prevalence of factor V Leiden and prothrombin G20210A mutations is more prominent in second trimester, primary fetal losses and it is independent of the existence of additional pathology predisposing to recurrent fetal losses.

Keywords: factor V Leiden/miscarriage/MTHFR C677T mutation/prothrombin G20210A mutation

Introduction

Recurrent fetal loss is a frequent health problem, with three or more affecting 1–2% and two or more affecting up to 5% of women in the reproductive age (Regan, 1998).

Although there is no consensus on the investigation of this condition, it is reasonable to offer a basic evaluation of couples with recurrent pregnancy losses. The tests to be considered are: (i) endocrine evaluation; (ii) hysterosalpingography (HSG); (iii) parental karyotypes; (iv) endometrial biopsy and (v) identification of Lupus anticoagulant, anticardiolipin antibodies and other autoantibodies (ACOG, 1995) (Clifford et al., 1994; Berry et al., 1995).

Thrombophilia is a multigenic disorder caused by inherited and acquired defects. Until now, the investigation of thrombophilia was confined to the deficiency of antithrombin (AT), protein C (PC), protein S (PS), while a number of acquired conditions is known to be related to thrombophilia (surgery, long immobilization, pregnancy, use of oral contraceptives, antiphospholipid syndrome, and obesity). Only recently was it discovered that the most common inherited defect is the resistance to activated protein C (aPCr), which is related, mainly, to the factor V Leiden mutation (Dahlback et al., 1993; Bertina et al., 1994). This defect is quite common in Caucasians (3–7%) (Rees et al., 1995; Lambropoulos et al., 1997) and is responsible for 20–25% of all the cases of isolated thrombotic events and for 40–45% of all the cases of familial thrombophilia. More recently, Poort et al. described a new polymorphism in the 3’untranslated region of the gene of prothrombin, the G20210A polymorphism (Poort et al., 1996). This polymorphism is believed to be related to high prothrombin levels in the plasma of these patients. The G20210A polymorphism is quite common in the normal population (0.7–4.0%) (Rosendaal et al., 1996). This polymorphism results in decreased synthesis of 5-methyltetrahydrofolate, the primary methyl donor in the conversion of homocysteine to methionine, and the resulting increase in plasma homocysteine concentrations is a risk factor for venous and arterial thromboses (Froos et al., 1995; de Franchis et al., 1996; Arruda et al., 1997; Margaglione et al., 1998).

Data accumulated over the past years suggest a possible association between thrombophilia and fetal loss (Brenner and Blumenfeld, 1997). A clear association has been established between fetal loss and certain thrombophilic states, such as antiphospholipid syndrome (APS), antithrombin (AT) deficiency and combined defects (Sanson et al., 1996; Regan, 1998).

While several reports have suggested an increase in the prevalence of aPCr (Rai et al., 1996; Brenner et al., 1997) and factor V Leiden (Grandone et al., 1997; Ridker et al., 1998; Brenner et al., 1999), not all reports concerning fetal loss and factor V Leiden mutation include data supporting this association (Preston et al., 1996; Balasch et al., 1997; Dizonz-Townson et al., 1997; Kutteh et al., 1998; Pauer et al., 1998). These uncertainties about the exact role of factor V Leiden in fetal loss may have resulted from bias concerning the selection of the patients regarding their prior evaluation of other known
causes of fetal loss, the number of previous miscarriages, and the type of miscarriage (first or second trimester, primary or secondary).

Moreover, there are a few reports (Kutteh et al., 1998; Brenner et al., 1999) evaluating the role of prothrombin G20210A mutation and MTHFR C677T polymorphisms and recurrent fetal losses. The aim of this case-control study was to investigate the relationship between recurrent miscarriages and factor V Leiden, prothrombin G20210A and C677T methylenetetrahydrofolate reductase mutations.

Materials and methods
In this case-control study the prevalence of factor V Leiden, prothrombin G20210A and C677T methylenetetrahydrofolate reductase mutations was determined in a consecutive series of 80 women referred for evaluation of recurrent spontaneous pregnancy loss (case patients) and 100 women with at least one successful pregnancy and no history of pregnancy loss (controls).

Recurrent miscarriage patients were Greek women (age range 28–42 years; mean 33), referred for evaluation at a university hospital recurrent miscarriage clinic. Eligibility criteria were a history of two or more spontaneous miscarriages. Thirty-five women had two abortions, 27 had three, and 18 had more than three. Sixty-one out of 80 had first trimester and the remaining 19 had second trimester miscarriages. On the other hand, 55 out of 80 had primary and the remaining 25 had secondary miscarriages. All women had been previously investigated for autoantibodies, hormonal status, coagulation disorders other than factor V Leiden and prothrombin G20210A polymorphism, uterine anatomical anomalies with hysterosalpingography (HSG) and karyotype. Of these one woman had an unbalanced reciprocal translocation [45,XX,t(13;14)(p11q11)] Robertsonian type], five were positive for APA, one had autoantibodies other than APA, nine had uterine septum, six had polycystic ovary syndrome (PCO), five had uterine fibroids. None of the patients had a history of thrombo-embolic event.

The control group consisted of 100 age-matched women (age range 30–45 years, mean 35) of the same ethnic origin. They were medical personnel or were hospitalized in the gynaecology department for causes not pertaining to miscarriages. They had no personal or family history of thrombosis.

All the subjects gave informed consent for the study.

Methods
DNA was extracted from whole blood as per standard protocols.

Factor V Leiden mutation
We performed a hot start polymerase chain reaction (PCR) using the primers FV1 and FV2 as previously described. The PCR product was digested by the restriction enzyme Mnl I (Beauchamp et al., 1994).

Prothrombin G20210A polymorphism
We performed a hot start PCR using the primers PT1 and PT2 followed by digestion of the product with the restriction enzyme Hind III (Makris et al., 1997).

Methylenetetrahydrofolate reductase C677T homozygosity
Amplification of a 198bp DNA fragment was performed, followed by Hinf I digestion, as described elsewhere (Kluijtmans et al., 1998).

Statistical analysis
The \( \chi^2 \) statistic was used to test the significance of any difference in the prevalence of factor V Leiden, the prothrombin G20210A mutation and the MTHFR C677T homozygosity between recurrent miscarriage patients and controls. The odds ratio was used as a measure of the strength of the association. All \( P \) values were two tailed, and 95% CI were calculated.

Results
Concerning the factor V Leiden mutation, 15 out of 80 recurrent miscarriage patients and four out of 100 controls carried the factor V Leiden mutation (19 versus 4%, \( P = 0.003, \) odds ratio 5.5, 95% CI: 1.7–17). No factor V Leiden homozygote was found in the whole setting.

Concerning the prothrombin G20210A polymorphism, seven out of 80 recurrent miscarriage patients and two out of 100 controls were carriers of the prothrombin G20210A mutation (9 versus 2%, \( P = 0.038, \) odds ratio 4.7, 95% CI: 0.9–23).

One recurrent miscarriage woman was compound heterozygote, i.e. carrier of both the factor V Leiden and prothrombin G20210A mutation.

Concerning the C677T methylenetetrahydrofolate reductase mutation, six out of 80 recurrent miscarriage women and 15 out of 100 controls were homozygotes for the C677T methylenetetrahydrofolate reductase mutation (8 versus 15%, \( P = 0.134, \) odds ratio: 0.4, 95% CI: 0.1–1.2). There was one case of a woman who was carrying both the MTHFR C677T and the prothrombin mutation.

These results suggest that factor V Leiden and prothrombin G20210A mutation, but not C677T methylenetetrahydrofolate reductase homozygosity, may be predisposing factors for recurrent miscarriages.

Of the 15 women who were carriers of the factor V Leiden mutation six had additional pathologies, which could potentially predispose them to recurrent miscarriage (two had fibroids, one had intrauterine septum, one had autoantibodies and two had PCO). Of the seven women who were carriers of the prothrombin G20210A polymorphism one had fibroids and PCO and one had intrauterine septum. The comparison of women without additional pathology and controls is shown in Table I.

Interestingly, there is no statistical difference in the prevalence of factor V Leiden and prothrombin G20210A mutations between women who have additional pathology and those who have not.

In order to investigate whether women with three or more miscarriages more frequently carry the factor V Leiden and prothrombin G20210A mutations than women with only two miscarriages, the prevalence of these two mutations is compared between the two groups of patients. Three out of 35 women with two recurrent fetal losses (8.5%) and 12 out of 45 with three or more (26.6%) carried the factor V Leiden mutation (\( P = 0.04 \)). Two out of 35 women with two recurrent fetal losses (5.7%) and four out of 45 with three or more (8.8%) carried the prothrombin G20210A polymorphism (\( P = 0.39 \)).

From the 61 women with first trimester abortions, nine (14.7%) were carrying the factor V Leiden and five (8.1%) the prothrombin mutation. From the 19 women with second trimester abortions six (31.5%) were carrying the factor V Leiden and two (10.5%) the prothrombin mutation (Table II).
These results suggest that the prevalence of factor V Leiden is more prominent in second trimester fetal loss, while the prevalence of prothrombin G20210A does not differ between the two groups.

Finally, the prevalence of these two mutations is compared between the 55 women who had primary and 25 with secondary recurrent miscarriage and controls (Table III). Both factor V Leiden and prothrombin G20210A constitute a significant risk factor if primary recurrent miscarriages are considered.

**Discussion**

The present report documents a clear association between factor V Leiden mutation and fetal loss (odds ratio = 5.5). The prevalence of factor V Leiden mutation is more prominent among women with primary, three or more, second trimester (although it is still statistically significant in first trimester) abortions, while it does not differ between women with and without additional pathology.

Factor V Leiden is a common mutation in Caucasians (Rees et al., 1995), and the prevalence of 4% in the control group is similar with the 4.3% reported for the population of Greece (Lambropoulos et al., 1997).

Three recent case-control studies have documented an association between factor V Leiden and fetal loss (Grandone et al., 1997; Ridker et al., 1998; Brenner et al., 1999) and demonstrated a 4-fold, 2.2-fold and 4-fold increase correspondingly in the prevalence of factor V Leiden in women with fetal loss compared to controls. The case patients were unselected in the work of Grandone et al. and Ridker et al. and selected in the study of Brenner et al. Both Grandone et al. and Brenner et al. found a more significant risk in second than in first trimester recurrent fetal losses.

On the other hand, there is a number of reports suggesting that factor V Leiden is not a predisposing factor for recurrent fetal loss (Balasch et al., 1997; Dizon-Townson et al., 1997; Kutteh et al., 1998; Pauer et al., 1998).

Dizon-Townson et al. found that none of the 40 selected women with three or more recurrent fetal losses (22 of first and 18 of second trimester) and none of the 25 female controls carried the factor V Leiden mutation. In contrast, the mutation was present in one male partner for each group (Dizon-Townson et al., 1997). Since the carrier status of the male partner does not affect the pregnancy outcome (Preston et al., 1996), the results are not relevant and larger numbers of cases and controls should be expected to be enrolled in the study.

Pauer et al. compared the prevalence of factor V Leiden between 84 unselected women with a history of two or more fetal losses (64 of first and 20 of second trimester) and 87 unselected controls and found that 10.7% of patients and 9.2% of controls were carriers of factor V Leiden, while the frequency of carriership was more prominent in the group of

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**Table I.** Comparison of the prevalence of factor V Leiden and prothrombin G20210A mutations between recurrent miscarriage women without additional pathology and controls

<table>
<thead>
<tr>
<th>Type of genetic defect</th>
<th>Recurrent miscarriage women without additional pathology (n = 53)</th>
<th>Controls (n = 100)</th>
<th>Odds ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor V Leiden mutation n (%)</td>
<td>9 (17)</td>
<td>4 (4)</td>
<td>4.90 (1.43–16.80)</td>
<td>0.006</td>
</tr>
<tr>
<td>Prothrombin G20210A mutation n (%)</td>
<td>5 (9)</td>
<td>2 (2)</td>
<td>5.10 (0.95–27.27)</td>
<td>0.035</td>
</tr>
<tr>
<td>Presence of either mutation n (%)</td>
<td>14 (26)</td>
<td>6 (6)</td>
<td>5.62 (2.01–15.70)</td>
<td>0.0006</td>
</tr>
</tbody>
</table>

CI = confidence interval.

**Table II.** Comparison of the prevalence of factor V Leiden and prothrombin G20210A mutations between women with recurrent miscarriages of first and second trimester and controls

<table>
<thead>
<tr>
<th>Type of genetic defect</th>
<th>First trimester miscarriages (n = 61)</th>
<th>Controls (n = 100)</th>
<th>Odds ratio (95% CI)</th>
<th>P value</th>
<th>Second trimester miscarriages (n = 19)</th>
<th>Controls (n = 100)</th>
<th>Odds ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVL mutation n (%)</td>
<td>9 (14.7)</td>
<td>4 (4)</td>
<td>4.1 (1.2–14)</td>
<td>0.01</td>
<td>6 (31.5)</td>
<td>4 (4)</td>
<td>11 (2.7–44)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>II G20210A mutation n (%)</td>
<td>5 (8.1)</td>
<td>2 (2)</td>
<td>4.3 (0.8–22)</td>
<td>0.06</td>
<td>2 (10.5)</td>
<td>2 (2)</td>
<td>5.7 (0.7–43)</td>
<td>0.06</td>
</tr>
<tr>
<td>Either mutation n (%)</td>
<td>14 (23)</td>
<td>6 (6)</td>
<td>4.6 (1.6–13)</td>
<td>0.001</td>
<td>8 (42.1)</td>
<td>6 (6)</td>
<td>11 (3.3–39)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Table III.** Comparison of the prevalence of factor V Leiden and prothrombin G20210A mutations between women with primary and secondary recurrent miscarriages and controls

<table>
<thead>
<tr>
<th>Type of genetic defect</th>
<th>First trimester miscarriages (n = 55)</th>
<th>Controls (n = 100)</th>
<th>Odds ratio (95% CI)</th>
<th>P value</th>
<th>Secondary miscarriages (n = 25)</th>
<th>Controls (n = 100)</th>
<th>Odds ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVL mutation n (%)</td>
<td>14 (25.4)</td>
<td>4 (4)</td>
<td>8.2 (2.5–26)</td>
<td>&lt;0.001</td>
<td>1 (4)</td>
<td>4 (4)</td>
<td>1.0 (0.1–9.3)</td>
<td>1.0</td>
</tr>
<tr>
<td>II G20210A mutation n (%)</td>
<td>6 (10.9)</td>
<td>2 (2)</td>
<td>6 (1.1–30)</td>
<td>0.02</td>
<td>1 (4)</td>
<td>2 (2)</td>
<td>2.0 (0.1–23)</td>
<td>0.5</td>
</tr>
<tr>
<td>Either mutation n (%)</td>
<td>20 (36.3)</td>
<td>6 (6)</td>
<td>8.9 (3.3–24)</td>
<td>&lt;0.001</td>
<td>2 (8)</td>
<td>6 (6)</td>
<td>1.3 (0.2–7.1)</td>
<td>0.7</td>
</tr>
</tbody>
</table>
second trimester fetal losses (15 versus 9.4%) (Pauer et al., 1998). This study might have possible bias in the control population, i.e. they were a group of unselected persons who could have suffered thrombosis, thus would be expected to have a higher prevalence of factor V Leiden.

Kutteh et al. studied 50 unselected women with three or more fetal losses of first (91.7%) and second trimester (8.3%). A total of 28 of them had primary and 22 secondary fetal losses. The control group consisted of 50 women without history of thrombosis or miscarriage. There was no statistical difference in the prevalence of factor V Leiden between the two groups, i.e. 2 versus 4% (Kutteh et al., 1998). The possible bias in this study could be in the case group, which consisted of women with mainly first trimester, secondary abortions, where the prevalence of the mutation seems to be much lower.

Balasch et al. studied 55 selected patients with two or more miscarriages of first trimester and 50 healthy controls. They found that one patient out of 55 and one control out of 50 had phenotypic activated protein C resistance (Balasch et al., 1997). They studied only first trimester abortions and this could limit the prevalence of the mutation in the study group.

Prothrombin G20210A was reported to be more common in Southern Europe (3%) (Rosendaal et al., 1998), while in Greece the rate is about 2.8% (unpublished data), which is in agreement with the prevalence of 2% in our controls. Prothrombin G20210A was significantly more common in women with fetal loss than in controls, accounting for a 4-fold risk. The prevalence of this polymorphism does not differ significantly between women with two and three or more fetal losses, while it confers a significant risk for primary miscarriages. Nevertheless, the association of prothrombin G20210A polymorphism and fetal losses does not reach significance if only first or second trimester fetal losses are considered. This discrepancy may be due to the size of the study groups, in view of the relatively low prevalence of this mutation. Studies involving a larger number of patients are needed to confirm the potential association of fetal loss and prothrombin G20210A polymorphism.

The present study is in partial agreement with Brenner et al., who found an odds ratio of 2.4 regarding the prevalence of prothrombin G20210A, but their results did not reach significance (Brenner et al., 1999). On the other hand, Kutteh et al. did not find any association between prothrombin G20210A polymorphism and fetal losses (Kutteh et al., 1998).

Concerning the MTHFR C677T homozygosity, our data suggest that it does not account for recurrent fetal loss and agree with that of Brenner et al. and Kutteh et al. (Kutteh et al., 1998; Brenner et al., 1999). This could be explained by the fact that the role of the MTHFR C677T homozygosity in the pathogenesis of venous or arterial thrombosis, although it is related to high homocysteine levels, is controversial (Frost et al., 1995; de Franchis et al., 1996; Arruda et al., 1997; Kluijtmans et al., 1998). Homozygosity for MTHFR in the absence of hyperhomocysteinemia is, if at all, a mild risk factor for venous thrombosis (Kluijtmans et al., 1998) and, therefore, it might be useful to determine the homocysteine levels in the study women (Wouters et al., 1993). On the other hand, hyperhomocysteinemia is a situation that is easily corrected by the use of folic acid during pregnancy, thus confounding the results.

Taken together the data of the present report suggest that factor V Leiden and prothrombin G20210A can be found in approximately one out of four women with a history of fetal loss, whether they carry additional pathology or not. The prevalence is more prominent when it comes to second trimester (one out of 2.5 women), or primary fetal loss (one out of three women). Nevertheless, in order to evaluate the necessity of routinely screening recurrent miscarriage women for thrombophilic mutations and intervening therapeutically, large-scale prospective studies should be performed.

References


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