Interaction Between the G20210A Mutation of the Prothrombin Gene and Oral Contraceptive Use in Deep Vein Thrombosis

Ida Martinelli, Emanuela Taioli, Paolo Bucciarelli, Sepideh Akhavan, Pier Mannuccio Mannucci

Abstract—Single-point mutations in the gene coding for prothrombin (factor II:A20210) or factor V (factor V:A1691) are associated with an increased risk of venous thromboembolism. The use of oral contraceptives is also a strong and independent risk factor for the disease, and the interaction between factor V:A1691 and oral contraceptives greatly increases the risk. No information is available about the interaction between oral contraceptives and mutant prothrombin. We investigated 148 women with a first, objectively confirmed episode of deep vein thrombosis and 277 healthy women as controls. Fourteen patients (9.4%) were carriers of factor II:A20210, 24 (16.2%) of factor V:A1691, and 4 (2.7%) of both defects. Among controls, the prevalence was 2.5% for either factor II:A20210 or factor V:A1691, and there was no carrier of both the mutations. The relative risk of thrombosis was 6-fold for factor II:A20210 and 9-fold for factor V:A1691. The most prevalent circumstantial risk factor in patients and the only one observed in controls was oral contraceptive use, which per se conferred a 6-fold increased risk of thrombosis. The risk increased to 16.3 and 20.0 when women with factor II:A20210 or factor V:A1691 who used oral contraceptives were compared with noncarriers and nonusers. These figures indicate a multiplicative interaction between the genetic risk factors and oral contraceptives. No difference in the type of oral contraceptives was observed between patients and controls, those of third generation being the most frequently used (73% and 80%). We conclude that carriers of the prothrombin mutation who use oral contraceptives have a markedly increased risk of deep vein thrombosis, much higher than the risk conferred by either factor alone. (Arterioscler Thromb Vasc Biol. 1999;19:700-703.)

Key Words: venous thrombosis □ prothrombin mutation □ factor V mutation □ oral contraceptives

In the last few years the discovery of 2 point mutations, one in the factor V gene that is responsible for resistance to activated protein C (factor V:A1691)1 and the other in the 3'-untranslated region of the prothrombin gene (factor II:A20210),2 has broadened the spectrum of inherited thrombophilia. These mutations are common in the general population (on average, 2% to 3%), and their prevalence varies in different countries, factor V:A1691 being more frequent in Northern Europe and factor II:A20210 in the south.3,4 They are also the most common genetic defects associated with venous thrombosis, accounting, respectively, for 12% to 20%5,6 and 5% to 19%7-13 of patients selected with varying criteria. Carriers of factor V:A1691 or factor II:A20210 have a moderately increased risk of venous thrombosis, which has been estimated to be 3- to 7-fold for factor V:A16911,14 and 2- to 12-fold for factor II:A20210.2,7-13 An important factor in the evaluation of the relative risk of venous thrombosis in carriers of thrombophilic defects is the concomitant presence of circumstantial risk factors. It has been reported that oral contraceptive users who are carriers of factor V:A1691 have a 30-fold increased risk of deep vein thrombosis relative to noncarriers and nonusers.15 No information on the effect of the combination of factor II:A20210 and oral contraceptive use is available so far. Therefore, we performed a case-control study to evaluate the interaction between genetic thrombophilic defects and circumstantial risk factor for deep vein thrombosis in carriers of the mutation, with special emphasis on the use of oral contraceptives.

Methods

Patients
One hundred sixty-two unrelated women with a first, objectively documented episode of deep vein thrombosis of the lower extremities were consecutively referred to our thrombosis center to be investigated for thrombophilia from April 1995 to April 1998. For 148 of them (86%), DNA was available for testing and therefore they were included in this study. Their median age was 30 years (range, 6 to 71 years) at the time of thrombosis and 35 years (range, 6 to 75 years) at the time of blood sampling. Deep vein thrombosis involved a lower limb in 142 patients (96%), and it was bilateral in 6 patients (4%). All patients were apparently free from cancer or autoimmune disorders.

Controls
The control population consisted of 277 healthy women who were friends or partners of the whole population of patients referred to our
thrombosis center in the same 3-year study period, with no genetic relationship with them. Their median age at the time of blood sampling was 46 years (range, 13 to 76 years). Previous thrombosis was excluded using a structured questionnaire validated for the retrospective diagnosis of venous thromboembolism.16

Information on oral contraceptive use at the time of thrombosis (for patients) or at the time of blood sampling (for controls) was recorded. Women were considered oral contraceptive users if they had taken the pill until 2 weeks or less before the thrombotic episode. One hundred twelve patients and 179 controls were of reproductive age (15 to 48 years); among them, 42 patients (64%) and 45 controls (25%) were oral contraceptive users. The type of oral contraceptive was also recorded, and the pills were classified into 3 categories, according with the dose of estrogen and the type of progestin: first generation (containing 50 μg or more of ethinyl-estradiol), second generation (30 μg of ethinyl-estradiol and levonorgestrel or norgestimate as progestin), and third generation (30 μg or less of ethinyl-estradiol and gestodene or desogestrel as progestin). In addition to oral contraceptive use, the presence of other circumstantial risk factors such as surgery, trauma, prolonged immobilization, pregnancy, or postpartum status was recorded in patients and controls.

Laboratory Tests
Blood samples were drawn into vacuum tubes containing 129 mmol/L sodium citrate as anticoagulant with a ratio of 9 parts blood and 1 part citrate solution. DNA analysis for the G20210A prothrombin and the G1691A factor V mutations were performed as previously described by Poort et al.17 and de Ronde and Bertina.18 Because a screening for thrombophilia includes also the search for deficiencies of the naturally occurring inhibitors and the antiphospholipid syndrome, antithrombin, protein C, and protein S plasma levels were measured, and the presence of lupus anticoagulant and anticardiolipin antibodies was looked for. Antithrombin heparin cofactor activity and protein C activity were tested; if low results were obtained, antigen levels and functional activity in the absence of heparin and by immunoelectrophoresis with or without heparin for antithrombin, and antigen levels for protein C were measured. Protein S was assayed by total and free antigen measurements.20 Protein C, protein S levels, and lupus anticoagulant were not evaluated in 22 patients (15%) who were on oral anticoagulant therapy at the time of blood sampling, because measurements of vitamin K-dependent proteins are affected by this treatment.

Statistical Analysis
Student’s t test was used to compare the age at thrombosis of patients with single or double thrombophilic defect. The χ2 test was used to compare the observed prevalence of the combined defect with the expected prevalence. Odds ratios were considered as an approximation of the relative risks and 95% CI were calculated according to Woolf.21 Using a logistic regression model, odds ratios were adjusted by age and the presence of other thrombophilic defects. In the same model we evaluated the interaction between oral contraceptive use and the presence of either factor II:A20210 or factor V:A1691. With this analysis, a relative risk close to 1.0 indicates a multiplicative interaction.22 Prevalence of oral contraceptive use and pregnancy or postpartum status was calculated on the number of women of reproductive age. Because oral contraceptives and pregnancy or postpartum condition mutually exclude each other, the prevalence and thrombotic risk associated with oral contraceptives were calculated excluding women who were pregnant or in the postpartum period at the time of thrombosis, and the prevalence of pregnancy or postpartum status excluding oral contraceptive users.

Results
Among the 277 controls, 7 (2.5%) were carriers of factor II:A20210 and 7 (2.5%) of factor V:A1691, none of them being homozygote or carrier of both the defects. Fourteen patients (9.4%) were heterozygous carriers of factor II:A20210 and 24 (16.2%) (2 homozygotes and 22 heterozygotes), were carriers of factor V:A1691. There was no homozygous carrier of factor II:A20210. Four patients (2.7%) were heterozygous carriers of both the mutations. This 2.7% prevalence was higher than the 0.06% prevalence expected by multiplying the frequencies of the single mutations in the control group. The median age at thrombosis was 30 years (range, 18 to 63 years) for carriers of factor II:A20210, 29 years (range, 15 to 57 years) for carriers of factor V:A1691, and 30 years (range, 18 to 39 years) for double carriers (differences not statistically significant). Sixteen patients (10.8%) and 3 controls (1%) had other thrombophilic defects. Among patients, 2 had antithrombin deficiency, 5 protein C deficiency, 2 protein S deficiency, and 7 lupus anticoagulant. Two controls had antithrombin deficiency and 1 had protein S deficiency.

Table 1 shows the relative risk of deep vein thrombosis for carriers of the prothrombin or factor V mutations. After adjustment for the presence of other thrombophilic conditions, the odds ratio for factor II:A20210 was 5.7 (95% CI, 2.2 to 14.6) and that for factor V:A1691 was 9.7 (95% CI, 3.9 to 23.8). Excluding from the analysis the 2 homozygous carriers of factor V:A1691, the risk associated with this mutation did not substantially change (odds ratio, 9.1 [95% CI, 3.6 to 23.1]). As none of the controls was a carrier of the combined defect, the risk of thrombosis for double carriers could not be calculated.

Table 2 shows that the distribution of circumstantial risk factors predisposing to deep vein thrombosis was roughly similar in carriers of factor II:A20210 and factor V:A1691 and in those with the combined defect, with oral contraceptive use being the most frequent. The effect of oral contraceptives in determining thrombosis and their interaction with genetic thrombophilic defects were analyzed in more details in 313 women of reproductive age, 179 controls and 134 patients. Among controls, 6 (3.4%) had factor II:A20210 and 5 (2.8%) had factor V:A1691. Among patients, 12 (9.0%) had factor II:A20210, 21 (15.7%) had factor V:A1691, and 4 (3.0%) had the combined defect. In these women, the adjusted odds ratio was 4.6 (95% CI, 1.7 to 12.7) for the prothrombin mutation and 6.0 (95% CI, 2.0 to 17.4) for the factor V mutation. After exclusion of 19 patients who were pregnant or in the postpartum period at the time of thrombosis, the prevalence of oral contraceptive users was 64% (74/115) among patients and 25% (45/179) among controls, and the risk conferred by oral contraceptives was 5.6 (95% CI, 3.3 to 9.6). To compare the type of oral contraceptives used by patients at the time of thrombosis with that used by controls at the time of blood sampling, we limited the analysis to patients who had thrombosis in the 3-year study period, because the prescription of oral contraceptives may have changed over time. Fifteen of the 74 patients (20%) on oral contraceptives were therefore excluded because they had thrombosis before 1995. Both in patients and controls the most frequently used oral contracept-

### Table 1. Risk of Deep Vein Thrombosis According to Genetic Status

<table>
<thead>
<tr>
<th></th>
<th>Patients (n=148)</th>
<th>Controls (n=277)</th>
<th>Odds Ratio (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No defect</td>
<td>106 (71.6%)</td>
<td>263 (94.9%)</td>
<td>1 (Reference)</td>
</tr>
<tr>
<td>Factor II:A20210</td>
<td>14 (9.4%)</td>
<td>7 (2.5%)</td>
<td>5.7 (2.2-14.6)</td>
</tr>
<tr>
<td>Factor V:A1691</td>
<td>24 (16.2%)</td>
<td>7 (2.5%)</td>
<td>9.7 (3.9-23.8)</td>
</tr>
<tr>
<td>Factor II:A20210 and factor V:A1691</td>
<td>4 (2.7%)</td>
<td>0</td>
<td>∞</td>
</tr>
</tbody>
</table>

*Adjusted for age and the presence of other thrombophilic defects.
Oral Contraceptives and Prothrombin Mutation in Deep Vein Thrombosis

TABLE 2. Distribution of Circumstantial Risk Factors at Time of Thrombosis in All Patients and in Those With Prothrombin Mutation, Factor V Mutation, or Both

<table>
<thead>
<tr>
<th></th>
<th>All Patients</th>
<th>Carriers of Factor II:A20210</th>
<th>Carriers of Factor V:A1691</th>
<th>Combined Carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=148)</td>
<td>(n=14)</td>
<td>(n=24)</td>
<td>(n=4)</td>
</tr>
<tr>
<td>None</td>
<td>35 (24%)</td>
<td>3 (21%)</td>
<td>4 (17%)</td>
<td>1 (25%)</td>
</tr>
<tr>
<td>Surgery</td>
<td>10 (18%)</td>
<td>1 (7%)</td>
<td>1 (4%)</td>
<td>0</td>
</tr>
<tr>
<td>Trauma/Immobilization</td>
<td>10 (18%)</td>
<td>1 (7%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Oral contraceptives*</td>
<td>74 (64%)</td>
<td>9 (75%)</td>
<td>11 (85%)</td>
<td>2 (67%)</td>
</tr>
<tr>
<td>Pregnancy/postpartum†</td>
<td>19 (32%)</td>
<td>0</td>
<td>8 (80%)</td>
<td>1 (50%)</td>
</tr>
</tbody>
</table>

*Prevalence is calculated on the number of women of reproductive age, excluding those who were pregnant or in the postpartum period at the time of thrombosis.
†Prevalence is calculated on the number of women of reproductive age, excluding those who were on oral contraceptives at the time of thrombosis.

Discussion

In this case-control study performed in women with a first episode of deep vein thrombosis, we confirm that the 2 most common genetic thrombophilic defects, ie, factor V:A1691 and factor II:A20210, are strongly associated with an increased risk for the disease. We also found that this risk increased markedly when oral contraceptive use was associated with carriernesship of either factor V or prothrombin mutation. Although the risk conferred by the presence of both the defects could not be estimated because of the absence of double carriers among controls, we found that the 2.7% prevalence of the combined defect observed in patients was 35 times higher than that expected, indicating a strong tendency to thrombosis in these patients.23–26

Oral contraceptive use is associated with a 4-fold increased risk of venous thrombosis, independent of the presence of other genetic or acquired risk factors.27 It has been reported that women with factor V mutation who use oral contraceptives have an increased risk of deep vein thrombosis (30-fold) in comparison with noncarriers and nonusers.15 We found a similar effect of oral contraceptives in carriers of the mutant prothrombin. This finding extends preliminary results recently obtained by us in a small, highly selected group of women with deep vein thrombosis.28 In this series of patients consecutively referred to us for a screening of thrombophilia, we observed that the majority of women had developed deep vein thrombosis in the presence of a circumstantial risk factor, such as oral contraceptive use or pregnancy or postpartum status. Women of reproductive age had a risk of thrombosis associated with the use of oral contraceptives that was quite similar to the risk associated with the presence of mutant prothrombin (odds ratios, 5.6 and 4.6, respectively). The risk of thrombosis for carriers of the mutation who used oral contraceptives was 16-fold, indicating a multiplicative interaction of the 2 risk factors. Obviously, this estimate has to be considered approximate, because it is affected by the small number of women (only 2 among controls) with both risk factors. It is likely that the same limitation has affected the estimates of the risks in carriers of either prothrombin or factor V mutation who did not use the pill (Table 3). However, the magnitude of the risk in carriers of the prothrombin mutation who used oral contraceptives was 65% higher than the additive effect of the 2 single risks, suggesting a strong interaction, although larger studies are needed to obtain a more reliable estimate of the risk. From a biochemical point of view, it has been reported that mutant prothrombin2 and oral contraceptives are independently associated with elevated plasma levels of prothrombin. A hypothesis that should be evaluated is whether the presence of both risk factors leads to particularly high plasma levels of prothrombin.

Another aim of this study was to look at the type of oral contraceptives, as those of third generation, which contain 30 μg or less of ethinyl-estradiol and gestodene or desogestrel as progestin, have been claimed to be associated with a 2-fold

TABLE 3. Risk of Thrombosis for Women, According to Genotype and Use of Oral Contraceptives

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
<th>Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal genotype without oral</td>
<td>35</td>
<td>127</td>
<td>1 (Reference)</td>
</tr>
<tr>
<td>contraceptives</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal genotype with oral</td>
<td>52</td>
<td>41</td>
<td>4.6 (2.6–8.0)</td>
</tr>
<tr>
<td>contraceptives</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor II:A20210 without oral</td>
<td>3</td>
<td>4</td>
<td>2.7 (0.6–12.7)</td>
</tr>
<tr>
<td>contraceptives</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor V:A1691 without oral</td>
<td>2</td>
<td>3</td>
<td>2.4 (0.4–15.1)</td>
</tr>
<tr>
<td>contraceptives</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor II:A20210 with oral</td>
<td>9</td>
<td>2</td>
<td>16.3 (3.4–79.1)</td>
</tr>
<tr>
<td>contraceptives</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor V:A1691 with oral</td>
<td>11</td>
<td>2</td>
<td>20.0 (4.2–94.3)</td>
</tr>
<tr>
<td>contraceptives</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Noncarriers and nonusers were the reference group.
increased risk of venous thromboembolism relative to the older second-generation preparations, containing levonorgestrel as progestin. We found no association between thrombosis and the type of oral contraceptives, those of third generation being the most frequently used both among patients and controls (73% and 80%, respectively). The high prevalence of third-generation pills in the control population is in contrast to that reported by investigators from other countries, but in agreement with the global pill consumption in the Italian general population.

Our findings of a strong interaction between oral contraceptive use and genetic defects raise the question whether or not screening for the prothrombin and factor V mutations should be recommended before prescribing oral contraceptives. It has already been stated that screening for mutant factor V is not cost-effective, because the absolute risk of venous thromboembolism is approximately 0.5/10,000 per year for women aged <45 years. This negative consideration can now be extended also to the mutant prothrombin, because to prevent one episode of deep vein thrombosis, oral contraceptives should be withheld from approximately 800 carriers of the mutations. Furthermore, 14,000 women should be tested to identify 800 carriers, which indicates that at present indiscriminate screening for both the mutations is not worthwhile. Currently, we recommend withholding oral contraceptives from carriers who have experienced thrombosis while taking the pill, considering alternative methods of contraception in those who have had thrombosis in the absence of the pill, and carefully evaluating each woman for the presence of other risk factors, such as a positive family history of thrombosis, that may contraindicate use of the pill.

References

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