Venous thromboembolism in young women

Role of thrombophilic mutations and oral contraceptive use

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Aims The interaction between the R506Q mutation of factor V and the G20210A mutation of prothrombin with oral contraceptives on venous thromboembolism was evaluated.

Methods and Results Three hundred and one women of reproductive age who had venous thromboembolism (140 while using oral contraceptives) and 650 healthy women (173 on oral contraceptives at presentation) were examined. Of the patients, 19.3% were carriers of R506Q (two homozygotes) and 9.6% were heterozygous carriers of G20210A; eight patients (2.7%) were heterozygous for both mutations. Among controls, 2.9% were carriers of R506Q, 3.1% of G20210A, while one case was a heterozygous carrier of both mutations. The relative risk (odds ratio) associated with carriership of R506Q or G20210A mutations was 10.3 and 4.7, respectively; it was 45.6 in carriers of both mutations. The odds ratio of using oral contraceptives in the absence of both mutations was 2.4. The odds ratios according to oral contraceptives use and the presence of R506Q or G20210A or both mutations were 41.0, 58.6 and 86.5, respectively. While the odds ratio for R506Q remains elevated (8.9) in non-oral contraceptive users, the odds ratio for G20210A was 2.0 and did not reach statistical significance.

Conclusions Our data showed a strong interaction between oral contraceptive use and the presence of either R506Q or G20210A mutations. In non-oral contraceptive users the risk of venous thromboembolism was significantly increased in carriers of R506Q but not in those with the G20210A mutation.


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Key Words: Factor V Leiden, G20210A prothrombin mutation, oral contraceptives, venous thromboembolism, risk factors.

Introduction

Patients with inherited thrombophilia typically present with venous thromboembolism, especially deep vein thrombosis of the lower limb and pulmonary embolism. The past few years have seen a whole series of discoveries regarding inherited thrombophilias. In 1994, Bertina et al.[1] described R506Q factor V mutation as a cause of the activated protein C resistance phenomenon. More recently, Poort et al.[2] reported that G20210A mutation in the prothrombin gene is associated with an increased risk of venous thrombosis.

At present, R506Q factor V and G20210A prothrombin mutations are the most common causes of familial thrombophilia. R506Q factor V mutation occurs in 3–5% of the general population and in about 20% of unselected patients suffering from venous thromboembolism. Based on case-control studies the risk of venous thromboembolism appears to increase five- to 10-fold in heterozygous carriers of this mutation[3]. In the general population, the prevalence of the G20210A prothrombin mutation is about 2–3%, although this variant seems to be more prevalent in some geographical areas[4]. Among unselected patients with previous venous thromboembolism the mutation has been found in 6%/2, and seems to be a moderate risk factor, increasing the risk two- to four-fold. In recent years many other groups[5–8] have confirmed the initial observation made by Poort et al.[2] on the association between venous thromboembolism and the
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Patients and methods

Patient group

Between September 1994 and July 2000, 760 unrelated women were consecutively referred to our department for investigation of possible thrombophilic states after experiencing at least one venous thromboembolism event. For the present analysis only women who had experienced venous thromboembolism events during reproductive age (n=471) were considered. After exclusion of cases with isolated pulmonary embolism (n=34), superficial thrombophlebitis (n=76), deep vein thrombosis in an upper limb (n=28) or unusual sites (n=32), 301 women with objectively confirmed deep vein thrombosis of a lower limb, with/without pulmonary embolism, were included (characteristics reported in Table 1). Deep vein thrombosis and pulmonary embolism were diagnosed by compression ultrasonography/venography and ventilation–perfusion lung scan, respectively. No subject had abnormal liver function and overt evidence of autoimmune or neoplastic disease at presentation. Blood was sampled at least 3 months after the only or last thrombotic episode and 3 weeks after withdrawal of any antithrombotic treatment. Data regarding family history of venous thrombosis and circumstantial triggering factors (recent surgery, trauma, immobilization, fracture, pregnancy/puerperium, oral contraceptives) at first deep vein thrombosis, were collected by personal interview.

Control group

During the same period, 919 apparently healthy women from the general population were given a complete thrombophilia work-up; 650 were of reproductive age at presentation and were used as control group (characteristics reported in Table 1). They were from the same geographical area as the patients but had no genetic or acquired risk factors. Against this background, we evaluated the prevalence of R506Q factor V mutation and G20210A prothrombin mutation in 301 women of reproductive age at first deep vein thrombosis, were considered. After exclusion of cases with isolated pulmonary embolism and oral contraceptive use are scanty. Recently, a multiplicative interaction was reported, the risk of venous thrombosis being increased about 16-fold.

Oral contraceptive use

Among the 301 patients, 140 (55.8%, after exclusion of those women who had thrombosis during pregnancy and puerperium) had experienced deep vein thrombosis while using oral contraceptives (Table 1). In the control group, 173 (26.6%) were oral contraceptive users at the time of blood sampling. The type of oral contraceptive was also recorded and the pills were classified into two categories, according to the type of progestin: second-generation (levonorgestrel or norgestrel) and third-generation (gestodene or desogestrel). Both in patients and controls the most frequently used oral contraceptives were third-generation, accounting, respectively, for 87.1% (122/140) and 87.9% (152/173).

Blood sampling and thrombophilia work-up

Blood was collected from the antecubital vein into 0.129 mmol·L⁻¹ trisodium citrate; plasma was prepared by centrifugation for 20 min at 2000 g at room temperature; plasma and blood for DNA extraction aliquots was snap frozen and stored at −70°C. The

G20210A prothrombin mutation. However, although the prevalence of G20210A prothrombin mutation has consistently been reported higher in general venous thromboembolism patients than in controls, it has been suggested by others that the mutation is associated with venous thromboembolism only in selected cases (e.g. in subsets of patients with idiopathic venous thromboembolism or recurrent thrombotic events). The low risk would not therefore justify the detection of this mutation as part of a thrombophilia work-up in unselected patients.
screening for thrombophilia included the following tests: prothrombin time; activated partial thromboplastin time; fibrinogen plasma levels, antithrombin III, protein C, protein S; activated protein C resistance, test for diagnosing lupus anticoagulant. DNA analysis for R506Q factor V mutation was performed in all cases with an activated protein C resistance normalized ratio <0·80; the presence of the G20210A mutation of the prothrombin gene was also tested.

All tests included in the thrombophilic screening were performed using standard methods: prothrombin time (Recombiplastin, Instrumentation Laboratory, Milan, Italy); activated partial thromboplastin time (automated aPTT, Organon Teknika, Rome, Italy); fibrinogen according to the Clauss method (Fibrinomat, bioMerieux, Lion, France); antithrombin III activity (Antithrombin, Instrumentation Laboratory; normal values: >80%); protein C activity (Coamate Protein C, Instrumentation Laboratory; normal values: >68%); protein S activity (Protein S, Instrumentation Laboratory; normal values: >62%); activated protein C resistance, according to de Ronde and Bertina [21] (normal values: >0·80 normalized ratio); factor V Leiden mutation [1] and the G20210A mutation of the prothrombin gene [2]. The presence of lupus anticoagulant was assessed according to the criteria of the International Society on Thrombosis and Haemostasis [22]: (a) diluted aPTT (1:15 PTT LA, Diagnostica Stago, Asnieres-sur-Seine, France) and (b) diluted Russell’s viper venom time (LA-Test and LA-Check, Organon Teknika, Rome, Italy); both tests were also performed after mixing with normal pool plasma (1:1) and repeated using higher phospholipid concentration. If low levels of antithrombin III, protein C or protein S were detected, antigen levels were also measured (Turbiquant Antithrombin, Dade Behring, Marburg, Germany; Vidas Protein C, bioMerieux; Asserachrom total and free Protein S; Diagnostica Stago).

### Statistical analysis

Continuous variables are presented as median and range. The Mann–Whitney U-test and the chi-square test were used for group comparisons of age and of carrier frequency, respectively; all P values less than 0·05 were considered to indicate statistical significance. Crude odds ratios and 95% confidence intervals (CI) were calculated with the approximation of Woolf [23] as estimates of the relative risk for deep vein thrombosis.
Table 2  Risk of deep vein thrombosis according to the presence of G20210A prothrombin mutation and R506Q factor V mutation

<table>
<thead>
<tr>
<th></th>
<th>Women with DVT</th>
<th>Healthy women</th>
<th>OR† (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No defect</td>
<td>204 (67.8%)</td>
<td>610 (93.8%)</td>
<td>1 (Reference)</td>
</tr>
<tr>
<td>G20210A prothrombin mutation</td>
<td>29 (9.6%)</td>
<td>20 (3.1%)</td>
<td>4.7 (2.5–8.6)</td>
</tr>
<tr>
<td>R506Q factor V mutation</td>
<td>56 (18.6%)</td>
<td>19 (2.9%)</td>
<td>10.3 (5.9–18.0)</td>
</tr>
<tr>
<td>G20210A prothrombin mutation + R506Q factor V mutation</td>
<td>8 (2.7%)</td>
<td>1 (0.2%)</td>
<td>45.6 (5.5–379)</td>
</tr>
<tr>
<td>Homozygous R506Q factor V mutation</td>
<td>2 (0.7%)</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Homozygous R506Q factor V + G20210A prothrombin mutation</td>
<td>2 (0.7%)</td>
<td>0</td>
<td>—</td>
</tr>
</tbody>
</table>

*Adjusted for age and presence of other thrombophilic defects. DVT= deep vein thrombosis.

Adjustment for age and the presence of other thrombophilic defects was performed by unconditional logistic-regression analysis with the SOLO software package (BMDP Statistical Software, Los Angeles, U.S.A.).

Results

The characteristics of the 301 patients and 650 controls are reported in Table 1. The median age at presentation was 37 years (16–58 years) and 33 years (15–49 years) for patients and controls, respectively (P<0.001). No significant difference, however, was found when the age at first deep vein thrombosis episode (30 years, 14–49 years) of patients was compared with the age of controls at presentation. The median time elapsed since the last thrombotic event was 13 months (3–21 months); deep vein thrombosis was proximal in 271 and distal in 30 cases. In 60/301 patients (19.9%) deep vein thrombosis was complicated by pulmonary embolism. In 120/301 patients (39.9%) a positive family history for venous thromboembolism was present, and 62 patients (20.6%) were heterozygous carriers of both mutations. In two patients (0.7%; 95% CI: 0.08–2.4%) the R506Q factor V and G20210A prothrombin mutations could not be detected during pregnancy or puerperium). The frequency of oral contraceptive use in patients was no different. There was no association between the presence of mutations and recurrence of thrombosis; only in the double carriers was recurrence of thrombosis slightly, although non-significantly, higher (28.6%) than in the other groups (17.4% and 19.0% in heterozygous carriers of R506Q factor V and G20210A prothrombin mutations, respectively).

A total of 19 controls (2.9%; 95% CI: 1.8–4.5%) were carriers of R506Q factor V, and 20 (3.1%; 95% CI: 1.9–4.7) of G20210A prothrombin mutation, none of them being a homozygous carrier. One case was a heterozygous carrier of both mutations (0.2%; 95% CI: 0.0–0.9%). Both the R506Q factor V and G20210A prothrombin mutations were therefore significantly over-represented in patients, as compared to controls (P<0.0001).

Other thrombophilic defects were diagnosed in 24 (8.0%) patients (five with antithrombin III, eight with protein C and five with protein S deficiencies; six with lupus anticoagulant) and in seven (1.1%) controls (two with protein C and one with protein S deficiencies; four with lupus anticoagulant).

The relative risks (odds ratio) of deep vein thrombosis associated with carriership of R506Q factor V and G20210A prothrombin mutations are reported in Table 2. Since at presentation patients were significantly older than controls, odds ratios were adjusted for age (and for other thrombophilic defects). After adjustment by logistic regression analysis, the estimated odds ratio of having deep vein thrombosis when carrying the G20210A prothrombin mutation was 4.7 (95% CI: 2.5–8.6); the adjusted odds ratio for R506Q factor V mutation was 10.3 (95% CI: 5.9–18.0). The odds ratio for double carriers was 45.6 (95% CI: 5.5–379). Odds ratios for homozygous carriers of R506Q factor V and G20210A prothrombin mutation and for carriers of homozygous R506Q factor V and G20210A prothrombin mutation could not be evaluated, since these conditions were found only in patients.

Oral contraceptive use, as reported in Table 1, was the most frequent circumstantial risk factor present at the first deep vein thrombosis episode (140/251, 55.8%, after exclusion of the 50 women who had deep vein thrombosis during pregnancy or puerperium). The frequency of oral contraceptive use in patients was no different.
among carriers of R506Q factor V (26/56, 46.4%) and G20210A prothrombin mutation (18/29, 62.1%), with the highest frequency being observed for carriers of both mutations (7/8, 87.5%). Since the prevalence of oral contraceptive users among controls was 26.6% (173/650), the risk conferred by oral contraceptives was 3.5 (95% CI: 2.6–4.7).

Table 3 shows the risk of deep vein thrombosis according to the use of oral contraceptives and the presence of the two mutations. Patients who were homozygotes for R506Q factor V mutation (n=2) and patients carrying homozygous R506Q factor V and G20210A prothrombin mutation (n=2) were excluded. Odds ratios were adjusted for age and the presence of other thrombophilic defects by logistic regression. The thrombotic risk of using oral contraceptives in the absence of both mutations was 2.4 (95% CI: 1.7–3.5). In oral contraceptive users, the estimated risk increased markedly with the presence of the mutations, being 41.0 (95% CI: 13.5–125) for R506Q factor V mutation, 58.6 (95% CI: 12.8–267) for G20210A prothrombin mutation and 86.5 (95% CI: 10.0–747) for double carriers. Surprisingly, while the odds ratio for R506Q factor V mutation remains high (8.9, 95% CI: 4.4–18.2) in non-users of oral contraceptives too, the odds ratio for the G20210A prothrombin mutation was 2.6 and did not reach statistical significance, with the 95% CI varying from 0.8 to 4.8. The odds ratio for the presence of G20210A prothrombin mutation was not different between women suffering from an idiopathic event and women with deep vein thrombosis in the presence of circumstantial risk factors other than oral contraceptives.

### Discussion

In this case-control study the prevalence of R506Q factor V and G20210A prothrombin mutations was evaluated in a series of 301 women of reproductive age suffering from deep vein thrombosis and consecutively referred to our department, and 650 healthy women. We focused on the interaction between the presence of the two most common thrombophilic mutations and the use of oral contraceptives. The high prevalence of the third-generation pills both in patients and controls (87–9% and 87–1%, respectively), in line with pill consumption in our country, did not allow us to check for a possible association between deep vein thrombosis and different oral contraceptives types.

The prevalence of heterozygotes for R506Q factor V and G20210A prothrombin mutations in our control population was consistent with that already reported in southern Europe.[24,25] The heterozygous R506Q factor V and G20210A prothrombin mutations were found in up to 18% and 9% in women with previous deep vein thrombosis, respectively. Our results confirm the strong association between the presence of R506Q factor V or G20210A prothrombin mutations and an increased risk for deep vein thrombosis (adjusted odds ratio 10.3 and 4.7, respectively). The presence of both mutations increased the risk about 45-fold, even if this figure should be considered as an approximation because of the small number of cases with double mutations. Because of the absence of homozygous carriers of R506Q factor V mutation in the control population, the risk conferred by the presence of homozygous R506Q factor V mutation could not be estimated.

Oral contraceptive use has been shown to be associated with a three- to four-fold increase in venous thromboembolism risk[14]. In our study the risk of deep vein thrombosis in women using oral contraceptives was 3.5, regardless of the presence of other genetic risk factors. In women using oral contraceptives who were not carriers of either R506Q factor V or G20210A prothrombin mutations the risk was 2.4. In line with previous published studies[15,16] our data show a gene–environmental interaction between the presence of R506Q factor V mutation and oral contraceptives use. This mutation increased the deep vein thrombosis risk about nine-fold in women not using oral contraceptives and 41-fold in oral contraceptive users, indicating a multiplicative interaction.

In line with data recently published[18–20], our study confirms that G20210A prothrombin mutation also acts
synergistically with oral contraceptive use on venous thromboembolism risk. In fact, the combination of oral contraceptive use and G20210A prothrombin mutation carrierhip increased the risk 80-fold.

The presence of both mutations in women using oral contraceptives seems to have an additive effect, the risk for deep vein thrombosis being more than 80-fold. However, since we found only seven and one oral contraceptive users carrying both mutations in patients and controls, respectively, the 95%CI are very large and the estimated risk should be considered an approximation.

Although a higher prevalence of G20210A prothrombin mutation has been consistently reported in venous thromboembolism patients than in controls[2,5-8], it has been suggested that this mutation may be a risk factor for venous thromboembolism only in selected cases[4,9-11]. Based on these data, some doubts have been raised about the detection of G20210A prothrombin mutation as part of the routine investigation in unselected patients[12,13]. Our study also failed to demonstrate that this mutation is a risk factor for deep vein thrombosis among non-users of oral contraceptives (odds ratio 2.0, 95%CI: 0.8-4.8).

In conclusion, the present study confirms that oral contraceptive use is the most frequent circumstantial risk factor for deep vein thrombosis in women of reproductive age and that oral contraceptive use strongly interacts with the two most common thrombophilic alterations. These findings raise the question whether it may be useful and feasible to screen for these mutations before prescribing oral contraceptives. Many arguments have been raised against general screening: (1) the low absolute risk of venous thromboembolism (0.5/10.000 per year for women aged under 45 years), (2) the high number of contraceptive users, (3) the corresponding economic costs, (4) the possibly induced use of less acceptable and less protective means of contraception, (5) the potential problems related to life insurance, (6) the psychological consequences deriving from awareness of having a ‘genetic defect’. For all these reasons it has been proposed to offer screening only to those women who have a personal or family history of venous thromboembolism[26,27]. However, previous published studies have clearly shown the unreliability of such a policy[28,29].

In the present study, data regarding the family history of deep vein thrombosis were also collected. A positive family history was present in 86/650 (13.1%) control women. Family history was positive in 8/40 carriers of R506Q factor V and/or G20210A prothrombin mutations. The sensitivity and positive predictive value of a first- and/or second-degree family history of deep vein thrombosis for these mutations were therefore very low (20.0% and 9.4%, respectively). Even though we agree that a general screening for these mutations would not be cost-effective, we share the opinion of other authors[20] that women should be informed of the increased thrombotic risk during oral contraceptive use if carriers of thrombophilic alterations. In this way, they will be able to decide for themselves whether to be screened before starting oral contraceptives treatment.

References


