Prothrombin 20210A and Oral Contraceptive Use as Risk Factors for Cerebral Venous Thrombosis

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Key Words
Cerebral venous thrombosis · Thrombophilia · FV 1691A · PT 20210A · MTHFR 677TT · Contraceptives, oral

Abstract
Background: This study investigates the association between cerebral vein thrombosis (CVT) and the mutations FV 1691A (factor V Leiden), PT 20210A and MTHFR 677TT and acquired factors including oral contraceptive (OC) use. Methods: 26 patients (21 females) and 217 healthy controls (134 females) were studied. Multiple regression analysis was performed. Results: The frequency of the three mutations in cases and controls were: PT 20210A, 23 versus 1%, odds ratio (OR) 21.40 (95% CI 4.29–118.75), p < 0.001; FV 1691A, 8 versus 1%, OR 5.94 (95% CI 0.66–46.9); MTHFR 677TT, 4 versus 7%, OR 0.54 (95% CI 0.03–4.08). OC use was more frequent in female patients over 14 years old than in controls (84 vs. 40%, OR 8.15, 95% CI 2.09–37.13, p < 0.001). The model that best explained the thrombotic risk included PT 20210A and OC use. Conclusions: PT 20210A and OC use are the main thrombophilic risk factors predisposing to CVT and should be routinely investigated in patients with this disease.

Introduction
Three highly prevalent mutations have been described in association with thrombosis: factor V Leiden (FV 1691A) [1], the prothrombin variant (PT 20210A) [2] and the homozygosity for the thermolabile methylene tetrahydrofolate reductase (MTHFR 677TT) [3]. These mutations increase the risk of thrombosis by different mechanisms: FV 1691A causes resistance to activated protein C; PT 20210A is associated with higher levels of plasmatic factor II; MTHFR 677TT may cause moderate hyperhomocysteinemia in the presence of vitamin deficiencies (folates, B6 or B12). Cerebral venous thrombosis (CVT) is an unusual and severe condition that is associated with hypercoagulability states [4–8]. Some studies have shown a higher frequency of PT 20210A in comparison with FV 1691A and other hereditary thrombophilias in patients with CVT [5, 9–12]. On the other hand, the use of oral contraceptives (OC) seems to be an important risk factor for CVT and, combined with PT 20210A, may strongly impair the normal coagulation [5]. The objective of this study was to evaluate the prevalence and the risk of thrombosis associated with these three thrombophilic mutations in patients with CVT, and the interaction between genetic and acquired factors.
Patients and Methods

Patients
Between January 1996 and January 2002, 31 patients younger than 50 years with a diagnosis of CVT confirmed by digital angiography or magnetic resonance angiography were referred to the hemostasis laboratory of a tertiary university hospital. For this study, we excluded by chart review 5 patients with major systemic diseases known to predispose to thrombosis, such as cancer, diabetes mellitus, infectious or collagen disease, or with any positive antiphospholipid antibody test. There were 21 female (81%) and 5 male patients, with a median age of 28.5 years (range 3–46 years). Twenty-one patients were Caucasians (81%), and 5 (19%) were African-Brazilians.

Control Group
All patients referred to the hemostasis laboratory are invited to bring at least one healthy control individual of the same age and racial background, and with no history of thrombosis (formally inquired) or genetic relationship. No female control was either pregnant or had had a recent delivery (up to 6 weeks). In addition, volunteering physicians and health care workers from the hospital also served as controls. The control group consisted of 217 individuals (134 women, i.e. 62%, and 83 men), with a median age of 29 years (range 15–62 years). There were 177 (82%) Caucasians and 40 (18%) African-Brazilians.

Laboratory Tests
After signing an informed consent, patients and controls had a sample of 10 ml blood collected in EDTA for DNA analysis. Detection of FV G1691A was carried out by polymerase chain reaction (PCR) of a fragment of exon 10 of the factor V gene and posterior digestion of FV G1691A was carried out by polymerase chain reaction (PCR) of a fragment of exon 10 of the factor V gene and posterior digestion with HinIII. The C677T substitution in the MTHFR gene was detected by HinfI cleavage of a PCR-amplified product obtained with the primers described by Froost et al. [3].

Risk Factors
The following variables were assessed as potential risk factors for CVT: gender, current cigarette smoking, current OC use (at the onset of the CVT or at the moment of blood collection for controls), body mass index (kg/m²) and the three mutations (FV 1691A, PT 20210A and MTHFR 677TT). No woman in the control group was in the puerperal period (up to the sixth week following delivery), so this was not included as a variable in the analysis.

Statistical Analysis
We compared the frequencies of the mutations and of acquired risk factors between cases and controls. The χ² or Fisher’s exact test was used for the comparison of dichotomous variables, and continuous variables were compared using the Wilcoxon test. The odds ratio (OR) and the 95% confidence intervals (CI) were calculated. Variables with a p value < 0.1 by univariate analysis were entered in a logistic regression analysis. Considering the known higher prevalence of CVT in women and to further explore the importance of OC in this group, an additional multivariate analysis was done including only female patients. The tests were performed using Epi-Info (Version 6.04b – January 1997, Centers for Disease Control and Prevention, Atlanta, Ga., USA) and SPSS 10.0 (SPSS 10.0 for Windows, SPSS Inc., Chicago, Ill., USA).

Results
The demographic characteristics, genotypes and coexisting exposures of patients and controls are shown in table 1. There were more females among cases. Two were in the puerperal period. The use of OC was more frequent in cases than in controls. A hereditary thrombophilia was diagnosed in 10 patients (38%): PT 20210A in 5 cases, antithrombin deficiency in 2 and 1 case each of MTHFR 677TT, FV 1691A and FV 1691A in association with PT 20210A. One patient with antithrombin deficiency was also at the first week postpartum. One patient with two mutations (FV 1691A and PT 20210A) was also using OC. Five patients were younger than 20 years, and 3 (60%) of them had hereditary thrombophilias: AT deficiency in 2 cases and FV 1691A in 1. No patient had either protein C or protein S deficiency.

The frequency of PT 20210A (all in the heterozygous form) was significantly higher in patients than in controls (table 1; 23 vs. 1%). The frequency of FV 1691A was also higher in patients than in controls (8 vs. 1%), but the difference was not statistically significant. The frequency of MTHFR 677TT was similar in the 2 groups. OC use among female carriers of PT 20210A was associated with a particularly high risk of developing CVT (p < 0.0001, OR = 35. 95% CI 3.34–892.36). One patient taking OC had FV 1691A, while this association was not found in the control group (p = 0.006).

Table 2 shows the results of the multiple regression analysis. Since the variable OC use was significant, we performed two separate multivariate analyses, one including all patients and excluding OC use, the other selecting females older than 14 years who were not in the puerperal period. In the multivariate model that included gender, FV 1691A and PT 20210A, the latter was the only variable associated with CVT. Matching patients and controls for gender distribution suggested a link between FV and CVT (p = 0.048, OR = 13.75; 95% CI 0.92–399 in univariate analysis) but did not change the results of the multivariate analysis (data not shown). In the second model, PT 20210A and OC use were both independently associated with CVT.
Table 1. Demographic characteristics and genotypes of patients and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients (n = 26)</th>
<th>Controls (n = 217)</th>
<th>p value</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (female: male)</td>
<td>21:5</td>
<td>134:83</td>
<td>0.056</td>
<td>2.60</td>
<td>0.88–8.21</td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>28.5</td>
<td>29</td>
<td>0.23</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>Range</td>
<td>3–46</td>
<td>15–62</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>2 (8)</td>
<td>36 (16)</td>
<td>0.18</td>
<td>0.42</td>
<td>0.07–1.95</td>
</tr>
<tr>
<td>Body mass index (median)</td>
<td>22.6</td>
<td>23.6</td>
<td>0.57</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>Use of OC¹</td>
<td>16/19 (84)</td>
<td>53/134 (40)</td>
<td>&lt;0.001</td>
<td>8.15</td>
<td>2.09–37.13</td>
</tr>
<tr>
<td>FV 1691A</td>
<td>2 (8)</td>
<td>3 (1)</td>
<td>0.09</td>
<td>5.94</td>
<td>0.66–46.9</td>
</tr>
<tr>
<td>PT 20210A</td>
<td>6 (23)</td>
<td>3 (1)</td>
<td>&lt;0.001</td>
<td>21.4</td>
<td>4.29–118.75</td>
</tr>
<tr>
<td>MTHFR 677TT</td>
<td>1 (4)</td>
<td>15 (7)</td>
<td>0.47</td>
<td>0.54</td>
<td>0.03–4.18</td>
</tr>
<tr>
<td>PT 20210A + OC¹</td>
<td>4 (21)</td>
<td>1 (1)</td>
<td>&lt;0.0001</td>
<td>35</td>
<td>3.34–892.36</td>
</tr>
</tbody>
</table>

Figures in parentheses indicate percentages. NA = Not applicable; FV 1691A = mutation 1691A in the factor V gene; PT 20210A = mutation 20210A in the prothrombin gene; MTHFR 677TT = homozygous for the mutation 677T in the gene of the enzyme methylene tetrahydrofolate reductase.

¹ 19 patients: only females included; postpartum and patients <15 years old also excluded.

Discussion

This study found a high prevalence of inherited thrombophilia in patients with CVT. PT 20210A was the most frequent hereditary disorder, showing the strongest association with CVT. We found an association with FV 1691A after matching by gender, but it was not significant on multivariate analysis. No association was found with MTHFR 677TT. The use of OC was also more prevalent in CVT patients, and in multivariate analysis, PT 20210A and OC use were independently associated with CVT in women of childbearing age. The main limitations of the present study relate to its nature (patient referral to a tertiary hospital specialized laboratory) and exclusion criteria (older patients and those with active infections or severe systemic diseases predisposing to thrombosis), which probably limit the generalization of our findings.

CVT is a relatively rare disorder, and there are few studies investigating its association with inherited thrombophilia. The frequency of FV 1691A in these patients varied from 3.7 to 25% [4, 5, 7–10, 12–15], whereas studies evaluating the prothrombin variant [5–7, 9, 10, 13, 16, 17] found frequencies varying from 0 [13] (in a series of 14 patients with no exclusion criteria) to 20% [5, 9]. A number of studies evaluating MTHFR 677TT [9, 10, 13, 17] found frequencies varying from 0 [9] to 36% [13]. These wide differences in frequency rates may be related to different racial backgrounds and entry criteria among studies. In the present series, the frequency of each of the three mutations was not very different from that shown in other studies, but PT 20210A was about threefold more common than FV 1691A. Some authors also found a greater frequency of PT 20210A than FV 1691A in patients with CVT [5, 9, 10, 13, 17]. In another recent Brazilian study, PT 20210A was fourfold more common than FV 1691A [11].

There are few studies assessing the magnitude of the association of different factors and CVT. The few case-control studies published so far have different designs and inclusion criteria. Our patients were less than 47 years old, and we did not exclude pediatric patients. The fre-
quency of thrombophilias in this young population is probably higher than that found in older patients with stroke, and this may explain, at least in part, the strong association between PT 20210A and CVT that we observed (OR ~21), compared to other studies [5, 6, 9, 11] (OR varying between 5.5 [11] and 10.2 [5]). The ethnic heterogeneity of our population may also help explain these differences. A study describing the geographic distribution of PT 20210A showed a greater prevalence of this mutation in the Iberian population than in North Europe [18]. Indeed, the Brazilian population is a result of a marked miscegenation, with many people coming from the Iberian Peninsula.

We observed a predominance of the female sex (4.2:1) in patients with CVT, as previously described [4, 5, 8]. In addition, women using OC were at greater risk of CVT by univariate and multivariate analyses. The association between OC use and thrombosis has been reported during the last three decades, with a four- to sevenfold increase in the risk of venous thromboembolism [19–23], but there are few studies evaluating patients with CVT. Two series of patients studied by Martinelli et al. [5, 24] showed an association between OC use and CVT, with ORs of 4 and 22, respectively, and the risk was markedly increased in the presence of both OC use and the prothrombin variant [5].

In no study evaluating the association between CVT and genetic disorders was a multivariate analysis performed. The study of Martinelli et al. [5] used a stratified analysis and, as mentioned, observed an association between the two variables. In the present study, the multivariate analysis confirmed the strong and independent association between CVT, PT 20210A and OC use.

In summary, our data suggest that PT 20210A is the most important genetic thrombophilic risk factor associated with CVT, and its presence must be investigated in any patient presenting with this disease. In addition, women known to be carriers of this mutation should be discouraged to use OC. Further epidemiological studies are needed to better define the interaction between genetic alterations and acquired risk factors.

References