Prevalence and Association of the Factor V Leiden and Prothrombin G20210A in Healthy Subjects and Patients with Venous Thromboembolism

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Aim. To determine the prevalences of factor V Leiden and the G20210A mutation in the prothrombin gene (PT20210A) and the frequency of their association in healthy subjects and in patients with venous thromboembolism (VTE).

Method. We studied 160 Croatian patients with at least one episode of VTE and 155 healthy subjects as a control group. Genomic DNA was extracted according to standard procedures and the presence of factor V Leiden and PT20210A were determined by polymerase chain reaction-restriction fragment length polymorphism method.

Results. The prevalences of factor V Leiden and PT20210A were in VTE patients 21% and 8% respectively, and 4% in controls for both mutations. Additionally, 4 patients were affected by double heterozygous defects, corresponding to a frequency of 3%, whereas none of the controls were double heterozygotes. The coexistence of the PT20210A in heterozygous carriers of factor V Leiden was 15% in VTE group. The results obtained for different subgroups of VTE patients showed that the carriers of analyzed mutations were identified only in subgroups of patients with deep venous thrombosis of lower extremities (in 30 patients with factor V Leiden and in 13 patients with PT20210A) and superficial venous thrombosis (in 3 patients with factor V Leiden).

Conclusion. The prevalences of factor V Leiden and PT20210A in analyzed population of VTE patients are higher than in the group of healthy subjects. High frequency of association between both mutations supports the need to perform simultaneous genetic analyses of factor V Leiden and PT20210A in all VTE patients.

Key words: blood coagulation factors; factor V; genes; point mutation; prothrombin; thromboembolism; thrombophilia; venous thrombosis
Here we present our results.

Evidence for venous thromboembolism, site of the thrombotic event, and age at onset had been documented in their medical records. Their median age was 39 years (range, 16-84 years) at the time of thrombosis and 45 years (range, 18-85 years) at the time of blood sampling. Patients were divided in 4 subgroups according to the site of the thrombotic event: 138 patients had deep venous thrombosis of the lower extremities, 12 patients had superficial venous thrombosis, 3 patients had a thrombotic event in unusual sites, and 7 patients had isolated pulmonary embolism.

Eight patients with deep venous thrombosis also experienced an episode of pulmonary embolism. Additional information regarding the circumstances under which they manifested the first event, and age at onset had been documented in their medical records. The control group consisted of 155 healthy subjects (45 men and 110 women) recruited from our hospital staff and their friends, aged 15-70 years. The inclusion criterion was the lack of any personal history of a thromboembolic disorder.

Methods

Whole blood for DNA analysis was collected in sodium citrate (0.129 mol/L) or EDTA. Genomic DNA was prepared according to standard procedures using either phenol/chloroform extraction with ethanol precipitation (16) or the salting out method (17).

The presence of factor V Leiden and the PT20210A was determined by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. A 287-bp fragment encompassing nucleotide position 1691 of factor V gene was amplified with primers, according to Zöller et al (18). Following the digestion with MnII (Stratagene, Austin, TX, USA), the wild type allele (1691G allele) resulted in 37-bp, 93-bp, and 157-bp fragments, whereas the mutant allele (1691A allele) resulted in 130-bp and 157-bp fragments. Analysis for PT20210A was performed according to the method described by Poort et al (3). After the digestion of amplified 345-bp fragments with Hind III (Roche Diagnostics, Mannheim, Germany), the mutant A allele was cleaved in two 23-bp and 322-bp fragments, whereas the wild type G allele remained undigested.

Digested PCR products were separated by electrophoresis on 1.5% agarose gels (Applied Biosystems, Foster City, CA, USA) for factor V Leiden (Fig. 1) and on Spreadex gels (Guest Elchrom Scientific, Charn, Switzerland) for PT20210A (Fig. 2).
group. In contrast, the frequency was higher in women (5%) than in men (2%) in the control group. The frequency of PT20210A was higher in women (10%) than in men (5%) among the patients, whereas it was higher in men (7%) than in women (3%) among healthy controls (Table 2).

Combined defects were identified only in the patient group; four patients (3%) were heterozygous carriers for both mutations (Table 1). The combined defect was observed in one man (2%) and three women (5%) (Table 2). The coexistence of PT20210A and factor V Leiden was identified only in heterozygous carriers of factor V Leiden with a prevalence of 15%, whereas it was not found in factor V Leiden homozygotes.

Table 3 shows the results obtained for different subgroups of patients with venous thromboembolism. In the subgroup of patients with deep venous thrombosis of the lower extremities 30 out of 138 patients (22%) were carriers of the factor V Leiden (24 heterozygous and 6 homozygous) and 13 out of 138 patients (9%) were carriers of the PT20210A. All four patients with combined heterozygosity belonged to this subgroup of patients with a corresponding prevalence of 3%. In the subgroup of patients with superficial venous thrombosis, the presence of factor V Leiden only was detected in 3 out of 12 patients (25%). All affected individuals were women and heterozygous carriers for factor V Leiden. We found neither factor V Leiden nor the PT20210A in the subgroup of patients with thrombosis in unusual sites or in the subgroup of patients with isolated PE.

**Discussion**

Factor V Leiden and PT20210A represent the two most common genetic risk factors for venous thromboembolism in Caucasians. Due to geographic and ethnic distribution of both mutations, their prevalence varies greatly in different countries (2-6,8,26). Factor V Leiden, as the most common inherited risk factor for venous thromboembolism, has been found at an average prevalence of 22%, ranging from 20% to 52% in patients with venous thromboembolism (19-22), whereas the prevalence of PT20210A is reported to be between 5% and 19% (3,23-26).

In this study, the prevalences (4%) of factor V Leiden and PT20210A in healthy subjects were the same. The observed prevalence for factor V Leiden corresponded to the reported prevalence (between 2% and 15%) in the healthy Caucasian population (5). The prevalence of PT20210A that we found was higher than the overall prevalence for this mutation reported by Rosendaal et al (26) to be about 2% (8), but similar to previously reported prevalence in southern European countries, particularly around the Mediterranean area.

In our study we found an overall prevalence of 21% for the factor V Leiden and 8% for PT20210A in analyzed population of patients with venous thromboembolism, which is close to the reported prevalence for both mutations in the same group of patients. After analyzing the incidence of these two mutations in the subgroups of patients (deep venous thrombosis of lower extremities, superficial venous thrombosis, thrombosis in unusual sites, and isolated pulmonary embolism), we found that the prevalence of factor V Leiden and PT20210A was higher in women (7%) than in men (5%) among the patients, whereas it was higher in men (5%) than in women (3%) among healthy controls (Table 2).

**Table 1.** Frequency of 1691 G/A genotypes in factor V gene and of 20210 G/A genotypes in the prothrombin gene in patients with venous thromboembolism (n=160) and in healthy control subjects (n=155)

<table>
<thead>
<tr>
<th>Genotype *</th>
<th>No. (%) of subjects</th>
<th>patients</th>
<th>healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor V</td>
<td>127 (79)</td>
<td>149 (96)</td>
<td></td>
</tr>
<tr>
<td>1691 GG</td>
<td>27 (17)</td>
<td>5 (3)</td>
<td></td>
</tr>
<tr>
<td>1691 GA</td>
<td>6 (4)</td>
<td>1 (1)</td>
<td></td>
</tr>
<tr>
<td>1691 AA</td>
<td>147 (92)</td>
<td>149 (96)</td>
<td></td>
</tr>
<tr>
<td>Prothrombin</td>
<td>13 (8)</td>
<td>6 (4)</td>
<td></td>
</tr>
<tr>
<td>20210 GG</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>20210 GA</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Combined defect</td>
<td>156 (97)</td>
<td>155 (100)</td>
<td></td>
</tr>
<tr>
<td>1691 AA and PT 20210 GG</td>
<td>4 (3)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>1691 GA and PT 20210 GA</td>
<td>110 (70)</td>
<td>10 (7)</td>
<td></td>
</tr>
</tbody>
</table>

\*GG – wild type; GA – heterozygous; AA homozygous.

**Table 2.** Prevalence of factor V Leiden, prothrombin gene mutation, and both mutations in patients with venous thromboembolism and healthy control subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No. of subjects</th>
<th>Factor V Leiden</th>
<th>prothrombin gene mutation</th>
<th>Factor V Leiden and prothrombin gene mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (total)</td>
<td>160</td>
<td>33 (21)</td>
<td>13 (8)</td>
<td>4 (3)</td>
</tr>
<tr>
<td>men</td>
<td>57</td>
<td>12 (21)</td>
<td>3 (5)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>women</td>
<td>103</td>
<td>21 (20)</td>
<td>10 (10)</td>
<td>3 (5)</td>
</tr>
<tr>
<td>Healthy controls (total)</td>
<td>155</td>
<td>6 (4)</td>
<td>6 (4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>men</td>
<td>45</td>
<td>1 (2)</td>
<td>3 (7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>women</td>
<td>110</td>
<td>5 (5)</td>
<td>3 (3)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

**Table 3.** Prevalence of factor V Leiden, prothrombin gene mutation, and both mutations in different subgroups of patients with venous thromboembolism

<table>
<thead>
<tr>
<th>Subgroup of patients with</th>
<th>No. of</th>
<th>factor V Leiden</th>
<th>prothrombin gene mutation</th>
<th>Factor V Leiden and prothrombin gene mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep venous thrombosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>of lower extremities</td>
<td>138</td>
<td>30 (22)</td>
<td>13 (9)</td>
<td>4 (3)</td>
</tr>
<tr>
<td>Superficial venous thrombosis</td>
<td>12</td>
<td>3 (25)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Thrombosis in unusual sites</td>
<td>3</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Isolated pulmonary embolism</td>
<td>7</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>
pulmonary embolism), we realized that all PT20210A carriers, as well as 30 of 33 identified factor V Leiden carriers were in the subgroup of patients with deep venous thrombosis of lower extremities. Accordingly, the coexistence of both mutations was attributable to this subgroup. The remaining three factor V Leiden carriers were found in the subgroup of patients with superficial venous thrombosis. This finding, together with no mutation detected in other 2 subgroups, suggested that the presence of factor V Leiden, PT20210A, or both, represented the main cause of deep venous thrombosis of lower extremities. Finding no factor V Leiden or PT20210A in cases with isolated pulmonary embolism corroborates the previous reports by Martinelli et al (27) and Ehrenforth et al (13), who found that the prevalence of factor V Leiden and PT20210A is not increased in patients with isolated pulmonary embolism.

The limitations of our study is the small number of patients in the subgroups. To obtain more informative results, a larger study is needed. We observed the difference in the prevalence of PT20210A between sexes in both groups studied. We can only speculate that women who were carriers of the mutation in our patient group (10%) were more prone to develop deep venous thrombosis, whereas men with PT20210A in the group of healthy subjects (7%) seemed to be more protected.

The combination of the most frequent genetic risk factors, factor V Leiden and PT20210A, has been frequently found in patients with venous thromboembolism (10-12,14). Ridker et al (28) found a prevalence of 0.4% of both mutations in individuals included in the physicians’ health study, of whom 1.5% had experienced venous thromboembolism and 0.3% had not. We demonstrated a high frequency of the association between both mutations (3%) among patients with deep venous thrombosis.

There are numerous reports (case/control studies) of unrelated individuals showing the additional inheritance of PT20210A with factor V Leiden (11-14). Our study results, showing that these two mutations tend to coexist in a heterozygous form (15%), are similar to those reported by Ehrenforth et al (11%) (13), Zöller et al (10%) (10), and Ferraresi et al (14%) (29).

In conclusion, the data presented here indicate that 29% of investigated patients with venous thromboembolism were carriers of these prothrombotic defects in contrast to 8% in controls. Additionally, our findings of frequently combined heterozygosity for these two gene defects support the need to perform simultaneous genetic analyses of factor V Leiden and PT20210A in all patients with venous thromboembolism, especially in those with deep venous thrombosis of lower extremities.

References
3 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.


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