Prevalence of the Factor II G 20210A Mutation in Symptomatic Patients with Inherited Thrombophilia

Dear Sir,

A novel inherited risk factor for venous thrombosis has been recently identified as a nucleotide substitution G to A at position 20210 of the 3'- untranslated region of the prothrombin gene (1). In a recent issue of Thromb Haemost, Makris et al. (2) reported that in 101 unrelated patients with previous venous thromboembolism and inherited thrombophilia (deficiency of antithrombin III, protein C, protein S or factor V Leiden) 8 (7.9%) were heterozygous for the factor II 20210A allele, at significant variance with the prevalence of factor II 20210A allele found in a control group of 150 individuals (n = 1, 0.7%). Both patients and controls came from Sheffield or the surrounding area (UK). Almost all the carriers of the factor II 20210A allele among the patients with inherited thrombophilia suffered from recurrent thromboembolic events (7 out of 8 patients with combined defects, 87%), whereas the rate of recurrence among the total index patients without the prothrombin mutation was 51% (48 out of 93 patients). The authors conclude that the prevalence of the factor II mutant allele is significantly greater in symptomatic patients with inherited thrombophilia and that the additional presence of the mutation increases the risk of venous thrombosis. Alenc-Gelas et al. (3) reported quite different results, being unable to find any carrier of the factor II A allele among 217 French subjects with factor V Leiden, 66 with previous thrombosis and 151 asymptomatic ones, whereas the prevalence of the carriers of the mutant factor II was 2.8% among 400 controls; however this latter investigation could have been biased by the relatively low number of kindreds included (n = 26). We investigated a cohort of 79 unrelated Italian patients with previous venous thrombotic disease and inherited thrombophilia: 3 with antithrombin deficiency, 11 with protein C deficiency, 4 with protein S deficiency, 60 with factor V Leiden mutation (55 heterozygotes, 5 homozygotes) and 1 with a double defect (antithrombin deficiency and heterozygous factor V Leiden genotype). Moreover we investigated 214 Italian control individuals with no history of vascular disease. In all the subjects the factor II 20210A allele was checked according to the original method of Poort et al. (1). Heterozygous mutant factor II genotype was detected in 6 thrombophilic patients (1 with antithrombin deficiency and 5 with heterozygous factor V Leiden) (7.6%, 95% CI 1.8 to 13.4) and in 6 controls (2.8%, 95% CI 0.6 to 5.0) (x² = 3.37, p = 0.0663). The frequency of the A allele was 3.8% (95% CI 0.8 to 6.8) in the patient group and 1.4% (95% CI 0.3 to 2.5) in the control group (x² = 3.30, p = 0.0692); a similar figure was obtained considering only the 61 individuals carrying the factor V Leiden mutation, showing a frequency of the A allele of 4% (95% CI 0.5 to 7.5). All the six patients with co-inheritance of the mutant factor II allele and another prothrombotic trait had recurrent episodes whereas only 29 of the remaining 72 patients with an isolated thrombophilic trait had recurrences (40.2%, x² = 7.39, p = 0.0047). Considering separately only the proband patients with inherited thrombophilia and recurrent episodes (n = 35), the prevalence of heterozygous carriers of factor II mutant genotype was significantly higher in comparison with the controls (x² = 13.48, p = 0.0002) (Table 1).

In our series the factor II A allele frequency among the patients with symptomatic thrombophilia was similar to that reported by Makris et al. (1) but not significantly different from that found in our control population, even though it could be expected that increasing the number of investigated subjects the significance level would be reached. However an overrepresentation of the factor II mutated A allele among symptomatic patients with inherited thrombophilia is questionable. The 79 index patients here reported were identified from a cohort of 393 patients with venous thrombotic disease consecutively referred to our center; in this cohort we identified 56 heterozygotes for factor V Leiden (14.2%) and 25 heterozygotes for mutant prothrombin gene (6.4%), so that the expected rate of double heterozygosity according to these percentages was 0.9%. The observed prevalence of individuals with combined factor V Leiden and mutant prothrombin was 1.3% (5 of 393), not supporting the hypothesis that individuals with double mutation should be more present among the patients suffering from venous thrombosis. On the other hand we fully confirm that the mutant factor II A allele is significantly more represented in patients with inherited thrombophilia and recurrent episodes than in patients with only one previous thrombotic episode: the mean age at the time of the blood drawing was comparable, being 46.8 years (median age 43) in the 6 individuals with mutant prothrombin gene associated with inherited thrombophilia and 45.9 years (median age 47) in the 72 individuals with isolated thrombophilic alteration.

The interpretation of these results is puzzling: the prevalence of the mutant factor II A allele among the proband patients here investigated is higher than in the controls but without reaching the statistical significance. On the other hand the concomitant presence of the mutant factor II A allele seems to render the patients with inherited thrombophilia more prone to recurrent thrombotic episodes. However the number of such individuals in our series is low, as well as in the investigation of Makris et al. (2); moreover more reliable informations about the interaction of mutant factor II A allele with other prothrombotic defects should be obtained from studies performed on all the individuals belonging to the thrombophilic kindreds, so that large cooperative studies are expected to clear this issue.

Table 1  Prevalence of factor II normal genotype (G/G) and mutant heterozygous (A/A) genotypes among proband patients with inherited thrombophilia and normal controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Inherited thrombophilia</th>
<th>Factor II G/A</th>
<th>Factor II A/A</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor II G/A</td>
<td>72 (52.4%)</td>
<td>6 (4.2%)</td>
<td>0</td>
<td>79</td>
</tr>
<tr>
<td>Factor II A/A</td>
<td>43 (30.9%)</td>
<td>0</td>
<td>0</td>
<td>43</td>
</tr>
<tr>
<td>Factor II G/A A/A</td>
<td>29 (83.9%)</td>
<td>6 (17.1%)</td>
<td>0</td>
<td>35</td>
</tr>
<tr>
<td>Controls</td>
<td>208 (52.3%)</td>
<td>6 (15.8%)</td>
<td>0</td>
<td>214</td>
</tr>
</tbody>
</table>

* one patient with double defect (A20210A deficiency and factor V Leiden) was excluded.
Letters to the Editor

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References

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The Mutation at Position 20210 in the 3’-Untranslated Region of the Prothrombin Gene Is Extremely Rare in Taiwanese Chinese Patients with Venous Thrombophilia

Dear Sir,

A mutation in the 3’-untranslated region of the prothrombin gene (the G to A mutation at nucleotide 20210) has been reported to be associated with an increase in the risk of venous thrombosis in Caucasians (1–5). The polymorphism is closely related to the prothrombin level, which in turn is associated with the risk of thrombosis (1). The prevalence of prothrombin 20210A mutation in Caucasians was shown to be present in 5–6% in unselected subjects with a history of venous thrombosis and 17–19% in venous thrombophila (1–5), and 1–2% in control subjects (1, 3–5). As far as we know, there was no report of prothrombin 20210A mutation within the Chinese population. Because there exists some racial difference in the incidence of factor V Leiden mutation between Caucasians and oriental populations (6–8), and no factor V Leiden mutation was found in Chinese venous thrombophilia (7), we aimed to determine the frequency of prothrombin 20210A in venous thrombophilic patients and healthy individuals in Taiwanese Chinese.

We have examined the prothrombin dimorphism among 111 verified Chinese venous thrombophilic patients composed of 55 males and 56 females, aged 47.4 ± 17.6 (Mean ± SD) years, at National Taiwan University Hospital, and 149 apparently healthy and age-matched individuals comprised of 78 males and 71 females, aged 43.9 ± 14.6 years (p = 0.08) without any history of venous thrombosis. The criteria of thrombophilia were defined according to our previous study (7).

DNA was extracted by conventional methods. A 345-bp fragment from exon 14 and the 3’-untranslated region of the prothrombin gene was amplified by polymerase chain reaction (PCR) using the primers described by Poort et al (1). Amplification performed for 35 cycles with annealing temperature of 56° C (Perkin Elmer Cetus, Norwalk, CT). Amplified DNA was digested with Hind III enzyme (Fermentas Lithuania) at 37° C and subjected to 2% agarose gel electrophoresis.

All the genomic DNA under investigation were shown with the genotype G/G in prothrombin 20210. We didn’t find any heterozygote (G/A) or homozygote (A/A) at position 20210 in venous thrombophilic patients and healthy individuals, i.e., the prevalence of the 3’-untranslated region of prothrombin mutation was 0% in 260 cases in our study.

The genotype G/G was confirmed by an automated fluorescence-based DNA sequence analysis of the amplified genomic DNA.

Prothrombin 20210A mutation might be extremely rare in Taiwanese Chinese. We concluded that prothrombin 20210 mutation was not an important cause of venous thrombophilia in Chinese similar to what has been reported with factor V Leiden mutation in Chinese. These unique findings might further explain that racial background plays a major role in the causes of inherited thrombophilia between Chinese and Caucasians.

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References

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