Study of the Prothrombin Gene 20201 GA Variant in FV:Q\textsuperscript{506} Carriers in Relationship to the Presence or Absence of Juvenile Venous Thromboembolism


Abstract—The G20210A transition of the prothrombin gene has been identified as a common but probably mild hereditary risk factor for venous thromboembolism (VTE). However, the prothrombin gene variant might contribute to the penetrance of thromboembolic disease in many patients with other prothrombotic defects, such as the FV:R506Q mutation. In this investigation, the A20210 allele was found in 9 of 450 healthy controls (2%). Among 89 asymptomatic FV:Q\textsuperscript{506} carriers, 3 subjects were doubly affected (3.4%). In contrast, of 263 unrelated carriers of the FV:Q\textsuperscript{506} mutant with a history of juvenile VTE, 30 also had the prothrombin gene G20210A variant (11.4%), including 25 of 220 patients who were heterozygous (11.4%) and 5 of 43 homozygous (11.6%) for FV:Q\textsuperscript{506}. Thus, the A20210 allele of the prothrombin gene is significantly overrepresented in symptomatic FV:Q\textsuperscript{506} carriers compared with healthy controls (\(P<0.0001\)) and asymptomatic relatives carrying the FV mutant (\(P=0.02\)). Persons homozygous for the 20210A allele were not found. A statistically significant increase in the prevalence of more unusual sites of venous thrombosis at clinical onset was found in doubly affected patients (9 of 30; 30%) compared with patients without the prothrombin gene variant (26 of 233; 11.1%) (\(P=0.004\)). First VTE occurred spontaneously in 53.3% of all doubly affected patients (16 of 30) and in 28.3% of all simply affected patients (66 of 233) (\(P=0.005\)). Among patients with VTE preceded by circumstantial risk factors, the A20210 allele was found in 7.7% (14 of 181). We conclude that the A20210 allele of the prothrombin gene is frequently coinherited in symptomatic FV:Q\textsuperscript{506} carriers and possibly influences age, site, and type of thrombotic onset manifestation in these patients. (Arterioscler Thromb Vasc Biol. 1999;19:276-280.)

Key Words: genes ■ variation (genetics) ■ prothrombin ■ factor V ■ mutation ■ thrombosis, venous

Within the last decade, several variant alleles of the genes encoding proteins regulating blood coagulation, such as protein C, protein S, antithrombin, and fibrinogen, have been shown to be relatively strong but uncommon risk factors for thrombosis.\textsuperscript{1–4} Resistance to activated protein C have been shown to be relatively strong but uncommon risk factors for thrombosis.\textsuperscript{1–4} Resistance to activated protein C has been investigated in greater detail, scant information is available concerning the coexistence of both of the most common prothrombotic gene variants, FII 20210 GA and FV 1691 GA. This prompted us to evaluate the prevalence of the prothrombin gene variant G20210A among patients with juvenile VTE and to assess its importance as an additional prothrombotic risk allele in patients carrying the FV:R506Q mutation.

Methods

Subjects

A total of 352 subjects carrying the FV:R506Q mutation were screened for the prothrombin gene A20210 allele. Two hundred sixty-three unrelated subjects (163 females and 100 males) had experienced an objectively confirmed first VTE \(\leq45\) years of age episode (juvenile VTE). Forty-three (28 females and 15 males) were known homozygous carriers of the FV:Q\textsuperscript{506} mutant, whereas 220 (135 females and 85 males) carried the FV mutation in a heterozygous form. Eighty-nine patients were asymptomatic relatives (56 females and 33 males; median age, 33 years; range, 16 to 74 years), carrying the FV:R506Q mutation in a heterozygous (\(n=79\)) or homozygous form (\(n=10\)). For controls, we screened 450 healthy persons (212 females and 238 males; age, 18 to 72 years; median age, 45 years) and to assess its importance as an additional prothrombotic risk allele in patients carrying the FV:R506Q mutation.

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Arterioscler Thromb Vasc Biol. is available at http://www.atvbaha.org
TABLE 1. Prevalence of the Prothrombin Gene 20210 GA Variant Among Healthy Controls and Symptomatic vs Asymptomatic Subjects Carrying the FV:Q506 Mutant

<table>
<thead>
<tr>
<th>FVQ506</th>
<th>FII20210GA</th>
<th>Healthy Controls, % (n=450)</th>
<th>FVQ506 → Juvenile VTE, %</th>
<th>Asymptomatic FVQ506, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+</td>
<td>+/−</td>
<td>11.6% (5) [3.9–25.1]</td>
<td>0.2 (1) [0.006–1.2]</td>
<td>7.5% (3) [0.8–10.7]</td>
</tr>
<tr>
<td>+/−</td>
<td>+/−</td>
<td>11.4 (25) [7.5–16.3]</td>
<td>7.3 (33) [5.1–10.1]</td>
<td>1.8 (8) [0.8–3.5]</td>
</tr>
<tr>
<td>−/−</td>
<td>+/−</td>
<td>3.8 (3) [0.8–10.7]</td>
<td>1.8 (8) [0.8–3.5]</td>
<td>7.5% (3) [0.8–10.7]</td>
</tr>
<tr>
<td>Σ F II 20210 GA</td>
<td>7.5 [5.3–10.4]</td>
<td>2.0 P&lt;0.0001 [0.9–3.7]</td>
<td>11.4 P&lt;0.02 [7.8–15.9]</td>
<td>3.4 [0.7–9.5]</td>
</tr>
</tbody>
</table>

Values are given as prevalences (in percentages), absolute numbers (in parentheses), and 95% confidence intervals (in brackets).

Age, 30 years) from the same geographic region for the presence of the prothrombin gene A20210 allele. The criterion for recruitment of control subjects was the lack of any history of a thromboembolic disorder.

In all of the symptomatic subjects enrolled, coexisting deficiencies of antithrombin, protein C, protein S, plasminogen, or antiphospholipid antibodies had been previously excluded by using conventional functional and immunological tests. From the medical records and the personal interview, information was obtained on characteristics of the thrombotic event, such as site, age at onset, and presence or absence of circumstantial risk factors known to be associated with an increased risk for venous thrombosis (recent surgery, trauma or immobilization, oral contraceptive intake, and pregnancy or postpartum period).

Blood Sampling

After subjects gave informed consent, venous blood was collected in EDTA-treated sample tubes (Sarstedt), from which cells were separated by centrifugation at 300g for 15 minutes. The buffy-coat layer was then removed and stored at −70°C until DNA extraction was performed by standard techniques.

DNA Analysis

The presence or absence of the 1691 G to A transition in the FV gene was determined by polymerase chain reaction and MnlI restriction analysis of PCR-amplified genomic FV DNA fragments. Screening of the G to A transition at nucleotide 20210 in the 3′-untranslated region of the prothrombin gene was carried out by HindIII cleavage of a 345-bp fragment amplified by polymerase chain reaction using a mutagenic primer, as described by Poort et al.1

Statistical Analysis

The Pearson-Mantel-Haenszel χ² test was used for group comparison of carrier frequency. P values and 95% confidence intervals (95% CI) were calculated. A P value <0.05 was considered significant. Statistical analysis was performed with BiAS software by Dr H. Ackermann, Department of Medical Statistics, University Frankfurt.

Results

Prevalence of the Prothrombin Gene 20210 GA Variant

In the group of 450 healthy controls, 9 (2%) were heterozygous carriers of the A20210 allele (95% CI, 0.9% to 3.7%), of whom 1 had also the FV:R506Q mutation, corresponding to a prevalence of 0.2% for the combined defect.

Of 43 symptomatic patients carrying the FV:R506Q mutation in a homozygous form, a coexistence of the prothrombin gene 20210 GA variant was detected in 5 subjects, corresponding to a prevalence of 11.6% (95% CI, 3.9% to 25.1%). In contrast, the G20210A transition of the prothrombin gene could not be detected in any of the 10 asymptomatic homozygotes for FV:Q506.

In subjects affected by the heterozygous FV:R506Q mutation, the frequency of the prothrombin gene 20210 GA variant was increased by 3-fold in symptomatic patients compared with asymptomatic relatives (P=0.04); of 220 symptomatic FV:Q506 carriers, 25 (11.4%) had also the A20210 allele (95% CI, 7.5% to 16.3%), whereas among 79 asymptomatic FV:Q506 heterozygotes, the prothrombin variant was detected in only 3 cases (3.8%; 95% CI, 0.8% to 10.7%). Persons homozygous for the A20210 allele were not found among the study group presented in this article.

Taken together, the A20210 allele of the prothrombin gene was significantly overrepresented in symptomatic carriers of the FV:Q506 mutant compared with healthy controls (P<0.0001) and asymptomatic relatives (P=0.02). The FII 20210 genotyping results are summarized in Table 1.

Age at Onset of VTE in Relation to the Genotype

In subjects carrying both FII 20210 GA and FV:R506Q, first VTE occurs at a younger age than simply affected patients, but the difference was not statistically significant (Table 2). The median age at clinical onset was 32 years of age for patients simply heterozygous for FV:Q506 (range, 18 to 45), 28 for double heterozygotes (range, 18 to 45), 28 for simply homozygous carriers of the FV:R506Q mutation (range, 18 to 41), and 25 for patients homozygous for FV:Q506 and heterozygous for FII A20210 (range, 18 to 43).
Site of First Clinical Manifestation

The onset manifestation in 263 symptomatic patients is shown in Table 2. It consisted of lower-extremity deep-vein thrombosis (DVT) (n = 214) and unusual sites of venous thrombosis (VT): subclavian or axillary (n = 13), central nervous (n = 8), mesenteric (n = 8), portal (n = 3), Budd-Chiari syndrome (n = 1), inferior caval (n = 2), and renal (n = 1) VT. No differences in site of VT was observed with respect to sex or age at onset. However, as shown in Table 2, a significant increase in the prevalence of more unusual sites of VT at clinical onset was observed in doubly affected patients (9 of 30; 30%) compared with patients without the prothrombin gene variant (26 of 233; 11.1%) (P = 0.004), including 4 of 38 homozygotes (10.5%) and 22 of 195 heterozygotes (11.3%) for FV:Q506. Conversely, we found a statistically significant increase in the prevalence of the A20210 allele in those subjects who had a more unusual manifestation of first VT (9 of 35; 25.7%) compared with subjects who had DVT (21 of 214; 9.8%) at clinical onset (P = 0.007).

Pulmonary embolism (PE) was detected in 48 cases: in 14 of these 48 cases, it occurred as an isolated event (29.2%), whereas in the remaining 34 cases, it was associated with DVT (70.8%). Patients homozygous for FV:Q506 had a significantly higher rate of concomitant PE (13 of 43; 30.2%) compared with heterozygotes (21 of 220; 9.5%) (P < 0.001). Among patients with symptoms of only PE, none had the A20210 allele of the prothrombin gene, whereas in patients with concomitant PE, the A20210 allele was found in 11.8% (4 of 34).

Type of First Clinical Manifestation

At clinical onset, circumstantial risk factors known to be associated with an increased risk of VTE were found in 181 patients (68.8%), whereas spontaneous VTE (ie, with no apparent triggering factor other than the congenital deficiency) occurred in the remaining 82 subjects (31.2%). In 44 patients (16.7%; 29 females and 15 males) the first thromboembolic event occurred in conjunction with recent surgery and in 55 patients (20.9%; 22 females and 33 males), with trauma and/or immobilization. Fifty-five of 163 female patients (33.7%) had taken oral contraceptives before first VTE. In 25.1% of females (n = 41), the first thromboembolic event was associated with pregnancy or the postpartum period. Fourteen women had more than 1 single risk factor. The events with a triggering factor were significantly more frequent than the spontaneous episodes in the female subjects versus the males (133 of 163 [81.6%] of females versus 48 of 100 [48%] of males; P < 0.001). When study was confined to all patients with spontaneous VTE (n = 82), the A20210 allele was present in 19.5% (n = 16), whereas among patients with VTE preceded by a triggering factor it was found in 14 of 181 (7.7%) (P = 0.005).

Furthermore, homozygous FV:Q506 carriers experienced significantly more of the spontaneous VTE at onset (20 of 43; 46.5%) than patients heterozygous for FV:Q506 (62 of 220; 28.2%) (P = 0.01). Among heterozygotes, the type of first thromboembolic event clearly differed between patients carrying the single FV:Q506 and those who were double heterozygotes (P = 0.005). In the latter patient group, VTE occurred spontaneously in 52% of cases (13 of 25) compared with 25.1% of patients simply heterozygous for FV:Q506 (49 of 195). In homozygotes of FV:Q506, the rate of spontaneous VTE did not differ significantly in relation to the FII 20210 genotype. Site and type of first thromboembolic event in relation to the genotype are listed in Table 2.

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### Table 2. Site and Type of First Clinical Manifestation in Relation to the Factor V and Factor II Genotype

<table>
<thead>
<tr>
<th>FII 20210 Genotype</th>
<th>FVQ506 Homozygous (n=43)</th>
<th>FVQ506 Heterozygous (n=220)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age at first VTE, y (range)</td>
<td>28 (18–41)</td>
<td>25 (18–43)</td>
</tr>
<tr>
<td>Site of onset manifestation, n (%)††</td>
<td>32 (84.2)</td>
<td>25 (80.8)</td>
</tr>
<tr>
<td>DVT (n=214) (81.4%)</td>
<td>32 (84.2)</td>
<td>25 (80.8)</td>
</tr>
<tr>
<td>Unusual presentation of VT† (n=35) (13.3%)</td>
<td>4 (10.5)</td>
<td>2 (8.4)</td>
</tr>
<tr>
<td>PE as an isolated event (n=14) (5.3%)</td>
<td>2 (5.3)</td>
<td>2 (9.1)</td>
</tr>
<tr>
<td>PE associated with DVT (n=34/214) (15.8%)</td>
<td>11 (34.4)</td>
<td>2 (6.6)</td>
</tr>
<tr>
<td>Sex (female [n=163]/male [n=160]), n</td>
<td>25/13</td>
<td>3/2</td>
</tr>
<tr>
<td>Type of onset manifestation, n/%*</td>
<td>18 (48.8%)</td>
<td>13 (59.1%)</td>
</tr>
<tr>
<td>Spontaneous (n=82; 31.2%)</td>
<td>17 (44.7)</td>
<td>13 (55.6)</td>
</tr>
<tr>
<td>Females (n=30; 18.4%)</td>
<td>8 (26.6)</td>
<td>6 (20.0)</td>
</tr>
<tr>
<td>Males (n=52; 52%)</td>
<td>9 (17.3)</td>
<td>7 (13.5)</td>
</tr>
<tr>
<td>Presence of triggering factors, (n=181; 68.8%)</td>
<td>21 (55.3)</td>
<td>12 (50.0)</td>
</tr>
<tr>
<td>Females (n=133; 81.6%)</td>
<td>17 (68)</td>
<td>9 (69.2)</td>
</tr>
<tr>
<td>Males (n=48; 48%)</td>
<td>4 (30.8)</td>
<td>3 (12.5)</td>
</tr>
</tbody>
</table>

*Percentages are related to the corresponding subgroup.
†No sex-related difference were observed regarding the site of onset manifestation.
‡Subclavian or axillary (n=13), central nervous (n=7), mesenteric (n=8), portal (n=3), Budd-Chiari syndrome (n=1), inferior caval (n=2), and renal (n=1) VT.
Discussion
Very recently, increased risk of venous thrombosis has been associated with a variation in the prothrombin gene due to a G to A transition at nucleotide 20210.\textsuperscript{13} As the precursor of thrombin that is known to exert procoagulant, anticoagulant, and antifibrinolytic activities,\textsuperscript{19,20} the zymogen prothrombin possesses an important role in the balance between procoagulation and anticoagulation.\textsuperscript{21} Due to the central role of prothrombin in the hemostatic system, it is obvious that variations in the prothrombin gene might predispose to thrombosis. Accordingly, heterozygosity for the prothrombin gene variant 20210 GA has been identified in \( \approx \)18\% of selected patients with a personal and family history of VTE and in 5\% to 7.1\% of unselected patients with VTE.\textsuperscript{13–17} For heterozygous carriers of the FII A20210 allele, odds ratios for thrombosis of \( \approx 3 \) to 4 have been reported.\textsuperscript{13–16} In comparison, the odds ratio for thrombosis has been calculated to be 3- to 8-fold for those carrying the FV mutation in a heterozygous form and 30- to 140-fold in homozygous individuals.\textsuperscript{5,6,9,10,22–24} Therefore, we might assume that the 20210 A allele of the prothrombin gene is a common but probably mild risk factor of venous thrombosis. However, since the discovery of the FV:R506Q mutation and its apparent cosegregation in \( \approx 15\% \) to 25\% of patients with heterozygous deficiencies for antithrombin,\textsuperscript{25} protein C,\textsuperscript{26–29} or protein S,\textsuperscript{30–33} evidence is accumulating that the association of multiple hemostatic defects greatly increases the penetrance of the thrombotic disease. Accordingly, patients affected by double or multiple heterozygous defects presented with thrombosis at a younger age and had a substantially higher frequency of VTE compared with patients suffering from a single heterozygous defect.\textsuperscript{25–28,30,33} This finding raises the question of whether the prothrombin 20210 A allele may also cosegregate with the common FV:R506Q mutation and contribute to the thrombotic manifestation in subjects affected by inherited APC resistance. Because of the relatively high prevalence of either the FV:R506Q mutation or the FII 20210 A allele in thrombophilic patients as well as in the general population, however, either FV:Q\textsuperscript{506} or FII 20210 A is likely to be frequently identified as an additional risk factor predisposing for thrombosis in carriers of the other mutant. This prompted us to assess the coexistence of the prothrombin 20210 G to A variant in symptomatic and asymptomatic carriers of FV:Q\textsuperscript{506}. The most marked finding of our investigation is that the prevalence of the prothrombin gene was significantly higher among FV:Q\textsuperscript{506} carriers with a history of juvenile VTE (11.4\%) compared with a group of healthy individuals (2\%) and asymptomatic relatives (3.4\%). With respect to the coexistence of the prothrombin gene variant 20210 GA in carriers of the FV:R506Q mutation, the rate observed in the present investigation of relatively young thrombophilic patients was clearly higher compared with results recently published for other populations.\textsuperscript{15,16,34,35} However, when it is assumed that a high proportion of combined inherited hemostatic abnormalities already predisposes for thrombophilia in young persons, the significance of the uncommon coinheritance of both FV:Q\textsuperscript{506} mutant and FII 20210 GA observed in previous studies is difficult to assess; either the age was not mentioned at all\textsuperscript{34} or the majority of patients investigated were \( \geq 60 \) years of age.\textsuperscript{15} Additionally, since both FII 20210 A and particularly FV:R506Q are shown to be highly prevalent hereditary risk factors for VTE in Germans,\textsuperscript{22} our findings are not surprising for our geographic region. The 20210 A allele of the prothrombin gene possibly has a similar distinctive racial and/or geographic distribution as has been described for the FV mutant.\textsuperscript{36} These observations need to be kept in mind for prediction of the risk of VTE emanating in different populations from either FV:R506Q mutation or FII 20210 GA variant or their coinheritance.

Similar to our findings, Ferraresi et al\textsuperscript{17} found a significant increase in the frequency of the 20210 GA genotype in thrombophilic patients doubly heterozygous for other known thrombophilic defects (14\%), including FV Leiden, but not in their asymptomatic relatives (3\%). Zöller et al\textsuperscript{37} reported that none of 78 thromboprotein S–deficient patients carried the FII 20210 GA variant, whereas of 29 FV:R506Q-positive index cases, 3 (10\%) were carriers of the 20210 A allele in heterozygous form. They calculated that the combined heterozygosity for the latter 2 gene defects led to earlier onset of thrombosis and tended to be more severe than single gene defects. In addition, we have observed a statistically significant increase in the prevalence of spontaneous events and more unusual sites of venous thrombosis at clinical onset among patients carrying both FV:Q\textsuperscript{506} and FII A20210 compared with patients simply affected by the FV mutation.

In summary, the data from our investigation as well as from previous studies\textsuperscript{13,17,37} underline the importance of the prothrombin 20210 GA variant as a common additional risk factor for venous thrombosis in carriers of the FV:Q\textsuperscript{506} mutant. The high frequency of double carriership for FV:Q\textsuperscript{506} and FII 20210 GA found in our patients who experienced juvenile VTE supports the hypothesis that the presence of 2 inherited prothrombotic risk factors might lead to thromboembolic manifestations at young ages with an increasing rate of spontaneous onset manifestations. Thus, comprehensive investigations of the prothrombin 20210 A allele are important for interpretation of the additional thrombotic risk in patients with other genetic defects predisposing for thrombosis.

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doi: 10.1161/01.ATV.19.2.276
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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