Different circumstances of the first venous thromboembolism among younger or older heterozygous carriers of the G20210A polymorphism in the prothrombin gene

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Background and Objectives. The G20210A polymorphism in the prothrombin gene is a common cause of inherited thrombophilia. Scarce information is available about the circumstances of the heralding thrombotic manifestation at different ages. The aim of this study was to determine the risk of spontaneous or secondary venous thromboembolism (VTE) among younger and older carriers of the G20210A prothrombin polymorphism.

Design and Methods. We performed a case-control study, investigating 650 patients with a first objectively documented deep venous thrombosis of the legs or pulmonary embolism and 703 individuals with no history of vascular disease. In all of them we carried out laboratory screening for antithrombin III, protein C and protein S deficiencies, and for the presence of the factor V Leiden and the G20210A prothrombin polymorphisms.

Results. After adjustment for other inherited causes of thrombophilia (deficiency of antithrombin III, protein C or S, factor V Leiden) the overall risk for VTE associated with the prothrombin polymorphism was 3.4 times higher than in the controls (95% CI, 2.0 to 5.8). Stratification according to the age and to the circumstances of the first event revealed an increased risk of spontaneous VTE only among the patients older than 45 years in comparison with age-matched controls (odds ratio 4.4, 95% CI 1.8 to 10.6); among the younger individuals the risk was increased for secondary VTE (odds ratio 4.8, 95% CI, 2.3 to 9.8) but not for spontaneous VTE.

Interpretation and Conclusions. The clinical penetrance of the thrombotic tendency associated with the G20210A prothrombin polymorphism is more expressed in the presence of a circumstantial risk factor (oral contraceptives, pregnancy, surgery, trauma) and in the presence of older age, which acts as an additional circumstantial risk factor. Accordingly, such situations should not discourage from carrying out laboratory screening.

Key words: venous thromboembolism, inherited thrombophilia, prothrombin G20210A, age.

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A G20210A transition in the 3'-untranslated region of the prothrombin gene is present in 2% of the general population and is a common cause of venous thromboembolism (VTE). A combined analysis of 14 case-control studies including 6,267 controls (3% heterozygous carriers of the mutant allele) and 3,356 patients with VTE (7.3% heterozygous carriers of the mutant allele, p < 0.001) produced a pooled odds ratio of 2.5 (95% CI 2.1 to 3.1). In spite of the large number of studies so far published, a thorough analysis of the clinical characteristics of the venous thromboembolic episodes occurring in the carriers of the prothrombin gene polymorphism is lacking. In general, most investigations were aimed to estimate the overall risk associated with the prothrombin gene polymorphism and no study analyzed the circumstances of the first VTE after stratification according to age at the time of the clinical event. In order to investigate whether the age of the carriers of the prothrombin gene polymorphism is relevant in producing different clinical presentations of a first VTE, we carried out a case-control study.

Design and Methods

Patients

We investigated 650 unrelated Italian patients (297 men and 353 women) who had previously had a deep vein thrombosis of the legs or a pulmonary embolism. Two hundred and seventy-two patients were consecutively admitted to our hospital from the emergency ward for an acute venous thrombotic event during the period November 1995 through February 2002; in the same period another 378 patients diagnosed in hospital by their physicians were consecutively referred to our Thrombosis Center for investigation of possible causes of thrombophilia. All patients were of Italian ancestry. A preliminary evaluation had ruled out patients with overt forms of neoplasia, autoimmune diseases (including primary antiphospholipid syndrome) and overt myeloproliferative disorders. All patients were interviewed by physicians who collected their medical history and evaluated the available objective documentation before any laboratory investigation, so that a diagnosis of VTE was recorded without knowledge of the results of the thrombophilia screening. The presence of circumstantial risk factors at the time of VTE, such as pregnancy or puerperium, oral contraceptive use, surgery, prolonged immobilization (bed rest for at least ten days, or plastering of the legs) and trauma was also recorded. A diagnosis of deep vein thrombosis was accepted if it had been established by objective methods such as phlebography or ultrasound.
examination; a diagnosis of pulmonary embolism was accepted if proven by perfusion lung scanning, computerized tomography, or nuclear magnetic resonance. Pulmonary embolism was diagnosed in 215 patients, 57 of whom were without objective evidence of deep vein thrombosis of the legs.

A control group of 703 unrelated individuals with Italian ancestry and with no history of previous thromboembolism (410 men and 293 women) was also investigated at the same time as the patients. The control subjects were recruited from among blood donors (n = 330), members of the hospital staff (n = 139), and outpatients with diagnoses other than malignant or autoimmune diseases (n = 234). Their mean age was 42 years (median 41, range 7 to 93).

**Laboratory tests**

Informed consent for laboratory screening was given by all the individuals (or by their parents in the case of children). The screening for thrombophilia has been previously described.\(^{16,17}\) Antithrombin heparin cofactor activity was measured by an amidolytic assay (Coamate Antithrombin, Chromogenix AB, Molndal, Sweden). Protein C activity was assayed by an amidolytic assay (Coamatic Protein C, Chromogenix) after Protac® activation. Total protein S antigen was measured by ELISA using polyclonal antibodies (Dako A/S, Glostrup, Denmark), and free protein S was measured in the same way after precipitation of the C4b-binding protein-protein S complex with polyethylene glycol 6000 (3.5% final concentration) or directly by ELISA using a commercial kit with a specific monoclonal antibody (Asserachrom® Free Protein S, Stago, Asnières, France). Diagnosis of antithrombin, protein C or protein S inherited deficiency was established if the plasma level was below the normal lower limit (75% for antithrombin and 70% for protein C and protein S) and at least one other first-degree relative of the proband had decreased plasma levels. DNA samples were analyzed for the presence of factor V Leiden and the G20210A prothrombin polymorphisms by specific amplification and digestion.\(^{2,18}\)

**Statistical analysis**

Differences between groups were estimated by Fisher’s exact test. Univariate odds ratios were calculated as an approximation of the relative risk for putative risk factors by simple cross-tabulation, with 95% confidence intervals.

**Results**

**Control groups**

Among the control individuals 21 were carriers of factor V Leiden (20 heterozygotes, 15 men and 5 women, and 1 female homozygote) (3%) and 21 were heterozygous carriers of the G20210A polymorphism in the prothrombin gene (12 men and 9 women, 3%). No control individual was homozygous for the prothrombin gene polymorphism or carried both factor V Leiden and the prothrombin gene polymorphisms.

**Clinical and laboratory features of the patients**

The main clinical and laboratory features of the population of patients are reported in Table 1. Thirty-eight per cent of the first venous thromboembolic episodes occurred spontaneously in the absence of a circumstantial risk factor (pregnancy and puerperium, oral contraceptives, surgery, trauma, bed rest). A condition associated with thrombophilia was detected in 33% of the cases; heterozygosity for the G20210A polymorphism in the prothrombin gene was found in 63 patients (9.7%), 16 of them (2.5%) carrying another thrombophilic trait (in 2 cases antithrombin deficiency, in 1 case protein S deficiency, in 1 case homozygosity for factor V Leiden and in 12 cases heterozygosity for factor V Leiden). These latter were excluded from further estimates concerning the prothrombin polymorphism, since the thrombotic risk could be attributed in part to the associated abnormality. Among the consecutive patients the percentage of patients with inherited thrombophilia was 26%, and among the referred patients it was 39% (p = 0.001). This difference was due to the higher percentage of patients with antithrombin, protein C, protein S deficiency or factor V Leiden among the referred patients (31%) in comparison with among the consecutive patients (19%, p < 0.001), whereas the carriers of the G20210A polymorphism in the prothrombin gene without other plasma abnormalities were similarly distributed between the two groups (7% among both the consecutive and the referred patients, p = 1.00).

The overall risk of VTE estimated in the whole patient cohort after adjustment for other causes of inherited thrombophilia was 3.4 times higher than that in controls (Table 2); the magnitude of the increase in risk did not change substantially for spontaneous VTE or for VTE associated with circumstantial risk factors (Table 2). The circumstantial risk factors associated with VTE among the individuals carrying the prothrombin gene polymorphism were surgery (n = 11), prolonged bed immobilization (n = 5), a previous trauma (n = 4), plastering of the legs (n = 2), oral contraceptive use (n = 4), puerperium (n = 4), and pregnancy (n = 1). One patient had a popliteal (Baker’s) cyst in the leg with venous thrombosis.

The first venous thromboembolic episode occurred in 441 patients before 45 years of age (180 males and 261 females, mean age at the first thrombosis 30 years, median 31, range 1 to 45); they were compared to the 422 controls younger than 45 years (242 males and 180 females, mean
age 32 years, median 32, range 7 to 45). The remaining 209 patients with a first VTE after 45 years (117 males and 92 females, mean age at the first thrombosis 58 years, median 57, range 46 to 84) were compared to the 281 controls older than 45 years (168 males and 113 females, mean age 57 years, median 56, range 46 to 93). The overall increase in risk of a first VTE associated with the G20210A prothrombin gene polymorphism was similar in both younger and older individuals (3.7-fold and 3.1-fold, respectively) (Table 2).

Further stratification according to the circumstances of the first VTE among younger or older patients in comparison with the respective age-matched control groups revealed, among the younger carriers of the G20210A prothrombin polymorphism, a significant increase in the risk of secondary VTE (odds ratio 4.8, 95% CI 2.3 to 9.8), but not of spontaneous VTE (Table 2). In contrast, the older individuals carrying the prothrombin polymorphism had a significantly increased risk of spontaneous VTE (odds ratio 4.4, 95% CI 1.8 to 10.6) but not of secondary VTE (Table 2).

Table 1. Clinical and laboratory characteristics of the patients with venous thromboembolism (VTE).

<table>
<thead>
<tr>
<th>Patients</th>
<th>Consecutive (n=272)</th>
<th>Referred (n=378)</th>
<th>Overall (n=650)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males/ females (ratio)</td>
<td>128/144 (0.88)</td>
<td>169/209 (0.80)</td>
<td>297/353 (0.80)</td>
</tr>
<tr>
<td>Mean age (median, range) - yrs at the first VTE</td>
<td>43 (41.2-80)</td>
<td>37 (34.1-84)</td>
<td>39 (37.1-84)</td>
</tr>
<tr>
<td>Mean age (median, range) - yrs at observation</td>
<td>43 (41.2-80)</td>
<td>47 (47.1-86)</td>
<td>45 (45.2-86)</td>
</tr>
<tr>
<td>Spontaneous first VTE, n (%)</td>
<td>104 (38)</td>
<td>143 (38)</td>
<td>247 (38)</td>
</tr>
<tr>
<td>Risk factors associated with first VTE, n (%): pregnancy or puerperium *</td>
<td>29 (10)</td>
<td>62 (30)</td>
<td>91 (26)</td>
</tr>
<tr>
<td>oral contraceptives *</td>
<td>36 (13)</td>
<td>38 (18)</td>
<td>74 (21)</td>
</tr>
<tr>
<td>surgery</td>
<td>39 (14)</td>
<td>60 (16)</td>
<td>99 (15)</td>
</tr>
<tr>
<td>trauma and plastering of the legs</td>
<td>19 (7)</td>
<td>39 (10)</td>
<td>58 (9)</td>
</tr>
<tr>
<td>bed rest</td>
<td>30 (11)</td>
<td>30 (8)</td>
<td>60 (10)</td>
</tr>
<tr>
<td>other causes</td>
<td>7 (3)</td>
<td>6 (1)</td>
<td>13 (2)</td>
</tr>
<tr>
<td>Total secondary first VTE, n (%)</td>
<td>168 (62)</td>
<td>235 (62)</td>
<td>403 (62)</td>
</tr>
<tr>
<td>Antithrombin deficiency, n (%)</td>
<td>6 (2)</td>
<td>2 (1)</td>
<td>8 (1)</td>
</tr>
<tr>
<td>Protein C deficiency, n (%)</td>
<td>8 (3)</td>
<td>15 (4)</td>
<td>23 (4)</td>
</tr>
<tr>
<td>Protein S deficiency, n (%)</td>
<td>4 (1)</td>
<td>7 (2)</td>
<td>11 (2)</td>
</tr>
<tr>
<td>Heterozygosity for factor V Leiden, n (%)</td>
<td>26 (10)</td>
<td>73 (19)</td>
<td>99 (15)</td>
</tr>
<tr>
<td>Homozygosity for factor V Leiden, n (%)</td>
<td>2 (1)</td>
<td>5 (1)</td>
<td>7 (1)</td>
</tr>
<tr>
<td>Heterozygosity for prothrombin G20210A, n (%)</td>
<td>20 (7)</td>
<td>27 (7)</td>
<td>47 (7)</td>
</tr>
<tr>
<td>Combined defects, n (%)</td>
<td>5 (2)</td>
<td>17 (5)</td>
<td>22 (3)</td>
</tr>
<tr>
<td>Total patients with thrombophilia, n (%)</td>
<td>71 (26)</td>
<td>146 (39)</td>
<td>217 (33)</td>
</tr>
</tbody>
</table>

*percentage of female patients.

Table 2. Relative risk for venous thromboembolism (VTE) associated with the heterozygous G20210A polymorphism in the prothrombin gene (odds ratio, OR, with 95% confidence interval, CI). The odds ratio was estimated after exclusion of the subjects with other causes of inherited thrombophilia.

<table>
<thead>
<tr>
<th>All thrombotic episodes</th>
<th>Spontaneous VTE</th>
<th>Secondary VTE</th>
</tr>
</thead>
<tbody>
<tr>
<td>All ages, OR (95% CI)</td>
<td>3.4 (2.0-5.8)</td>
<td>3.1 (1.5-6.1)</td>
</tr>
<tr>
<td>Age ≤ 45 years, OR (95% CI)</td>
<td>3.7 (1.8-7.6)</td>
<td>1.3 (0.4-4.8)</td>
</tr>
<tr>
<td>Age &gt; 45 years, OR (95% CI)</td>
<td>3.1 (1.4-6.8)</td>
<td>4.4 (1.8-10.6)</td>
</tr>
</tbody>
</table>

Discussion

Conflicting data are available about the circumstances of the first VTE among heterozygous carriers of the G20210A prothrombin gene polymorphism. The risk of first VTE has been suggested to be significant only in the elderly population6 but in other studies the risk associated with the mutant genotype was increased in all age groups2,9,11,13 or was marginally increased only in the younger population.5,19 Analysis of the circumstances of the first VTE (spontaneous or secondary to circumstantial risk situations such as pregnancy, surgery, oral contraceptive use) did not show any variation in the increased risk in two stud-
ies, whereas the risk was increased only for secondary thromboses and not for spontaneous ones in another study.

A reason for such discrepancies could be a difference in the selection procedures of the series of patients, including patients referred for screening to specialized Thrombosis Centers or consecutive patients or patients derived from population-based studies. Moreover, several studies excluded individuals aged more than 60 to 70 years, so that their conclusions are biased regarding the effect of thrombophilic traits among the elderly population.

In our study the prevalence of inherited thrombophilia among the individuals referred to our Thrombosis Center was higher than that among the patients consecutively admitted to our hospital; yet this difference was due to an overrepresentation of individuals with deficiency in antithrombin, protein C, or protein S or resistance to activated protein C. Such alterations can nowadays be easily diagnosed by commercial kits and a number of patients had been probably referred by their physicians not only on the basis of the clinical history but also on the basis of an abnormal preliminary plasma screening. On the other hand the prevalence of the G20210A polymorphism in the prothrombin gene was similar among referred and consecutive patients; this might be due to the fact that DNA analysis is available only in specialized laboratories, so that a referral bias related to preliminary laboratory results can be excluded. Since the thrombotic risk associated with the G20210A prothrombin polymorphism was estimated after exclusion of all the individuals carrying other plasma or gene abnormalities, the effect of the aforementioned referral bias was considered negligible.

Our findings fit with most previous reports, since stratification for age or for the circumstances of the first VTE did not produce any significant variation in the 3.4-fold increase in thrombotic risk associated with the prothrombin polymorphism (Table 2). Yet further stratification according to both age and circumstances of the first VTE revealed that among the younger carriers of the prothrombin gene polymorphism, VTE is for the most part associated with circumstantial risk factors, with a 4.8-fold increase in risk of secondary events. This suggests that the G20210A polymorphism in the prothrombin gene is a relatively weak risk factor which needs a concomitant acquired risk circumstance for clinical expression. In contrast, carriers of the prothrombin polymorphism are significantly more prone to spontaneous VTE only in an older age range, with a 4.4-fold increase in risk after 45 years and 19.6-fold increase in risk after 60 years. The age at the first spontaneous VTE was before 45 years in the large majority of patients with deficiency of antithrombin, protein C or protein S; on the other hand, a substantial portion (43%) of the carriers of factor V Leiden with spontaneous VTE had their first episode after 45 years. This is in agreement with previous observations that factor V Leiden is associated with a less severe thrombotic risk than that due to deficiency of antithrombin, protein C, or protein S. The first spontaneous VTE occurred after 45 years in 75% of the symptomatic carriers of the G20210A prothrombin polymorphism. In the general population the risk of VTE increases with age, accordingly, in carriers of the prothrombin polymorphism, advanced age (i.e. the prolonged exposure to the mutant genotype) seems to acts as a concomitant acquired risk circumstance, leading to an increased risk of spontaneous VTE. A similar positive interaction between age and a genetic risk factor has been previously reported for factor V Leiden.

Our findings contrast with those of two studies, which reported only a marginally increased risk of VTE associated with the prothrombin polymorphism. The first, derived from the cohort enrolled in the Physician's Health Study, found a modest increase in risk of primary or secondary VTE among male individuals aged 40 to 84 years carrying the prothrombin polymorphism. The general design of this investigation, enrolling a special group of male physicians, who self-reported thrombotic events, should induce caution when considering such negative results. Moreover, at variance with most studies concerning this issue, cancer as a cause of secondary VTE was not excluded, so that the impact of the mutant genotype could have been reduced in comparison with a strong prothrombotic factor such as neoplasia; finally, the estimate of the relative risk was not adjusted for other causes of inherited thrombophilia, so that the ratio between the venous thrombotic episodes occurring among the carriers of the prothrombin polymorphism alone and those occurring among the individuals with no detected cause of thrombophilia was likely underestimated. Another study was a cross-sectional investigation on a large random population sample; only among individuals younger than 45 years did the carriers of the prothrombin polymorphism have a marginally increased risk of spontaneous VTE. Yet this investigation excluded all the inhabitants aged less than 18 years and more than 65 years, probably leading to an underestimation of the role of the prothrombin polymorphism among elderly individuals. In our series, out of 47 heterozygotes for the prothrombin polymorphism, 10 (21%) had a first VTE outside this age range (4 before 18 years and 6 between 66 and 73 years of age).

In conclusion we confirmed that the G20210A polymorphism in the prothrombin gene is associated with an increased risk of VTE; such a risk is rel-
Prothrombin G20210A and age at thrombosis

...tatively weak, so that the clinical penetrance of the defect is fully expressed in the presence of a concomitant acquired risk circumstance, including older age. Therefore laboratory screening should not be selective, since exclusion of elderly patients may lead to a significant risk for VTE associated with exposure to triggering situations in the young should prompt family screening in order to identify the asymptomatic carriers of the prothrombin polymorphism and offer them short-term antithrombotic prophylaxis during risk situations.

References

10. Margagnone M, Brancaccio V, Giuliani N, D'Andrea G, Capr...
Pre-publication Report & Outcomes of Peer Review

Contributions
VD was responsible for the conception of the study, data analysis, and development of the text; ER contributed to the conception of the study and to the interpretation of the data. VD and ER should be equally considered as the principal authors. VD, GC, EM, RP, ER, and KP selected the patients and were responsible for their clinical management; VD, ER, and KP performed the medical interviews of the patients; ER and KP selected the controls and performed their medical interviews; AD, ER, and KP performed the laboratory tests; ER was responsible for the overall clinical and laboratory data collection and the statistical analysis; GL supervised the study and critically reviewed the manuscript giving important intellectual contribution as senior author.

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Redundant publications: no substantial overlapping with previous papers.

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This manuscript was peer-reviewed by two external referees and by Professor Vicente Vicente, Deputy Editor. The final decision to accept this paper for publication was taken jointly by Professor Vicente and the Editors. Manuscript received August 30, 2002; accepted November 13, 2002. In the following paragraphs, professor Vicente summarizes the peer-review process and its outcomes.

What is already known on this topic
The G20210A polymorphism of the prothrombin gene has been associated with a thrombophilic status. However, an accurate evaluation of risk of venous thromboembolism among carriers of this polymorphism has not been established.

What this study adds
This study confirms that the thrombotic risk associated with the G20210A polymorphism is weak. However, the clinical penetrance of this polymorphism is clearly more relevant in the presence of concomitant acquired risk circumstances, such as oral contraceptive use, pregnancy, surgery, and trauma, especially in older patients.