Factor V Leiden Prevalence in Venous Thromboembolism Patients*

Christophe Leroyer, MD; Bernard Mercier, MD; Martine Escoffre, MD; Claude Férec, MD; and Dominique Mottier, MD

Background: Recent findings have demonstrated a high frequency of activated protein C resistance in patients suffering from deep venous thrombosis (DVT). This abnormality has been related to a mutation in the factor V gene (at nucleotide position 1,691, guanine to adenine [G→A] substitution).

Aim: To assess the frequency of the mutation in unselected inpatients with a proved DVT. To study the clinical characteristics of such patients.

Methods: All consecutive patients admitted to the hospital because of a clinical suspicion of DVT were eligible. Diagnosis of DVT with the help of venous ultrasound imaging or venography. Ventilation and perfusion lung scan was performed in all patients, and interpreted according to the Prospective Investigation of Pulmonary Embolism Diagnosis criteria; in patients with a low- or intermediate-probability lung scan, pulmonary angiography was requested. Polymerase chain reaction amplification was performed in patients with a proved DVT. A control group consisted of bone marrow volunteer donors.

Results: From July 1994 to November 1995, 165 patients were included. Thrombosis was considered as distal in 77 and proximal in 88; an associated pulmonary embolism (PE) was found in 75 patients. Of 165 patients, 24 (14.5%) showed the factor V gene mutation (95% confidence interval, 9.4 to 19.8); the mutation was present in 3.5% of 200 bone marrow volunteer donors; odds ratio for having DVT in the presence of the mutation was 4.1. No difference in the level of DVT, or the presence of an associated PE was observed according to the presence of the mutation. Patients with the mutation have a significantly more frequent history of DVT (p=0.04) and more previous reported episodes (1.1 vs 0.6; p=0.04).

Conclusion: The factor V mutation is frequent in unselected DVT patients. No difference in the severity of the thrombosis episode was observed in these patients.

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Key words: embolism; factor V; risk factor; pulmonary; thrombophilia; thrombosis, vein

Abbreviations: APC=activated protein C; CI=confidence interval; DVT=deep venous thrombosis; PE=pulmonary embolism; VTE=venous thromboembolism

The single point mutation in the factor V gene (at nucleotide position 1,691, guanine to adenine [G→A] substitution) has been recently recognized as the genetic basis for poor anticoagulant response to activated protein C (APC).2-5 The assessment of the mutation may appear preferable to the study of APC resistance: this latter may be biased due to underlying conditions, such as anticoagulant therapy, prolonged baseline activated partial thromboplastin time, or false-positive results.6,7 Whether unselected patients suffering from venous thromboembolism (VTE) should be tested for these possible abnormalities remains debatable.8 The aims of this study were (1) to evaluate the frequency of the mutation in the factor V gene in unselected inpatients with deep venous thrombosis (DVT), and (2) to assess the clinical characteristics associated with the presence of the mutation, including the age of occurrence and the severity of the disease.

Materials and Methods

Patients and Control Subjects

Since March 1994, data have been collected prospectively on a register of all consecutive patients with a clinically suspected...
DVT admitted to the Department of Internal Medicine and Chest Diseases. Patients who developed DVT during the course of hospitalization were not included.

For all patients, clinical data were registered at admission to the Department by two clinicians. Questions were asked on history of VTE, number of previous episodes, age of the first episode, and family history of VTE in first-degree relatives (parents, sibling, or child). The following risk factors were registered: prolonged immobilization (>72 h), recent surgery or trauma within the last 3 months, varicose veins, congestive heart failure, malignancy, obesity (20% over ideal body weight), COPD, and treatment with estrogens.

DNA samples were performed in all patients with a proved DVT. A control group consisted of 200 healthy bone marrow volunteer donors to estimate the incidence of the mutation in our population. The study protocol was approved by the Ethics Committee of our University Hospital, and written informed consent was obtained from all participants.

**Diagnostic Methods**

All patients had objective tests within 48 h of hospital admission to confirm the diagnosis of DVT. The diagnosis of DVT was performed using either venous ultrasound imaging or venography. Venous ultrasound imaging was the standard for diagnosis; venography was undertaken only in the case of inconclusive assessment of the deep veins by venous ultrasound imaging.

Venous ultrasound imaging (B-mode, duplex, and color Doppler) was performed by two trained physicians using a high-resolution (7.5 MHz) transducer. The diagnosis of DVT was based on the finding of a direct intraluminal image, the absence of venous compressibility, and abnormalities in the Doppler signal. If there was no intraluminal defect, a full venous compressibility, and a normal flow, the test result was considered as normal. In other cases, results of venous ultrasound were considered as inconclusive, and a venography was performed.

Ascending radiographic bilateral venography was performed using a bolus injection of low-osmolar nonionic contrast medium (Omipaque; Nycemed Imaging As; Askar, Norway), via scalp needles inserted in a dorsal vein; tourniquets were placed around the lower third of the calf, and around the lower third of the thigh. Six conventional 30.120-cm radiographs were taken while the tourniquets were being removed sequentially. If iliac veins and/or vena cava were not clearly visible, digitalized venograms were taken after a unilateral or bilateral distal contrast medium injection. If the vena cava remained invisible, a digitalized proximal cavography was performed after a unilateral femoral injection. Interpretation was undertaken by a trained radiologist. The diagnosis of a recent thrombus was based on the appearance of a constant intraluminal filling defect within an occluded vein.

Thrombosis was considered as distal if involving only the calf veins (anterior tibial veins, posterior tibial veins, peroneal veins) below the trifurcation of the popliteal vein. Thrombosis was considered as proximal if involving the deep veins in the pelvis, the thigh, and popliteal region proximal to the trifurcation, with or without calf-vein thrombosis. If patients had bilateral vein thrombosis, they were classified according to the most proximal extension of the thrombus.

A ventilation-perfusion scan was performed in all patients with a proved DVT. Ventilation studies were performed with 1,100 MBq of nebulized phylate technecne; perfusion scans were then performed immediately, using microspheres labeled with 99mTc albumin, injected IV; for each technique, six views were obtained. Interpretation was performed by a trained physician, according to the Prospective Investigation of Pulmonary Embolism criteria. Patients with a normal lung scan were considered as free from pulmonary embolism (PE); patients with a high-probability scan were considered as having PE; in patients with low- or intermediate-probability lung scans, a pulmonary angiogram was requested within 48 h of hospital admission. The femoral vein Seldinger technique with a 5 F to 7 F pigtail catheter was used; selected injections in both right and left pulmonary arteries were performed. A mask of the region under study was done prior to injection, and then withdrawn to the obtained angiograms, according to the digital technique. If no embolism was detected on anterior projections, a lateral view was carried out on both sides. Interpretation was performed by a trained radiologist. PE was proved if either a constant intraluminal defect was present or a cup-shaped stop in the contrast medium progression was observed in an artery >2 mm.

**PCR Amplification**

DNA was extracted according to standard salting out procedure.10 Polymerase chain reactions were performed in a 50-μL reaction mixture containing the following: 10 mmol/L Tris Hcl at a pH of 8.3, 50 mmol/L KCl, 1.5 mmol/L MgCl2, 200 μmol/L dNTPs, 25 pmol of primers FV - 10 ε5 -C (AGTCCTTA-CAAGACATACATGACGACATGACGACGATCGATCTGCCC), and FV-10 ε3 (AAATCTCAGAATTCT-GAAGGTTA), 100 to 500 ng of genomic DNA, and 1 U of Taq polymerase. Amplifications were performed (model 9600; Perkin Elmer Corp; Norwalk, Conn): 3 min at 94°C followed by 40 cycles of 30 s at 94°C, 30 s at 57°C, 30 s at 72°C, and a final extension of 5 min at 94°C, and 15 min at 68°C. The amplified fragments were then digested by Mnl I restriction endonuclease and analyzed by electrophoresis on a 1.4% agarose gel. The Mnl I restriction fragments were 105+54 base pairs for the normal allele and 159 base pairs for the mutated allele. This analysis allows the identification of the normal heterozygotes and homozygotes. For all the patients carrying at least one factor V Leiden allele, another amplification was performed to confirm the results.1

**Statistical Analysis**

To calculate sample size, two proportions were selected from previous studies: the frequency of the factor V mutation in patients with a history of VTE (21%), and the frequency of associated PE in case of DVT (41% in a similar group of patients from our study center). Based on these outcomes, we estimated that at least 150 patients will be required to show a significant 40% increase of associated PE in DVT patients with the mutation, assuming a type I error of 5% and a power of 80%.

Analyses were performed using a statistical software package (Epi-info; CDC; Atlanta). Analyses were accomplished using x2 or Student’s t test, when appropriate. A p value ≤0.05 was considered significant.

**Results**

From July 1994 to November 1995, 165 patients, 75 men and 90 women, were included in the study. The mean (±SD) age was 57.4±19.1 years (range, 15 to 88). Thrombosis was considered as distal in 77 (46.6%) and as proximal in 88. Diagnosis was performed with the help of a venous Doppler ultrasound in 140 patients and of venography in the remaining 25. An associated PE was found in 75 patients (45.4%). Diagnosis was performed with the
help of pulmonary scintigraphy in 44; for the remaining 31 patients, diagnosis was performed with additional pulmonary angiography.

Twenty-four of 165 patients (14.5%) showed the mutation of the factor V gene (at nucleotide position 1691, G→A substitution). The 95% confidence interval (CI) was 9.4 to 19.8. In the 53 patients younger than 50 years old, as many as 25.6% showed the mutation. All subjects were heterozygous for the mutation. In contrast, the mutation was present in 3.5% (95% CI, 1 to 6) of the 200 healthy bone marrow volunteer donors (mean age, 40 years; range, 20 to 55 years). All DVT patients and control subjects were white. Odds ratio for having DVT in the presence of the mutation was 4.1 (95% CI, 1.8 to 9.4).

Table 1 shows the clinical characteristics of the 24 patients with the mutation, compared to the 141 DVT patients without the mutation. Mean (±SD) age of patients with the mutation was significantly lower: 51.2±20.2 vs 59.9±18.6 years (p=0.04). Age ranged from 20 to 79 years in patients with the mutation, and from 15 to 88 years in DVT control subjects. The proportion of patients with the mutation was not significantly different among subgroups of patients, divided according to the level of the thrombosis process (ie, distal or proximal) or the occurrence of an associated PE. The presence of reported previous VTE episodes was significantly more frequent in patients with the mutation (p=0.04). The number of reported previous VTE episodes was higher in patients with the mutation (1.1±1.4 vs 0.6±1.1; p=0.04). In addition, the age of the reported first VTE episode was lower in patients with the mutation (37.1±24 vs 45.2±27), and the familial history of VTE was more frequent in patients with the mutation (41.6% vs 25.5%); these differences, however, did not reach the level of significance (p=0.12 and p=0.1, respectively). Lastly, the presence of associated risk factors was frequent in both patients with and without the mutation (70.8% vs 70.9%; p=0.95). The most frequent risk factors were recent surgery or trauma in 65 (39.4%) patients, prolonged immobilization in 47 (28.4%) patients, and malignancy in 36 (21.8%) patients. The prevalence of the mutation was not different in these subgroups.

**Table 1—Clinical Characteristics of the 165 DVT Patients With and Without the Factor V Gene Mutation**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Factor V Mutation</th>
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<tbody>
<tr>
<td></td>
<td>Presence (n = 24)</td>
<td>Absence (n = 141)</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>9/15</td>
<td>66/75</td>
</tr>
<tr>
<td>Age, yr, mean±SD</td>
<td>51.2±20.2</td>
<td>59.9±18.6</td>
</tr>
<tr>
<td>Location of DVT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distal/proximal</td>
<td>13/11</td>
<td>64/77</td>
</tr>
<tr>
<td>Associated PE</td>
<td>11</td>
<td>64</td>
</tr>
<tr>
<td>Previous VTE, No. (%)</td>
<td>14 (58.3)</td>
<td>51 (36.1)</td>
</tr>
<tr>
<td>Age, yr, of first episode, mean±SD</td>
<td>37.1±24</td>
<td>45.2±27</td>
</tr>
<tr>
<td>No. of previous episodes</td>
<td>1.1±1.4</td>
<td>0.6±1.1</td>
</tr>
<tr>
<td>Familial history of VTE, No. (%)</td>
<td>10 (41.6)</td>
<td>36 (25.5)</td>
</tr>
<tr>
<td>Associated risk factor, No. (%)</td>
<td>17 (70.8)</td>
<td>100 (70.9)</td>
</tr>
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</table>

**DISCUSSION**

Three conclusions can be drawn from our study that evaluate the frequency of the factor V mutation in unselected inpatients with a proved DVT. First, as expected from previous results on APC resistance, we found a high frequency of 14.5%, and an estimated odds ratio of 4.1 for having DVT in the presence of the abnormality. In addition, the presence of the mutation was significantly associated with a history of VTE and with more previous episodes. Second, no difference in the severity of the thrombosis process was observed according to the presence of the mutation. Third, an associated risk factor was frequently detected, both in patients with and without the mutation.

Only a few case-control designed studies have been performed so far to assess the frequency of the factor V mutation in VTE patients. Bertina et al studied 301 selected patients with a first episode of DVT, referred for anticoagulant treatment; the presence of the mutation was detected in 17.6%, and explained 82% of the observed APC resistance. Rosendaal et al determined the factor V Leiden genotype in 471 patients aged younger than 70 years with a first episode of DVT; the presence of the mutation was detected in 19.5%. In contrast, the presence of the mutation was detected in, respectively, 0% and 2.9% of the control subjects. These results are consistent with the recent findings by Ridker et al13,14 based on the Physicians’ Health Study cohort of apparently healthy men (age range, 40 to 84 years). During a mean follow-up period of 8.6 years, 121 subjects of this cohort had a confirmed VTE disease. Using a case-control design, they showed that the presence of the factor V mutation (12 heterozygous subjects) was associated with an increased risk of venous thrombosis. Taken together, these findings suggest that screening for factor V mutation at the first presentation of DVT may be useful.
REFERENCES


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