Brief Report

Prevalence of Hereditary Thrombophilia in Patients Older Than 75 Years With Venous Thromboembolism Referred for Thrombophilia Screening

Virginie Siguret,1,2 Joseph Emmerich,1,3 Tiphaine Belleville,1 Jean-Louis Golmard,4 Elisabeth Mazoyer,5 Isabelle Gouin-Thibault,1,5 and Eric Pautas1,6,7

1Université Paris Descartes, Sorbonne Paris Cité, INSERM UMR-S-1140, France. 2Assistance Publique Hôpitaux de Paris, Service d’Hématologie biologique, Hôpital Européen Georges Pompidou, France. 3Assistance Publique Hôpitaux de Paris, Service de Médecine vasculaire-HTA, Hôpital Européen Georges Pompidou, France. 4Assistance Publique Hôpitaux de Paris, Département de Biostatistiques, GH Pitié-Salpêtrière-Charles Foix, France. 5Assistance Publique Hôpitaux de Paris, Service d’Hématologie biologique, Hôpital Cochin, France. 6Assistance Publique Hôpitaux de Paris, Court séjour gériatrique, DHU FAST, GH Pitié-Salpêtrière-Charles Foix, Ivry sur Seine, France. 7Université Pierre et Marie Curie, Faculté de Médecine, Paris, France.

Address correspondence to Virginie Siguret, PhD, INSERM UMR-S-1140, 4 av de l’Observatoire, 75006 Paris, France. Email: virginie.siguret@parisdescartes.fr

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Abstract

Background. Few studies focused on genetic risk factors for venous thromboembolism (VTE) in the very elderly people. In patients aged 75 years and older with VTE referred for laboratory screening tests for thrombophilia, we aimed: (i) to estimate the F5G1691A and F2G20210A mutation prevalence; (ii) to compare prevalence rates with those of a control group; and (iii) to compare the prevalence rates between patient subgroups, defined as with one or multiple VTE episodes and with provoked/unprovoked VTE.

Methods. Data were extracted from two prospective thrombophilia registries according to the following inclusion criteria: Caucasian patients aged 75 years and older presenting with at least one confirmed VTE episode. Associated VTE risk factors had been recorded using a standardized questionnaire. Laboratory tests included plasma antithrombin, protein C, and protein S activity measurements and F5G1691A and F2G20210A genotyping.

Results. Of the 312 patients (mean age: 84 ± 6 years; 245 women and 67 men), 47.1% had two or more VTE episodes and 63.5% patients had unprovoked VTE. None had deficiencies in antithrombin, protein C, or protein S. The F5G1691A and F2G20210A mutations were found in 29 (9.3%, 95% confidence interval [CI]: 6.3–13.1) and 18 (5.8%, 95% CI: 3.5–9.0) patients, respectively, versus 3.4% (95% CI: 1.9–4.9) and 3.1% (95% CI: 2.6–3.5) in control subjects (p = .0002 and p = .0082, respectively). Overall, 45 (14.4%) patients carried at least one mutated allele. No associations were found between F5G1691A/F2G20210A, unprovoked VTE or recurrence (p > .05).
Conclusions. Our study provides new data on genetic risk factors for VTE in the very elderly people. Whether identification of hereditary thrombophilia in elderly patients may influence patient’s management in this age group remains unanswered.

Key words: Venous thromboembolism—F5 G1691A mutation—F2 G20210A mutation—Risk factor

Venous thromboembolism (VTE) is a common disorder in the elderly people with an annual incidence close to 1% in individuals aged 75 years and older (1,2). This high incidence is related to the high prevalence of acquired hypercoagulable or thrombophilic states in the elderly people (1–4). While clinical risk factors for VTE were widely investigated in the advanced age population, few studies focused on genetic risk factors in this age group, especially in patients with unprovoked VTE (5–11). Among inherited factors for venous thrombosis, deficiencies of the natural anticoagulants (antithrombin [AT], protein C [PC], and protein S [PS]) identified in the 1980s as strong risk factors for VTE are rare and patients with such deficiencies usually experience VTE before the age of 40 years. In contrast, F5 G1691A (factor V Leiden) and F2 G20210A mutations are very common and may be viewed as moderate risk factors for VTE throughout the life (11). The F5 G1691A mutation affects one of the cleavage sites in factor V, thus leading to resistance to activated protein C (12). F2 G20210A mutation, located in the 3'-untranslated part of the prothrombin gene, is associated with increased prothrombin levels (13). Given the paucity of data in the very elderly people, we sought to assess the prevalence of inherited factors for VTE, namely AT, PC, and PS deficiencies and F5 G1691A (factor V Leiden) and F2 G20210A mutations in patients aged 75 years and older who were referred for laboratory screening tests for thrombophilia and who were presenting with at least one confirmed VTE episode. We compared F5 G1691A and F2 G20210A prevalence rates to those of a control group including Caucasian healthy subjects without thrombosis history. Finally, we compared the prevalence rates of both mutations between patient subgroups, defined as with one or multiple VTE episodes and with provoked or unprovoked VTE.

Methods

Patients

Data were extracted from two prospective thrombophilia registries. Consecutive patients were either registered between January 1999 and June 2010 in the thrombophilia screening database of Charles Foix Geriatrics Teaching Hospital (Paris area) or registered between 2003 and 2008 in the FARIVE study database at the European Georges Pompidou Teaching Hospital (Paris) (3,14). Patients were eligible for inclusion in the present study if they were Caucasians aged 75 years and older, living in the Paris area, and presenting with at least one confirmed episode of proximal lower limb deep vein thrombosis or pulmonary embolism. The methods for diagnosing deep vein thrombosis were compression ultrasonography or venography, and those for pulmonary embolism were spiral computed tomography or high-probability ventilation-perfusion lung scan or compatible physical findings in a patient with proven proximal deep vein thrombosis. Before sampling, informed consent was obtained from all patients and the attending physician completed a standardized questionnaire on the history of thrombotic disease and acquired clinical risk factors at the last VTE episode (recent surgery, malignancy, stroke, myocardial infarction, heart failure, acute infection, acute dehydration; hormonal replacement therapy). The questionnaire was double checked by two experts through systematic review of medical charts. Exclusion criterion was failure to complete the study questionnaire. In addition, patients with antiphospholipid syndrome or with genetic data lacking were excluded from the analysis. The study was approved by Charles Foix Hospital review board.

Control Group

The control group consisted in a large sample of Caucasian healthy adults up to 80 years (n = 6 154) without thrombotic disease history enrolled in the French FITE-NAT study that evaluated the F5 G1691A and F2 G20210A mutation prevalence rates across different regions of France (15). Of note, the FITE-NAT elderly subgroup comprised patients aged 65–80 years (n = 776), with a prevalence rate of 3.7% for F5 G1691A mutation and 3.3% for F2 G20210A mutation. No significant difference was found in the F5 G1691A and F2 G20210A prevalence rates according to age or sex in the whole FITE-NAT study (15). Since a north-east/south-west gradient was observed in the F5 G1691A distribution in FITE-NAT study, we considered the prevalence of the F5 G1691A determined in subjects living in the Paris area, ie, 3.4% (95% confidence interval [CI]: 1.9–4.9). The prevalence of F2 G20210A in the whole FITE-NAT study was of 3.1% (95% CI: 2.6–3.5), without any difference in the geographical distribution (15).

Laboratory Tests

Blood samples were collected in 0.105M trisodium citrate. Plasma was prepared by centrifugation for 20 minutes at 2,000g at 18°C and plasma aliquots were frozen at −30°C for less than 2 weeks until assays. Genomic DNA was isolated from leukocytes by standard methods (6). Thrombophilia screening included assays of plasma antithrombin, protein C, and protein S activity (Diagnostica Stago, Asnières sur Seine, France), screening for lupus anticoagulant (Staclot LA, Diagnostica Stago and diluted Russell viper venom time using Larc Screen and Lac Confirm-IL, Lexington, MA), antiphospholipid antibodies (Coaliza anticardiolipin Biogenic, Maugio, France), and genetic testing for F5 G1691A and F2 G20210A as previously described (16).

Statistical Analysis

Descriptive statistics were mean ± SD for the age and numbers and percentages for the other (qualitative) variables. F5 G1691A and F2 G20210A prevalence rates were estimated by the Clopper-Pearson method. The 95% CI of the differences of the mutation rates were estimated using the Monte-Carlo method. F5 G1691A and F2 G20210A prevalence rates were compared to those reported in the French FITE-NAT study using Pearson Chi-square tests. Comparisons of the F5 G1691A and F2 G20210A frequencies between subgroups were performed using Pearson Chi-square tests. All tests were two-sided, and the computations were performed using the SAS V9 statistical package.

Results

Of 318 Caucasian consecutive patients who were eligible for the study, 3 with antiphospholipid syndrome and 3 whose genetic data were lacking were excluded from the analysis, leaving 312 patients (245
women and 67 men), mean age 84 ± 6 years (75–103). Main characteristics of patients are summarized in Table 1. Of the 312 patients, 165 (52.9%) had a single VTE episode and 147 had 2 or more episodes (2 episodes, n = 58; 3 episodes, n = 33; 4–10 episodes, n = 56). The proportion of patients with recurrent VTE was higher among females than among males (51.4% vs 31.3%, p = .0035). At least one clinical risk factor for VTE was identified at the time of the single or last episode in 114 (36.5%) patients. The remaining 198 (63.5%) patients had unprovoked VTE. The proportion of patients with multiple VTE episodes was significantly higher in the group without than with at least one clinical associated VTE risk factor (54.0% vs 35.1%, p = .0012).

None of the 312 patients had deficiencies in AT, PC, or PS. The F5 G1691A mutation was found in 29 (9.3%, 95% CI: 6.3–13.1) patients, of whom 28 were heterozygous and 1 was homozygous (Table 2). The F2 G20210A mutation was found in 18 (5.8%, 95% CI: 3.5–9.0) patients, all of whom were heterozygous. Two patients were double heterozygotes. Overall, 45 (14.4%, 95% CI: 10.7–18.8) patients carried at least one mutated allele (Table 2).

We compared these prevalence rates to those of FITE-NAT study which included French healthy adults without thrombotic disease history, ie, 3.4% (95% CI: 1.9–4.9) for F5 G1691A and 3.1% (95% CI: 2.6–3.5) for F2 G20210A, and found significantly higher rates in our patients with VTE (p = .0002 and p = .0082, respectively) than in the control group.

Looking at the potential association between the number of VTE episodes in our cohort and the prevalence of genetic defects, the F5 G1691A mutation was found in 10.2% (95% CI: 5.8–16.3) of patients with multiple VTE episodes versus 8.5% (95% CI: 4.7–13.8) of patients with a single VTE episode (p = .602). The F2 G20210A mutation was found in 7.5% (95% CI: 3.8–13.0) of patients with multiple VTE episodes versus 4.2% (95% CI: 1.7–8.6) of patients with a single VTE episode, p = .220. The 95% CI of the mutation rate differences between patients with single and those with multiple VTE episodes were (−8.2, 4.8) and (−8.4, 2.0) for F5 G1691A and F2 G20210A, respectively.

Finally, F5 G1691A mutation was present in 10.6% (95% CI: 6.7–15.8) of patients with unprovoked VTE versus 7.0% (95% CI: 3.1–13.4) with clinical risk factor-associated VTE (provoked VTE) (p = .293). The F2 G20210A mutation was found in 6.1% (95% CI: 3.2–10.4) of patients with unprovoked VTE versus 5.3% (95% CI: 2.0–11.1) with clinical risk factor-associated VTE (p = .771). The 95% CI of the differences of the mutation rates between patients with unprovoked/clinical risk factor-associated VTE were (−10.2, 2.0–11.1).

### Table 1. Characteristics of the 312 Patients of the Patient Cohort

<table>
<thead>
<tr>
<th>Variable</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age ± SD (years)</td>
<td>84 ± 6.3</td>
</tr>
<tr>
<td>Female</td>
<td>245 (78.5)</td>
</tr>
<tr>
<td>Prior VTE (DVT and/or PE)</td>
<td>147 (47.1)</td>
</tr>
<tr>
<td>Unprovoked VTE</td>
<td>198 (63.5)</td>
</tr>
<tr>
<td>Associated VTE risk factors</td>
<td></td>
</tr>
<tr>
<td>Isolated recent immobilization</td>
<td>27 (8.6)</td>
</tr>
<tr>
<td>Recent surgery</td>
<td>40 (12.8)</td>
</tr>
<tr>
<td>Active malignancy</td>
<td>18 (5.8)</td>
</tr>
<tr>
<td>Recent stroke or myocardial infarction</td>
<td>11 (3.5)</td>
</tr>
<tr>
<td>Acute heart failure</td>
<td>11 (3.5)</td>
</tr>
<tr>
<td>Acute infection</td>
<td>19 (6.1)</td>
</tr>
<tr>
<td>Acute dehydration</td>
<td>2 (0.6)</td>
</tr>
</tbody>
</table>

Note: Recent: defined as <3 months. DVT = deep vein thrombosis; PE = pulmonary embolism; VTE = venous thromboembolism.

### Table 2. Prevalence of F5 G1691A and F2 G20210A Mutations in the Control Subjects and in the 312 Elderly Patients Included in Our Study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control Group (n = 615)</th>
<th>Patients With Unprovoked VTE (n = 198)</th>
<th>Patients With Provoked VTE (n = 312)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F5 G1691A Wild-type, %</td>
<td>3,099 (98.4)</td>
<td>160 (80.8)</td>
<td>38 (19.2)</td>
</tr>
<tr>
<td>F5 G1691A Mutated, %</td>
<td>30 (0.94)</td>
<td>5 (2.56)</td>
<td>25 (7.89)</td>
</tr>
<tr>
<td>F2 G20210A Wild-type, %</td>
<td>2,693 (84.6)</td>
<td>245 (78.5)</td>
<td>200 (63.8)</td>
</tr>
<tr>
<td>F2 G20210A Mutated, %</td>
<td>462 (15.4)</td>
<td>53 (17.5)</td>
<td>41 (13.2)</td>
</tr>
</tbody>
</table>

Note: VTE = venous thromboembolism.

Restriction to Paris area.

Of whom two had combined F5 G1691A and F2 G20210A mutations.

All of whom were heterozygous.
3.3) and (−5.9, 4.8) for F5 G1691A and F2 G20210A, respectively. In addition, no association was found between unprovoked VTE and sex (p = .196).

Discussion

In this study, we specifically focused on genetic risk factors for VTE in a cohort of very elderly patients (mean age: 84 years, 78.5% of women) with proximal deep vein thrombosis and/or pulmonary embolism, consecutively recorded in two thrombophilia registries. Most patients (63.5%) had unprovoked VTE, reflecting a selection of patients who were referred to us by physicians for thrombophilia screening. Indeed, patients with multiple acquired clinical risk factors for VTE are usually not referred for thrombophilia screening in agreement with current recommendation for not testing patients with provoked VTE in this age group (1,2,10,17). However, less is known in elderly patients with unprovoked VTE, and our study provides new data on this topic.

Deficiencies in natural coagulation inhibitors usually lead to VTE at a younger age and were not found in our population. In our cohort, the prevalence rates of the F5 G1691A and F2 G20210A mutations were 9.3% and 5.8%, respectively. Only a few studies provided data on the prevalence of both F5 G1691A and in older patients with VTE, especially in octogenarians. Results of main studies are summarized in Table 3. The apparent discrepancies of results across studies regarding mutation prevalence rates reflect differences in patient selection criteria and study designs (Table 3). When older patients are highly selected for thrombophilia screening, including a large proportion of patients with unprovoked VTE, the prevalence rates of both F5 G1691A and F2 G20210A are significantly higher than in controls, as we found in our study (p = .0002 and p = .0082, respectively), in keeping with results of previous studies (10). Conversely, the less elderly patients are selected, the lower the F5 G1691A and F2 G20210A prevalence rates are, as illustrated in Oger’s study (7).

Large population-based studies indicate that both F5 G1691A and F2 G20210A are independent risk factors for VTE throughout life (1,5,8–11). However, in the aforementioned geriatric cohorts, F5 G1691A and F2 G20210A had lower prevalences than in cohorts of younger patients with VTE. The impact of both mutations on VTE in less evident, as VTE is a multifactorial disease. The population-attributable risk associated with F5 G1691A and F2 G20210A remains to be estimated in large studies focused specifically on the very elderly people. The relative contributions of these risk factors to the incidence of VTE are particularly difficult to assess, as numerous permanent and transient conventional clinical risk factors accumulate with advancing age (1). Neither mutation significantly influenced the number of VTE episodes in our study suggesting that laboratory screening for thrombophilia in elderly patients experiencing a first VTE episode fails to identify patients at high risk for recurrence. Previous data failed to show a substantial increase in the VTE recurrence rate in patients who are heterozygous for F5 G1691A or F2 G20210A (18). Thus, there is no evidence that testing patients for F5 G1691A or F2 G20210A may guide the management of VTE in elderly patients including those with multiple episodes (19). Indeed, the American College of Chest Physicians recommends that decisions regarding duration of anticoagulation should be made with reference to whether an episode of VTE was provoked and the presence of other risk factors, regardless of whether heritable thrombophilic defect is present (19).

Table 3. Prevalence of F5 G1691A and F2 G20210A Mutation in Studies Providing Specific Data in Older Patients With VTE

<table>
<thead>
<tr>
<th>Study (type of study)</th>
<th>Patient Selection</th>
<th>Geographic Origin</th>
<th>Ethnicity</th>
<th>Prevalence (%): F5 G1691A</th>
<th>Prevalence (%): F2 G20210A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oger et al. (7)</td>
<td>Nonorthopedic</td>
<td>Caucasian/Paris area</td>
<td>White/USA</td>
<td>5.7±</td>
<td>10.4</td>
</tr>
<tr>
<td>Folson et al. (8,9)</td>
<td>LITE study</td>
<td>Caucasian/Paris area</td>
<td>USA</td>
<td>13.3±</td>
<td>13.0</td>
</tr>
<tr>
<td>Rosendaal et al. (10)</td>
<td>MAISTHRO registry</td>
<td>Netherlands</td>
<td>Caucasian/</td>
<td>13.3±</td>
<td>13.0</td>
</tr>
<tr>
<td>Siguret et al. (11)</td>
<td>MASTERO registry</td>
<td>France/Lille area</td>
<td>Caucasian/</td>
<td>13.3±</td>
<td>13.0</td>
</tr>
<tr>
<td>Andre et al. (6)</td>
<td>Unprovoked VTE</td>
<td>French/Brittany/France</td>
<td>White/USA</td>
<td>13.0±</td>
<td>13.2</td>
</tr>
<tr>
<td>Weingarz et al. (12)</td>
<td>Unprovoked VTE</td>
<td>Germany</td>
<td>Caucasian/</td>
<td>13.3±</td>
<td>13.0</td>
</tr>
</tbody>
</table>

Notes: LITE = Longitudinal Investigation of Thromboembolism Etiology; MAISTHRO = Main-ISar-THROmbosis; NA = not available; ND = not determined; VTE = venous thromboembolism.
In elderly patients with unprovoked VTE, multiple VTE episodes were more common than in patients with clinical risk factors, in keeping with reports in younger patients (18). Interestingly, the proportion of patients with multiple episodes was higher in women than in men. Age did not explain this difference, as mean age was 84.6 years in females (n = 126) and 85.2 years in males (n = 21). In contrast, several studies showed that young men are more likely to experience recurrent episodes than are women but this sex-related difference is no longer detectable after 50–60 years of age (18,20–21). Of note, no specific data are available in octogenarians. Our results need to be confirmed in further studies.

Our study has several limitations, first regarding our control group which consisted in a large sample of healthy adults up to 80 years selected for no thrombotic disease history across different regions of France. Even though patients and controls were not matched in age and sex, no difference of both F5 G1691A and F2 G20210A prevalence rates in the age and sex distribution was found in the control group as previously shown (15). Moreover, we took into account the geographic distribution of subjects thus minimizing biases because we know that prevalence rates for both mutations vary considerably in the general population between studies of Caucasians from different ethnic backgrounds. Secondly, the size of our study is limited and thus is underpowered to detect a potential small difference between the prevalence of both mutations in patients with multiple VTE and those without, and in patients with unprovoked VTE and those without. It can be added that if any difference exists, it should not be strong and thus of limited clinically relevance.

In conclusion, our study provides new data on the genetic risk factors for thrombosis in patients older than 75 years with one or more episodes of VTE. Whether the identification of hereditary thrombophilia in elderly patients may influence the patient’s management remains unanswered.

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Conflict of Interest
None.

References