ABO blood group but not haemostasis genetic polymorphisms significantly influence thrombotic risk: a study of 180 homozygotes for the Factor V Leiden mutation

Procare-GEHT Group*

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Summary

Limited data exist on the impact of additional genetic risk factors on the clinical manifestations of factor (F) V Leiden homozygotes. A retrospective multi-centre cohort study was performed to assess the role of the FII G20210A gene mutation, the protein C (PC) promoter CG haplotype, the combination of two PC polymorphisms (A-1641G, C-1654T), the FXIII Val34Leu polymorphism, two thrombin-activatable fibrinolysis inhibitor polymorphisms (Thr325Ile, Ala147Thr), two plasminogen activator inhibitor-1 polymorphisms (-675 4G/5G, A-844G), the methylene-tetrahydrofolate reductase (MTHFR) C677T polymorphism and the ABO blood group on the thrombotic phenotype in FV Leiden homozygotes. 127 subjects with venous thrombosis and 53 asymptomatic subjects were analysed. The T allele of MTHFR C677T was more frequent in symptomatic subjects than in asymptomatic ones (68% vs. 45%, P = 0.02; odds ratio (OR) 2.8, 95% CI 1.3–5.8, after adjustment for potential confounders). For the other polymorphisms, no difference was observed between symptomatic and asymptomatic subjects. The non-O blood group was more frequent among symptomatic carriers (84% vs. 57%, P = 0.0002; OR 4.1, 95% CI 1.7–9.7). In conclusion, except for the ABO blood group, none of the polymorphisms studied contribute strongly to the thrombotic risk in FV Leiden homozygotes.

Keywords: methylene-tetrahydrofolate reductase, ABO blood group, venous thromboembolism, Factor V Leiden homozygotes.

Factor (F) V Leiden homozygotes are far from rare (0.06 to 0.25%) in the general population (Emmerich et al, 1997). The thrombotic risk associated with the FV Leiden homozygosity is usually considered to be strong. However, this risk has differed widely among studies, from 80 in the Leiden case–control study (Rosendaal et al, 1995) to 18 in the prospective Copenhagen cohort (Juul et al, 2004).

It is well known that thrombophilia results from the interaction of multiple genetic and environmental factors. If the multigenic model proves to be correct, additional low and frequent genetic risk factors could play a role in thrombotic phenotype. However, given the low prevalence of the defect, limited data exist concerning the impact of such risk factors on the clinical manifestations of FV Leiden homozygotes (Greengard et al, 1994; Samama et al, 1995; Rintelen et al, 1996; Emmerich et al, 1997; Ehrenforth et al, 2004). Among the polymorphisms that could contribute to the thrombotic risk of FV Leiden homozygotes, those associated with quantitative or qualitative modifications of proteins involved in the haemostatic process are particularly of interest (Lane & Molica, 2002). We have therefore selected several candidate polymorphisms for the present study: the FII G20210A, a well-known risk factor for venous thromboembolism (VTE), affects the 3’-untranslated region of the mRNA and causes elevated prothrombin plasma concentrations (Poort et al, 1996). The FXIII Val34Leu partly alters the clot-stabilising propensity of FXIII and renders the fibrin clot less porous with thinner fibres (Catto et al, 1999). The Protein C (PC) promoter CG
The aim of this study was to evaluate the impact of these polymorphisms on the risk of venous thrombosis (VTE) in a large cohort of 180 subjects that were homozygous for FV Leiden mutation. In addition, we compared the frequency of non-O blood groups in symptomatic homozygotes and in asymptomatic ones.

**Design and methods**

**Study design**

Factor V Leiden homozygotes were enrolled from the Procare Study. Detailed descriptions of the Procare Study, biological methods and the main results have been previously reported (Procare group, 2000, 2004). Briefly, the study was conducted as a large multi-centre trial with 23 participating French centres (Appendix 1). Between December 1995 and March 1996, consecutive subjects with FV Leiden mutation, irrespective of their clinical history (patients with an objectively proven venous thrombosis history or family members), were eligible for the study. Comprehensive clinical data forms were completed for each subject with attention to risk periods, thrombotic events and antithrombotic treatments. Blood groups were recorded. A positive family history was defined as at least one first-degree or two second-degree relatives with VTE. All the subjects were screened for inherited or acquired thrombophilic abnormalities: antithrombin, PC, protein S, lupus anticoagulant, IgG and IgM anticardiolipin antibodies, activated PC-sensitivity and G20210A prothrombin polymorphism. Of the 566 cases included in the Procare Study, all the homozygotes were asked to participate in the present study. Inclusion was extended until April 1st 2002. Therefore, patients designated as symptomatic FV Leiden homozygous carriers were enrolled as well as asymptomatic carriers. Written informed consent was obtained from each subject.

**Symptomatic carriers**

Among the symptomatic carriers, only the referred probands were included in the study. Related family members with a history of thrombosis were excluded to avoid bias. Probands with an established congenital or acquired prothrombotic condition were excluded from the study.

**Asymptomatic carriers**

Among the asymptomatic carriers, only unrelated subjects were included in the study. When multiple asymptomatic carriers from the same family were identified, only one carrier was retained for the analysis based on the age and sex (sex and age similar to those of the propositus when possible).

**Biological analysis**

Genomic DNA was prepared by using the standard salting-out techniques. Genotyping of polymorphisms was carried out by using previously described polymerase chain reaction techniques (Frosst et al, 1995; Rosendaal et al, 1995; Poort et al, 1996; Henry et al, 1998, 2001; Le Cam-Duchez et al, 1999; Canavy et al, 2000) in two centres (Haematology Laboratories, Marseille and Rouen).

**Statistical analysis**

Statistical analysis was performed by P.-E. Morange using sas software (v. 8.01). Continuous variables were expressed as mean ± SD. Categorical variables were expressed as frequencies and percentages. The Wilcoxon rank-sum test was used to compare continuous variables. Categorical variables were compared by using the chi-squared test or Fisher’s exact test when frequencies were below five. The effect of polymorphisms on the risk of thrombosis was assessed by using logistic regression after adjustment for age and sex. Further adjustment for the number of prothrombotic conditions was also performed. This number corresponded to the number of environmental conditions (oral contraceptive intake, pregnancy, major surgery, immobilisation) known to induce VTE experienced by each subject. It was calculated until the first VTE for symptomatic subjects and until inclusion for asymptomatic ones.

Results are given as odds ratios (OR) and 95% CI. $P<0.05$ was considered significant.

**Results**

**Main characteristics of the population studied**

A total of 180 unrelated subjects carrying the homozygous FV Leiden mutation were analysed. The main characteristics...
According to the history of thrombosis are reported in Table I. Fifty-three unrelated subjects were asymptomatic. Whereas the mean age at inclusion was significantly lower in asymptomatic subjects compared with those with a history of thrombosis ($P < 0.0001$), no significant difference with the mean age at onset of first VTE was observed ($P = 0.32$).

The sex ratio was similar in the two groups of subjects ($P = 0.16$).

Among the 127 unrelated subjects with a history of VTE, 119 had a history of deep vein thrombosis and/or pulmonary embolism and eight suffered only from isolated superficial venous thrombosis (SVT). Table II lists the characteristics of the first VTE according to sex. Only 35 women (41%) developed the first VTE without any environmental risk factors, unlike the men (64%; $27/42$; $P = 0.01$). Women developed VTE significantly earlier than men ($P = 0.002$). Women experienced more prothrombotic conditions before VTE than men; the mean number of prothrombotic conditions before VTE was three in women and 0.57 in men ($P < 0.0001$).

### Effect of genetic polymorphisms and the ABO blood group on the risk of VTE

A significant difference was observed for the MTHFR C677T polymorphism; T allele carriers were more frequent amongst subjects with a history of thrombosis than those without ($P = 0.02$; Table III). A total of 110 T allele carriers, including 23 homozygous (677TT) and 87 heterozygous (677CT) subjects were identified; 86 (18 homozygotes) were symptomatic carriers (68%) and 24 (five homozygotes) were asymptomatic (45%). T allele carriers were thus more frequent amongst subjects with VTE than those without (Table III). This leads to an OR of 2.6 (95% CI 1.3–5.2; $P = 0.0048$) after

<table>
<thead>
<tr>
<th>Table I. Main characteristics of the 180 FV Leiden homozygotes.</th>
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<tr>
<td>Patients with venous thrombosis $(n = 127)$</td>
</tr>
<tr>
<td>Age at inclusion, years mean (±SD)</td>
</tr>
<tr>
<td>Sex, male (%)</td>
</tr>
<tr>
<td>Age (years) at first thrombosis mean (±SD)</td>
</tr>
<tr>
<td>Type of events</td>
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<tr>
<td>DVT (±PE)</td>
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<tr>
<td>Superficial vein thrombosis</td>
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</tbody>
</table>

†$P$ for difference between the mean age at onset of first thrombosis and the mean age of asymptomatic subjects at inclusion.

DVT, deep vein thrombosis; PE, pulmonary embolism.

<table>
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<tr>
<th>Table II. Clinical characteristics of the 127 patients with history of VTE according to gender.</th>
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<tbody>
<tr>
<td>Site of first VTE</td>
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<tr>
<td>DVT alone†, $n$ (%)</td>
</tr>
<tr>
<td>DVT + PE, $n$ (%)</td>
</tr>
<tr>
<td>Isolated PE, $n$ (%)</td>
</tr>
<tr>
<td>Superficial vein thrombosis, $n$ (%)</td>
</tr>
</tbody>
</table>

Prothrombotic conditions of first VTE

| None (%)                                                                                     | 35 (41)                      | 27 (64)       | 0.01 |
| Major surgery, $n$ (%)                                                                       | 5 (5-9)                      | 10 (23-8)     | 0.0035 |
| Pregnancy, $n$ (%)                                                                           | 16 (18-8)                    | –             |      |
| Oral contraceptives, $n$ (%)                                                                 | 27 (31-8)                    | –             |      |
| Immobilisation, $n$ (%)                                                                      | 2                             | 3 (16-7)      | NS  |

Age at first VTE (mean ± SD)                                                                  | 31 ± 13                      | 40 ± 17       | 0.002 |

Number of prothrombotic conditions (mean ± SD)‡

| Recurrence, $n$ (%)                                                                          | 48 (56-5)                    | 30 (71-4)     | 0.15 |

| Includes four axillary thrombosis.                                                              |
| Includes oral contraceptive intake, pregnancy, major surgery, immobilisation.                   |

VTE, venous thromboembolism; DVT, deep vein thrombosis; PE, pulmonary embolism; SD, standard deviation; NS, not significant.

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age and sex adjustment (Table IV). Further adjustment did not affect the outcome (OR 2.8, 95% CI 1.3–5.8; \( P = 0.008 \); Table IV). Homozygosity for the MTHFR C677T was more prevalent in symptomatic FV Leiden homozygotes (14%) than in asymptomatic ones (9%) but the difference was not significant (\( P = 0.38 \)). When comparing TT carriers versus C carriers in the fully adjusted model, the OR was 2.47 (95% CI 1.6–5.26; \( P = 0.019 \)).

No difference in genotype distribution was observed for all the other polymorphisms (Table III). The FII 20210A allele tended to be more frequent in subjects with a history of thrombosis, but the difference was not significant (\( P = 0.37 \)).

Non-O groups were also more frequent in subjects with a history of thrombosis compared with those without (77% vs. 46%; \( P = 0.0002 \), Table III) leading to an OR of 4.1 (95% CI 1.9–8.9; \( P = 0.0003 \)) after age and sex adjustment. Further adjustment did not affect the results.

Table IV. Impact of the methylene-tetrahydrofolate reductase (MTHFR) C677T polymorphisms and the ABO blood groups on the risk of VTE.

<table>
<thead>
<tr>
<th>MTHFR C677T</th>
<th>VTE present ((n = 127))</th>
<th>VTE absent ((n = 53))</th>
<th>( P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>41 (32%)</td>
<td>29 (55%)</td>
<td></td>
</tr>
<tr>
<td>CT + TT</td>
<td>86 (68%)</td>
<td>24 (45%)</td>
<td></td>
</tr>
<tr>
<td>OR (95 CI)</td>
<td>2.6 (1.3–5.2)†</td>
<td>2.8 (1.3–5.8)‡</td>
<td>0.0048</td>
</tr>
</tbody>
</table>

ABO blood groups

| O            | 18 (46)         | 21 (54)         |         |
| Non-O        | 109 (77)        | 32 (23)         |         |
| OR (95% CI)  | 4.1 (1.9–8.9)† | 4.1 (1.7–9.7)‡ | 0.0003  |

†After adjustment for age and sex.
‡Further adjustment for the number of prothrombotic conditions (including oral contraceptive intake, pregnancy, major surgery, immobilisation).

VTE, venous thromboembolism.

**Discussion**

Because of the relative rarity of FV Leiden homozygosity, only a specific study including a sufficient number of subjects could be used to analyse the impact of polymorphisms located in genes coding for the proteins involved in coagulation and fibrinolysis on the risk of thrombosis in these subjects. Therefore, we performed a large multi-centre cohort study including 180 homozygotes, of whom 127 had an objective history of VTE. To minimise possible selection bias, none of the subjects, symptomatic or not, were related.

The prevalence of the main candidate gene polymorphisms in the asymptomatic population was no different to that usually found in the Caucasian population (Poort et al, 1996; Tosseto et al, 1997; Morelli et al, 2005). To our knowledge, only Ehrenforth et al (2004) have assessed the contribution of genetic risk factors on an FVL homozygous population with, however, several differences in the study design and in the characteristics of the subjects included. They studied 165 FV Leiden homozygotes including 129 subjects with VTE. A concomitant natural inhibitor deficiency was identified in six symptomatic patients. Among the 36 asymptomatic homozygotes, seven had SVT. Only FII20210A and MTHFR C677T were determined.

It is widely accepted that the pathogenesis of VTE is multifactorial. Circumstantial factors may concur with genotype in the development of VTE in homozygous carriers of FV Leiden (Greengard et al, 1994; Samama et al, 1995; Rintelen et al, 1997).
with reduced MTHFR activity (Tosseto et al., 1997; Morelli et al., 2005). However, several studies have identified the role of MTHFR C677T on the risk of VTE in subjects heterozygous for FV Leiden or FII G20210A mutations (De Stefano et al., 2000; Keijzer et al., 2002). These data suggest a small role for MTHFR C677T that may be difficult to identify because individually it conveys so small a risk, unless combined with other genetic risk factors. The impact of the MTHFR C677T on FV Leiden homozygotes has been poorly assessed. In the present study, the T allele (either 677TT or 677CT) was more prevalent in symptomatic homozygous FV Leiden than in asymptomatic subjects, leading to an increased risk for VTE of 2.8, after adjustment for possible confounders. This finding differed from that of Ehrenfort et al. (2004), where no association was found between the risk of VTE and the MTHFR 677TT in a homozygous FV Leiden population. However, it is to be noted that in that study, only 105 of the 129 FV Leiden homozygotes with VTE were genotyped for the MTHFR C677T. Authors compared the genotype distribution of MTHFR C677T in this group of symptomatic FV Leiden homozygotes to that observed in a group of subjects who had no history of arterial or venous thrombosis and were not carriers of FV Leiden as a proxy of the prevalence among FV Leiden homozygotes without thrombosis. This could explain the discrepancy observed between the two studies. However, our results should be interpreted with caution since the pathogenesis remains unclear. It has been shown that TT carriers have higher plasma homocysteine levels than CT and CC carriers, with no differences between CT and CC carriers (Almawi et al., 2005). If the MTHFR C677T polymorphism affects the risk of VTE through the modification of homocysteine plasma levels, the prevalence of TT carriers should be higher in subjects with a history of VTE when compared with those without. Indeed, in clinical studies, only the MTHFR 677TT genotype was associated with a higher risk (Den Heijer et al., 2005). In our study, we observed a higher frequency of the homozygous MTHFR 677TT genotype in patients with VTE compared with asymptomatic subjects. Unfortunately, the difference was not significant. Thus, the association we observed between the MTHFR C677T polymorphism and thrombotic risk could be spurious because of a small sample size or multiple testing. On the other hand, both CT and TT genotypes were associated with reduced MTHFR activity (Tosseto et al., 1997). The question can be raised as to whether the decrease in MTHFR activity, even in its heterozygous state, could contribute to the development of VTE in FV Leiden homozygotes. The design of the present study did not permit the assessment of plasma homocysteine levels.

None of the other studied polymorphisms were associated with the risk of thrombosis. However, given the relatively low power of the study, we cannot exclude a slight effect that has not been detected. One of our most important findings was that non-O blood group was a risk factor for VTE in FV Leiden homozygotes. To our knowledge, this is the first study to assess the effect of blood group on thrombotic risk in a large cohort of FV Leiden homozygotes. This finding extends previous data (Robert et al., 2000; Morelli et al., 2005): among FV Leiden heterozygotes, the risk of thrombosis was increased two- to fourfold in non-O blood groups compared with the O blood group, which is similar to the results observed in our study in FV Leiden homozygotes.

In conclusion, there is no clear evidence that the polymorphisms studied have any impact on thrombotic risk in homozygous carriers of the FV Leiden mutation. On the other hand, our data suggest that the non-O blood group could influence the risk of thrombosis in a population already considered at high risk (Rosendaal et al., 1995). However, these points remain to be confirmed in a larger cohort of patients.

References


Appendix 1

The Procare-GEHT group for this study includes the following Haematology Laboratories and participants in France:

2. Lille: Anne Bauters, Brigitte Jude.
5. Bordeaux: Christine Vergnes.
7. Colombr: Dominique De Frost.