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Co-segregation of Thrombophilic Disorders in Factor V Leiden Mutation Carriers; the Contributions of Factor VIII, Factor XI, TAFI and Lipoprotein (a) to the Absolute Risk of Venous Thromboembolism

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Abstract

The clinical expression of factor V Leiden mutation varies widely within and between families and only a minority of carriers will ever develop venous thromboembolism (VTE). Co-segregation of thrombophilic disorders is a possible explanation. Our aim was to assess the contribution of high levels of factor VIII:C, factor XI:C, thrombin activable fibrinolysis inhibitor (TAFI) and lipoprotein (a) (Lp(a)) to the risk of VTE in factor V Leiden carriers.

Levels of the four proteins were measured, in addition to tests of deficiencies for antithrombin, protein C and protein S, and the prothrombin G20210A mutation, in 153 factor V Leiden carriers, derived from a family cohort study. The (adjusted) relative risk and absolute risk of VTE for high levels of each protein were calculated.

Of carriers, 60% had one or more concomitant thrombophilic disorders. Crude odds ratios (95% CI) of VTE for high protein levels were: 3.2 (1.1-9.3) (factor VIII:C); 1.7 (0.6-4.9) (factor XI:C); 3.0 (1.1-8.2) (TAFI); and 1.9 (0.7-5.7) (Lp(a)). Adjusted for age, sex, other concomitant thrombophilic disorders and exogenous risk factors, the odds ratio for VTE were 2.7 (0.8-8.7) for high factor VIII:C levels and 1.8 (0.6-5.3) for high TAFI levels. Annual incidences in subgroups of carriers were 0.35% (0.09-0.89), 0.44% (0.05-1.57) and 0.94% (0.35-2.05) for concomitance of high levels of factor VIII:C, TAFI and both, respectively, as compared to 0.09% (0.00-0.48) in single factor V Leiden carriers and 1.11% (0.30-2.82) for other concomitant disorders.

High levels of factor VIII:C and TAFI, in contrast with factor XI:C and Lp(a), are mild risk factors for VTE, that substantially contribute to the risk of VTE in factor V Leiden carriers. Our data support the hypothesis that the clinical expression of factor V Leiden depends on co-segregation of thrombophilic disorders.
Introduction

Resistance to activated protein C, due to the factor V:Q506 mutation (factor V Leiden), is the most common known heritable thrombophilic defect. It is found in approximately 5% of caucasians and, depending on selection, in 20 to 50% of patients with venous thromboembolism (VTE). Only 20 to 30% of factor V Leiden carriers will ever experience VTE during their lifetime. Moreover, there is a wide intra- and interfamilial variation in its clinical expression. These observations suggest that the occurrence of VTE depends on the concomitance of factor V Leiden with exogenous risk factors or other genetic defects. The contributions of inherited deficiencies of antithrombin, protein C and protein S will be limited because the chance of co-segregation is small, given their low prevalences. The prothrombin G20210A mutation, elevated factor VIII activity (factor VIII:C) and mild hyperhomocysteinemia are more prevalent thrombophilic disorders. Previously we demonstrated that the risk of VTE in factor V Leiden carriers increased only 1.3 fold if they also carried the prothrombin mutation. The risk was approximately 4-fold higher in homozygous factor V Leiden carriers and 17.5-fold higher if inherited protein C or S deficiencies were the concomitant thrombophilic disorders. However, only 20% of symptomatic factor V Leiden carriers showed co-segregation with these thrombophilic disorders. More recently, high levels of factor XI activity (factor XI:C) and thrombin activatable fibrinolysis inhibitor (TAFI) have been identified as risk factors for VTE. Elevated levels of lipoprotein a (Lp(a)) may also be involved in the pathogenesis of VTE, due to its homology to plasminogen.

In the present study we assessed the contribution of factor VIII:C, factor XI:C, TAFI and Lp(a) to the risk of VTE in factor V Leiden carriers.

Design and Methods

In a previous family cohort study, designed to estimate the absolute risk of VTE in factor V Leiden carriers, we enrolled consecutive patients with VTE and factor V Leiden (proband) and their first-degree relatives older than 15 years. Probands were referred to the out-patient clinics of the three participating university hospitals. Their living first degree relatives were identified through pedigree analysis. After informed consent had been obtained, each relative was interviewed by one of the investigators using a standardized and validated medical history form. Detailed information was collected about previous episodes of VTE, surgical interventions, trauma, periods of immobilisation and prophylactic or therapeutic use of anticoagulant drugs. For women, the use of oral contraceptives and the
obstetric history were also documented. At the end of the outpatient visit a blood sample was taken for DNA testing of factor V Leiden.

In the present study, in a single center we performed additional tests on stored plasma and DNA in 153 of the previously enrolled 269 relatives, who carried factor V Leiden. The remaining 116 carrier relatives had to be excluded, because of insufficient amounts of stored plasma. Comparing included and excluded carriers, there were no differences in their clinical characteristics or the prevalence of VTE (11.1% versus 10.3%, p=1.00), suggesting absence of selection bias. Carriers were additionally tested for the prothrombin mutation and inherited deficiencies of antithrombin, protein C, and protein S, and plasma levels of factor VIII:C, factor XI:C, TAFI, and Lp(a) were measured. The study was approved by the institutional review board of the hospital and all participants gave informed consent.

Definitions
A previous episode of deep vein thrombosis or pulmonary embolism was considered to have occurred if confirmed by compression ultrasound, venography, ventilation/perfusion lung scanning, or pulmonary angiography, or if the patient had received full dose heparin and oral anticoagulants for at least 3 months without objective testing at a time when these techniques were not yet available. For this classification the patients’ charts were reviewed. VTE was classified secondary if it had occurred within 3 months after exposure to one or more exogenous risk factors including surgery, trauma, immobilisation for more than 7 days, oral contraceptive use, pregnancy or malignancy. VTE that occurred in the absence of any exogenous risk factor was considered spontaneous.

Laboratory Studies
Factor V Leiden and the prothrombin mutation were demonstrated by polymerase chain reaction, as described previously.3,17

Protein C and total protein S antigen levels were measured by ELISA (reagents obtained from DAKO, Glostrup, Denmark), protein C activity (Berichrom Protein C, Behring, Marburg, Germany) and antithrombin activity (Coatest TM, Chromogenix AB, Mölndal, Sweden) by chromogenic substrate assays.

Inherited deficiencies of antithrombin, protein C or protein S were defined as a plasma level below the lower limit of the normal range at two separate measurements and in at least two relatives. Protein S deficiency was considered to be acquired due to pregnancy or oral contraceptive use unless it was established by repeated measurement at least three months after delivery and discontinued
oral contraceptive use, respectively. Factor VIII:C and factor XI:C were measured by one-stage clotting assays. TAFI activity was determined with the substrate hippuryl-L-arginine, using HPLC-assisted measurement of the released hippuric acid as described previously.\textsuperscript{18} Lp(a) was measured by ELISA (TintElize, Biopool International, US, Denver, Colorado, USA). Levels of factor XI:C, TAFI and Lp(a) above the 75th percentile of their distribution in factor V Leiden carrier-relatives were defined as high. As in other studies, we used the upper limit of its normal range, i.e. 150%, as the cut-off point for high levels of factor VIII:C, to enable a comparison of results between our and other studies. Antithrombin, protein C, protein S, factor VIII:C, factor XI:C and TAFI were expressed as percentage of the levels measured in pooled normal plasma set at 100%.

Statistics
The risk of VTE was estimated for FVIII:C, FXI:C, TAFI and Lp(a) by univariate analysis. We assessed the influence of age, sex and hormonal state, i.e. premenopause, oral contraceptive use and pregnancy on their plasma levels. The relative risk of VTE in subgroups with combined disorders, as compared within factor V Leiden carriers without concomitant disorders, was calculated from annual incidences. The annual incidences were calculated by dividing the number of persons with VTE and the total number of observation years in each subgroup. Observation years were defined as the period from the age of 15 years until the first episode of VTE, or until the date of study entry in asymptomatic carriers, considering that venous thrombosis is rarely found before the age of 15 years. Hazard ratios of thrombophilic disorders were calculated using a multivariate Cox proportional hazard model, adjusted for age and sex.
Crude odds ratios were calculated by simple cross tabulation. Continuous variables were analysed by the Mann-Whitney-U-test and presented as median values and their ranges. Categorical variables were analysed by Fisher’s exact test or the Chi-square test, as appropriate. A two-tailed p-value of less than 0.05 was considered to indicate statistical significance. Analysis was performed using SAS software, version 6.12 (SAS-Institute Inc., Cary, North Carolina, USA).

Results
The characteristics of 153 factor V Leiden carriers in this study are summarised in Table I. Seventeen carriers (11%) had a history of VTE, which occurred at a median age of 33 years (range 17-63). Exposure to exogenous risk factors was
similarly distributed among symptomatic and asymptomatic carriers. VTE was
classified as spontaneous in 41% of cases. Symptomatic carriers had higher median
plasma levels of factor VIII:C, factor XI:C, TAFI and Lp(a) than did asymptomatic
carriers. Only differences in levels of factor VIII:C and TAFI were statistically
significant (Table I).
Odds ratios (95% CI) for VTE were calculated by comparing carriers with FVIII:C
levels ≥ 150% and < 150%, respectively. For FXI:C, TAFI and Lp(a), high levels
were defined as values above the 75th percentile measured in carriers. Odds
ratios were 3.2 (1.1-9.3) in carriers with FVIII:C levels ≥150%; 1.7 (0.6-4.9) for
FXI:C levels ≥111%; 3.0 (1.1-8.2) for TAFI levels ≥116%, and 1.9 (0.7-5.7) for
Lp(a) levels ≥216 mg/L.
Levels of FVIII:C (p<0.001), TAFI (p=0.02), FXI:C (p=0.001) and Lp(a) (p=0.06)
increased with age (data not shown). There were no differences between
premenopausal women and men younger than 50 years of age, and between
postmenopausal women and men of the same age. Women on oral contraceptives
(n=25) had higher levels of FXI:C (median 102% versus 94%, p=0.03) and TAFI
(111% versus 101%, p=0.02) than non-users younger than 50 years of age (n=20),
whereas observed differences in levels of FVIII:C (120% versus 146%, p=0.13)
and Lp(a) (median 72 mg/L versus 50 mg/L, p=0.40) were not significant.

Table 1 Characteristics of 153 factor V Leiden carriers.

<table>
<thead>
<tr>
<th></th>
<th>VTE (17)</th>
<th>No VTE (136)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women (%)</td>
<td>11 (65)</td>
<td>68 (50)</td>
<td>0.3</td>
</tr>
<tr>
<td>Median age at study entry, yr (range)</td>
<td>54 (19-80)</td>
<td>38 (15-81)</td>
<td>0.006</td>
</tr>
<tr>
<td>Exposure to exogenous risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral contraception (%) women</td>
<td>5 (1.5)</td>
<td>17 (60)</td>
<td>0.17</td>
</tr>
<tr>
<td>Pregnancy (%) women</td>
<td>10 (91)</td>
<td>14 (60)</td>
<td>0.09</td>
</tr>
<tr>
<td>Surgery, trauma, immobilisation (%)</td>
<td>27</td>
<td>127</td>
<td>0.1</td>
</tr>
<tr>
<td>First episode of VTE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median age at onset, yr (range)</td>
<td>33 (17-63)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spontaneous (%)</td>
<td>7 (41)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary to</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral contraception (%) women</td>
<td>1 (36)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy (%) women</td>
<td>3 (27)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery, trauma, immobilisation (%)</td>
<td>3 (27)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median plasma levels (range)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVIII:C, (%)</td>
<td>106 (47-300)</td>
<td>139 (62-318)</td>
<td>0.94</td>
</tr>
<tr>
<td>FXI:C, (%)</td>
<td>102 (80-152)</td>
<td>99 (63-186)</td>
<td>0.12</td>
</tr>
<tr>
<td>TAFI, (%)</td>
<td>113 (87-116)</td>
<td>103 (77-193)</td>
<td>0.009</td>
</tr>
<tr>
<td>Lp(a), (mg L)</td>
<td>72.5 (6-1321)</td>
<td>38 (1-917)</td>
<td>0.20</td>
</tr>
</tbody>
</table>
Table 2 Frequency of a first episode of VTE in 153 factor V Leiden carriers related with concomitance of high levels of factor VIII:C and/or TAFI, and other combinations of thrombophilic disorders.

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVL only (n=62)</td>
<td>FVL-FVIII (n=11)</td>
<td>FVL-TAFI (n=17)</td>
<td>FVL-FVIII - TAFI (n=20)</td>
<td>Other combinations (n=13)</td>
</tr>
<tr>
<td>Patients with VTE, (n)</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Observation period, (yr)</td>
<td>11.50</td>
<td>11.49</td>
<td>4.59</td>
<td>6.35</td>
</tr>
<tr>
<td>Annual incidence (95%CI)</td>
<td>0.09 (0.00-0.18)</td>
<td>0.35 (0.09-0.89)</td>
<td>0.14 (0.05-0.57)</td>
<td>0.91 (0.35-2.65)</td>
</tr>
<tr>
<td>Relative risk (95% CI)</td>
<td>1.0 (reference)</td>
<td>4.0 (0.5-30.1)</td>
<td>5.0 (0.6-43.6)</td>
<td>10.9 (2.0-59.1)</td>
</tr>
</tbody>
</table>

FVL, heterozygous factor V Leiden; FVIII, factor VIII:C ≥150%; TAFI, ≥116%; other combinations included the prothrombin gene mutation, homozygous factor V Leiden, and protein S deficiency.

Factor V Leiden carriers were divided into subgroups in accordance with concomitance of the thrombophilic disorders that were identified as risk factors for VTE (Table 2).

Group 1 (n=62) contained carriers without a concomitant disorder; group 2 (n=41), carriers with FVIII:C levels ≥150%; group 3 (n=17), carriers with TAFI levels ≥116%; and group 4 (n=20), carriers with levels of FVIII:C ≥150% and TAFI ≥116%. The remaining factor V Leiden carriers (group 5, n=13) had any of the following less frequently found concomitant disorders: the prothrombin gene mutation (n=3); the prothrombin gene mutation and a TAFI level ≥116% (n=1); the prothrombin gene mutation and a FVIII:C level ≥150% (n=1); the prothrombin gene mutation, a TAFI level ≥116% and a FVIII:C level ≥150% (n=1); protein S deficiency (n=1); homozygous factor V Leiden (n=2); and homozygous factor V Leiden and a FVIII:C level ≥150% (n=1).

The annual incidence of VTE was 0.09 in group 1; 0.44 in group 2; 0.35 in group 3; and 0.94 in group 4 (Table 2). In group 5 it was 1.11. The additional risks of separate concomitant disorders are presented in Table 3. Because

Table 3 The influence of concomitant thrombophilic disorders and exogenous factors on the risk for VTE in 153 factor V Leiden carriers.

<table>
<thead>
<tr>
<th>Concomitant risk factors</th>
<th>Crude OR (95% CI)</th>
<th>Hazard ratio (95% CI) *</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVIII:C ≥150%</td>
<td>3.2 (1.4-9.3)</td>
<td>2.7 (0.8-8.7)</td>
</tr>
<tr>
<td>FVL:C ≥116%</td>
<td>1.7 (0.6-4.9)</td>
<td>0.8 (0.3-2.5)</td>
</tr>
<tr>
<td>TAFI ≥116%</td>
<td>3.0 (1.4-8.2)</td>
<td>1.8 (0.6-5.3)</td>
</tr>
<tr>
<td>Lp(a) ≥216 mg L</td>
<td>1.9 (0.7-5.7)</td>
<td>1.3 (0.4-3.8)</td>
</tr>
<tr>
<td>Any exogenous factor #</td>
<td>3.1 (0.7 - 13.3)</td>
<td>1.5 (0.3 - 7.6)</td>
</tr>
</tbody>
</table>

OR denotes odds ratio. * Also adjusted for age, sex and other concomitant thrombophilic disorders and exogenous risk factors. # ever exposed versus never exposed to risk factors, including surgery, trauma, immobilization, oral contraceptive use or pregnancy.
interactions between exogenous risk factors and thrombophilic disorders were not demonstrated, the former were considered as a composite variable in the multivariate model. Adjusted hazard ratios were 2.7 (0.8-8.7) for FVIII:C ≥150%; 0.8 (0.3-2.5) for FXI:C ≥111%; 1.8 (0.6-5.3) for TAFI ≥116%; 1.3 (0.4-3.8) for Lp(a) >176 ≥216 mg/L; and 1.5 (0.3-7.6) for exposure to any of the mentioned exogenous risk factors.

Discussion
We found that high levels of factor VIII:C (≥150%) or TAFI (≥116%, the 75th percentile of the distribution of values in factor V Leiden carrier-relatives) were associated with an increased risk for VTE in factor V Leiden carriers. High levels of factor XI:C (=111%, the 75th percentile) and Lp(a) (=216 mg/L, the 75th percentile) were not identified as risk factors. Using the current definitions, one or more concomitant thrombophilic disorders were demonstrated in about 60% of heterozygous factor V Leiden carriers. These were high levels of factor VIII:C and TAFI, deficiencies of antithrombin, protein C and protein S, the prothrombin G20210A mutation and homozygosity for factor V Leiden. VTE occurred in 17.6% of carriers with concomitant disorders, compared with 1.6% of single factor V Leiden carriers (p=0.001). Our data support the hypothesis that the wide spectrum of clinical expression of factor V Leiden depends on clustering of thrombophilic disorders.

The estimated risk of VTE at high levels of factor VIII:C and TAFI, respectively, is in line with the results of previous studies. A recent study in unselected families showed an absolute annual risk (0.27%) in factor V Leiden carriers who also had factor VIII:C levels ≥150%, corresponding with the here reported risk (0.3%) 

The relative risk of VTE associated with high levels of TAFI (3.0, 1.1-8.2) is comparable with the finding of van Tilburg et al., but they did not demonstrate an additional risk of high TAFI levels in factor V Leiden carriers. Although high levels of factor XI:C were associated with an increased risk of VTE, comparable to the relative risk reported from a recent case-control study, we did not demonstrate statistical significance (odds ratio 1.7, 95% CI 0.6-1.9). This might be explained by the smaller size of our study group.

High Lp(a) levels were not associated with an increased risk of VTE (odds ratio 1.93, 95% CI 0.7-5.7). In a previous analysis of a larger number (392) of factor V Leiden carriers and non-carriers, that included the present study population, we found no differences in Lp(a) levels comparing subjects with (median 80 mg/L)
and those without (69 mg/L) VTE \((p=0.24)^{20}\). Although these findings are in agreement with the results of two case-control studies,\(^{21,22}\) two other studies demonstrated an increased risk of VTE at \(Lp(a)\) levels \(>300 \text{ mg/L}\).\(^{11,13}\) To estimate the contribution of high levels of factor VIII:C, factor XI:C, TAFI and \(Lp(a)\), separately, relative risks were adjusted for age, sex, other concomitant disorders and exposure to exogenous risk factors. Adjusted hazard ratios, though not statistically significant, suggested a 2.7 and 1.8-fold higher risk in carriers who had high levels of factor VIII:C and TAFI, respectively. High levels of factor XI:C and \(Lp(a)\) did not influence the risk of VTE in carriers of factor V Leiden. The clinical implications of concomitant risk factors depend on the absolute rather than the relative risk of VTE. The absolute annual risk as we have reported from the original family cohort study, was 0.45\% in relatives who carried factor V Leiden, as compared with 0.10\% in non-carrier relatives.\(^1\) The here presented results show annual incidences in carriers, ranging from 0.35\% to 1.11\% for various concomitant disorders. Single factor V Leiden carriers will consequently have a lower absolute risk. The annual incidence in this subgroup which contained 40\% of carriers, was 0.09\% and hence comparable to the absolute annual risk in non-carriers\(^1\) and to the risk in the general population.\(^{23}\) This finding may explain why many factor V Leiden carriers will never develop VTE. It emphasizes the need for risk stratification and, accordingly, recommendations concerning prophylaxis and treatment of VTE in factor V Leiden carriers.

Our study has obvious limitations. First, we were able to analyse only some of the factor V Leiden carriers, who enrolled in the original study, but overt selection bias seems unlikely. Second, as non-carriers were excluded from additional testing, we missed the opportunity to compare the distribution of concomitant thrombophilic disorders in carriers and non-carriers and to assess clustering in non-carriers. Considering the low absolute annual risk of VTE in single factor V Leiden carriers, and the high prevalences and relative risks of high factor VIII:C and TAFI levels, the question raises whether the latter disorders are less frequently found in non-carriers.

It will be clear that the presented results must be interpreted cautiously. However, they support the supposed co-segregation and interactions of an increasing number of prevalent, mild risk factors in symptomatic factor V Leiden carriers. Further studies are warranted to establish our findings. Extensive testing of concomitant disorders in carriers will become important in clinical practice if it enables us to identify carriers who are at either low or high risk for VTE.
In conclusion, high levels of factor VIII:C and TAFI are mild risk factors for VTE, that are frequently found in factor V Leiden carriers and substantially contribute to their risk for VTE. This could not be demonstrated for high levels of factor XI:C and Lp(a).

Acknowledgments
We would like to thank JCM Meijers (Academical Medical Center, Amsterdam) for performing measurements of factor XI:C and TAFI and JLP Brouwer for collecting the plasma samples.

References