Mechanisms of the Factor V Leiden Paradox


Objective—Carriers of the factor V Leiden mutation (FVL-carriers) have a substantially increased risk of deep venous thrombosis (DVT), whereas the risk of pulmonary embolism (PE) is only mildly increased compared with noncarriers. So far few studies have investigated possible mechanisms for this so-called FVL paradox.

Methods and Results—Consecutive patients with a first DVT or PE were included in a large population-based case-control study (MEGA study). Patients, aged 18 to 70 years, provided a questionnaire, DNA (n = 3313), or plasma (n = 1474). Surgery, injury, and travel were considered thrombosis-provocative. Of 2063 patients with isolated DVT, 20% were FVL-carrier, as were 8% of the 885 patients with isolated PE. Among DVT patients, FVL-carriers had their thrombi more often proximal and a higher number of affected veins than noncarriers. No differences were observed between FVL-carriers and noncarriers in time between provocation and diagnosis, in vitro coagulation time, and thrombus density. Compared with patients with both DVT and PE, isolated DVT patients more often had thrombi located distally and had a similar number of affected veins. Compared with isolated PE patients, isolated DVT patients had a similar time between provocation and diagnosis, and similar in vitro coagulation time and thrombus density.

Conclusion—Although some effects were differential for FVL-carriers and noncarriers, and some were differential for PE and DVT patients, none of the potential mechanisms offered a clear explanation. (Arterioscler Thromb Vasc Biol. 2008;28:1872-1877)

Key Words: factor V Leiden ■ pulmonary embolism ■ deep venous thrombosis ■ thrombus location ■ coagulation ■ thrombus density

The incidence of venous thrombosis is about 1 to 3 per 1000 individuals per year and is associated with life-threatening pulmonary embolism (PE).1 Both autopsy2 and clinical3,4 studies have shown that approximately 90% of the pulmonary emboli arise from thrombi in the deep veins of the lower limbs. Moreover, asymptomatic PE can be found in about half the patients presenting with deep venous thrombosis (DVT).5 Therefore, in general DVT and PE are considered as two entities of a single disease and referred to as venous thrombosis or venous thromboembolism.

However, several studies have shown that the prevalence of some risk factors differs in patients with DVT compared with those with PE.6-9 The factor V Leiden mutation, the most prevalent genetic factor known to increase the risk of venous thrombosis, has repeatedly been shown to be a strong risk factor for DVT, but at most a weak risk factor for PE. Shortly after the discovery of the Factor V Leiden mutation, it was hypothesized that the presence of Factor V Leiden would often lead to fatal PE, resulting in a lower number of Factor V Leiden–positive subjects among those surviving PE. This would explain the weak effect of Factor V Leiden on the risk of PE found in studies of survivors of venous thrombosis, such as case-control studies. However, this hypothesis was rejected as autopsy studies have shown that among patients with fatal PE, the proportion of individuals with Factor V Leiden was no different from that in PE survivors or from that in the general population.10,11

The differential effect of Factor V Leiden on DVT and PE is known as the “Factor V Leiden paradox.”12 Although this paradox has been reported repeatedly,5,13,14 some still doubt whether it exists. We therefore studied the prevalence of Factor V Leiden among patients with an isolated DVT, isolated PE, or a combination of DVT and PE. Furthermore, we studied whether the effect was specific for Factor V Leiden, by assessing the effect of the prothrombin 20210A mutation, another well known factor involved in the risk of venous thrombosis.

So far, few studies have investigated mechanisms that could lead to the Factor V Leiden paradox, except for a possible difference in thrombus location. In this study, we sought to investigate several potential explanations for the paradox. First we studied the difference in location. Second, we focused on differences in number of affected veins. A third possible mechanism was a difference in time interval...
between the provocation of thrombus formation and the actual diagnosis. The fourth possible mechanism was a difference in growth speed as expressed by in vitro coagulation time. A fifth, related, mechanism was a difference in clot structure with lower chances of thrombus breaking, which might be expressed as a difference in in vitro clot density.

In this study we investigated these 5 possible mechanisms by determining (1) whether Factor V Leiden affects thrombus location, number of affected veins, time until diagnosis, growth speed, or clot density and (2) whether these factors differ in prevalence between patients with isolated deep venous thrombosis of the leg compared to patients with isolated pulmonary embolism or combined deep venous thrombosis and pulmonary embolism.

Materials and Methods
All analyses were done as part of the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA study), a large population-based case–control study. Between March 1999 and September 2004 all consecutive patients with a first episode of venous thrombosis were recruited from 6 anticoagulation clinics in the Netherlands. These clinics monitor the anticoagulant treatment of all outpatients within a well-defined geographical area. Eligible participants were between 18 and 70 years of age at the time of their inclusion. Patients who died (n=280) and those who were at the end stage of disease (n=82) and were therefore unable to fill in a questionnaire were excluded. Of the 5969 eligible patients, 5051 (84.5%) were willing to participate.

Control subjects were recruited from 2 sources: first, by inviting partners of patients (82% of the partners participated), and second by using a random digit dialing method (69% of the eligible individuals participated). All participants provided informed consent in which they agreed to participate. This study was approved by the Medical Ethics Committee of the Leiden University Medical Center, Leiden, the Netherlands.

Data Collection
Risk factors for venous thrombosis including surgery, injury, and travel were reported in a standardized mailed questionnaire covering a period of 1 year before the venous thrombotic event. The questionnaire included a permission form to obtain information regarding the diagnostic procedure of the thrombotic event from existing medical records. Informed consent to obtain medical records was obtained by phone no written permission form 337. No permission to obtain medical records 186.

Did not provide DNA 250

Genotyping Factor V Leiden or FII 20210A failed 11

DNA samples where genotyping of Factor V Leiden failed (11 patients) were excluded from the present analyses, resulting in a total of 3313 patients who were eligible for analysis (Figure 1).

Participating patients 5051

Mini questionnaire by phone no written permission form 337

Permission to obtain medical records 4528

DVT or PE objectively confirmed 3574

No venous thrombosis 861

Did not provide DNA 250

Genotyping Factor V Leiden or FII 20210A failed 11

DNA 3313

Only DNA 1620

Used anticoagulants at time of blood draw 219

Plasma 1474

Figure 1. Flowchart of patients.

DNA Collection and Laboratory Analyses
Patient included between March 1999 and May 2002 were asked to provide a blood sample at least 3 months after discontinuation of anticoagulant treatment (median time until blood draw 123 days), whereas those who were unable or unwilling to come to the anticoagulation clinic for a blood draw were sent a cotton swab for the collection of buccal cell DNA. From May 2002 onwards DNA was collected through buccal swab samples only. Assessment of the Factor V Leiden mutation. Information regarding the location of the thrombus in the leg was available for 2083 patients with DVT, but obviously not for patients with an isolated PE. A thrombus in the calf veins only was defined as located distally, whereas a thrombus in any of the other veins was defined as proximal. For calculation of time between the onset of thrombus formation and diagnosis only patients who had surgery, an injury, or had traveled in the 100 days before the diagnosis confirmed venous thrombosis were included. In these patients we assumed that thrombus formation started shortly after provocation.
subsequent lysis by measuring the optical density at 405 nm every 20 seconds. In vitro coagulation time was defined as time from adding the buffer until the midpoint between the start of clot formation (minimum turbidity) and maximum clot size (maximum turbid transition). Thrombus density was defined as the difference in light absorbance between the maximum turbidity minus the light absorbance at minimal turbidity, measured in optical densities (OD). For the calculation of in vitro coagulation time and thrombus density only those patients who donated plasma but did not receive anticoagulant treatment at time of blood draw were included in the analysis (n=1474).

Statistical Analyses
Odds ratios and 95% confidence were calculated as estimated of the relative risk by using unconditional logistic regression analysis. Percentages and 95% confidence intervals (95% CI) were calculated using the exact method. Differences in time between provocation and diagnosis of venous thrombosis were determined using a log-rank test. All analyses were performed in SPSS for windows 14.0 (SPSS Inc).

Results
A total of 3313 patients were included in the present analysis, of whom 2063 were objectively diagnosed with DVT, 885 with PE, and 365 with both. The characteristics of these 3 groups and the control subjects are shown in Table 1. Of the patients with DVT, 415 carried the Factor V Leiden mutation (20%), 60 patients with both DVT and PE carried the Factor V Leiden mutation (16%), and 75 patients with PE (8%) carried Factor V Leiden, compared with 256 control subjects (5%). Therefore the risk of DVT was 4.5-fold increased (OR 3.2 95% CI 2.4 to 4.2) and for PE (OR 2.3 95% CI 1.5 to 3.3).

<table>
<thead>
<tr>
<th>Location</th>
<th>FVL</th>
<th>Total</th>
<th>Percentage FVL*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal thrombosis†</td>
<td>318</td>
<td>1559</td>
<td>20% (18–22)</td>
</tr>
<tr>
<td>Isolated inferior cava</td>
<td>1</td>
<td>7</td>
<td>14% (−14–42)</td>
</tr>
<tr>
<td>Isolated iliocaval vein</td>
<td>6</td>
<td>49</td>
<td>12% (4–25)</td>
</tr>
<tr>
<td>Iliofemoral vein</td>
<td>12</td>
<td>61</td>
<td>20% (11–32)</td>
</tr>
<tr>
<td>Isolated femoral vein</td>
<td>40</td>
<td>170</td>
<td>24% (17–30)</td>
</tr>
<tr>
<td>Popliteal-iliofemoral vein</td>
<td>11</td>
<td>58</td>
<td>19% (9–29)</td>
</tr>
<tr>
<td>Popliteal-femoral vein</td>
<td>79</td>
<td>356</td>
<td>22% (18–27)</td>
</tr>
<tr>
<td>Isolated popliteal vein</td>
<td>169</td>
<td>858</td>
<td>20% (17–22)</td>
</tr>
<tr>
<td>Distal thrombosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isolated calf veins</td>
<td>53</td>
<td>329</td>
<td>16% (12–20)</td>
</tr>
<tr>
<td>No. of affected veins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One vein</td>
<td>216</td>
<td>1208</td>
<td>18% (16–20)</td>
</tr>
<tr>
<td>≥2 veins</td>
<td>175</td>
<td>750</td>
<td>23% (20–26)</td>
</tr>
</tbody>
</table>

*FVL indicates Carrier of the Factor V Leiden mutation; †Other locations for 70 patients; ‡On occasion combined with a thrombus in the calf veins.

This differential effect was specific for Factor V Leiden and not for prothrombin 20210A mutation, which was present in 121 of 2063 patients with DVT (5.9%) and 38 of 885 patients with PE (4.3%). Odds ratios for carriers of the prothrombin 20210A mutation were clearly elevated with overlapping confidence intervals for both DVT (OR 3.2 95% CI 2.4 to 4.2) and for PE (OR 2.3 95% CI 1.5 to 3.3).

Location
Differences in Factor V Leiden prevalence between patients with proximal and distal DVT were small. Among those with proximal DVT, 318 of 1559 (20%) carried the Factor V Leiden mutation, whereas this was 53 of 329 (16%) patients with distal DVT; difference 4% (95% CI 0% to 9%; Table 2). Patients with DVT more often had distally located thrombi (302 of 1635 patients, 19%) compared with patients with both PE and DVT (27 of 253 patients, 11%), difference 8% (95% CI 3% to 12%).

Number of Affected Veins
Of the 750 patients who had multiple veins affected, 175 carried the Factor V Leiden mutation (23%), whereas this was 216 of 1208 (18%) patients who had only 1 vein affected, a difference of 5% (95% CI 2% to 9%; Table 2). The number of affected veins was similar in patients who had an isolated DVT as in patients with a combination of DVT and PE, 650 of 1690 (39%) had 2 or more veins affected, whereas this was 100 of 268 patients who had combination of DVT and PE (38%), a difference of 1% (95% CI −7 to 5%).

Time Interval Between Provocation and Diagnosis
We studied the time interval between Factor V Leiden carriers versus noncarriers in patients who were diagnosed with DVT or PE within the first 100 days after provocation of thrombus formation (n=1048). Within this time window,
carriers of the Factor V Leiden mutation had a similar time interval between provocation and the diagnosis as noncarriers (Figure 2A). Patients with PE seemed to be diagnosed slightly later after provocation; however, results were not significant (Figure 2B).

**In Vitro Coagulation Time**
In vitro coagulation time was similar in patients with Factor V Leiden (2.45 minutes) and noncarriers (2.46 minutes), a difference of 0.01 minutes (95% CI -0.07 to 0.08). Also no differences were observed in coagulation time between patients with DVT (2.45 minutes) and PE (2.47 minutes), difference 0.02 minutes (95% CI -0.04 to 0.09).

**Thrombus Density**
Factor V Leiden carriers had a slightly lower thrombus density (mean OD 0.46) compared with noncarriers (mean OD 0.48), difference 0.02 (95% CI 0.01 to 0.04). However, thrombus density was similar in patients with isolated DVT (mean OD 0.47) and isolated PE (mean OD 0.47), difference 0.00 (95% CI -0.01 to 0.01).

**Discussion**
The prevalence of Factor V Leiden is substantially higher in patients with DVT, in presence or absence of a concomitant PE, than in patients with isolated PE. In fact, Factor V Leiden is only a mild risk factor for isolated PE, whereas the risk of DVT is substantially increased by this mutation. We studied multiple explanatory mechanisms for the differential effect of Factor V Leiden on the risk of DVT and PE: thrombus location, number of affected veins, time between provocation and diagnosis, in vitro clot formation, and in vitro clot density. Although some effects were different for Factor V Leiden carriers and noncarriers, and some were different for patients with PE and patients with DVT, none of the mechanisms offered a clear explanation.

**Location**
So far, studies have been inconsistent on whether the thrombus location is different in Factor V Leiden carriers compared with noncarriers. Some studies, including ours, showed that the presence of Factor V Leiden leads to an increased risk of thrombosis in the proximal veins,\(^{17,18}\) whereas others have shown the opposite,\(^{19-21}\) or found no difference in location.\(^{22,23}\)

More distal located thrombi are less likely to be accompanied by PE, which is in agreement with other studies.\(^{1,24,25}\) Therefore, if Factor V Leiden would lead to more distal located thrombi, and proximal located thrombi would lead to PE, one would expect that Factor V Leiden carriers were at lower risk for PE. However, as the results in the literature regarding the location of thrombi in Factor V Leiden carriers are inconsistent, and we even found an increased risk of a proximally located thrombus for Factor V Leiden carriers, it is unlikely that the location of the thrombus in the leg explains the risk difference of Factor V Leiden in DVT and PE risk.

**Thrombus Size**
Murine models have shown that mice homozygous for the Factor V Leiden mutation had a larger thrombus volume compared with wild-type mice.\(^{26}\) This is in line with our results as we showed that carriers of the Factor V Leiden mutation more often had multiple veins affected compared with noncarriers. It seems logical that when each thrombus has a certain probability of embolizing, the overall likelihood would increase with the number of veins involved. From this finding it does not logically follow that DVT patients with Factor V Leiden have a decreased incidence of PE. Moreover, the number of affected veins was not different in patients with isolated DVT or both DVT and PE.

It should be noted that it is impossible to study the effect of the location and thrombus size in patients with isolated PE and that individuals with both DVT and PE have been used as a surrogate for the isolated PE population. Furthermore, for the group of DVT and PE we are probably studying the part of the residual thrombus as part of the thrombus was detached. Therefore we believe that this may have resulted in an underestimation of the number of proximal thrombosis and number of affected veins in those with a combination of DVT and PE.

**Growth Speed**
Factor V Leiden mice had faster growing thrombi compared with non–Factor V Leiden mice.\(^{26}\) We studied growth speed in 2 ways, both epidemiologically and in vitro. First, we studied whether time between a clear thrombus provocation such as surgery, injury, or travel and diagnosis was similar in carriers versus noncarriers and found no difference. Although not significant, it seemed to take even more time to diagnose
PE than to diagnose DVT. As a consequence it will be unlikely that the presence of Factor V Leiden will have resulted in earlier treatment and a reduction in risk of embolization. Secondly, we studied the growth speed by measuring clotting time in vitro. No differences were found between Factor V Leiden carriers and noncarriers in clotting time, nor was there a difference between PE and DVT patients. However, care should be taken in interpreting these results, as the in vitro clotting was performed without the presence of activated protein C (APC). Thus the effect of Factor V Leiden may not have become apparent by using this assay. Furthermore, on average patients with a DVT were treated for 4 months with anticoagulant therapy, whereas patients with a PE were treated for 6 months. Although in both cases blood draws were performed on average 4 months after the last therapy, these differences might have affected the in vitro thrombus density and the coagulation time. Because of this limitation we cannot exclude a possible difference in growth speed of the thrombus as an explanation for the Factor V Leiden paradox. As mouse models have shown an increased speed of thrombus formation in Factor V Leiden mice and patients with PE had a longer time interval between provocation and diagnosis, there might be a relation. It should therefore be investigated more extensively whether the duration of thrombus formation could explain the Factor V Leiden paradox.

Thrombus Density
Finally, we studied whether a difference in thrombus density could shed light on the Factor V Leiden paradox. We found that Factor V Leiden carriers had a slightly lower thrombus density than noncarriers. The results combined with the higher number of affected veins might suggest a different composition of the thrombus. Yet, no differences in thrombus density were found between patients with DVT or PE. Therefore, thrombus density does not seem to offer an explanation for the Factor V Leiden paradox. However, it should be noted that for both the thrombus density and in vitro coagulation time differences were small.

Drawbacks
We studied the possible mechanisms in patients between 18 and 70 years who did not have an acute fatal PE or died shortly after the thrombosis. Previous studies have shown that the presence of FVL was only slightly less among elderly patients compared with younger patients, whereas no differences were found between those with a fatal PE compared with nonfatal PE. Furthermore, relatively few patients died because of an acute PE. Therefore, we do not believe that, if we would have been able to include these patients, different results would have been obtained.

The diagnosis of PE, DVT, and the combination was based on methods used in clinical practice by hospitals in diagnosing venous thrombosis. Patients were probably not routinely screened for both DVT and PE. This might have resulted in a possible misclassification as some of the combined diagnosis might have been missed. However, there was a clear difference in the prevalence of factor V Leiden between patients with an isolated DVT, an isolated PE, or a combined diagnosis. We believe that although we may have underestimated the effects of possible mechanisms attributable to this misclassification, if there would have been an important effect we would have been able to find it in our large study. As none of the mechanisms showed even a small effect we believe that misclassification could not explain the absence of any associations. This is confirmed by our analyses in the subgroup of patients who had at least 2 diagnostic tests.

Conclusion
These results confirm the existence of the Factor V Leiden paradox. However, none of the above mechanisms seems to be a solid explanation of the Factor V Leiden paradox. Future research might focus on a possible difference in growth speed and composition of the thrombus as these represent the most promising explanation.

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Disclosures
None.

References


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