Influence of single nucleotide polymorphisms on thrombin generation in factor V Leiden heterozygotes

Olivier Segers1; Paolo Simioni2; Daniela Tormene2; Elisabetta Castoldi1

1Department of Biochemistry, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University Medical Centre, Maastricht, the Netherlands; 2Department of Cardiologic, Thoracic and Vascular Sciences, 2nd Chair of Internal Medicine, University of Padua Medical School, Padua, Italy

Summary

Carriership of the factor V (FV) Leiden mutation increases the risk of venous thromboembolism (VTE) ~4-fold, but the individual risk of each FV Leiden carrier depends on several co-inherited risk and protective factors. Under the hypothesis that thrombin generation might serve as an intermediate phenotype to identify genetic modulators of VTE risk, we enrolled 188 FV Leiden heterozygotes (11 with VTE) and determined the following parameters: thrombin generation in the absence and presence of activated protein C (APC); plasma levels of prothrombin, factor X, antithrombin, protein S and tissue factor pathway inhibitor; and the genotypes of 24 SNPs located in the genes encoding these coagulation factors and inhibitors. Multiple regression analysis was subsequently applied to identify the (genetic) determinants of thrombin generation. The endogenous thrombin potential (ETP) showed a striking inter-individual variability among different FV Leiden carriers and, especially when measured in the presence of APC, correlated with VTE risk. Several SNPs in the F2 (rs1799963, rs3136516), F10 (rs693335), SERPINC1 (rs2227589), PROS1 (Heerlen polymorphism) and TFPI (rs5940) genes significantly affected the ETP. APC and/or the ETP,APC in FV Leiden carriers. Most of these SNPs have shown an association with VTE risk in conventional epidemiological studies, suggesting that the genetic dissection of thrombin generation leads to the detection of clinically relevant SNPs. In conclusion, we have identified several SNPs that modulate thrombin generation in FV Leiden heterozygotes. These SNPs may help explain the large variability in VTE risk observed among different FV Leiden carriers.

Key words

Factor V Leiden, intermediate phenotype, single nucleotide polymorphism, thrombin generation, venous thromboembolism

Introduction

Approximately 5% of Caucasians carry a missense mutation in the coagulation factor V (FV) gene (Arg506→Gln, FV Leiden) (1), which increases VTE risk ~4-fold in heterozygous carriers and ~11-fold in homozygous carriers (2). This is attributable to a deregangement of the protein C pathway, the anticoagulant system responsible for the proteolytic inactivation of activated factors V (FVa) and VIII (FVIIIa). In fact, the FV Leiden mutation abolishes one of the cleavage sites recognised by activated protein C (APC) on FVa(a), making FVa(a)Leiden less susceptible to APC-mediated inactivation (3, 4) and a poor APC-cofactor in FVIIIa inactivation (5, 6). As a consequence, plasma from FV Leiden carriers shows an impaired anticoagulant response to the addition of APC in vitro, a condition known as APC resistance (7).

Although the FV Leiden mutation is an established risk factor for VTE, not all carriers of the mutation are exposed to the same VTE risk. While most FV Leiden carriers remain asymptomatic throughout their lives (8, 9), others (e.g. those belonging to “thrombophilic” families) experience severe and recurrent thrombotic episodes already at a young age. In line with the multifactorial aetiology of VTE (10), this wide variation in clinical penetrance is likely due to the co-inheritance of additional risk and protective factors. While the role of FV genetic variation in modulating VTE risk in FV Leiden carriers has been addressed in several studies (11-13), extragenic modulators remain largely elusive, and so far only few thrombophilic defects have been reported to synergise with FV Leiden in increasing VTE risk (14-17).

Recent genome-wide association studies have shown that numerous single nucleotide polymorphisms (SNPs) that are common in the general population contribute to the risk of VTE (18-20). However, their role in FV Leiden carriers has not been investigated, possibly because of the large number of FV Leiden carriers that would be required to detect such subtle risk modifiers in conventional association studies. On the other hand, statistical power can be considerably increased by using an inter-
mediate phenotype (a quantitative trait that closely reflects disease susceptibility), instead of disease occurrence (a dichotomous variable), as the end-point of the analysis (21). In the last few years, the ability of a plasma sample to generate thrombin following in vitro activation of coagulation, as determined with the thrombin generation assay, has emerged as a promising intermediate phenotype for linkage and association analyses on VTE (22, 23), as demonstrated by its strong correlation with VTE risk in numerous retrospective and prospective studies from different laboratories (24-33).

Building on these findings, we have used the thrombin generation test to screen haemostasis-related candidate genes for potential modulators of VTE risk in FV Leiden carriers.

Materials and methods

Study population

The study population has been described in detail elsewhere (13). Briefly, probands with FV Leiden were identified at Padua Academic Hospital (Italy) among consecutive patients who underwent thrombophilia screening following a thrombotic event. Probands and their family members (with or without VTE) who carried FV Leiden in the heterozygous state, but no deficiency of antithrombin, protein C or protein S, were invited to participate in the study. Pregnant women as well as users of oral contraceptives, hormone replacement therapy, oral anticoagulant therapy or heparin at the time of blood collection were excluded, since these conditions are known to influence thrombin generation. FV Leiden pseudo-homozygotes (11), identified in a previous study on the same population (13), were also excluded. A total of 188 FV Leiden heterozygotes belonging to 78 different families were eventually enrolled. Of these, 11 had experienced at least one episode of VTE (deep-vein thrombosis, pulmonary embolism or VTE at an unusual site).

Blood was drawn in 3.8% sodium citrate (9/1 vol/vol) and platelet-poor plasma was prepared by centrifugation at 2,000g for 15 minutes (min). Aliquots were stored at -80°C until use. Buffy coats were kept at -20°C for later isolation of genomic DNA. The study was approved by the Ethical Committee of Padua Academic Hospital and conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants.

Thrombin generation

Thrombin generation was measured using Calibrated Automated Thrombography (CAT) (34). The assay was performed in the absence and presence of APC, essentially as described (35). Briefly, plasma was incubated with tissue factor (TF; Innovin; Dade Behring, Leusden, The Netherlands) and synthetic phospholipid vesicles (DOPS/DOPC/DOPE, 20/60/20 mol/mol/mol) for 10 min at 37°C. Subsequently, buffer or purified APC (Innovative Research, Southfield, MI, USA) was added and coagulation was immediately started with a mixture of CaCl$_2$ and fluorogenic substrate for thrombin (Z-Gly-Gly-Arg-AMC; Bachem, Bubendorf, Switzerland). Final concentrations in the well were 6.8 pM TF, 30 μM phospholipids, ~10 nM APC, 16 mM added CaCl$_2$ and 300 μM thrombin substrate. Fluorescence was read in a Fluoroskan Ascent reader (Thermo Labsystems, Helsinki, Finland) and thrombin generation parameters were calculated using the Thrombinoscope software (Thrombinoscope, Maastricht, The Netherlands).

Pooled plasma from two healthy FV Leiden heterozygotes measured in parallel served as FV Leiden reference plasma. The APC concentration was arbitrarily chosen such as to reduce the endogenous thrombin potential (ETP) of the FV Leiden reference plasma to ~25% of the ETP obtained in the absence of APC. However, the FV Leiden carriers under study turned out to be generally more resistant to APC than the FV Leiden reference plasma (possibly due to the fact that they belonged to thrombo-philic families), with an average %rest (ETP$_{APC}$/ETP$_{noAPC}$) of 42.6%.

The inter-assay variation, determined by measuring the FV Leiden reference plasma in triplicate on 13 different plates and expressed as coefficient of variation (%CV), was 3.7% for the ETP$_{APC}$ and 9.8% for the ETP$_{noAPC}$.

Measurement of factor levels in plasma

Prothrombin (PT) levels were measured with an in-house chromogenic assay, as previously described (35). Factor X (FX) and antithrombin (AT) activity levels were measured with the commercial Biophen Factor X kit (Hyphen BioMed, Neuville-sur-Oise, France) and Coamatic® Antithrombin kit (Chromogenix, Mölndal, Sweden), respectively, according to the manufacturers’ instructions. Total and free protein S as well as full-length tissue factor pathway inhibitor (TFPI) levels were measured using in-house ELISAs, as previously described (35). Plasma levels of coagulation factors and inhibitors were expressed as percentages of normal pooled plasma.

Genetic analysis

Five haemostasis-related genes (F2, F10, SERPINC1, PROS1 and TFPI) encoding major determinants of thrombin generation in normal individuals (36) were selected as candidate modulators of thrombin generation in FV Leiden carriers. Non-redundant SNPs capturing most common genetic variation in these genes were identified based on the information available in public databases such as HapMap (www.hapmap.org) and SeattleSNPs (http://pga.mbt.washington.edu/). A few SNPs with allele frequencies <0.05 were also included because of their established or suspected association with VTE risk. In total, 24 SNPs (6 in F2, 5 in F10, 5 in SERPINC1, 4 in PROS1 and 4 in TFPI) were selected for the study (Suppl. Table 1, available online at www.thrombosis-on line.com).

Genomic DNA was isolated from buffy coats using the QiaAmp Blood Mini Kit (QIAGEN, Venlo, the Netherlands). SNP genotyping was performed by PCR-mediated amplification and restriction analysis or using commercial 5’ nuclease (TaqMan®) as-
Statistical analysis

Data are presented as absolute numbers for categorical variables (sex, VTE) and as mean ± standard deviation for continuous variables (age, coagulation factor levels, thrombin generation). Means were compared using Student’s t-test. Correlations were expressed as Pearson’s coefficients (r).

The association between thrombin generation and VTE was determined by logistic regression analysis after stratifying the study population in tertiles of thrombin generation. The first tertile was taken as a reference and risk estimates for the other tertiles were expressed as age- and sex-adjusted odds ratios (OR) and 95% confidence intervals (95% CIs).

The effects of individual haplotypes on factor levels and thrombin generation (ETP_{APC} and ETP_{APC}) were investigated by multiple regression analysis, essentially as described (23). The regression model for factor levels included age, sex (0=male, 1=female) and all haplotypes (0, 1 or 2 copies) of the corresponding gene as independent variables. The regression model for thrombin generation also included the levels of all plasma factors (except the one for which haplotypes were entered) as additional independent variables. The effect of each haplotype on factor level or thrombin generation was expressed as unstandardised regression coefficient (B), representing the absolute change in factor level or ETP per haplotype copy.

Statistical analysis was performed using SPSS 20.0.0 software for Windows (SPSS Inc., Chicago IL, USA).

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Mean ± SD</th>
<th>ETP_{APC}</th>
<th>ETP_{APC}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>p</td>
<td>B</td>
</tr>
<tr>
<td>Age (years)</td>
<td>38.4 ± 16.5</td>
<td>1.13a</td>
<td>0.03b</td>
</tr>
<tr>
<td>Sex (males/females)</td>
<td>82/106*</td>
<td>33.8a</td>
<td>26.4b</td>
</tr>
<tr>
<td>VTE (n)</td>
<td>11*</td>
<td>81.1a</td>
<td>30.4b</td>
</tr>
<tr>
<td>PT (%)</td>
<td>106.7 ± 14.1</td>
<td>6.51</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FX (%)</td>
<td>103.7 ± 20.2</td>
<td>1.99</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AT (%)</td>
<td>102.7 ± 10.1</td>
<td>-5.81</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total PS (%)</td>
<td>99.6 ± 19.2</td>
<td>0.45</td>
<td>0.325</td>
</tr>
<tr>
<td>Free PS (%)</td>
<td>99.7 ± 23.8</td>
<td>0.42</td>
<td>0.270</td>
</tr>
<tr>
<td>Full-length TFPI (%)</td>
<td>116.2 ± 33.8</td>
<td>-0.80</td>
<td>0.002</td>
</tr>
</tbody>
</table>

*These categorical variables are expressed as absolute numbers instead of as mean ± SD. B, unstandardised regression coefficient. aRegression model 1, including age, sex and VTE history as independent variables. bRegression model 2, including age, sex, VTE history as well as the levels of coagulation factors and inhibitors as independent variables.

Table 1: Determinants of thrombin generation in FV Leiden carriers.

Results

Thrombin generation and VTE risk

The demographic characteristics and plasma factor levels of the 188 FV Leiden heterozygotes under study are summarised in Table 1. None of them was pregnant or on oral contraceptives, hormone replacement therapy or antiocoagulant therapy at the time of blood collection.

Thrombin generation determined in the absence and presence of APC showed a large inter-individual variability. The ETP_{APC} (651 ± 137 nM.min) had an inter-individual variation of 21.0% vs an inter-assay variation of 3.7% and the ETP_{APC} (285 ± 169 nM.min) had an inter-individual variation of 59.4% vs an inter-assay variation of 9.8%. The ETP_{APC} and the ETP_{APC} were significantly correlated (r=0.488, p<0.001). Females had a higher ETP_{APC} than males (330 ± 166 nM.min vs 225 ± 154 nM.min; p<0.001), whereas no difference was observed in the ETP_{APC} (666 ± 140 nM.min vs 632 ± 131 nM.min; p=0.086). Thrombin generation was higher in subjects that had experienced VTE than in healthy subjects, but only the difference in ETP_{APC} reached statistical significance (ETP_{APC} 744 ± 125 nM.min vs 645 ± 136 nM.min, p=0.027; ETP_{APC} 393 ± 225 nM.min vs 278 ± 164 nM.min, p=0.123). However, after correction for age and sex, VTE history was a major determinant of both the ETP_{APC} (B=81.1 nM.min, p=0.059) and the ETP_{APC} (B=121.7 nM.min, p=0.015) (Table 1, regression model 1).

A relationship between thrombin generation and VTE risk was further suggested by logistic regression analysis. When FV Leiden carriers were stratified in tertiles of ETP_{APC} the age- and sex-adjusted OR for VTE increased from 1.00 in the first (reference) tertile to 2.55 (95%CI 0.25-25.84) in the second tertile and 6.27 (95%CI 0.73-53.79) in the third tertile. For the ETP_{APC} the age-
and sex-adjusted OR for VTE was 1.00 in the first (reference) tertile, 5.76 (95%CI 0.61-54.18) in the second tertile and 8.11 (95%CI 0.79-82.92) in the third tertile. Despite the wide confidence intervals, which are likely due to the low number of VTE cases in our study population, these trends were close to significance (p=0.094 for the third tertile of ETP_{APC}, p=0.078 for the third tertile of ETP_{APC}), suggesting that thrombin generation, especially when measured in the presence of APC, may be a marker of VTE risk in FV Leiden carriers.

**Determinants of thrombin generation in FV Leiden heterozygotes**

A multiple regression analysis including age, sex, VTE history and the levels of coagulation factors and inhibitors as independent variables showed that the major determinants of the ETP_{APC} in FV Leiden carriers are PT (B=6.51 nM.min/1% increase in PT level; p<0.001) and AT (B=-5.81 nM.min/1% increase in AT level; p<0.001), followed by FX (B=1.99 nM.min/1% increase in FX level; p<0.001) and TFPI (B=-0.80 nM.min/1% increase in TFPI level; p=0.002) (Table 1). Interestingly, when the levels of these coagulation factors and inhibitors were included in the regression model, the effect of VTE history on the ETP_{APC} was completely lost (Table 1, regression model 2), suggesting that altered levels of these factors are responsible for this association.

The most important determinants of the ETP_{APC} were PT (B=4.08 nM.min/1% increase in PT level; p<0.001), TFPI (B=-2.97 nM.min/1% increase in TFPI level; p<0.001) and FX (B=1.60 nM.min/1% increase in FX level; p=0.012) (Table 1). However, inclusion of factor levels in the regression model did not abolish the effect of VTE history on the ETP_{APC}, but only slightly weakened it (Table 1, regression model 2), suggesting that this effect is only partially mediated by increased/decreased levels of these factors and that qualitative alterations in protein function (and/or al-

### Table 2: Effects of F2 haplotypes on PT levels and on thrombin generation in FV Leiden carriers.

<table>
<thead>
<tr>
<th>Haplotype frequency</th>
<th>Effect on PT levels</th>
<th>Effect on ETP_{APC}</th>
<th>Effect on ETP_{APC}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>p</td>
<td>B</td>
</tr>
<tr>
<td>H1</td>
<td>C</td>
<td>G</td>
<td>0.537</td>
</tr>
<tr>
<td>H2</td>
<td>C</td>
<td>C</td>
<td>4.25</td>
</tr>
<tr>
<td>H3</td>
<td>G</td>
<td>A</td>
<td>0.098</td>
</tr>
<tr>
<td>H4</td>
<td>C</td>
<td>G</td>
<td>4.67</td>
</tr>
<tr>
<td>H5</td>
<td>T</td>
<td>G</td>
<td>6.69</td>
</tr>
<tr>
<td>H6</td>
<td>C</td>
<td>C</td>
<td>23.14</td>
</tr>
<tr>
<td>H7</td>
<td>C</td>
<td>C</td>
<td>0.016</td>
</tr>
<tr>
<td>H8</td>
<td>T</td>
<td>G</td>
<td>0.005</td>
</tr>
<tr>
<td>MAF</td>
<td>0.263</td>
<td>0.074</td>
<td>0.101</td>
</tr>
</tbody>
</table>

Nucleotide numbering according to Degen & Davie (1987). MAF, minor allele frequency; B, unstandardised regression coefficient.

### Table 3: Effects of F10 haplotypes on FX levels and on thrombin generation in FV Leiden carriers.

<table>
<thead>
<tr>
<th>Haplotype frequency</th>
<th>Effect on FX levels</th>
<th>Effect on ETP_{APC}</th>
<th>Effect on ETP_{APC}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>p</td>
<td>B</td>
</tr>
<tr>
<td>H1</td>
<td>0.457</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>H2</td>
<td>-4.03</td>
<td>0.131</td>
<td>-6.27</td>
</tr>
<tr>
<td>H3</td>
<td>-6.71</td>
<td>0.025</td>
<td>-20.41</td>
</tr>
<tr>
<td>H4</td>
<td>-0.40</td>
<td>0.911</td>
<td>8.67</td>
</tr>
<tr>
<td>H5</td>
<td>-0.90</td>
<td>0.869</td>
<td>-11.92</td>
</tr>
<tr>
<td>H6</td>
<td>29.02</td>
<td>0.013</td>
<td>40.94</td>
</tr>
<tr>
<td>MAF</td>
<td>0.096</td>
<td>0.404</td>
<td>0.285</td>
</tr>
</tbody>
</table>

Nucleotide numbering of the cDNA according to Leytus et al. (1986). Intronic nucleotides are numbered from the 5’ end of the intron. MAF, minor allele frequency; B, unstandardised regression coefficient.
terations in other factors, not included in our analysis) may play a major role in this association.

SNP genotyping and haplotype construction

All FV Leiden heterozygotes were genotyped for 24 SNPs located in the genes coding for FT (F2), FX (F10), AT (SERPINC1), protein S (PROS1) and TFPI (TFPI) (Suppl. Table 1, available online at www.thrombosis-online.com). Two SNPs (rs3211772 in PROS1 and rs2227624 in SERPINC1) were never observed in our population and were excluded from subsequent analysis. Phasing of genotype data yielded 8 haplotypes in F2, 6 haplotypes in F10 and 5 haplotypes each in SERPINC1, PROS1 and TFPI. The SNP composition of these haplotypes and their observed frequencies in our study population are reported in Table 2-6.

Effects of SNPs on factor levels and thrombin generation

The relationship between haplotypes in the F2, F10, SERPINC1, PROS1 and TFPI genes and thrombin generation was investigated by multiple regression analysis. Since genetic variation can affect thrombin generation by modifying not only protein levels, but also protein function, all haplotypes were tested for their association with the ETP_{APC} and the ETP_{+APC}, even if the levels of the corresponding proteins were not determinants of these phenotypes.

PT (F2 gene)

The selected F2 SNPs defined eight haplotypes in our population, three of which significantly increased PT levels and thrombin generation. The most pronounced effects on PT level (B=-23.1% PT/haplotype copy, p<0.001), ETP_{APC} (B=199.4 nM.min/haplotype copy, p<0.001) and ETP_{+APC} (B=158.5 nM.min/haplotype copy, p<0.001) were associated with haplotype H6, which contains the well-known G20210A mutation (38). However, haplotypes H2, H4 and H5 also increased PT levels and the ETP_{APC} and haplotype H2 also increased the ETP_{+APC}. Remarkably, these haplotypes all share the A19911G SNP, which has been previously shown to predict slightly increased PT levels (39), thrombin generation (23, 40) and VTE risk (41, 42).

Table 4: Effects of SERPINC1 haplotypes on antithrombin levels and on thrombin generation in FV Leiden carriers.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Effect on AT levels</th>
<th>Effect on ETP_{APC}</th>
<th>Effect on ETP_{+APC}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>p</td>
<td>B</td>
</tr>
<tr>
<td>H1</td>
<td>0.561</td>
<td>Reference</td>
<td>8.84</td>
</tr>
<tr>
<td>H2</td>
<td>0.197</td>
<td>1.30</td>
<td>0.346</td>
</tr>
<tr>
<td>H3</td>
<td>0.128</td>
<td>-3.03</td>
<td>0.066</td>
</tr>
<tr>
<td>H4</td>
<td>0.112</td>
<td>-3.55</td>
<td>0.042</td>
</tr>
<tr>
<td>H5</td>
<td>0.003</td>
<td>3.29</td>
<td>0.737</td>
</tr>
</tbody>
</table>

Table 5: Effects of PROS1 haplotypes on protein S levels and on thrombin generation in FV Leiden carriers.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Effect on total PS levels</th>
<th>Effect on free PS levels</th>
<th>Effect on ETP_{APC}</th>
<th>Effect on ETP_{+APC}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>p</td>
<td>B</td>
<td>p</td>
</tr>
<tr>
<td>H1</td>
<td>-1.18</td>
<td>0.672</td>
<td>-4.48</td>
<td>0.177</td>
</tr>
<tr>
<td>H2</td>
<td>0.247</td>
<td>0.751</td>
<td>-0.44</td>
<td>0.895</td>
</tr>
<tr>
<td>H3</td>
<td>0.184</td>
<td>0.272</td>
<td>-5.16</td>
<td>0.175</td>
</tr>
<tr>
<td>H4</td>
<td>0.176</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>H5</td>
<td>0.013</td>
<td>0.695</td>
<td>-14.93</td>
<td>0.148</td>
</tr>
</tbody>
</table>

Nucleotide numbering according to Olds et al. (1993). MAF, minor allele frequency; B, unstandardised regression coefficient.

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Six haplotypes were identified in the F10 gene (Table 3). Haplotypes H2 and H3, which share the common IVS2 C+517G SNP (18), decreased FX levels and the ETP\(_{\text{APC}}\) (B=−49.0 nM.min/haplotype copy, p=0.005 for H2; B=−50.8 nM.min/haplotype copy, p=0.010 for H3). Haplotype H6, tagged by the rare T930G SNP, considerably increased FX levels (B=14.9% FX/haplotype copy, p=0.148), which how-
The factor V (FV) Leiden mutation is an established risk factor for venous thromboembolism. The risk for venous thromboembolism varies among different FV Leiden carriers. Several (common) single nucleotide polymorphisms in genes encoding coagulation factors and inhibitors influence thrombin generation and may explain at least part of the variation in the risk of venous thromboembolism among different FV Leiden carriers.

What is known about this topic?
- The factor V (FV) Leiden mutation is an established risk factor for venous thromboembolism.
- The risk for venous thromboembolism varies among different FV Leiden carriers.

What does this paper add?
- Thrombin generation varies widely among different FV Leiden carriers.
- Thrombin generation, especially when measured in the presence of activated protein C (APC), may be a marker of venous thrombosis risk in FV Leiden carriers.
- Several (common) single nucleotide polymorphisms in genes encoding coagulation factors and inhibitors influence thrombin generation and may explain at least part of the variation in the risk of venous thromboembolism among different FV Leiden carriers.

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the effects of common SNPs in coagulation-related genes on thrombin generation parameters in a normal population have been previously reported (23). Finally, by challenging the protein C pathway with pre-formed APC instead of thrombomodulin, we could not assess the effects of protein C SNPs on thrombin generation and investigate their possible synergic interaction with the FV Leiden mutation.

In conclusion, we have presented suggestive evidence that, in FV Leiden carriers, thrombin generation (especially when measured in the presence of APC) correlates with VTE risk and is modulated by several common SNPs in haemostasis-related genes. Because of their prevalence in the general population, these SNPs may substantially contribute to the variation in thrombin generation and VTE risk observed among different FV Leiden carriers.

Acknowledgements
The authors would like to thank Prof. J. Rosing for helpful suggestions and for critically reading the manuscript.

Conflicts of interest
None declared.

References