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Short running title: PAI-1 polymorphism and risk of VTE recurrence

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Abstract:

BACKGROUND: Plasminogen-activator inhibitor (PAI)-1 is an important inhibitor of the plasminogen/plasmin system. PAI-1 levels are influenced by the 4G/5G polymorphism in the PAI-1 promoter. We investigated the relationship between the PAI-1 polymorphism and VTE recurrence, and its possible modification by factor V Leiden (FVL) and Prothrombin (PTM) mutations.

METHODS AND RESULTS: Patients (n=1069) from the Malmö Thrombophilia Study were followed from discontinuation of anticoagulant treatment until diagnosis of VTE recurrence or the end of the study (maximum follow-up 9.8 years). 127 patients (11.9%) had VTE recurrence. PAI-1 was genotyped by TaqMan PCR. Cox regression analysis adjusted for age, sex and acquired risk factors of VTE showed no evidence of an association between PAI-1 genotype and risk of VTE recurrence in the study population as a whole. However, by including an interaction term in the analysis we showed that FVL but not PTM modified the effect of PAI-1 genotype: patients with the 4G allele plus FVL had a higher risk of VTE recurrence [hazard ratio (HR) =2.3, 95% confidence interval (CI) =1.5-3.3] compared to patients with the 4G allele but no FVL (reference group) or FVL irrespective of PAI-1 genotype (HR=1.8, 95% CI=1.3-2.5). Compared to reference group, 5G allele irrespective of FVL was associated with lower risk of VTE recurrence only when compared with 4G allele together with FVL.

CONCLUSIONS: FVL has a modifying effect on PAI-1 polymorphism in relation to risk of VTE recurrence. The role of PAI-1 polymorphism as a risk factor of recurrent VTE may be FVL dependent.

Keywords: Fibrinolysis, plasminogen, PAI-1 polymorphism, recurrence, venous thromboembolism.

INTRODUCTION

Venous thromboembolism (VTE), which includes deep vein thrombosis (DVT) and pulmonary embolism (PE), is a common cardiovascular disease (CVD) that frequently recurs (1). The risk of recurrence varies over time and is highest during the first 6 to 12 months after diagnosis (2). VTE is considered a chronic disease as the cumulative risk of VTE recurrence after a first episode of deep vein thrombosis (DVT) has been shown to be about 25% at 5 years and 30% at 10 years (3, 4). Treatment with oral anticoagulants such as vitamin K antagonists prevents most episodes of recurrence, albeit at the cost of an increased risk of major bleeding (5). Both acquired factors (older age, malignancy, trauma, major surgery, immobilization, female hormone therapy and pregnancy) and inherited factors [major thrombophilias: factor V Leiden (FVL) (rs6025) and prothrombin (PTM) G20210A (rs1799963) mutations and protein C, protein S and antithrombin deficiencies] have been identified as risk factors for primary VTE (6). Overall, ≈25% to 50% of patients with first-time VTE have an idiopathic condition, without a readily identifiable risk factor (7). Furthermore, there is an increased risk of recurrence in patients with no identifiable acquired risk factors (unprovoked recurrence), compared to patients with known acquired risk factors (provoked) (8). Moreover, the risk factor for VTE recurrence is not always the same as for primary VTE. The risk of recurrent thrombosis after stopping treatment is not easily predicted, in spite of a number of identified risk factors, such as male sex, elevated D-dimer levels and residual thrombosis (9).

Thrombotic risk assessment is necessary before continuation of anticoagulant treatment to limit the treatment duration in patients with a lower risk of recurrence. Notably, the emergence of new anticoagulants with potentially improved risk-benefit ratios requires better risk stratification to avoid overprescription.

Inherited thrombophilic defects are associated with a higher risk of VTE recurrence; however,

the risk is lower than for primary VTE (10, 11). Furthermore, the clinical importance of these inherited genetic defects in VTE recurrence risk is controversial (9, 12, 13). The heterogeneity of results suggests that additional factors contribute to the thrombophilic phenotype.

Fibrinolysis is of vital importance for dissolution of the thrombus, and a link between fibrinolysis and the coagulation system has been suggested (14). Hypofibrinolysis has been suggested as a risk factor for recurrent venous thrombosis (15). Plasminogen activator inhibitor (PAI)-1 is a glycoprotein of the serine protease inhibitor (SERPIN) superfamily, which plays a crucial role in hypofibrinolysis and may thus promote thrombotic events.

The most-studied SNP of *PAI*-1 gene (rs1799768) is a single guanosine nucleotide insertion/deletion (4G/5G) variation situated within the promoter region, 675 base pairs upstream of the transcription start site, where one allele has a sequence of four guanosines (4G) and the other has five guanosines (5G) (16). When compared to the 4G allele, the 5G allele is associated with a lower transcription of PAI-1 and the presence of a 5G allele may thereby lead to a lower risk of thrombotic events (16). The thrombotic risk of carrying a 4G allele of the *PAI*-1 gene is controversial. Segui et al (17) showed that the presence of a 4G allele significantly increased the risk of primary venous thrombosis, but only in patients with other thrombophilic defects. Patients with protein S deficiency combined with homozygosity for the 4G allele (4G/4G) had a higher risk of pulmonary embolism (PE) (18). On the other hand, Stegnar et al (19) found that the PAI-1 polymorphism (4G/4G, 4G/5G or 5G/5G) is not a major risk factor for VTE. To our knowledge, no study so far has investigated the relationship between risk of VTE recurrence and the PAI-1 polymorphism.

The aim of the present study was to investigate whether the PAI-1 polymorphism influences the risk of VTE recurrence and to determine whether the association differs between patients with and without the two most common thrombophilic defects, i.e., FVL and PTM mutations.

MATERIAL AND METHODS

Study population

Participants were selected from the Malmö Thrombophilia Study (MATS), a prospective population-based study conducted at Skåne University Hospital in Sweden from March 1998 to December 2008 on 1465 consecutive unselected VTE patients were collected. MATS is a well-characterised cohort which has been used previously to investigate the risk of VTE recurrence (20-22). It includes 70% of all patients diagnosed at the Emergency Department with VTE (DVT and/or PE). The remaining 30% were excluded due to unwillingness to participate, language problems, dementia or other illness. Included patients were required to leave blood samples, answer a questionnaire and were evaluated concerning risk factors for VTE. Risk factors such as malignancies diagnosed prior to or at the diagnosis of VTE, heredity (defined as a history of VTE in first-degree relatives), and use of contraceptive pills, hormonal therapy, pregnancy and postpartum period (defined as first 6 weeks after delivery) among women were assessed. The location of VTE at inclusion, VTE events prior to study inclusion, and all VTE recurrences during follow-up was recorded. Diagnosis of DVT and PE was objectively confirmed by phlebography, duplex ultrasonography, computed tomography (CT), lung scintigraphy or magnetic resonance imaging (MRI) (20). All VTE patients in MATS were treated in accordance with the standard protocol of Malmö University Hospital, i.e., low molecular weight heparin (LMH) or unfractionated heparin (UFH) during the initiation of oral anticoagulants (until INR value is $\geq$ 2.0 but at least 5 days). FVL mutation and the PTM as well as the levels of antithrombin, free protein S and protein C were analysed (see below). Antithrombin levels below 0.70 kIE/L, free protein S (women <0.5 kIE/L, men <0.65 kIE/L) and protein C (<0.7 kIE/L) were defined as deficiencies.

Patients for whom complete information on recurrence was missing (n=51), patients who had thrombotic events before inclusion (n=25) were excluded. Patients who had recurrence or

died during anticoagulant therapy (n=311) were excluded because the risk of recurrence mainly occurs after stopping the anticoagulant treatment. The aim of our study was to investigate the risk of recurrence and patients were only followed after stopping the anticoagulant treatment. Therefore, only patients who completed the scheduled period of anticoagulation without experiencing VTE recurrence were included in the study.

In total 1069 patients were included in the study, 127 (11.9 %) suffered from VTE recurrence. The primary end-point of the study was confirmed diagnosis of DVT and/or PE during the follow-up period. Patients were censored if they were free of DVT and PE throughout the follow-up period.

All participants provided written informed consent according to the Deceleration of Helsinki before their inclusion in the study and the study was approved by the ethics committee of Lund University (LU 237/2007).

Laboratory methods

DNA was isolated from whole blood using the QiAmp 96 DNA Blood Kit (Qiagen, Hilden, Germany). The PAI-1 polymorphism was genotyped using a method described previously (23). TaqMan® probes were obtained from Applied Biosystems (Carlsbad, CA, USA). PCR was run with 10 ng of genomic DNA on a CFX-384 Real-Time PCR Detection System (Bio-Rad) according to the manufacturer's recommendations. The probe and primer sequences were as follows: VIC probe, VIC-CTGACTCCCCCACGT; FAM probe, 6-FAM-ACTCCCCACGTGTC; forward primer, GCCAGACAAGGTTGTTGACACA; and reverse primer, GCCGCCTCCGATGATACA. DNA mutations for FVL and PTM G20210A were analysed by TaqMan allele discrimination assays (Applied Biosystems) as described previously (24). Protein C was analysed by a chromogenic method using the Berichrom® Protein C reagent (Siemens Healthcare Diagnostics, Upplands Väsby, Sweden) (25). Free Protein S was analysed by latex immunoassay with Coamatic® Protein S-Free (Chromogenix,

Haemochrom Diagnostica AB, Gothenburg, Sweden) (26). Antithrombin was analysed with a thrombin-based method using Berichrom Antithrombin (Siemens Healthcare Diagnostics) (27). All analyses were performed using a BCS-XP coagulation analyser (Siemens Healthcare Diagnostics). Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2).

Statistical analysis

To account for different follow up time, survival methods were used to analyse the data. Survival curves for time to recurrent VTE by FVL and PAI-1 genotypes are presented and the log-rank test was used to compare recurrence-free survival between groups. Interaction term analysis was performed to test for an interaction between PAI-1 and FVL and PAI-1 and PTM. Univariate and multivariate analyses, adjusting for age, sex, and acquired risk factors for VTE (malignancy, trauma, major surgery, immobilization, female hormone therapy and pregnancy, postpartum period) were performed using Cox proportional hazards models and hazard ratios were calculated for each group of patients. The fit of the proportional hazards model was checked visually by plotting the recurrence rates over time and by calculating Schoenfeld (partial) residuals. Schoenfeld residuals were used as the dependent variable and time as the independent variable in order to assess the proportional hazards assumption. Statistical analyses were performed using IBM SPSS 21 (IBM, Armonk, NY, USA).

RESULTS

Baseline characteristics are presented in Table 1. Of the patients with recurrent VTE, 47% had FVL, compared to 31% of patients without recurrence P<0.0001). PTM was the second most common thrombophilic defect after FVL and was present in 6% of the patients with recurrence compared to 4% in patients without recurrence; however, this difference was not statistically significant (p=0.40). There were no major differences in distribution in protein C, protein S, antithrombin, age, sex, BMI, type of thrombosis or malignancy between patients with non-recurrent VTE and those with VTE recurrence (Table 1).

Distribution of PAI-1 genotypes and VTE recurrence

The PAI-1 genotype distribution did not differ between recurrent and non-recurrent VTE in the whole study population (p=0.34) (Table 1). However, stratification of the data according to FVL status showed that of the patients with recurrent VTE, 53% had both presence of at least one 4G allele (i.e., 4G/4G or 4G/5G, which will be described as the 4G allele in the following text) and FVL, compared to 30% of patients without recurrence (p<0.0001) (Table 1). There was no difference in distribution of presence of two 5G alleles (5G/5G, which will be described as 5G alleles in the following text) and FVL between patients with non-recurrent VTE and those with VTE recurrence (p=0.99) (Table 1). Stratification of data according to PTM status showed no significant differences in distribution of PAI-1 genotypes in recurrent and non-recurrent VTE (data not shown).

PAI-1 polymorphism and risk of VTE recurrence

Univariate and multivariate Cox regression analyses adjusted for age, sex, and acquired risk factors of VTE were performed to calculate risk of VTE recurrence. FVL was associated with an increased risk of VTE recurrence [hazard ratio (HR) =1.8, 95% confidence interval (CI) =1.3-2.5, p=0.001]. This association was not confounded by age, sex, or acquired risk factors (adjusted HR=1.8, 95% CI=1.3-2.5, p=0.001) (Table 2). PTM was not significantly associated

with risk of VTE recurrence in univariate (HR=1.6, 95% CI=0.8-3.5, p=0.21) or multivariate (HR=1.7, 95% CI=0.8-3.6, p=0.19) analyses adjusted for age, sex and acquired risk factors of VTE.

For PAI-1 genotype there was no evidence of an association with risk of VTE recurrence in the whole population (HR=1.2, 95% CI=0.8-1.8, p=0.31) (Table 2). However, inclusion of an interaction term between PAI-1 genotype and FVL in the model showed a modifying effect of FVL on PAI-1 genotype (PAI-1 polymorphism*FVL: HR=0.4, 95% CI=0.2-0.9, p=0.04). In the presence of FVL, the 4G allele was associated with a higher risk of VTE recurrence (HR=1.8, 95% CI=1.1-2.9, p=0.029). Interestingly the 4G allele was an additional risk factor for VTE recurrence in patients with FVL in the interaction model: the recurrence risk was increased to 2.2 (95% CI=1.6-3.9, p<0.0001), compared to 1.8 (95% CI=1.3-2.5, p=0.001) when FVL was analysed without taking PAI-1 genotype into consideration (Table 2). We also performed a separate interaction analysis for PTM and PAI-1 polymorphism but did not find any significant interaction between PTM and PAI-1 (PAI-1 polymorphism*PTM: HR=1.7, 95% CI=0.4-8.0, p=0.51. No significant effect of PTM was found on association between PAI-1 and risk of recurrent VTE (HR=1.2, 95% CI=0.8-1.8, p=0.37). Furthermore, sensitivity analysis in the presence of PTM, protein S, protein C and antithrombin deficiencies show that the results were driven mainly by the presence of FVL and it was the strongest thrombophilic defect in the study (data not shown). Therefore further stratification analysis was performed only for PAI-1 and FVL.

HRs for each PAI-1 genotype in the presence and absence of FVL were calculated by Cox regression analysis and are shown in Table 2. Presence of the 4G allele plus FVL was associated with a significantly higher risk of VTE recurrence compared to presence of the 4G allele without FVL (reference group), independent of age, sex, and acquired risk factors of VTE (HR=2.3, 95% CI=1.5-3.3, p<0.0001) (Table 2). Furthermore, compared to the reference

group, having 5G alleles was associated with a higher risk of recurrence in the presence (HR=1.7, 95% CI=0.85-3.2, p=0.14) and absence (HR=1.8, 95% CI=1.1-2.9, p=0.029) of FVL (Table 2). The fit of the proportional hazards model was checked and we found no evidence for time dependence of the main predictors in the Cox regression models (data not shown). We also performed a sensitivity analysis by excluding the patients with high-risk thrombophilia (n=41) ((homozygous carriers or patients with natural anticoagulants deficiencies (antithrombin, protein C and protein S) or carriers of multiple abnormalities have been excluded)) and the results remained almost unaltered (data not shown).

A survival analysis was performed to determine whether the PAI-1 polymorphism influences recurrence-free survival. Data on PAI-1 genotype were stratified according to FVL status into four groups (A-D), as shown in Figure 1. In FVL free patients, presence of the 4G allele was associated with significantly longer recurrence-free survival compared to patients with 5G alleles (Figure 1, compare A with B). An opposite trend was observed in patients with FVL: presence of the 4G allele was associated with shorter recurrence-free survival compared to presence of 5G alleles (Figure 1, compare C with D).

Interestingly, recurrence-free survival in patients with 5G alleles did not differ according to the presence or absence of FVL (Figure 1, B and C). Patients with the 4G allele without FVL had the longest recurrence-free survival (Figure 1; $p<0.0001$, log-rank test).

PAI-1 polymorphism and risk of unprovoked VTE recurrence

We performed a sub-analysis on patients with unprovoked VTE recurrence. Patients with a recorded acquired risk factor for VTE, such as surgical intervention, immobilization or cast therapy within the last month, malignancies diagnosed prior to or at diagnosis of the first VTE event, use of contraceptives pills, female hormone therapy, current pregnancy and postpartum period (first 6 weeks after delivery), were excluded from the analysis. Cox regression analysis was performed on the remaining 632 patients, who had unprovoked VTE without any

acquired risk factors at the time of inclusion. 81 patients had recurrence during the follow-up period. Patients with the 4G allele plus FVL had a significantly higher risk of VTE recurrence (HR=2.5, 95% CI=1.5-4.2, $p<0.0001$) compared to patients with the 4G allele but no FVL (reference group), independent of age and sex (Supplementary Table 1). Finally, we calculated risk per 100 person-years (py) for VTE recurrence. Patients with the 4G allele but no FVL had a lower risk of VTE recurrence [2.0 per 100 py (95% CI=1.5-2.7)] compared to patients with the 4G allele plus FVL [4.3 per 100 py (95% CI=3.2-5.6)] and patients with 5G alleles without [3.6 per 100 py (95% CI=2.4-5.4)] and with FVL [3.3 per 100 py (95% CI=1.7-5.7)] (Table 3). Risk per 100 py for VTE recurrence was also calculated separately for patients who had first unprovoked VTE and results are presented in Table 3.

DISCUSSION

We have shown that the role of PAI-1 4G allele in VTE recurrence is FVL dependent and is a risk factor for VTE recurrence in patients with FVL. Moreover we show that 4G allele may have a protective role against recurrence in the absence of FVL. Our results indicate that combined analysis of both PAI-1 polymorphism and FVL may be required for better risk assessment of risk of VTE recurrence.

The PAI-1 polymorphism has been investigated in various CVDs, with controversial results (28, 29). However, to our knowledge no study so far has studied the PAI-1 polymorphism in VTE recurrence. In one previous study, PAI-1 4G/4G homozygotes had a markedly reduced risk of cerebrovascular mortality compared with PAI-1 5G/5G homozygotes in older stroke patients (30). In a second study, the PAI-1 4G/4G genotype was found to be more prevalent in a control group than in younger ischemic stroke patients (31). By contrast, others showed that the 4G allele was associated with an increased risk of stroke (32). Finally, a recent study on cerebral venous thrombosis (CVT) showed no association between PAI-1 polymorphism and risk of CVT (33). In agreement with that study, we were also unable to observe any association between PAI-1 polymorphism and risk of VTE recurrence, when FVL was not included in the analysis. We found a significant interaction between PAI-1 and FVL but not between PAI-1 and PTM, suggesting a modifying effect of FVL on PAI-1 polymorphism. In the presence of FVL, 4G allele was associated with a higher risk of VTE recurrence, whereas 5G allele was not affected by the presence of FVL. A case-control study on primary DVT also showed that the presence of the 4G allele significantly increased the risk of thrombosis in patients with thrombophilic defects (17). Furthermore, the 4G allele has been reported to be more prevalent in thrombosis patients carrying FVL compared to control subjects (34). In agreement with our results, two meta-analyses performed on 22 (35) and 34 (36) studies also showed that the 4G allele was associated with higher risk of venous thrombosis in patients

with other genetic thrombophilic defects. However, to the best of our knowledge, this is the first prospective study in which the role of PAI-1 polymorphism in the presence of common thrombophilic defects has been investigated in recurrent VTE. Our results indicate that the PAI-1 4G allele may have a dual role in VTE recurrence depending on the presence or absence of FVL.

It remains a challenge to predict the individual risk of recurrence in unprovoked VTE (without any known acquired risk factors). We have previously shown that lower levels of apolipoprotein M is a risk factor for VTE recurrence in first unprovoked VTE in men but not in women (21). VTE recurrence rates are reported to be higher in patients with inherited genetic defects (10, 24). However, the clinical importance of these inherited genetic defects in VTE recurrence risk is controversial (13, 37). Our results show that the risk of recurrence was even higher in patients with unprovoked VTE with both FVL and the 4G allele compared to all VTE patients with both FVL and the 4G allele. These results suggest that the presence of the 4G allele plus FVL significantly increases the risk of VTE recurrence as whole as well as in unprovoked VTE and may therefore be used to identify patients with recurrence in this group of high-risk patients.

The molecular mechanism underlying the role of the PAI-1 polymorphism in CVDs is not well understood and needs to be elucidated. The PAI-1 polymorphism is located in the promoter region of the *PAI*-1 gene and there is a consensus that it is responsible for increased transcription of PAI-1 in CVDs (23, 38, 39), including venous thrombosis (19, 40). However, its role in vascular tissue remodelling is still controversial. PAI-1 has been shown to both promote and prevent vascular remodelling processes, a phenomenon sometimes referred to as the “PAI-1 paradox” (41). It is possible that the role of PAI-1 depends on the vascular bed, type of lesion, experimental/clinical conditions and different molecules interacting with it. Decreased fibrinolytic capacity in coronary heart disease is associated with increased plasma

PAI-1 concentrations (42). However, the role of PAI-1 has been questioned because other factors that potentially explain the association between fibrinolysis and atherosclerotic disease, such as diabetes mellitus, hypertension, obesity and dyslipidaemia, are often present (43). Our results show that the association between PAI-1 polymorphism and risk of VTE recurrence is independent of acquired risk factors associated with VTE but dependent on FVL, a hereditary risk factor for VTE recurrence. We speculate, based on previous studies that high levels of PAI-1 associated with presence of the 4G allele may be required for remodelling of the vascular bed after thrombosis and may therefore protect against recurrence of VTE. Furthermore, the protective effect may be lost in the presence of additional hereditary risk factors such as FVL. However, this needs to be investigated in future studies.

VTE patients can be protected from recurrence by continued anticoagulant treatment. However, the VTE recurrence rate must be weighed against the risk of bleeding when deciding whether to keep patients on prolonged anticoagulant treatment. Patients with acquired risk factors for VTE are known to be at lower risk of recurrence compared to those with no acquired risk factors (unprovoked VTE) (44) and therefore may not require long-term anticoagulation. Patients with the highest risk of recurrence, such as those with metastases, require lifelong thromboprophylaxis. However, assessing the relative risks of VTE and bleeding in patients at intermediate risk of VTE recurrence for extended anticoagulation with vitamin K antagonists is a challenge. The increase in the risk of major bleeding in patients treated with vitamin K antagonists such as warfarin compared to controls is low in well-controlled patients (45). However, higher annual rates of major haemorrhage in patients treated with warfarin in routine clinical practice have been reported: 1.7% in a prospective cohort of 402 patients (46) and 3.4% in a retrospective study of 505 patients (47). Our results indicate predictive importance of the combined analysis of PAI-1 polymorphism and FVL in VTE recurrence. These results may form the basis for clinical decision making regarding

extended anticoagulation especially in patients with both the 4G allele and FVL as the risk of VTE recurrence seems to be higher in these patients compared to bleeding risk found in controlled settings as well as in clinical practice.

A potential limitation is the lack of genetic analyses on Protein S, protein C and antithrombin. However, as these thrombophilic defects are very rare, they are therefore unlikely to have affected the results of the study. Another limitation is the lack of information on PAI-1 levels therefore our findings should be interpreted with caution. Our study is the first to show a modifying effect of FVL on PAI-1 in VTE recurrence. Further prospective studies addressing the role of PAI-1 together with thrombophilic defects in recurrent VTE are indicated.

In conclusion, we suggest a dual role of 4G allele of the PAI-1 in VTE recurrence. PAI-1 does not seem to act as a risk factor alone. However, our results indicate a significant interaction between FVL and PAI-1 and risk of VTE recurrence. Our results may shed light on the controversies involving the fibrinolytic system in relation to VTE.

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DISCLOSURE

Authors declare no conflict of interest

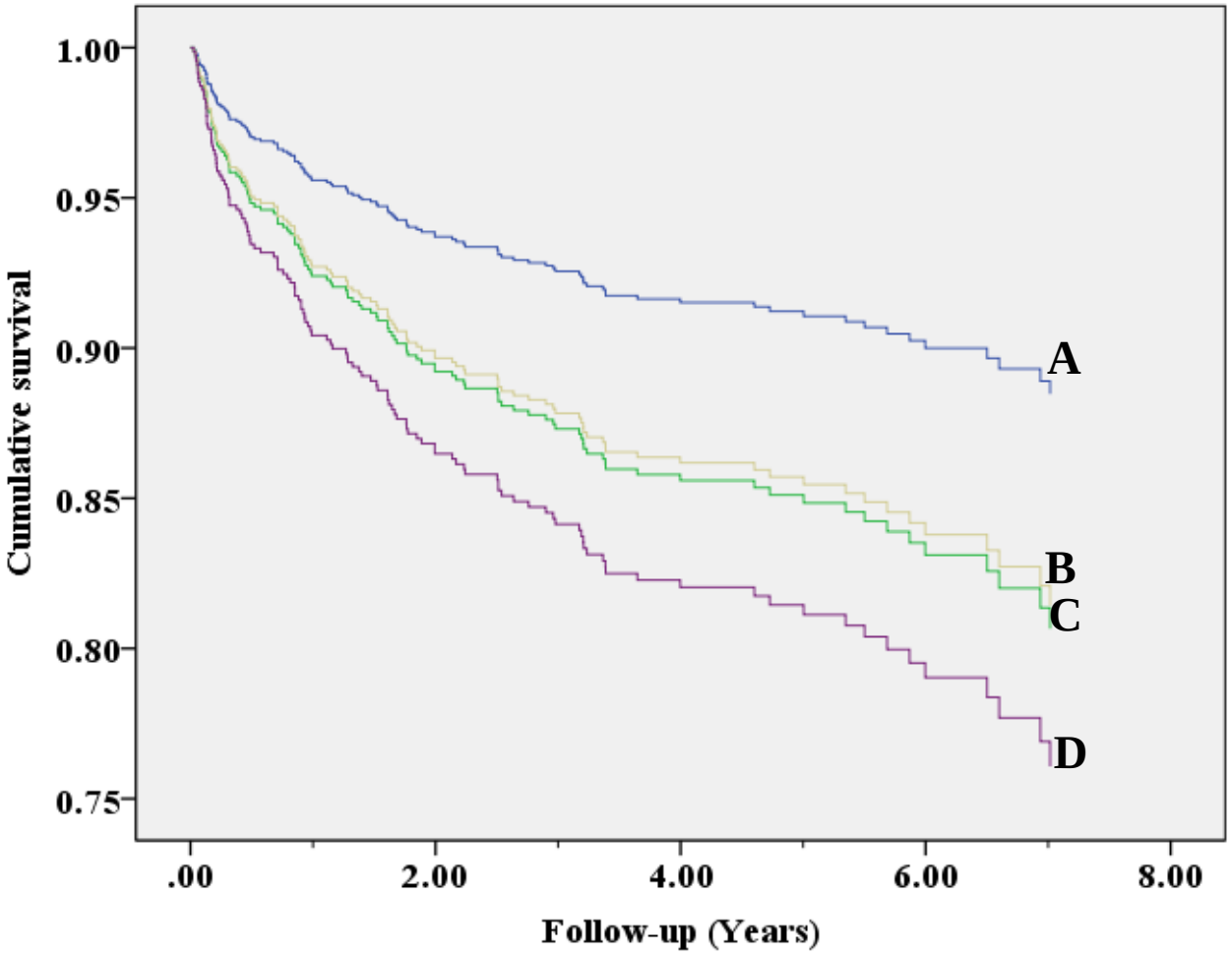
FIGURE LEGEND

Figure 1. Survival curves demonstrating recurrence-free survival for various combinations of the PAI-1 polymorphism and FVL in VTE patients. The table shows the combinations analysed and number of patients. $p<0.0001$ (log-rank difference in survival between all groups).

1. 'What is known on this topic'
    - Plasminogen activator inhibitor-1 (PAI-1) is one of the most important inhibitors of the plasminogen/plasmin system.
    - Levels of PAI-1 are influenced by the 4G allele of a [-675] 4G/5G polymorphism in PAI-1 promoter region.
    - However to our knowledge no study has so far investigated the relationship between PAI-1 polymorphism and recurrent VTE.

2. 'What this paper adds'.
    - The PAI-1 4G allele is an additional risk factor for VTE recurrence in patients with factor V Leiden (FVL) mutation and may have a protective role in patients without FVL mutation.
    - Our results shed light on the controversies involving the fibrinolytic system in relation to VTE recurrence.

Figure 1



	FVL mutation	4G/4G or 4G/5G	5G/5G	No. of patients
A	-	+	-	550
B	-	-	+	169
C	+	-	+	81
D	+	+	-	269

Table 1. Baseline characteristics of the study population and distribution of PAI-1 genotypes in non-recurrent and recurrent VTE patients.

Variables	Mean (±SD) or %			
	Non-recurrent VTE (n=942)	Recurrent VTE (n=127)	Total (n = 1,069)	¶P-value
Age at inclusion, (years), mean ± SD	62.4±17.4	61.5±15.5	62.3±17.2	0.32*
Sex				
Men (529)	49%	51%	49%	0.71
Women (540)	51%	49%	50%	
BMI, (kg/m$^{2)}$, mean ± SD	26.5±4.7	27.3±4.9	26.6±4.7	0.11*
FVL mutation				
Yes	31%	47%	33%	<0.0001
No	69%	53%	67%	
Prothrombin mutation				
Yes	4%	6%	04%	0.40
No	96%	94%	96%	
Protein C deficiency				
Yes	2%	00%	1.3%	0.20
No	98%	100%	98.7%	
Protein S deficiency				
Yes	1.3%	0.8%	1.2%	0.67
No	98.7%	99.2%	98.8%	
Antithrombin deficiency				
Yes	0.7%	0.8%	0.7%	0.96
No	99.3%	99.2%	99.3%	
Thrombosis type				
DVT	74%	73%	75%	0.43
PE	21%	20%	21%	
DVT+PE	5%	7%	6%	
Malignancy				
Yes	10%	7%	9%	0.40
No	90%	93%	91%	
PAI-1 genotype				
4G/4G or 4G/5G	77%	73%	77%	0.34
5G/5G	23%	27%	23%	
Stratified according to FVL status				
4G/4G or 4G/5G				
FVL mutation	30%	53%	33%	<0.0001
No FVL mutation	70%	47%	67%	
5G/5G				
FVL mutation	32%	32%	32%	0.99
No FVL mutation	68%	68%	68%	

FVL, Factor V Leiden; DVT, deep venous thrombosis; PE, pulmonary embolism; BMI, body mass index. P-value, Chi-square test until unless indicated. *Mann-Whitney *U* test, ¶comparing non-recurrent with recurrent VTE

Supplementary Table 1. Hazard ratios (HRs) with 95% confidence intervals (CIs) for risk of unprovoked recurrent VTE according to thrombophilia status and PAI-1 genotype

	Unadjusted		Adjusted*	
Group	HR (95%CI)	p value	HR (95%CI)	p value
No FVL mutation and 4G/5G or 4G/4G	1 (reference group)	-	-	-
No FVL mutation and 5G/5G	1.5 (0.7-3.1)	0.26	1.5 (0.8-3.1)	0.023
FVL mutation and 5G/5G	2.4 (1.1-4.9)	0.02	2.3 (1.1-4.8)	0.03
FVL mutation and 4G/5G or 4G/4G	2.6 (1.5-4.3)	<0.0001	2.5 (1.5-4.2)	<0.0001

*Adjusted for age and sex

Table 2. Hazard ratios (HRs) with 95% confidence intervals (CIs) for FVL mutation, prothrombin mutation and PAI-1 polymorphism in patients with VTE recurrence

Group	Unadjusted HR (95%CI)	p value	Adjusted* HR (95%CI)	p value*	Adjusted¶ HR (95%CI)	p value
FVL mutation						
No FVL mutation	1 (reference group)					
FVL mutation	1.8 (1.3-2.5)	0.001	1.8 (1.3-2.6)	0.001	1.8 (1.3-2.5)	0.001
Prothrombin mutation						
No Prothrombin mutation	1 (reference group)					
Prothrombin mutation	1.6 (0.8-3.5)	0.21	1.7 (0.8-3.6)	0.19	1.7 (0.8-3.6)	0.19
PAI-1 polymorphism						
Genotype 4G/5G or 4G/4G	1 (reference group)					
Genotype 5G/5G	1.2 (0.8-1.8)	0.31	1.2 (0.8-1.8)	0.31	1.2 (0.8-1.8)	0.31
FVL mutation and PAI-1 polymorphism						
No FVL mutation and 4G/5G or 4G/4G	1 (reference group)					
No FVL mutation and 5G/5G	1.8 (1.1-2.9)	0.029	1.8 (1.1-2.9)	0.029	1.8 (1.1-2.9)	0.029
FVL mutation and 5G/5G	1.7 (0.86-3.2)	0.12	1.7 (0.87-3.3)	0.12	1.7 (0.85-3.2)	0.14
FVL mutation and 4G/5G or 4G/4G	2.2 (1.5-3.4)	<0.0001	2.3 (1.5-3.4)	<0.0001	2.3 (1.5-3.3)	<0.0001

*Adjusted for age and sex. ¶Adjusted for age, sex and acquired risk factors associated with VTE (malignancy, trauma, major surgery, immobilization, female hormone therapy and pregnancy)

FVL, Factor V Leiden.

Table 3. Risk of VTE recurrence per 100 person-years for different PAI-1 genotypes and in all VTE and unprovoked VTE patients according to FVL mutation status

	VTE recurrence (n)	Person-years	Recurrence rate (95% CI)
All VTE	**127**	**4272**	**2.6 (1.9-3.4)**
No FVL mutation and 4G/5G or 4G/4G	44	2155	2.0 (1.5-2.7)
No FVL mutation and 5G/5G	23	633	3.6 (2.4-5.4)
FVL mutation and 5G/5G	11	333	3.3 (1.7-5.7)
FVL mutation and 4G/5G or 4G/4G	49	1151	4.3 (3.2-5.6)
Sub-analysis of unprovoked VTE	**81**	**2468**	**3.3 (2.6-4.1)**
No FVL mutation and 4G/5G or 4G/4G	25	1203	2.1 (1.4-3.0)
No FVL mutation and 5G/5G	11	354	3.2 (1.7-5.5)
FVL mutation and 5G/5G	10	210	4.8 (2.4-8.5)
FVL mutation and 4G/5G or 4G/4G	35	700	5.0 (3.5-6.9)

*Unprovoked VTE: Cases without any acquired risk factor for VTE. FVL, Factor V Leiden.