

The 4G/5G Sequence Polymorphism in the Promoter of Plasminogen Activator Inhibitor-1 (PAI-1) Gene: Relationship to Plasma PAI-1 Level in Venous Thromboembolism

Mojca Stegnar^{1,2}, Pavel Uhrin², Polona Peternel¹, Alenka Mavri¹, Barbara Salobir-Pajnič¹, Janez Stare³, Bernd R. Binder²

From the ¹University Medical Centre, Department of Angiology, Ljubljana, Slovenia, ²University of Vienna, Department of Vascular Biology and Thrombosis Research, Vienna, Austria; and ³University of Ljubljana, Medical Faculty, Institute of Biomedical Statistics, Ljubljana, Slovenia

Summary

Impaired fibrinolysis due to increased plasminogen activator inhibitor-1 (PAI-1) is observed in up to 40% of patients with venous thromboembolism and might be causally related to the disease. There is evidence that genetic variations in the promoter of the PAI-1 gene and metabolic factors contribute to increased plasma PAI-1 levels.

A single nucleotide insertion/deletion (4G/5G) polymorphism in the promoter region of the PAI-1 gene and metabolic factors were studied in 158 unrelated patients below the age of 61 years (43 ± 11 years, mean \pm standard deviation) with history of objectively confirmed venous thromboembolism and in 145 apparently healthy controls.

Patients had on average two times higher PAI activity (11.9 vs. 6.1 IU/ml) and by 40% higher PAI-1 antigen (14.8 vs. 10.7 ng/ml) than healthy controls, and also higher body mass index, lipid levels, fasting glucose and insulin. Patients differed significantly from healthy controls neither in the frequency of the 4G and 5G alleles (0.57/0.43 in patients and 0.52/0.48 in controls) nor in the distribution of the 4G/5G genotypes. Possession of the 4G/4G or the 4G/5G genotype did not increase relative risk for venous thromboembolic disease and the distribution of the 4G/5G genotypes was neither associated with recurrent nor with spontaneous disease. In patients association between the 4G/5G genotypes and PAI activity (adjusted for body mass index, triglyceride and glucose level) was observed, with the highest PAI activity values in the 4G/4G genotype (14.6 IU/ml), intermediate in the 4G/5G genotype (13.3 IU/ml) and the lowest in the 5G/5G genotype (5.2 IU/ml, all values means). Association between PAI activity and triglyceride level was the strongest in the 4G/4G genotype (correlation coefficient $r = 0.47$, $p < 0.01$) and the weakest in the 5G/5G genotype ($r = -0.04$, not significant).

In conclusion, the present case-control study shows an association between the 4G/5G polymorphism in the promoter of the PAI-1 gene and plasma PAI-1 levels in patients with venous thromboembolism. Similar distribution of the 4G/5G genotypes in patients and healthy controls suggests that this genetic variation by itself is not a major risk factor for venous thromboembolism.

Introduction

Plasminogen activator inhibitor-1 (PAI-1) is a glycoprotein with a molecular weight of approximately 50 kD and is a member of the serine protease inhibitor super-family. PAI-1 is a rapid and specific inhibitor of both tissue-type plasminogen activator and urokinase and is considered as the primary regulator of plasminogen activation *in vivo* (for review see 1). Abnormally high concentration of PAI-1 in blood observed in atherothrombotic and other diseases seem to result from changes in the rate of PAI-1 gene expression in tissues rather than from release of stored PAI-1 in the cells (2). The diversity of the molecules that modulate PAI-1 biosynthesis implies that the PAI-1 gene must be unusually complex, containing DNA sequences that are, either directly or indirectly, responsive to these molecules and thus contribute to PAI-1 gene expression.

The 4G/5G polymorphism in the promoter of the PAI-1 gene seems to be one of the DNA sequence variation, which has functional importance in regulating expression of the PAI-1 gene. The 4G/5G polymorphism is located -675 base pairs upstream from the start of transcription site of the PAI-1 gene and is characterized by a single guanosine deletion/insertion, resulting in two alleles containing either 4 or 5 guanosines (G) in a row (3). *In vitro* assays of PAI-1 gene promoter activity demonstrated significantly higher activity of the 4G than the 5G allele under conditions of cytokine stimulation, such as are found in the acute phase (3). The 4G/5G polymorphism might influence also basal levels of PAI-1 gene transcription since sequence-specific binding to the 4G and 5G alleles of two factors present in a nuclear cell extracts was observed in unstimulated cells (4).

The 4G/5G polymorphism has been shown to be associated with plasma PAI-1 levels in several groups of patients: after myocardial infarction (3-5), in non-insulin-dependent diabetes mellitus (6) and recently also in cerebrovascular disease (7). The highest PAI-1 levels were observed in subjects with the 4G/4G genotypes and the lowest in the 5G/5G genotypes. Furthermore, in young patients after myocardial infarction the prevalence of the unfavorable 4G allele was higher than in healthy controls (4). Association between the 4G/4G genotype and coronary artery disease was established also in patients with non-insulin-dependent diabetes mellitus (8), suggesting pathogenetic role of this DNA sequence variation in vascular disease.

Recently, relationship between expression of PAI-1 gene and the 4G/5G polymorphism was described by us also in patients with deep vein thrombosis (9). However, the results were obtained in a relatively small group of subjects. Therefore, in the present study over 300 subjects with and without a history of venous thromboembolism were in-

Correspondence to: Dr. M. Stegnar, University Medical Centre, Department of Angiology, Riharjeva 24, 1000 Ljubljana, Slovenia - Tel.: +386 61 333 500; FAX Number: +386 61 333 155; E-mail: mojca.stegnar@trnovo.kclj.si

investigated. The 4G/5G polymorphism was determined with an allele specific polymerase chain reaction, which is considered reliable and useful in screening for this polymorphism in larger population studies (10).

Subjects and Methods

Subjects

Subjects participated in the study after they had given their full informed consent. All originated from Central Europe and were not related to each other. None of the women included in the study was taking oral contraceptives or hormonal replacement therapy. The study was approved by the State Ethical Committee.

Subjects with history of venous thromboembolic disease were recruited from consecutive patients treated at the Department of Angiology in Ljubljana, in the period from January 1992 to April 1996. Hundred and fifty-eight patients 19 to 60 years old (62 women and 96 men) were included in the study at least three months (on average 16 ± 9 months, mean \pm standard deviation) after objectively confirmed acute deep vein thrombosis and/or pulmonary embolism. Most patients (92%) had history of leg deep vein thrombosis. History of arm deep vein thrombosis was present in eight patients, pulmonary embolism without signs of deep vein thrombosis was observed in three patients and thrombosis of the vena cava in one patient. Eighty-three percent of patients had a history of a single event. In 44% of patients no common factors predisposing for venous thromboembolism could be established. In the rest venous thromboembolism was secondary to predisposing factors such as surgery (N = 29), trauma (N = 21), immobilization (N = 15) and bed-rest (N = 14). In women the most frequent predisposing factors were oral contraceptives (N = 21), puerperium (N = 6), pregnancy (N = 4) and hormonal replacement therapy (N = 4). At the time of blood sampling 30% of patients were receiving oral anticoagulant treatment.

Hundred and forty-five apparently healthy subjects 19 to 60 years of age (82 women and 63 men) were asked to participate as controls. They were mainly hospital staff, medical students and their acquaintances. None had history of thromboembolic disease.

Blood Sampling and DNA Isolation

Blood samples were obtained between 7 and 9 a.m after overnight fast and 20 min rest. Blood was sampled from an antecubital vein, in most cases without application of a tourniquet.

For DNA analysis blood was sampled into K₃-EDTA vacuum tubes, thoroughly mixed with the anticoagulant by inverting the tube several times and stored at -70° C. DNA was isolated using commercially available genomic DNA purification kit (Wizard™, Promega, Madison, WI, USA).

For measurement of PAI-1 blood flowed directly into precooled siliconized glass vacuum tubes with 0.13 mol/l trisodium citrate (1 volume of citrate to 9 volumes of blood). Tubes were placed in ice water and centrifuged within one hour for 30 min at 2000 g and 4° C. Platelet poor plasma was transferred to small plastic vials, frozen in liquid nitrogen and stored at -70° C until analyzed. For biochemical assays blood was collected into vacuum tubes without an anticoagulant; after one hour serum was harvested and analyzed the same day.

Allele Specific Polymerase Chain Reaction for Detection of the 4G/5G Polymorphism

Allele specific polymerase chain reaction was performed according to Falk and coworkers (10) with some modifications. For each genomic DNA two polymerase chain reactions were performed with two 17-mer allele specific primers: 5'-GTC TGG ACA CGT GGG GG-3' (for the 5G allele) and 5'-GTC TGG ACA CGT GGG GA-3' (for the 4G allele) in combination with a downstream primer 5'-TGC AGC CAG CCA CGT GAT TGT CTA G-3'. In the 5G/5G genotype polymerase chain reactions resulted in amplification of 140 base pairs DNA fragment with 5G specific primer only, in the 4G/4G genotype

only 139 base pairs DNA fragment was amplified with the 4G specific primer, and in heterozygotes both polymerase chain reactions resulted in amplification of the 140 and 139 base pairs DNA fragments. As a positive control for the polymerase chain reaction a 256 base pairs DNA fragment was generated using a fourth primer 5'-AAG CTT TTA CCA TGG TAA CCC CTG GT-3' located upstream of the polymorphic region in combination with the downstream primer. Each DNA amplification was performed in a total volume of 25 μ L using 150-200 ng of genomic DNA. The reaction mixture contained 50 mM Tris-HCl buffer, pH 9.0, 1.5 mM MgCl₂, 0.2 mM of each deoxyribonucleoside triphosphate, 0.6 μ M of each primer, except for the upstream control primer which were 0.12 μ M and 1.0 U of DNA polymerase (Dynazyme, Finnzymes Oy, Finland). The mixture was subjected to 35 step cycles of 94° C (35 s), 65° C (45 s), 72° C (75 s). Amplified DNA fragments were analyzed by 2% agarose gel electrophoresis. Genotyping was performed by two independent investigators and results agreed by 97%. In case of disagreement the analysis was repeated.

PAI-1 and Biochemical Assays

PAI-1 antigen was determined by an enzyme-linked immunosorbent assay and PAI activity by a chromogenic substrate assay utilizing commercially available kits (Imulyse™ PAI-1, Biopool, Sweden and Spectrolyse®/fibrin, Biopool, Sweden, respectively). Glucose, triglyceride, total-cholesterol and high-density lipoprotein (HDL)-cholesterol were determined by biochemical analyzer (Ektachem 250, Kodak, Rochester, NY, USA). Low-density lipoprotein (LDL)-cholesterol was calculated from total-cholesterol, HDL-cholesterol and triglyceride levels (11). Insulin was determined with radioimmunoassay kit (Sorin, Biomedica, Italy).

Statistical Methods

The skewness of PAI-1 antigen, triglyceride, glucose and insulin distribution was normalized by log-transformation, while PAI activity was square-rooted, in order to permit use of parametric methods. In the tables the transformed values are presented as anti-log means with 95% confidence intervals (PAI-1 antigen, triglyceride) or squared means with 95% confidence intervals (PAI activity). The t-test and analysis of variance were used to compare values between the two groups of subjects and between different genotypes. Pearson's correlation coefficient was used to evaluate associations between PAI-1 and other variables. To compare PAI-1 antigen and PAI activity levels in different genotypes, these values were adjusted for body mass index, triglyceride and glucose levels with the use of analysis of covariance.

The χ^2 -test was used to analyze deviation of the genotype distribution from the distribution that would be expected if the alleles were in the Hardy-Weinberg equilibrium and to test differences in frequencies of genotypes between patients and controls and subgroups of patients.

Calculations were performed using the Statistica programme (StatSoft™, USA). P values of <0.05 were taken as statistically significant.

Results

Subjects with a history of venous thromboembolic disease were on average 3 years older, had higher body mass index, higher lipid and glucose levels and higher PAI-1 antigen and PAI activity than apparently healthy controls (Table 1).

Distribution of the 4G/5G genotypes and frequencies of the 4G and the 5G alleles are shown in Table 2. Although in the patient group frequency of the 4G allele was higher than in controls and more individuals were homozygous for the 4G allele and less individuals homozygous for the 5G allele, the differences between groups did not reach the level of statistical significance. If patients were subgrouped according to history of single versus recurrent event or spontaneous versus secondary venous thromboembolism no significant distribution of genotypes within subgroups or compared to healthy controls was observed.

The observed frequencies of the genotypes did not differ significantly from the frequencies that would be expected if alleles were in the Hardy-Weinberg equilibrium neither in patients (χ^2 -test = 2.94, degree of freedom = 1) nor in controls (χ^2 -test = 0.37, degree of freedom = 1).

Odds ratio as a measure of relative risk of venous thromboembolism was for subjects with the 4G/5G genotype 1.50 (95% confidence interval: 0.81-2.77) and for subjects with the 4G/4G genotype 1.56 (95% confidence interval: 0.79-3.11) compared to the subjects with the 5G/5G genotype ($p = 0.380$).

In all subjects investigated PAI activity and PAI-1 antigen significantly correlated with age ($r = 0.23$ and $r = 0.34$, respectively), body mass index ($r = 0.46$ and $r = 0.54$, respectively), total cholesterol ($r = 0.33$ and $r = 0.26$, respectively), triglyceride ($r = 0.46$ and $r = 0.51$, respectively), glucose ($r = 0.32$ and $r = 0.35$, respectively) and insulin ($r = 0.32$ and $r = 0.32$, respectively) levels. In a multiple regression analysis model body mass index, triglycerides and glucose explained 31% of variability in PAI activity and 41% of variability in PAI-1 antigen. The results of regression analysis were not altered significantly if other variables were included in the model. Therefore, PAI activity and PAI-1 antigen levels were adjusted for body mass index, triglyceride and glucose level.

In patients analysis of covariance revealed significant interactions between the 4G/5G polymorphism and PAI activity ($F = 5.35$, $p = 0.006$). The highest level of PAI activity was observed in the 4G/4G genotype, intermediate level in the 4G/5G genotype and the lowest level in subjects with the 5G/5G genotype. PAI-1 antigen showed similar association with the genotype as PAI activity, although interactions were not significant ($F = 1.95$, $p = 0.147$). In the control group significant interactions were observed neither between the genotype and PAI activity ($F = 2.02$, $p = 0.136$) nor between the genotype and PAI-1 antigen ($F = 2.00$, $p = 0.139$) (Table 3).

Interactions between triglyceride levels and PAI-1 in relation to the 4G/5G genotype were analyzed in each group of subjects using linear regression models. The strongest association between PAI activity and triglyceride level was observed in patients with the 4G/4G genotype and the weakest in patients with the 5G/5G genotype. (Table 4). Regression slopes for these associations significantly differed between groups of patients with different genotypes ($p = 0.015$), but not in healthy controls.

Discussion

Defective fibrinolysis due to increased PAI-1 is observed in up to 40% of patients with venous thromboembolism (12-17). Increased PAI-1 correlates also with the development of future thrombotic events (18) and might therefore be causally related to the disease. The mechanism of increased PAI-1 is not elucidated, but the present study showed that increased PAI-1 in venous thromboembolism should be at least partially attributed to the 4G/5G polymorphism in the promoter of the PAI-1 gene, since relationship between the 4G/5G genotype and expression of PAI-1 levels in plasma was observed in patients with this disease. Increased levels of PAI-1 were determined in those patients who possessed the 4G allele. After adjustment for body mass index and metabolic variables (triglycerides and glucose level) patients with the 4G allele had on average twice higher level of PAI activity and about 50% higher PAI-1 antigen than healthy controls with the same genotype.

In contrast to the consistent association between the 4G/5G genotype and plasma PAI-1 levels in patients with vascular disease and diabetes, this association seems to be less pronounced or absent in healthy

Table 1 Anthropometric, metabolic and fibrinolytic data in patients with venous thromboembolism and in healthy controls. Means with standard deviations or 95% confidence intervals in parenthesis are shown

	Units	Patients N=158	Controls N=145	P
Age	years	43 (11)	40 (11)	= 0.003
BMI ¹	kg/m ²	26.9 (4.2)	25.1 (4.0)	= 0.001
Total Cholesterol	mmol/L	6.1 (1.2)	5.2 (1.2)	< 0.001
HDL-Cholesterol	mmol/L	1.2 (0.4)	1.3 (0.4)	= 0.494
LDL-Cholesterol	mmol/L	4.0 (1.0)	3.3 (1.1)	< 0.001
Triglycerides	mmol/L	1.6 (1.5-1.7)	1.3 (1.2-1.4)	< 0.001
Glucose	mmol/L	5.3 (5.1-5.4)	5.0 (4.9-5.1)	= 0.006
Insulin	mU/L	7.7 (7.0-8.4)	6.3 (5.5-7.2)	= 0.013
PAI activity	U/mL	11.9 (9.9-14.1)	6.1 (4.7- 7.7)	= 0.001
PAI-1 antigen	ng/mL	14.8 (12.9-17.1)	10.7 (9.1-12.6)	= 0.003

¹ BMI: Body mass index = (body weight in kg)/(body height in m)²

Table 2 Distribution of the 4G/5G genotypes and frequency of the 4G and 5G alleles in patients with venous thromboembolism and healthy controls. Allele frequencies were obtained by gene counting

	Patients N=158	Controls N=145	χ^2 -test
N (%) with 4G/4G genotype	46 (29.1)	38 (26.2)	1.97
N (%) with 4G/5G genotype	88 (55.7)	76 (52.4)	$p=0.372$
N (%) with 5G/5G genotype	24 (15.2)	31 (21.4)	
Frequency of 4G allele	0.57	0.52	1.26
Frequency of 5G allele	0.43	0.48	$p=0.261$

Table 3 PAI activity and PAI-1 antigen levels in patients with thromboembolism and in healthy controls with different 4G/5G genotypes, adjusted for body mass index, triglyceride and glucose levels (means with 95% confidence intervals)

Genotype	PAI activity IU/mL		PAI-1 antigen ng/mL	
	Patients	Controls	Patients	Controls
4G/4G	14.6 (10.5-19.1)	7.1 (4.0-11.1)	16.5 (12.3-22.0)	10.8 (7.8-15.0)
4G/5G	13.3 (10.3-16.7)	5.3 (3.5- 7.4)	15.7 (12.4-19.7)	10.0 (7.9-12.4)
5G/5G	5.2 (2.1- 9.6)	7.1 (4.2-10.8)	10.7 (7.6-14.7)	12.4 (8.3-18.2)
All	12.3 (10.1-14.7)	5.2 (4.7- 7.7)	15.3 (12.7-17.6)	10.6 (8.9-12.6)

Table 4 Correlation coefficients (r) and slopes of the linear regression line (B) and for the associations between PAI activity and triglyceride levels in patients and in healthy controls with three different 4G/5G genotypes. In patients but not in controls, slopes of the regression lines significantly differed ($p = 0.015$)

	4G/4G		4G/5G		5G/5G	
	r	B	r	B	r	B
Patients	0.47**	1.58	0.42**	1.46	-0.04	0.33
Controls	0.47**	1.57	0.43**	1.52	0.52**	1.60

* $p < 0.05$, ** $p < 0.01$

subjects (3, 19, 20) as observed also in the present study. It was recently reported that in healthy subjects genotype contributes only 1.2% to the total variance in the PAI-1 level (7). The discrepancy between patients and healthy subjects probably indicates that patients, although studied several months after the acute disease, are likely to suffer some low-grade-acute-phase response with production of cytokines, which differentially affect PAI-1 promoter, thus exaggerating differences between the two alleles and magnifying the effect of the genotype.

Increased plasma levels of PAI-1 are related to several anthropometric and metabolic factors of which body constitution, plasma levels of triglycerides and insulin seem to be the most important (21-23). Significant associations between PAI-1 and body mass index, total cholesterol, triglycerides, glucose and insulin were observed also in the present study. However, in a multiple regression analysis model triglycerides and glucose, but not insulin, explained the largest part of the PAI-1 variability. It should be emphasized that most of the insulin measurements (98%) were in the normal range while elevated triglyceride levels (>2.3 mmol/l) were observed in over 20% of patients. It has been observed that the influence of triglyceride on PAI-1 seems to be genotype dependent (6, 24). Genotype dependent association between triglyceride level and PAI-1 was established also in the present study in which the steepest regression slope was calculated for patients with the 4G/4G genotype. In healthy controls the genotype did not affect this association (Table 4). These results might suggest that such gene-environmental interactions have different levels of importance in different subject groups and might be more important in those groups of patients that have increased levels of triglycerides. Triglyceride levels or its composition may alter the level of protein(s) that bind to the polymorphic site in the PAI-1 promoter and hence affect PAI-1 production as suggested by Panahloo and coworkers (6).

A difference in genotype distribution between subjects with and without venous thromboembolism would support a causal role for PAI-1 in the pathogenesis of this disorder. However, the present study did not support this possible cause-effect relationship, since distribution of the 4G/5G genotypes and prevalence of the unfavourable 4G allele in patients with venous thromboembolism was not different from the distribution and prevalence observed in healthy controls. Different genotypes were also not associated with the severity of the disease, since the distribution was different neither between patients with single or recurrent DVT nor between patients with spontaneous or secondary DVT. Possession of at least one 4G allele did not increase significantly relative risk for venous thromboembolic disease. These results are in agreement with the results of Ridker and coworkers, who recently found no association between the 4G/5G polymorphism and development of venous thromboembolism in middle-aged men participating in the Physicians' Health Study (25).

It was previously observed that the frequency of apparently healthy subjects homozygous for the 4G allele is significantly higher in younger groups as opposed to older groups of subjects (10). No such age-dependent distribution of the 4G/5G genotypes was observed in the present study. In this study genotype distribution was also not gender-dependent (data not shown), supporting published data (10).

It can be concluded that possession of a 4G allele may contribute to defective fibrinolytic activity due to elevated PAI-1 in patients and thus possibly to an increased risk for venous thromboembolism. Lack of association between the 4G/5G polymorphism and risk of venous thromboembolism suggests no major pathogenetic role of this polymorphism by itself. However, the results of the study imply that this genetic variation might be important in conjunction with some other factor(s) such as elevated triglyceride levels.

Acknowledgements

The study was conducted while M. Stegnar was the recipient of a Slovenian Science Foundation Fellowship. Technical assistance of Mrs. M. Tehovnik and Mr. M. Zeyda is gratefully acknowledged. The study was supported by the Slovenian Ministry of Science and Technology (grant No. J3-7822), Austrian Science Foundation and Austrian National Bank (grant No. 5566).

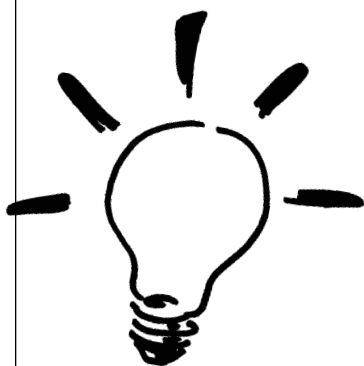
References

1. Wiman B. Plasminogen activator inhibitor 1 (PAI-1) in plasma: Its role in thrombotic disease. *Thromb Haemost* 1995; 74: 71-6.
2. Loskutoff DJ. Regulation of PAI-1 gene expression. *Fibrinolysis* 1991; 5: 197-206.
3. Dawson SJ, Wiman B, Hamsten A, Green F, Humphries S, Henney AM. The two allele sequences of a common polymorphism in the promoter of the plasminogen activator inhibitor-1 (PAI-1) gene respond differently to interleukin-1 in HepG2 cells. *J Biol Chem* 1993; 268: 10739-45.
4. Eriksson P, Kallin B, Van 't Hooft FM, Bavenholm P, Hamsten A. Allele-specific increase in basal transcription of the plasminogen-activator inhibitor 1 gene is associated with myocardial infarction. *Proc Natl Acad Sci USA* 1995; 92: 1851-5.
5. Ye S, Green FR, Scarabin PY, Nicaud V, Bara L, Dawson SJ, Humphries SE, Evans A, Luc G, Cambou JP, Arveiler D, Henney AM, Cambien F. The 4G/5G genetic polymorphism in the promoter of the plasminogen activator inhibitor-1 (PAI-1) gene is associated with differences in plasma PAI-1 but not with risk of myocardial infarction in the ECTIM study. *Thromb Haemost* 1995; 74: 837-41.
6. Panahloo A, Mohamed-Ali V, Lane A, Green F, Humphries SE, Yudkin JS. Determinants of plasminogen activator inhibitor 1 activity in treated NIDDM and its relation to a polymorphism in the plasminogen activator inhibitor 1 gene. *Diabetes* 1995; 44: 37-42.
7. Catto AJ, Carter AM, Strickland M, Bamford JM, Davies JA, Grant PJ. Plasminogen activator inhibitor-1 (PAI-1) 4G/5G promoter polymorphism and levels in subjects with cerebrovascular disease. *Thromb Haemost* 1997; 77: 730-4.
8. Mansfield MW, Stickland MH, Grant PJ. Plasminogen activator inhibitor-1 (PAI-1) promoter polymorphism and coronary artery disease in non-insulin-dependent diabetes. *Thromb Haemost* 1995; 74: 1032-4.
9. Grubič N, Stegnar M, Peternel P, Kaider A, Binder BR. A novel G/A and the 4G/5G polymorphism within the promoter of the plasminogen activator inhibitor-1 gene in patients with deep vein thrombosis. *Thromb Res* 1996; 84: 431-43.
10. Falk G, Almqvist A, Nordenhem A, Svensson H, Wiman B. Allele specific PCR for detection of a sequence polymorphism in the promoter region of the plasminogen activator inhibitor-1 (PAI-1) gene. *Fibrinolysis* 1995; 9: 170-4.
11. Friedewald WT, Levy RI, Fredrickson DS. Estimation of plasma low density lipoprotein-cholesterol concentration without use of the preparation ultracentrifuge. *Clin Chem* 1972; 18: 499-502.
12. Isacson S, Nilsson IM. Defective fibrinolysis in blood and vein walls in recurrent „idiopathic“ venous thrombosis. *Acta Chir Scand* 1972; 138: 313-9.
13. Nilsson IM, Ljungner H, Tengborn L. Two different mechanisms in patients with venous thrombosis and defective fibrinolysis: low concentration of plasminogen activator or increased concentration of plasminogen activator inhibitor. *Br Med J* 1985; 290: 1453-6.
14. Jorgensen M, Bonnevie-Nielsen V. Increased concentration of the fast-acting plasminogen activator inhibitor in plasma associated with familial venous thrombosis. *Br J Haematol* 1987; 65: 175-80.
15. Juhan-Vague I, Valadier J, Alessi MC, Aillaud MF, Ansaldo J, Philip-Joet C, Holvoet P, Serradimigni A, Collen D. Deficient t-PA release and elevated PA inhibitor levels in patients with spontaneous or recurrent deep venous thrombosis. *Thromb Haemost* 1987; 57: 67-72.

16. Malm J, Laurell M, Nilsson IM, Dahlbäck B. Thromboembolic disease – critical evaluation of laboratory investigation. *Thromb Haemost* 1992; 68: 7-13.
17. Gram J, Sidelmann J, Jespersen J. Does low protein concentration of tissue-type plasminogen activator predict a low risk of spontaneous deep vein thrombosis? *Thromb Haemost* 1995; 74: 718-21.
18. Schulman S, Wiman B and the Duration of Anticoagulation (DURAC) Trial Study Group. The significance of hypofibrinolysis for the risk of recurrence of venous thromboembolism. *Thromb Haemost* 1996; 75: 607-11.
19. Mansfield MW, Strickland MH, Grant PJ. PAI-1 concentrations in the first-degree relatives of patients with non-insulin-dependent diabetes: metabolic and genetic associations. *Thromb Haemost* 1997; 77: 357-61.
20. Henry M, Chomiki N, Scarabin PY, Alessi MC, Peiretti F, Arveiller D, Ferrieres J, Evans A, Amouyel P, Poirier O, Cambien F, Juhan-Vague I. Five frequent polymorphisms of the PAI-1 gene. Lack of association between genotypes, PAI activity, and triglyceride levels in a healthy population. *Arterioscler Thromb Vasc Biol* 1997; 17: 851-8.
21. Vague P, Juhan-Vague I, Aillaud MF, Badier C, Viard R, Alessi MC, Collen D. Correlation between blood fibrinolytic activity, plasminogen activator inhibitor level, plasma insulin level, and relative body weight in normal and obese subjects. *Metabolism* 1986; 35: 250-3.
22. Juhan-Vague I, Vague P, Alessi MC, Badier C, Valadier J, Aillaud MF, Atlan C. Relationships between plasma insulin, triglyceride, body mass index, and plasminogen activator inhibitor 1. *Diabete Metab* 1987; 13: 331-6.
23. Juhan-Vague I, Thompson SG, Jespersen J, on the behalf of the ECAT Angina Pectoris Study Group. Involvement of the hemostatic system in the insulin resistance syndrome, a study of 1500 patients with angina pectoris. *Arterioscler Throm* 1993; 13: 1865-73.
24. Mansfield MW, Strickland MH, Grant PJ. Environmental and genetic factors in relation to elevated circulating levels of plasminogen activator inhibitor-1 in caucasian patients with non-insulin-dependent diabetes mellitus. *Thromb Haemost* 1995; 74: 842-7.
25. Ridker PM, Hennekens CH, Lindpaintner K, Stampfer MJ, Miletich JP. Arterial and venous thrombosis is not associated with the 4G/5G polymorphism in the promoter of the plasminogen activator inhibitor gene in a large cohort of US men. *Circulation* 1997; 95: 59-62.

Received October 30, 1997 Accepted after revision January 16, 1998

THINK REPRINTS



- Articles appearing in *Thrombosis and Haemostasis* are the respected source of information for state-of-the-art information. Think of the many venues where reprints will reinforce your message:

THINK REPRINTS

- when you want to reach the potential product users

THINK REPRINTS

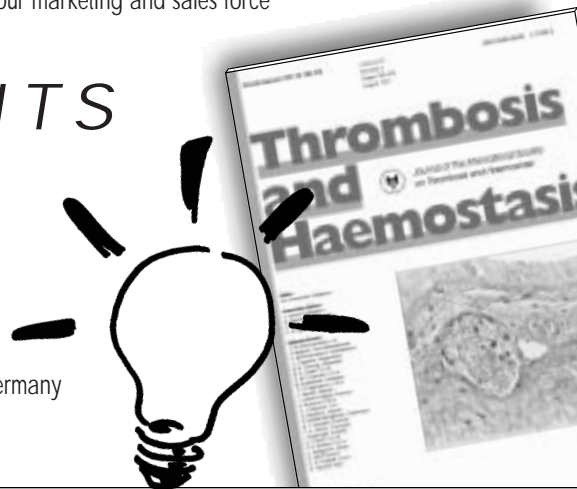
- when you want to affirm the message of your marketing and sales force

THINK REPRINTS

- when you want to supplement seminars and classroom presentations

THINK REPRINTS

- and ask for information on quantities and prices:
F. K. Schattauer Verlag GmbH
P.O.Box 10 45 43, D-70045 Stuttgart, Germany
Phone: + 49-711-2 29 87 14
Fax: + 49-711-2 29 87 50



 **Schattauer**
<http://www.schattauer.com>

