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ORIGINAL ARTICLE

Low protein Z levels and risk of occurrence of deep vein thrombosis

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Summary. *Background:* Protein Z (PZ) serves as a cofactor for activated factor X inhibition by the PZ-dependent protease inhibitor. *In vivo* and *in vitro* studies aimed at investigating the role of PZ levels in venous thombosis have produced conflicting results. *Objectives:* We investigated whether reduced PZ levels and PZ gene common variants are associated deep vein thrombosis (DVT). *Patients and methods:* In 197 patients with DVT and in 197 age-matched and sex-matched controls, PZ plasma levels and gene polymorphisms were evaluated by means of an enzyme-linked immunosorbent assay and direct cycle sequence analysis. *Results:* Similar PZ levels were found in controls (1.44; SD 0.63 μg mL^{-1}) and in patients (1.44; SD 0.96 μg mL^{-1}). The incidence of PZ levels below the 5.0 (0.52 μg mL^{-1}) or the 2.5 percentile of controls (0.47 μg mL^{-1}) was higher in patients (10.2% and 8.7%, respectively) than in controls {4.1% [odds ratio (OR) 2.7, 95% confidence interval (CI) 1.2–7.3], and 2.0% (OR 4.6, 95% CI 1.5–13.9), respectively}. This relationship was independent of the effect of age, sex, and factor V Leiden and FII A^{20210} alleles [OR 2.8 (95% CI 1.1–7.3), and OR 4.9 (95% CI 1.4–17.3)]. PZ levels were associated with the intron C G-42A and the intron F G79A polymorphisms in cases ($r^2 = 0.129$) and in controls ($r^2 = 0.140$). However, frequencies of the PZ gene polymorphisms were similar in the two groups and were not associated with very low PZ levels. *Conclusions:* The present data suggest an association between very low PZ plasma levels and the occurrence of DVT, with PZ gene polymorphisms contributing little to this relationship.

Keywords: deep vein thrombosis, gene, polymorphisms, protein Z.

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Introduction

Venous thrombosis is the third most common cardiovascular affliction after ischemic heart disease and stroke [1]. It is common in Caucasians, affecting 1 in 1000 individuals every year, and is strongly associated with life-threatening pulmonary embolism. The pathogenesis of venous thrombosis is multifactorial, involving acquired and genetic factors. In addition to circumstantial predisposing factors (e.g. surgery, pregnancy, immobilization, malignancy), genetic predisposition because of molecular abnormalities of components of the coagulation pathway has been found in subjects who have suffered from thromboembolic disease [2]. Abnormalities within the gene loci coding for natural anticoagulants (antithrombin, protein C, and protein S) and for fibrinogen have been shown to be rather uncommon risk factors for venous thrombosis [3]. In patients of European ancestry, a common mutation within the gene of the coagulation factor V (FV Leiden mutation) and one within the prothrombin gene (a G > A transition at nucleotide position 20 210) have been shown to account for a large number of cases of thromboembolism [2,3]. Although our knowledge of a series of major risk factors for venous thrombosis has been greatly improved, there exist many thrombotic events whose pathogenesis is unclear.

Protein Z (PZ) is a member of the coagulation cascade known for many years, but its role has remained obscure until recently. PZ is a vitamin K-dependent glycoprotein with an important role in the regulation of the coagulation cascade because of the PZ-dependent protease inhibitor [4]. The PZ-dependent protease inhibitor in the presence of PZ causes rapid inactivation of factor Xa (FXa). Recently, reduced circulating levels of PZ have been suggested to play a role in the occurrence of bleeding [5] and deep vein thrombosis (DVT) [6,7], as well as in unexplained abortions with early fetal loss [8], late fetal demise and intrauterine growth restriction [9], acute coronary syndromes [10], and stroke [11]. However, these data have been disputed [12–17]. A series of variants naturally occurring within the PZ gene locus have been identified, and some of them have been found to modulate PZ plasma levels [17,18], and to be associated with the occurrence of stroke, although high rather

than low PZ plasma levels were predictive of stroke [17], but not with the risk of DVT [19]. On the other hand, in a patient presenting with pelvic thrombosis and carrying the FV Leiden allele, a homozygous missense mutation within the PZ locus and reduced PZ plasma concentrations were found [20].

In a cohort of patients with documented DVT and in age-matched and sex-matched controls, we decided to determine whether very low PZ plasma levels, and the presence of PZ polymorphisms known to modulate PZ plasma levels, are risk factors for the occurrence of venous thrombosis. Reduced PZ levels, below the 5.0 or the 2.5 percentile, were more frequent in patients, suggesting a possible role of PZ, via a severe reduction of plasma values, in the occurrence of DVT.

Materials and methods

Patients and controls

Between January 2003 and December 2004, we investigated 197 non-anticoagulated patients (median age 48 years; range: 13–81 years), 91 men and 106 women, with a documented first venous thrombosis, who were referred at least 3 months after the thrombotic episode (range: 3–78 months) for a work-up to two thrombosis centers, one at the IRCCS 'Casa Sollievo della Sofferenza', San Giovanni Rotondo (FG), and the other at the 'A. Cardarelli' Hospital, Naples. Both centers are located in the south of Italy. The median age (range) at the time of the first thrombotic episode was 45.0 years (range: 12–81 years). The presenting thrombotic episode was DVT in one leg in 172 patients (81 men and 91 women), or at unusual sites (upper extremities, isolated mesenteric veins, jugular veins) in 25 patients (15 men and 10 women). Among these patients, 38 individuals (15 men and 23 women) with an objectively confirmed DVT also experienced an episode of pulmonary embolism.

A complete clinical summary with emphasis on personal and family history for thromboembolic disease, and circumstantial vascular risk factors (surgery, immobilization, pregnancy, postpartum, trauma, oral contraception, varicose veins and malignancy), was obtained from all subjects by specially trained staff. DVT was diagnosed by ultrasonography, and pulmonary embolism by angiography or high-probability ventilation/perfusion lung scan.

One hundred and ninety-seven apparently healthy subjects (98 men and 99 women; median age 44.0 years, range: 21–73 years) randomly selected from a southern Italian general population of employees of the 'Casa Sollievo della Sofferenza' Hospital, S. Giovanni Rotondo, without a history of venous thromboembolism, served as controls. Briefly, among employees who previously gave their informed consent to participate in case–control studies [21], the first subject who matched for sex and age with a patient and consented to participate in the study was enrolled. All subjects who reported a personal history of clinical venous thrombosis were excluded from the study. Both cases and controls were Caucasian and they were from the same region. The two groups were comparable for sex, social status, and age.

After approval of the local Ethics Committees, the study was carried out according to the Principles of the Declaration of Helsinki; informed consent was obtained from all subjects.

Blood collection and coagulation tests

Blood samples were collected in vacuum plastic tubes containing 1/10th volume 3.8% trisodium citrate and centrifuged at 2000 $\times g$ for 15 min to obtain platelet-poor plasma. The latter was frozen and stored in small aliquots at −70 °C until tested. Antiphospholipid antibodies – lupus anticoagulant and anticardiolipin IgG (ELISA, Byk Gulden, Italy) – antithrombin, protein C, amidolytic and immunologic (Behring, Marburg, Germany) and total and free protein S antigen (ELISA, Diagnostica Stago, Asnières, France) were determined in all patients, as reported elsewhere [22]. Clotting assays were performed on a KC4 Amelung coagulometer (Amelung, Germany). Interassay and intra-assay coefficients of all the variables never exceeded 8.0% and 5.0%, respectively. PZ plasma levels were evaluated by means of an enzyme-linked immunosorbent assay (Asserachrom Protein Z; Diagnostica Stago, Asnières, France).

DNA extraction and analysis

DNA was extracted from peripheral blood leukocytes according to standard protocols [21]. A 220 bp DNA fragment of the FV gene that includes nucleotide 1691 was amplified and digested with *Mnl*I, as previously described [23]. To identify the G > A mutation of the prothrombin gene, a 345 bp fragment was obtained and then digested using the *Hin*dIII endonuclease, as previously reported [24]. Amplifications of regions of the PZ gene containing the intron A G-103A (intron 1; dbSNP no. rs17880587) and intron F G79A (intron 6; dbSNP no. rs17882561) polymorphisms were achieved using sense and antisense oligonucleotides designed on the basis of known sequences of the PZ gene locus (Genbank accession number AF440358) [18]. In addition, a polymorphism was investigated, a 41 bp insertion/deletion previously recognized within exon 4 and now mapped in intron C 42 bp (G-42A) before the beginning of exon 4 (intron 3, nucleotide 1 313 287, according to contig NT_027140). The oligonucleotide custom synthesis system was from Life Technologies (Paisley, UK). PCR was carried out on 50-μL samples in a Perkin-Elmer Cetus thermal cycler (Perkin-Elmer Cetus, Norwalk, CT, USA). Each sample contained 0.1 μg of genomic DNA, 10 pmol of each primer, 125 μM dNTP, 5 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, and 1 U of *Taq* polymerase. The solution was overlaid with 50 μL of mineral oil and, after an initial denaturation step (3 min at 95 °C), was subjected to 30 cycles, each consisting of 1 min at 95 °C, 1 min at 56–60 °C and 2 min at 72 °C. Then, 5-μL samples of the amplification products were separated by 2% agarose gel electrophoresis in TAE buffer (40 mM Tris–acetate, 1 mM EDTA, pH 7.7) containing 0.5 μg mL^{-1} ethidium bromide, and visualized under UV light. Then, amplified DNA fragments were subjected to direct cycle sequence

analysis using the *Taq* dye-deoxy terminator method and an ABI PRISM 310 Genetic Analyzer sequencer (PE Biosystems, Foster City, CA, USA).

Statistical analysis

All the analyses were performed according to the Statistical Package for Social Science (SPSS 11.0 for Windows, Chicago, IL, USA). The significance of any difference in means was evaluated by non-parametric test, and the significance of any difference in proportions was tested by chi-squared statistics. The allele frequencies were estimated by gene counting, and genotypes were scored. The observed numbers of each FV, prothrombin or PZ genotype were compared with those expected for a population in Hardy–Weinberg equilibrium using a chi-squared test. Haplotype frequencies were estimated using the expectation maximization approach, as implemented in Arlequin (http://lgb.unige.ch/arlequin/). The significance of the difference in observed alleles and genotypes between the groups was tested using chi-squared analysis after grouping homozygous and heterozygous carriers of the FV Leiden mutation and homozygous and heterozygous carriers of the A^{20210} prothrombin allele. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. Multiple linear regression analysis with stepwise selection of the variables adjusted for age and sex, in which '*P* to enter' and 'tolerance' values were set at 0.05 and 0.01, respectively, was used to evaluate those PZ gene polymorphisms related to plasma PZ concentrations.

Adjusted ORs and 95% CIs were calculated with logistic-regression models that controlled for age, sex, FV Leiden and A^{20210} prothrombin mutation. Statistical significance was taken as $P < 0.05$.

Results

Demographic characteristics and the incidence of circumstantial and thrombophilic risk factors in both patients and controls are shown in Table 1. As expected, patients had a higher incidence of FV Leiden and FII A^{20210} mutations.

PZ plasma levels in patients with DVT and controls

In controls, the mean (SD) PZ plasma concentration was 1.44 (0.63)μg mL^{-1}, with a total range: of 0.21–3.44 μg mL^{-1} and a median value of 1.39 μg mL^{-1}. PZ plasma levels were not higher in men (mean 1.51; SD 0.66 μg mL^{-1}) than in women (mean 1.38; SD 0.61 μg mL^{-1}; Mann–Whitney *U*-test, $P = 0.164$). In patients, the mean (SD) PZ plasma concentration was 1.44 (0.96) μg mL^{-1}, with a total range: of 0.17–6.80 μg mL^{-1} and a median value of 1.26 μg mL^{-1}. PZ plasma levels did not differ between men (mean 1.36; SD 0.73 μg mL^{-1}) and women (mean 1.52; SD 1.14 μg mL^{-1}; Mann–Whitney *U*-test, $P = 0.503$). The mean PZ plasma concentration did not differ between patients and controls (Mann–Whitney *U*-test, $P = 0.662$). In healthy men, plasma PZ levels were similar to those found in men with a previous venous thrombosis (Mann–Whitney *U*-test, $P = 0.132$). Likewise, in women, PZ plasma concentration did not differ between patients and controls (Mann–Whitney *U*-test, $P = 0.887$). Taking the 5.0 percentile of the normal distribution in the control group as the cut-off value for low PZ (0.52 μg mL^{-1}), reduced PZ plasma levels were found in 20 (10.2%) patients and in eight (4.1%) controls. This difference reached significance (OR 2.7; 95% CI 1.2–6.3). Taking the 2.5 percentile of the normal distribution in the control group as the cut-off value for very low PZ (0.47 μg mL^{-1}), reduced PZ plasma levels were found in 17 (8.5%) patients and in four (2.0%) controls (OR 4.6; 95% CI 1.5–13.9). These findings were similar after taking into account the presence of circumstantial risk factors. Actually, in patients with a positive history of venous thromboembolism, very low PZ levels were found in 10 (OR 5.1; 95% CI 1.7–15.9) and 11 (OR 2.8; 95% CI 1.1–7.0) subjects, according to the 2.5 and 5.0 percentiles, respectively. Among patients without a circumstantial risk factor, very low PZ levels were found in seven (OR 3.9; 95% CI 1.2–12.9) and nine (OR 2.5; 95% CI 1.0–6.6) subjects, according to the 2.5 and 5.0 percentiles, respectively. Likewise, in patients without genetic thrombophilic risk factors, a relationship was found with the 2.5 percentile (OR 5.8; 95% CI 1.6–20.7) and 5.0 percentile (OR 3.3; 95% CI 1.3–8.7). Overall, plasma PZ values below the 2.5 percentile were more frequently found in patients, regardless of the presence of a thrombophilic risk factor (OR 5.6, 95% CI 1.6–19.1, and OR 3.8, 95% CI 1.1–13.3, in patients with idiopathic and secondary venous thromboembolism, respectively). PZ levels below the 5.0 percentile were only associated with idiopathic venous thromboembolism (OR 3.1; 95% CI 1.2–8.4). No difference was observed according to age, sex, and presence of specific circumstantial risk factors (e.g. surgery, immobilization,

Table 1 Clinical characteristics and thrombophilic risk factors in controls and patients investigated

Variables	Controls	Patients
Age in years [median (range)]	44.0 (21–73)	45.0 (12–81)
Men [*n* (%)]	98 (48.8)	95 (47.3)
Lower limb DVT (%)	–	172 (85.6)
Pulmonary embolism (%)	–	38 (18.9)
Circumstantial risk factor + ve (%)	–	104 (52.8)
PZ in μg mL^{-1} [mean (SD)]	1.44 (0.63)	1.44 (0.96)
FV Leiden (%)	7 (3.5)	28 (13.9)*
FII A^{20210} allele (%)	6 (3.0)	19 (9.5)†
AT deficiency (< 80%)	NA	0
PC deficiency (< 50%)	NA	0
PS deficiency (< 50%)	NA	1 (0.5)
LAC positivity	NA	5 (2.5)
aCL IgG > 14 GPL	NA	4 (2.0)
Homocysteine > 95 percentile (< 20 μmol L^{-1})	NA	24 (11.9)

SD, standard deviation; DVT, deep vein thrombosis; PZ, protein Z; AT, antithrombin; PC, protein C; PS, protein S; LAC, lupus anticoagulant; GPL, IgG phospholipid units; aCL, anticardiolipin; NA, not assessed. *OR (95% CI): 4.5 (2.0–10.3). †OR (95% CI): 3.4 (1.4–8.4).

Table 2 Factors independently associated with the occurrence of deep vein thrombosis

Variable	*P*-value	OR	95% CI
PZ < 5.0 percentile			
Factor V Leiden (FV Leiden)	0.004	8.26	2.75–38.22
FII A^{20210} allele	0.007	5.66	1.62–19.81
PZ	0.038	2.78	1.06–7.32
PZ < 2.5 percentile			
FV Leiden	0.004	7.93	2.39–37.25
FII A^{20210} allele	0.007	5.58	1.59–19.55
PZ	0.015	4.98	1.37–17.28

Variables for which the model was adjusted but not associated with the venous thromboembolic event: age and sex. PZ, protein Z.

pregnancy, postpartum, trauma, oral contraception). After adjustment for age, sex, and carrier status for the FV Leiden and FII A^{20210} mutation, the estimated risk remained similar when low (< 5.0 percentile) (OR 2.8, 95% CI 1.1–7.3) or very low (< 2.5 percentile) (OR 5.0, 95% CI 1.4–17.3) PZ levels were considered. Multiple logistic analysis (Table 2) demonstrated that the association was mainly determined by the carrier status for the FV Leiden and FII A^{20210} mutation, with an additional contribution of PZ plasma levels below the 5.0 or the 2.5 percentiles (OR 2.78, 95% CI 1.06–7.32, and OR 4.98, 95% CI 1.37–17.28, respectively). After exclusion of patients with a deficiency of natural anticoagulants, antiphospholipid antibodies, or circulating homocysteine levels > 95 percentile, the relationship with plasma levels below the 5.0 or the 2.5 percentiles still remained significant (data not shown).

Analysis of the PZ gene polymorphisms

In both the patient and control groups, the intron A G-103A, intron C G-42A and intron F G79A PZ gene polymorphisms were determined by means of direct cycle sequence analysis. The allele and genotype frequencies found are reported in Table 3. The genotype distribution for all polymorphisms was in Hardy–Weinberg equilibrium. The frequencies of the less common alleles of all polymorphisms were as previously reported for a Caucasian population [17,19]. No differences were found in allele and genotype frequencies between patients and controls (Table 3).

There was a strong linkage disequilibrium (LD) between the intron A G-103A and intron F G79A PZ gene polymorphisms in both controls and patients (D′ 0.5394; $P < 0.001$), whereas no LD was found with the intron C G-42A polymorphism (D′ 0.2373 and 0.3782, respectively). Although a small increase of the haplotype containing the intron A 103A, intron C −42A and intron F 79A alleles was recorded in patients (0.016 vs. 0.011), estimated haplotype frequencies were comparable between patients and controls.

Effect of gene polymorphisms on PZ plasma levels

An association with circulating PZ levels was shown for the intron A G-103A, intron C G-42A and intron F G79A polymorphisms (Table 3). Actually, in controls, heterozygous carriers of the intron A A-103 allele, as well as heterozygous subjects with the intron C A-42 or intron F A79 allele, showed mean PZ levels (1.16, 1.24 and 1.29 μg mL^{-1}) lower than those in individuals with the GG genotype (1.53, 1.51 and 1.51 μg mL^{-1}; respectively). In those presenting with the AA genotype, the plasma concentration was further reduced (0.86, 0.62 and 0.97 μg mL^{-1}, respectively). Likewise, heterozygous patients with the intron A A-103, intron C A-42 or intron F A79 allele displayed lower PZ plasma levels (1.39, 1.20 and 1.19 μg mL^{-1}) than those measured in carriers of the GG genotype (1.44, 1.54 and 1.56 μg mL^{-1}, respectively). Patients with the AA genotype had the lowest PZ plasma concentrations (0.75, 0.96, and 0.87 μg mL^{-1}, respectively).

A stepwise model was used to determine the effects of gene polymorphisms, adjusted for age and sex, on PZ plasma levels (dependent variable). In the model, the intron C G-42A and intron F G79A gene polymorphisms independently predicted plasma PZ antigen levels in both controls ($r^2 = 0.08$ and 0.06, respectively) and patients ($r^2 = 0.04$ and 0.09, respectively). However, the higher frequency of carriers of PZ plasma levels below the 5.0 and the 2.5 percentiles among patients was unaffected by any of the PZ gene variants investigated (data not shown).

Discussion

PZ plays an important role in regulation of the coagulation pathway, because of the PZ-dependent inactivation of FXa on phospholipid surfaces through the formation of a complex with a recently described serpin, the Z-dependent protease inhibitor [4,25]. Recent data suggest that low PZ levels may play a role in the pathogenesis of ischemic stroke [10,26] and venous thrombosis [6,7]. In the present study, we investigated the role of PZ plasma levels in patients who were not taking oral anticoagulants, referred for DVT, and in age-matched and sex-matched apparently healthy controls. In agreement with previous studies [8,10–13,16,26,27], PZ concentrations showed a wide range in controls as well as in patients. In controls, mean values were different from those measured in some studies [8,11,27] but in agreement with those measured in others [12,13,26], including in different Italian settings [10,16]. In this study, we have found that very low PZ plasma concentrations, below the 5.0 or the 2.5 percentiles, occur more frequently among patients with documented DVT. This association was similar in patients with and without circumstantial risk factors, and no difference was observed according to age, sex, and the presence of specific circumstantial risk factors (e.g. surgery, immobilization, pregnancy, postpartum, trauma, oral contraception). These data suggest that a severe reduction in PZ plasma levels may be associated with the risk of DVT. The association was independent of the effects of other variables, such as age, sex, and carrier status with regard to the FV Leiden or the FII A^{20210} mutations. As there is no assay with which to evaluate PZ activity, we could not investigate the role of

Table 3 Mean protein Z (PZ) plasma levels in controls and in patients according to gene polymorphisms investigated

Gene variant		Controls		Patients	
		n (%)	Mean (SD)	*n* (%)	Mean (SD)
Intron A G-103A					
Allele	G	346 (87.8)	–	343 (87.1)	–
	A	48 (12.2)	–	51 (12.9)	–
Genotype	GG	154 (78.2)	1.53 (0.63)	150 (76.1)	1.44 (0.80)**
	AG	38 (19.3)	1.16 (0.54)*	43 (21.8)	1.39 (1.11)**
	AA	5 (2.5)	0.86 (0.35)*	4 (2.1)	0.75 (0.20)
Intron C G-42A					
Allele	G	345 (87.6)	–	323 (87.1)	–
	A	49 (12.4)	–	71 (18.0)	–
Genotype	GG	151 (76.7)	1.51 (0.64)***	136 (69.1)	1.54 (0.97)
	AG	43 (21.8)	1.24 (0.64)***	51 (25.8)	1.20 (1.01)****
	AA	3 (1.5)	0.62 (0.09)	10 (5.1)	0.96 (0.37)****
Intron F G79A					
Allele	G	321 (81.5)	–	316 (80.2)	–
	A	73 (18.5)	–	78 (19.8)	–
Genotype	GG	135 (68.5)	1.51 (0.63)	126 (64.0)	1.56 (0.87)
	AG	51 (25.9)	1.29 (0.62)*****	64 (32.5)	1.19 (0.90)*****
	AA	11 (5.6)	0.97 (0.54)*****	7 (3.5)	0.87 (0.30)*****

Mean (SD) PZ plasma levels are expressed in μg mL^{-1}. By *post hoc* Dunnet *C*-test for multiple comparisons: *$P < 0.05$ vs. the intron A −103GG genotype; **$P < 0.05$ vs. the intron A −103AA genotype; ***$P < 0.05$ vs. the intron C –42AA genotype; ****$P < 0.05$ vs. the intron C –42GG genotype; *****$P < 0.05$ vs. the intron F 79GG genotype.

dysfunctional variants of PZ. Thus, the measurement of PZ antigen could have missed individuals with abnormal PZ. In the present study, patients were referred. Theoretically, referral could represent a limitation, and another source of bias may be the measurement of PZ levels from months to years after the thrombotic event. However, PZ concentrations seem to be unaffected by an increase in age [27].

A relationship between venous thrombosis and PZ plasma levels has been excluded in two case–control studies, in which similar levels of the protein in patients and in controls were found. In addition, both studies indicated that reduced PZ levels (< 10th percentile) are not a risk factor for venous thrombosis [16,28]. In keeping with this, also in the present study, mean PZ plasma levels as low as below the 10th percentile (data not shown) were comparable in patients and in controls. However, at variance with those studies, a further reduction of PZ plasma levels, < 5.0 or the 2.5 percentile, was independently associated with the occurrence of venous thrombosis (OR 2.78 and 4.98, respectively). These findings are in agreement with previous investigations, which have indicated a high frequency of PZ deficiency (> 2 SD) among patients with ischemic cerebrovascular events [11,29] but not in patients with a previous history of DVT [11]. In the latter report, a small cohort of patients was investigated, and mean PZ values in controls (2.29 μg mL^{-1}) and mean age at analysis in patients with DVT (37.3 years) were different from mean control PZ values (1.36 μg mL^{-1}) and mean age of patients (46.5 years) in the present study. Although PZ concentrations are unaffected by increase in age [27], the presence of confounding variables, such as untreated hypertension, diabetes, and hyperlipidemia [30], may well explain the different findings.

Studies in mice and in human beings have shown that PZ levels modulate the prothrombotic tendency associated with FV Leiden carriership [6,7]. We could not address this issue, because among the subjects analyzed, only two individuals, both of them patients, carried the FV Leiden mutation and had very low PZ plasma concentrations.

Genetic factors may be an important determinant of the wide normal range of PZ plasma concentrations [31]. A series of common and rare gene variants have been reported in the PZ locus [19]. In the present work, we investigated the intron A G-103A and the intron F G79A polymorphisms in all patients and controls enrolled. Interestingly, in controls and in patients, the intron A G-103A polymorphism as well as the intron F G79A polymorphism was found to be associated with PZ plasma levels, carriers of the rare allele showing lower concentrations. These data are in agreement with recent results obtained for the intron F G79A polymorphism [15,17]. In addition, we have identified the molecular cause of a previously reported insertion/deletion PZ gene polymorphism [19]. This was a G > A transition at position −42 in intron C that confers a perfect repetition of about 40 bp. This gene variation did not show LD with the other polymorphisms analyzed. Homozygosity for the A allele was found to be associated with reduced PZ plasma levels in controls ($r^2 = 0.08$) and in patients ($r^2 = 0.04$). However, the PZ allelic variants investigated, alone or in combination, did not display different frequencies between patients and controls, and nor did their frequencies differ in subjects whose PZ plasma levels were below the 5.0 or the 2.5 percentiles.

None of these gene polymorphisms is likely to have a direct effect on gene expression. Thus, it is conceivable that they are in LD with unknown allelic variants that modulate PZ gene

expression. Nonetheless, these findings emphasize the importance of common genetic variations within the PZ locus in the modulation of plasma concentrations, and help to explain, to some extent, the wide range observed in the general population as well as inconsistencies in mean plasma levels observed in different populations.

In conclusion, the results of the present investigation confirm the wide range of PZ plasma values and show that genetic factors within the PZ locus may explain, at least in part, this interindividual variability. In addition, the present data suggest that very low PZ concentrations could be associated with the occurrence of DVT. Larger studies are needed to address whether measuring PZ antigen levels help to better define a risk profile for DVT.

Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

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