Polymorphisms of the protein Z-dependent protease inhibitor (ZPI) gene and the risk of venous thromboembolism

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Dear Sir,

Protein Z-dependent protease inhibitor (ZPI), together with its cofactor protein Z (PZ), inhibits activated factor Xa (Xa) (1–3). Studies in mice showed that the PZ null genotype increases the mortality in utero of homozygous factor V Leiden mice due to vascular thrombosis and hepatic fibrin deposition (4), suggesting a role for the PZ-ZPI pathway in the pathogenesis of venous thromboembolism (VTE) (3).

While several studies investigated the association between PZ levels and VTE, which gave rise to contrasting results (5–8), only two studies evaluated the role of ZPI (7, 9). In the first (9), the coding region of the ZPI gene was sequenced in patients with VTE and in healthy controls, and two nonsense polymorphisms producing stop codons (R67X and W303X) were associated with a 6-fold increased risk of VTE, suggesting a role of ZPI deficiency in the pathogenesis of the disease. The second study (7) measured ZPI levels in 426 patients and 471 controls, and did not find any association with VTE.

This study investigated the prevalence of the ZPI polymorphisms R67X and W303X in a large group of Italian patients with VTE and in healthy subjects. In addition, the interaction between the two polymorphisms and thrombophilia or plasma levels of PZ antigen was evaluated.

Criteria for patient selection were previously described (8). Briefly, from January 1999 to August 2004, 742 patients with a first, objectively documented episode of VTE were referred to the Thrombosis Center for thrombophilia screening. Two hundred ninety-nine patients were excluded from the study because of conditions that might potentially affect plasma PZ levels (oral anticoagulant therapy, oral contraceptive use, pregnancy, liver or renal disease) or because no plasma aliquot was left. Of 60 additional patients no DNA aliquot was left. Therefore, 383 patients remained in the study. Of these, 365 were analyzed for R67X, 368 for W303X and 350 for both polymorphisms. For the remaining patients no DNA amplification was obtained.

The control group was formed by 416 healthy individuals, partners or friends of the population of patients, who voluntarily agreed to be investigated for thrombophilia screening in the same period as patients. All subjects gave their written informed consent to the study. The study was approved by the Institutional Review Board of the University of Milan.

The two polymorphisms in the ZPI gene (R67X and W303X) were analyzed by bidirectional allele-specific PCR (Van de Water et al. 2004). PZ plasma levels were measured using a commercial kit (Asserachrom Protein Z, Diagnostica Stago, Asnières, France) following the manufacturers’ instructions. Thrombophilia screening included DNA analyses for factor V Leiden and prothrombin mutation, functional and antigenic assays for antithrombin, protein C and protein S, the search for antiphospholipid antibodies (only in patients) and fasting and post-methionine load total plasma homocysteine measurements (10).

Continuous variables are presented as median and range. Differences between patients and controls were calculated by the Mann-Whitney U test for continuous variables and by Chi²-test for categorical variables. Odds ratios and 95% confidence intervals (CI) were used as a measure of the association between the ZPI polymorphisms or thrombophilia markers and VTE. Using an unconditional logistic regression analysis, odds ratios were adjusted for possible confounders, such as age, sex and the presence of markers of thrombophilia. Statistical significance was set at p<0.05.

Patients had a similar sex distribution (male/female: 145/238 in patients and 172/244 in controls) and were younger than controls (median age, range: 39 years, 14–76 for patients at the time of VTE, and 42 years, 16–84 for controls at the time of blood sampling, p=0.02).

All the causes of inherited thrombophilia and hyperhomocysteinemia were associated with a statistically significant increased risk of VTE, for an odds ratio (adjusted for age, sex, and each for the other causes of thrombophilia) of 5.9 (95% CI 3.0–11.2) for factor V Leiden, 2.4 (1.3–4.4) for prothrombin G20210A, 5.0 (2.0–12.5) for antithrombin, protein C and protein S deficiencies taken together, and 3.4 (2.1–5.4) for hyperhomocysteinemia. Table 1 shows that the prevalence of the two ZPI polymorphisms was similar in patients and controls, and there was no increased risk of VTE associated with either polymorphism.

When the study population was stratified according to the presence or absence of the polymorphisms R67X or W303X and inherited thrombophilia or hyperhomocysteinemia, the risk of VTE remained statistically not significant (data not shown). Also, no interaction was observed between the presence of the polymorphisms and low PZ levels (below the 25th percentile of the distribution among controls, i.e. 1.34 µg/ml) (data not shown).

In this case-control study, we investigated the role of the two ZPI polymorphisms as risk factors for VTE, finding no associ-
The table shows the distribution of the R67X and W303X ZPI polymorphisms in patients and controls and the risk of venous thromboembolism. Compared to the study by Van de Water et al., we found a higher prevalence of the R67X (4.1% vs. 1.2%), a lower prevalence of the W303X (0.5% vs. 3.2%) polymorphism in patients, and the same 4.4% overall prevalence. Among controls, we found a higher prevalence of the polymorphisms (5.3% vs. 0.8%). The reasons of the different prevalence among controls in our study and in that of Van de Water et al. may be the different selection criteria of controls (friends or partners of patients in this study and blood donors in the other), or a random effect due to the relatively small number of controls analyzed in the New Zealand study. Since VTE is a multifactorial disease resulting from the combination of various risk factors, we investigated the effect of the combined presence of the two polymorphisms in the ZPI gene and inherited thrombophilia or hyperhomocysteinemia, finding no interaction. However, since the stratification of the study population gave odds ratios with wide confidence intervals, further studies are needed to address this issue. Finally, we investigated whether or not the ZPI polymorphisms, together with low levels of PZ, are associated with an increased risk of VTE. The prevalence of ZPI polymorphisms was similar in patients and controls in the four quartiles of PZ levels.

In conclusion, the presence of the R67X or W303X polymorphism in the ZPI gene does not increase the risk of VTE or affect PZ levels. Our results, together with the lack of an independent association between low PZ levels and VTE already reported by some of us (8) and others (7), do not support a mechanistic role of the ZPI-PZ complex in patients with VTE.

### References