Protein Z-Dependent Regulation of Coagulation

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Key words

Factor Xa, factor Xla, serpin, proteinase inhibitor

Summary

Protein Z (PZ) is a 62 kDa vitamin K-dependent plasma protein that serves as a cofactor for the inhibition of factor Xa by protein Z-dependent protease inhibitor (ZPI). ZPI is a recently identified 72 kDa member of the serpin superfamily of proteinase inhibitors that contains a tyrosine at its reactive center. PZ circulates in plasma in a complex with ZPI. Inhibition of factor Xa by ZPI in the presence of phospholipids and Ca++ is enhanced 1000-fold by PZ, but ZPI also inhibits factor Xla in a process that does not require PZ, phospholipids or Ca++. ZPI activity is consumed during coagulation through proteolysis mediated by factor Xa with PZ and factor Xla. Concomitant PZ deficiency dramatically increases the severity of the prothrombotic phenotype of factor V Leiden mice. Studies to determine the potential roles of PZ and ZPI deficiency in human thrombosis are in progress.

Introduction

In 1977, Prowse and Esnouf identified an additional vitamin K-dependent protein circulating in bovine plasma and named it protein Z (PZ) as it was the last of the vitamin K-dependent proteins to elute during anion exchange chromatography (1, and P. Esnouf, personal communication). The human counterpart to bovine PZ was isolated in 1984 (2). The organization of the PZ gene, at chromosome 13q34, and the structure of the PZ molecule are very similar to those of coagulation factors VII, IX, X, and protein C (3-5). In contrast to these serine protease zymogens, however, in PZ the region around the typical “activation site” is absent and the histidine and serine residues of the canonical catalytic triad are missing (4, 5). Thus, PZ, like protein S, does not serve a proteolytic function.

Human PZ is a 62,000 MW glycoprotein that has a plasma t1/2 of ~2.5 days (2, 6). Plasma PZ levels in 450 Red Cross donors span a broad range (0.6-5.7 g/mL) with a mean concentration of 2.9 ± 1.0 µg/mL in EDTA anticoagulated plasma (~2.6 µg/mL, 40 nM, in citrated plasma) (6). Preliminary reports suggest PZ behaves as a negative acute phase reactant, which may contribute to the wide range in PZ plasma values (7, 8). Like the other vitamin K-dependent coagulation factors, the plasma level of PZ is low in newborn infants (9, 10). Individuals with disseminated intravascular coagulation (DIC), liver disease, and amyloidosis have low plasma levels of PZ (11-13), whereas patients on chronic hemodialysis and with idiopathic thrombocytopenic purpura reportedly have high plasma levels of PZ (14, 15). Warfarin therapy reduces both the PZ antigen level (1-16%) and its degree of γ-carboxylation much more than other vitamin-K dependent factors (6). Immunoreactive PZ has been detected in atherosclerotic plaques (16).

McDonald et al. (17) have reported that the kinetics of the binding of human and bovine PZ to PC/PS (75%/25%) vesicles is dramatically different from that of the other vitamin K-dependent coagulation factors:

<table>
<thead>
<tr>
<th>Protein</th>
<th>k_{assn} (10^{-5} s^{-1} M^{-1})</th>
<th>k_{dissn} (s^{-1})</th>
<th>K_{d} (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine PZ</td>
<td>1.95</td>
<td>0.0063</td>
<td>32</td>
</tr>
<tr>
<td>Human PZ</td>
<td>3.36</td>
<td>0.057</td>
<td>170</td>
</tr>
<tr>
<td>Bovine prothrombin</td>
<td>176.0</td>
<td>1.9</td>
<td>230</td>
</tr>
</tbody>
</table>

Both the association and dissociation rate constants for the PZ’s are significantly slower than those of prothrombin, and the dissociation of bovine PZ from the phospholipids is significantly slower than that of human PZ.

In vitro, bovine PZ has been shown to interact with diisopropylphosphoryl (DIP)-thrombin (K_{d} = 0.15 µM) and mediate the binding of DIP-thrombin to phospholipids (18). Human PZ, however, binds thrombin poorly (K_{d} = 8.9 µM) and has a minimal impact on thrombin’s association with phospholipids (19). Additional studies showed that the enhanced binding of thrombin to bovine PZ required a 36 amino acid C-terminal extension present in bovine, but absent in human PZ (19). Thrombin cleavage of bovine PZ at arginine 365 (R365) releases this C-terminal peptide (19, 20).

PZ Deficiency and Hemorrhage

It has been suggested that PZ deficiency is associated with a hemorrhagic disorder, perhaps related to capillary fragility (21). Thirty-six individuals with bleeding disorders of unknown etiology were studied: many of who had a positive Rumpel-Leede test (83%) and a prolonged bleeding time (43%). The mean PZ level in the patients was 52% (range 22-112%). Additional studies have also reported a potential relationship between hemorrhagic symptoms and PZ deficiency and prothrombin complex concentrate, which contains PZ, has been used to prevent perioperative hemorrhage in individuals with a bleeding history and perceived PZ deficiency (see ref. 10 for review). Against a role for PZ deficiency in the bleeding seen in these individuals is the fact that 10% of apparently normal people (Red Cross blood donors) have PZ levels of <50% (6) and the fact that individuals taking oral anticoagulants, whose PZ levels are very low (6), do not have abnormal bleeding times. Two additional studies have failed to detect a relationship between PZ deficiency and a bleeding tendency (22, 23). Consistent with these latter reports, PZ null mice have normal bleeding times and do not have a hemorrhagic phenotype (24).
PZ is a Cofactor for Factor Xa Inhibition

More recently, it was discovered that the procoagulant activity of factor Xa is first incubated with PZ (25). This inhibitory effect requires the presence of phospholipids and Ca²⁺ ions and is time dependent (maximal at 120 s) apparently reflecting the slow association of PZ with phospholipids. PZ that was proteolytically cleaved at arginine 43 (R43), thereby separating its Gla domain from the remainder of the molecule, lacked inhibitory activity (25). These results suggested that an interaction between factor Xa and PZ occurs at the phospholipid surface. Consistent with this notion, the rate of inhibition of factor Xa by antithrombin was slowed by PZ in the presence of phospholipids and Ca²⁺ (25). Additional work showed that the inhibitory effect of PZ on factor Xa activity in the one-stage coagulation assay was due at least in part to a plasma proteinase inhibitor that recognizes the factor Xa-PZ complex.

A two-stage coagulation assay for PZ-dependent factor Xa inhibition was used to isolate a previously unidentified 72,000 MW single chain glycoprotein from human plasma, which was named PZ-dependent protease inhibitor (ZPI) (25). In a system using purified components, the factor Xa inhibition by ZPI is rapid (>95% within 1 min by coagulation assay) and requires the presence of PZ, phospholipids, and Ca²⁺. Indirect evidence strongly suggests that the inhibitory process involves the formation of a stoichiometric complex of factor Xa-ZPI-PZ at the phospholipid surface (25).

ZPI Is a Serpin

ZPI cDNA was isolated and cloned from a human liver cDNA library (26). The ZPI cDNA is 2.44 kb in length and has a relatively long 5' region (466 nt) that contains six potential ATG translation start codons. ATG's 1-4 are followed by short open reading frames, whereas ATG₅ and ATG₆ are in an uninterrupted open reading frame that includes the encoded ZPI protein. In vitro experiments show that ATG₆ is sufficient for the expression of rZPI in cultured Chinese hamster ovary (CHO) cells. Northern analysis suggests that the liver is a major site of ZPI synthesis (26). The predicted 423 residue amino acid sequence of the mature ZPI protein is 25-35% homologous with members of the serpin superfamily of protease inhibitors and is 78% identical to the amino acid sequence predicted by a previously described cDNA isolated from rat liver, regeneration-associated serpin protein-1 (rasp-1) (27). Thus, ZPI is likely the human homologue of rat rasp-1, which was identified as a gene whose transcription is increased following subtotal hepatectomy in rats (27). Alignment of the amino acid sequence of ZPI with those of other serpins predicts that Y387 is the P₁ residue at the reactive center of the ZPI molecule. Consistent with this notion, rZPI(Y387A), an altered form of ZPI in which tyrosine 387 has been changed to alanine, lacks PZ-dependent factor Xa inhibitory activity (26).

In the presence of phospholipids and Ca²⁺, the rate of factor Xa inhibition by ZPI is enhanced >1000-fold (t₁/₂ <10 s vs. 210 min) by PZ (28). Heparin (0.25 U/mL) does not affect ZPI-mediated inhibition of factor Xa in the presence of PZ and only modestly increases factor Xa inhibition by ZPI in the absence of PZ (factor Xa inhibition at 60 min.: 61 ± 8% with heparin; 29 ± 10% without heparin). The combination of PZ and ZPI dramatically delays the initiation and reduces the ultimate rate of thrombin generation in mixtures containing prothrombin, factor V, phospholipids, and Ca²⁺ (Fig. 1A) (28). In similar mixtures containing factor Va, however, PZ and ZPI do not inhibit thrombin generation (Fig. 1B). Thus, the anticoagulant action of PZ and ZPI presumably must precede the activation of factor V and formation of the prothrombinase complex or, perhaps, follow the consumption of prothrombin at local sites.

ZPI Inhibition of Factor XIa

ZPI does not produce significant inhibition of thrombin, meizothrombin, factor VIIa, factor IXa, factor XIIa, kallikrein, activated protein C, t-PA, u-PA, plasmin, trypsin, leukocyte elastase, chymotrypsin or cathepsin G in the presence or absence of PZ, phospholipids, and Ca²⁺ (26). Factor XIa, however, is inactivated by ZPI in a reaction that does not require the presence of PZ, phospholipids or Ca²⁺, and is not affected by the presence of high molecular weight kinogen (28). Heparin (0.20 U/mL) increases the rate (t₁/₂ 25 s vs. 50 s) and the extent (99% vs. 93%) of the factor XIa inhibition produced by ZPI (28).

Whether factor XIa inhibition produced by ZPI is physiologically relevant is not known, but studies comparing the phenotypes of PZ and ZPI gene-deleted mice could help address this issue. It is important to note, however, that an apparent interaction between factor XIa and ZPI is detectable in the plasma milieu (see below), at least when relatively large quantities of factor XIa are produced through kaolin-induced contact activation. Under these in vitro conditions, therefore, ZPI appears to compete effectively with other factor XIa inhibitors (e.g. α₂-antitrypsin, C₁ esterase inhibitor) and the substrate factor IX in plasma for the active site of factor XIa.
Instability of ZPI Complexes with Factors Xa and Xla

As is typical for members of the serpin superfamily of proteinase inhibitors, ZPI is proteolytically cleaved during its inhibition of factor Xa and factor Xla with a reduction in its size from 72 kDa to 68 kDa. The N-terminal amino acid sequences of the peptides (4.2 kDa) released from ZPI following its interaction with factor Xa and factor Xla are identical, SMPP-VIKVDRPF, and correspond to the amino acid sequence in the ZPI molecule following Y387 (28). Thus the reactive center of ZPI that is involved in its inactivation of both factors Xa and Xla is Y387-Ser388 (P1-P1’).

The factor Xa-ZPI and factor Xla-ZPI inhibitory complexes, however, are dramatically less stable than other enzyme-serpin complexes. In contrast to the thrombin-antithrombin interaction, for example, the factor Xa-ZPI and factor Xla-ZPI complexes do not survive SDS-PAGE, but can be detected in the less denaturing conditions of native-PAGE (without SDS) (28). Dissociation of the thrombin-antithrombin complex is very slow (~2.5 x 10^-6 s^-1) and appears to proceed exclusively through the cleavage of antithrombin (29). Dissociation of the factor Xa-ZPI complex is much more rapid (1.7 x 10^-4 s^-1) and likely also occurs through the cleavage of ZPI (28). In this regard, therefore, ZPI behaves as a substrate for the factor Xa-PZ-phospholipid complex, albeit a very poor one.

ZPI Is Consumed during Coagulation

Serum produced from plasma by the induction of coagulation with kaolin, phospholipids and Ca^2+ or tissue factor and Ca^2+ contains little ZPI functional activity. Western blot analysis shows that, during coagulation of plasma, ZPI is proteolytically cleaved at its C-terminus with reduction in its apparent molecular weight from 72 kDa to 68 kDa (28). Subsequent studies have shown that factor Xa (in the presence of PZ) is responsible for the consumption of ZPI with tissue factor induced coagulation. Factor Xla also contributes, however, when coagulation is initiated by direct contact activation with kaolin and relatively large concentrations of factor Xla are generated.

As the interaction of factor Xla with ZPI produces cleaved, inactive ZPI, an additional effect of this interaction would be to reduce the level of active ZPI available for PZ-dependent factor Xa inhibition. The levels of factor Xla produced during normal, in contrast to kaolin-induced, coagulation of plasma are likely to be very low (30), however, suggesting that the potential reduction in ZPI activity produced by factor Xla could only be physiologically important at local sites of coagulation. On the other hand, the consumption of ZPI produced by factor Xa and PZ during tissue factor induced coagulation would prevent subsequent ZPI inhibition of factor Xla.

PZ Circulates in Plasma in a Complex with ZPI

Purified PZ and ZPI form a complex that is detectable by gel filtration chromatography and native-PAGE (31). This interaction does not require the presence of Ca^2+ or phospholipids and reduces the rate and extent of factor Xla inhibition produced by ZPI. In pooled normal plasma, which contains excess ZPI, all the PZ appears to be complexed with ZPI (Fig. 2). Whether the plasma half-life of the PZ-ZPI complex differs from that of free ZPI (or free PZ) is not known. In the plasma samples from 13 individuals on chronic warfarin anticoagulation, which contain low PZ antigenic levels (9.2 ± 3.5%), the antigenic levels of ZPI were 55 ± 12.6% that of pooled normal plasma (31). This suggests that PZ does affect the plasma concentration of ZPI, either through an effect on ZPI secretion, localization or clearance.

PZ, a Serpin Cofactor

PZ is not the only protein that has been shown to function as a cofactor to enhance the inhibitory activity of a serpin toward an enzyme. Thrombomodulin increases the rate of thrombin inhibition by protein C inhibitor ~140-fold (32). This effect of thrombomodulin reportedly depends primarily on the interaction between thrombin and thrombomodulin. Vitronectin increases the rate of thrombin inhibition by plasminogen activator inhibitor-1 (PAI-1) about 200-fold (33-35). Vitronectin appears to produce this enhancement by both binding PAI-1, thereby inducing a conformational change at its reactive center, and through a protein-protein interaction with thrombin (35-38). Similarly, the cofactor action of PZ presumably involves its ability both to bind (31) and to bring ZPI to the phospholipid surface, as well as its ability to interact with factor Xa at this surface (25).

Two potential pathways for PZ-dependent factor Xa inhibition by ZPI are shown in Fig. 3. On the left, PZ and factor Xa first form a complex at the phospholipid surface and this complex is subsequently recognized by ZPI. On the right, a preformed PZ-ZPI complex is directed to the phospholipid surface via its PZ moiety and binds factor Xa. The final result of either pathway is the formation of a Ca^2+-dependent complex at the phospholipid surface that contains PZ, factor Xa and ZPI. Since PZ circulates bound to ZPI, the pathway on the right presumably reflects the inhibitory mechanism that occurs in the plasma milieu.
The effect of PZ on factor X activity and thrombin generation during coagulation was determined using pooled normal human plasma that had been depleted of PZ (and other vitamin K-dependent coagulation factors) by barium salt adsorption. In this barium adsorbed plasma replenished with factor X, but not prothrombin (to prevent significant thrombin generation), the factor Xa activity produced following the induction of coagulation by factor IXa was dramatically reduced in the presence of PZ (Fig. 4A) (24). Thrombin generation was examined in the same system by the addition of prothrombin as well as factor X to the plasma. In the presence of PZ, thrombin generation was significantly delayed and the peak thrombin concentration was reduced >50% (Fig. 4B) (24).

**Prothrombotic Phenotype of Murine PZ Deficiency**

To investigate the *in vivo* consequences of PZ deficiency, the PZ gene was disrupted in mice (24). In the standard mixed C57Bl/6 × 129Sv genetic background, genotyping of 303 progeny derived from PZ(+/−) intercrosses showed that PZ null mice were born in expected numbers: 23% PZ(+/+), 54% PZ(+/−), and 23% PZ(−/−). Immunohistological analysis of the tissues from PZ(−/−) mice did not demonstrate vascular thrombosis or hepatic fibrin deposition. Thus, in the mixed C57Bl/6 × 129Sv genetic background and in the absence of an additional challenge, murine PZ deficiency produces a grossly normal phenotype (24). To further evaluate the potential prothrombotic risk associated with PZ deficiency, PZ gene-disrupted mice were crossbred with mice carrying the factor V*Leiden* mutation. Mice homozygous for the FV*Leiden* mutation [FV(+/+)] express a strain-specific thrombotic phenotype (39). In the mixed C57Bl/6 × 129Sv genetic background ~20% of the FV(+/+) mice die during the neonatal period with microvascular thrombosis.

The genotypes of six week old mice derived from a variety of FV*Leiden/PZ* mating strategies are shown in Table 1. The PZ(−/−) genotype markedly increases the mortality of FV(+/+) mice (Table 1A) and only a single FV(+/+)/PZ(−/−) animal survived to adulthood (Table 1C). The PZ(+/−) genotype also increases mortality in FV(+/+) mice as the

**Tab. 1** Progeny of FV*Leiden/PZ* matings

<table>
<thead>
<tr>
<th>Mating Pairs</th>
<th>Observed</th>
<th>Expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. FV(+/+)/PZ(+/-) x FV(+/+)/PZ(+/-)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 6 weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FV(+/+):PZ(+/+)</td>
<td>40 (52%)</td>
<td>25%</td>
</tr>
<tr>
<td>FV(+/+):PZ(+/-)</td>
<td>37 (48%)</td>
<td>50% *p&lt;0.01</td>
</tr>
<tr>
<td>FV(+/+):PZ(−/−)</td>
<td>0 (0%)</td>
<td>25% p&lt;0.01</td>
</tr>
<tr>
<td>At E17.5-E18.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FV(+/+):PZ(+/-)</td>
<td>7 (21%)</td>
<td>25%</td>
</tr>
<tr>
<td>FV(+/+):PZ(+/-)</td>
<td>18 (53%)</td>
<td>50%</td>
</tr>
<tr>
<td>FV(+/+):PZ(−/−)</td>
<td>9 (26%)</td>
<td>25%</td>
</tr>
<tr>
<td>B. FV(+/+)/PZ(+/+) x FV(+/-)+PZ(+//)</td>
<td>6 weeks</td>
<td></td>
</tr>
<tr>
<td>FV(+/+):PZ(+/-)</td>
<td>28 (74%)</td>
<td>50%</td>
</tr>
<tr>
<td>FV(+/+):PZ(+/-)</td>
<td>10 (26%)</td>
<td>50% *p&lt;0.01</td>
</tr>
<tr>
<td>C. FV(+/+)/PZ(−/-)+ x FV(+/+)/PZ(+/-)</td>
<td>6 weeks</td>
<td></td>
</tr>
<tr>
<td>FV(+/+):PZ(+/-)</td>
<td>16 (100%)</td>
<td>50%</td>
</tr>
<tr>
<td>FV(+/+):PZ(−/-)</td>
<td>0 (0%)</td>
<td>50% p&lt;0.01</td>
</tr>
</tbody>
</table>

* Difference between observed and expected frequencies derived by the confidence interval for binomial distributions
# Single FV (+/)/PZ(−/−) male, derived from a F(+/+)/PZ(+/-) intercross, mated with 5 females
ratio of FV(+/+)/PZ(+/+) to FV(+/+)/PZ(−/−) progeny derived from FV(+/+)/PZ(+/−) intercrosses is 1:1, instead of the anticipated 1:2 (p < 0.01) (Table 1A). Moreover, the survival of FV(+/+)/PZ(−/−) mice is significantly less than that of FV(+/+)/PZ(+/−) animals (p < 0.01) (Table 1B) showing that the level of PZ affects the phenotype of heterozygous FV\textit{Leiden} mice as well.

Of the nine pups found dead during the six weeks following birth, one had the FV(+/+)/PZ(−/−) genotype, three the FV(+/+)/PZ(+/−) genotype and five the FV(+/+)/PZ(+/+) genotype, implying that many of the FV(+/+)/PZ(−/−) and FV(+/+)/PZ(+/−) mice were lost in utero or during the perinatal period. To determine whether the PZ genotype affected the intrauterine survival of FV(+/+)/ mice and to obtain appropriate tissues for histological analysis, embryos derived from FV(+/+)/PZ(+/−) intercrosses were examined at late gestation. FV(+/+)/PZ(−/−) and FV(+/+)/PZ(+/+) embryos were present at embryonic day E17.5-E18.5 in expected numbers, but eight of the nine FV(+/+)/PZ(−/−) showed obvious signs of hemorrhage by visual inspection (Table 1A). Anti-fibrinogen immunohistochemical analysis of the FV(+/+)/ embryos showed vascular thrombosis and hepatic fibrin deposition, the scope of which was directly related to the PZ genotype [PZ(−/−)>PZ(+/−)>PZ(+/+)].

In summary, when combined with the homozygous factor V\textit{Leiden} genotype, PZ deficiency causes intrauterine and perinatal thrombosis and an apparent consumptive coagulopathy that leads to near absolute mortality. The genetic combinations FV(+/+)/PZ(+/−) and FV(+/+)/PZ(−/−) produce smaller, although significant reductions in survival. The results are consistent with human data showing that a combination of prothrombotic traits significantly increases the risk of thrombosis and underscores the multigenic nature of thrombophilia.

**PZ/ZPI and Human Thrombosis**

The \textit{in vitro} and \textit{in vivo} studies suggest that PZ plays an important role in dampening coagulation. The cofactor effect of PZ for the inactivation of factor Xa by ZPI is presumably an important part of this regulatory action of PZ, but additional, ZPI-independent, effects of PZ have not been excluded. In contrast to TFPI null and protein C null mice (40, 41), which develop lethal disseminated intravascular coagulation, homozygous PZ deficient mice have an apparently normal phenotype, at least in a mixed C57Bl/6 × 129SV genetic background and in the absence of a thrombotic challenge. In this regard they are similar to FV(+/+), TFPI(+/−), and PC(+/−) mice which are also asymptomatic in the unchallenged state (39-41). Like the PZ(+/−) genotype, the TFPI(+/−) genotype in combination with the FV(+/+)/ genotype produces near absolute mortality (42). Thus, the thrombotic risk associated with homozygous PZ deficiency appears similar to that of heterozygous TFPI deficiency in the mouse. Based on the data from the murine gene-deletion models, it has been speculated that PZ deficiency could be a modest thrombotic risk factor in humans (42).

Vasse et al. have reported a significant association between prior ischemic stroke and PZ deficiency (<1.0 µg/mL) (43). In contrast, they found no association between venous thromboembolism and PZ deficiency. A relationship between PZ deficiency and arterial, but not venous, thrombosis would be of interest. Although inherited defects in other natural anticoagulant pathways, for example deficiencies of antithrombin or protein C, and the factor V\textit{Leiden} genotype, have readily been shown to increase the risk of venous thromboembolism, their effect on arterial thrombosis has been more difficult to demonstrate and remains controversial (44). In part this disparity may reflect an overriding contribution of atherosclerotic vascular disease to arterial thrombosis in older individuals. Studies that have reported positive results have frequently involved selected groups of young patients (45, 46).

The stroke patients studied by Vasse et al. were also a very select group (43). Their mean age was 33 years, 65% were female, and none had hypertension or dyslipidemia. The study was retrospective in nature and PZ levels were determined after the index stroke. There is no reason, however, to suspect that PZ deficiency improves survival following stroke or that the antithrombotic therapy that many of these patients were likely receiving would affect their plasma concentration of PZ. The patients reportedly were not receiving oral anticoagulants and this is critical in view of the marked depression in PZ plasma levels produced by these drugs. Comparison of the relative frequencies of low PZ levels in the control group and in the stroke patients suggested that PZ deficiency increases the risk of stroke about 4-fold. These selected patients, however, represent only a small fraction of all stroke patients and it seems likely that the relative risk of stroke associated with PZ deficiency may be lower in older subjects with vascular disease and a variety of medical conditions. Nevertheless, given the high incidence of stroke and the fact that 10% of a general population (Red Cross blood donors) have PZ levels of <50% (6), even a small increase in the risk of stroke with PZ deficiency in older individuals could be important in public health terms. In the Vasse et al. study, PZ deficiency did not appear to affect the risk of venous thrombosis. Only a relatively small number of selected subjects were evaluated, however, and a preliminary report suggests that concomitant PZ deficiency may reduce the age of onset and increase the number of venous thrombotic episodes in factor V\textit{Leiden} patients (47).

The report of Vasse et al. clearly requires confirmation (43). Moreover, additional clinical studies will be required to define the potential roles of both PZ and ZPI in human thromboembolic disease.

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