Protein Z, an anticoagulant protein with expanding role in reproductive biology

Wassim Y Almawi, Fatima S Al-Shaikh, Ohannes K Melemedjian and Ahmad W Almawi

Department of Medical Biochemistry, College of Medicine and Medical Sciences, Arabian Gulf University, PO Box 22979, Manama, Bahrain, Department of Pharmacology, University of Arizona, Tucson, Arizona, USA and Department of Biochemistry, McMaster University, Hamilton, Ontario, Canada

Correspondence should be addressed to W Y Almawi; Email: wassim@agu.edu.bh

Abstract

Protein Z (PZ) is a vitamin K-dependent factor characterized by its homology to other vitamin K-dependent factors (factors VII, IX, and X, protein C and protein S), but lacks any enzymatic activity. Instead, PZ acts as a cofactor for the inhibition of factor Xa through the serpin PZ-dependent protease inhibitor (ZPI). PZ deficiency is associated with a procoagulant state, highlighted by excessive FXa secretion and thrombin production, and is linked with several thrombotic disorders, including arterial vascular and venous thromboembolic diseases. A role for the PZ–ZPI complex in the regulation of physiological pregnancy has been demonstrated, highlighted by the progressive elevation in PZ levels in the first trimester of gestation, which then steadily decline toward delivery. An association between altered plasma PZ concentrations and adverse pregnancy outcomes (recurrent miscarriage, stillbirth, preeclampsia, intrauterine growth restriction, and placental abruption) has been reported. The mechanism by which PZ deficiency leads to adverse pregnancy outcomes is not clear, but it is multifactorial. It may be attributed to the anti-PZ IgG and IgM autoantibodies, which apparently act independently of classical antiphospholipid antibodies (lupus anticoagulant, anticardiolipin, and anti-

Introduction

The vitamin K-dependent anticoagulant plasma glycoprotein protein Z (PZ) was first isolated from bovine plasma in 1977 and from human plasma in 1984 and was shown to play an important role in the regulation of the coagulation cascade (Broze 2001, Vasse 2008). While in vitro studies have shown that bovine PZ could promote the assembly of thrombin with phospholipid surfaces, thereby enhancing coagulation, the human PZ form binds to thrombin poorly, with very little effect on the association of thrombin binding with phospholipids. More recent studies have shown that PZ forms a calcium ion-dependent complex with factor Xa on phospholipid surfaces, thereby serving as a cofactor for the inhibition of factor (F) Xa through a PZ-dependent protease inhibitor (ZPI; Huang et al. 2012).

Deficiency in PZ secretion and/or function is linked with a procoagulant state and several thrombotic disorders, including arterial and venous thrombosis. As the outcome of pregnancy is dictated to a large extent by the maintenance of adequate maternal and fetal blood circulation and as coagulation abnormalities are associated with adverse pregnancy outcomes, a role for the PZ–ZPI complex in the regulation of pregnancy has been suggested, and an association between altered plasma PZ levels and adverse pregnancy outcomes has been reported, often with apparently contradictory conclusions. This review summarizes the relationship between adverse pregnancy outcomes and acquired and constitutional PZ–ZPI deficiency, in order to understand whether or not PZ deficiency could be considered as a risk factor for poor pregnancy outcomes.

Biochemistry of PZ

PZ is a 62 kDa vitamin K-dependent single-chain glycoprotein, consisting of 360 amino acids containing a N-terminal γ-carboxyglutamic acid (Gla) domain necessary for its effective secretion (Souri et al. 2009), followed by two epidermal growth factor-like domains (light chain homolog), and a C-terminal pseudo-catalytic domain (heavy chain homolog) (Vasse 2008; Fig. 1).
endothelial cells (Kusanovic et al. 2007), have been reported on the production of PZ by human placental cells (Kemkes-Matthes & Matthes 1995). Conflicting findings are reported regarding the level of PZ in patients with chronic liver diseases (Ahmed et al. 2009). The liver is the main source of PZ, and plasma PZ levels are reduced in patients with chronic liver diseases (Koren-Michowitz et al. 2006). Several mechanisms by which PZ acts as a cofactor in the modulation of the activity of ZPI, which include direct interaction of PZ with both FXa and ZPI at phospholipid surfaces (Dayer et al. 2012), have been postulated. Specific interactions between the PZ Gla domain and the FXa Gla domain (Gla–Gla interaction) have been suggested to accelerate the inhibition rate (Huang et al. 2010, 2012, Dayer et al. 2012). PZ has also been suggested to induce structural changes in ZPI (Huang et al. 2012), whereby PZ aligns the inhibitory site of ZPI with the active site of FXa (Huang et al. 2010, Karimi et al. 2012; Fig. 3). This alters the secretion, localization, and clearance of ZPI (Broze 2001), hence facilitating the interaction between ZPI and FXa. Irrespective of the mechanism, the PZ–ZPI complex prevents thrombin generation in the early phases of coagulation, before the formation of the prothrombinase complex (Huang et al. 2010, 2012).

The PZ–ZPI complex exerts its anticoagulant effect through the inactivation of phospholipid-bound FXa. While ZPI can inhibit FXa, its complexing with PZ accelerates the ZPI-mediated inhibition of FXa by 1000-fold (Al-Shanqeeti et al. 2005, Koren-Michowitz et al. 2006). Several mechanisms by which PZ acts as a cofactor in the modulation of the activity of ZPI, which include direct interaction of PZ with both FXa and ZPI at phospholipid surfaces have been postulated (Fig. 3). Specific interactions between the PZ Gla domain and the FXa Gla domain (Gla–Gla interaction) have been suggested to accelerate the inhibition rate (Huang et al. 2010, 2012, Dayer et al. 2012). PZ has also been suggested to induce structural changes in ZPI (Huang et al. 2012), whereby PZ aligns the inhibitory site of ZPI with the active site of FXa (Huang et al. 2010, Karimi et al. 2012; Fig. 3). This alters the secretion, localization, and clearance of ZPI (Broze 2001), hence facilitating the interaction between ZPI and FXa. Irrespective of the mechanism, the PZ–ZPI complex prevents thrombin generation in the early phases of coagulation, before the formation of the prothrombinase complex (Huang et al. 2010, 2012).

Although structurally related to other coagulation serine proteases (FVIIa, FIXa, FXa, and activated protein C), PZ lacks significant protease activity due to the presence of only Asp in its active center and lack of histidine and serine residues in the catalytic triad (replaced by Ala and Thr residues respectively).

PZ acts as a cofactor for the 72 kDa serpin ZPI, which rapidly (1/2 < 10 s) inhibits FXa and FXa (Heeb et al. 2005, Huang et al. 2012), hence reducing thrombin generation (Koren-Michowitz et al. 2006, Vasse 2008, 2011; Fig. 2). Relative to PZ, ZPI is present in the plasma at higher levels (Han et al. 2000, Tabatabai et al. 2001), where it binds to all PZ at a 1:1 ratio (Han et al. 2000, Tabatabai et al. 2001), hence circulating as a stable PZ–ZPI complex, with virtually no free PZ being detected (Corral et al. 2007, Vasse 2008). Compared with other vitamin K-dependent factors, PZ exhibits 100-fold slower membrane binding and dissociation kinetics, due to the presence of an additional Gla residue at position 11 in the PROZ (PZ) protein (Vasse 2008, Souri et al. 2009).

Human PROZ gene is located on chromosome 13q34, in proximity to F8 (FVIII) and TSTA3 (FX) genes (Fig. 1). The PROZ gene spans 15 kb and is organized into nine exons, including an alternative exon (Souri et al. 2009). The liver is the main source of PZ, and plasma PZ levels are reduced in patients with chronic liver diseases (Kemkes-Matthes & Matthes 1995). Conflicting findings have been reported on the production of PZ by human endothelial cells (Kusanovic et al. 2007). In the liver, 30% of the synthesized PZ is converted to Gla inside the cells, before it is secreted into the plasma in a vitamin K- and Gla-30-dependent process (Vasse 2008, Souri et al. 2009). While PZ has a long half-life (2.5 days; Miletich & Broze 1987, Kusanovic et al. 2007), the half-life of the PZ–ZPI complex compared with that of either free ZPI or free PZ remains to be established (Kusanovic et al. 2007).

**Figure 1** Organization of the PZ (PROZ) and ZPI genes and protein products.

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**Figure 2** ZPI–PZ complex. (A) Crystal structure of the ZPI (blue)–PZ (purple) complex (PDB ID: 3H5C). (B) ZPI–PZ complex interaction domains showing amino acids interacting at the interface. Labeled interacting amino acid residues (shown as stick representations) of ZPI and PZ are K68, D74, D238, K239, N261, D292, and T296, while those of PZ are H210, R212, E219, E244, H250, R298, R350 and Q357 and are colored by specific elements (carbon, green; nitrogen, blue; and oxygen, red). Images were prepared using PyMol.
The interaction between ZPI and FXa is a reversible event (Han et al. 2000, Heeb et al. 2005). In contrast to other serpin complexes, ZPI is proteolytically and rapidly cleaved at its C-terminus, which reduces its size from 72 to 68 kDa, resulting in an inactive ZPI with little or no functional activity (Broze 2001, Huang et al. 2010, 2012), and hence low free FXa levels for ZPI binding (Broze 2001). The resultant ZPI constitutes a poor substrate for the FXa–PZ complex (Broze 2001, Huang et al. 2012).

**PZ synthesis and plasma levels**

Mean plasma PZ levels range from 1.16 to 2.71 μg/ml (Miletich & Broze 1987) and are influenced by genetic and non-genetic factors, which include chronic liver diseases, age, gender, vitamin K levels, concurrent use of anticoagulants, and pregnancy (Al-Shanqeeti et al. 2005, Vasse 2008, Souri et al. 2009). Variations in PZ levels have been reported with regard to age, gender, and ethnic origin. Plasma PZ levels rapidly increase during the first few months of childhood and then slowly taper off, with adult levels being reached during puberty (Miletich & Broze 1987, Gowri et al. 2011), and higher PZ levels have been reported in men than in women (Miletich & Broze 1987). PZ levels are reduced in prothrombotic states, associated with excessive FXa secretion and thrombin production (Al-Shanqeeti et al. 2005, Koren-Michowitz et al. 2006). A recent meta-analysis has demonstrated that reduced PZ levels are linked with an increased risk of thrombotic events, such as arterial vascular and venous thromboembolic diseases (Sofi et al. 2010), and pregnancy complications. The latter include preeclampsia (PE; Erez et al. 2007, 2009), early fetal death (Vasse 2011), intrauterine growth restriction (Bretelle et al. 2005), and recurrent spontaneous miscarriage (Topalidou et al. 2009, Alshaikh et al. 2013).

Whether PZ deficiency constitutes an independent risk factor for thrombosis is inconclusive (Vasse et al. 2002, Al-Shanqeeti et al. 2005, Martinelli et al. 2005), since the association of PZ deficiency with an increased risk of thrombosis has been reported in the presence of other prothrombotic risk factors such as FV Leiden (Kemkes-Matthes & Matthes 1995, Martinelli et al. 2005), prothrombin G20210A mutation, and hyperhomocysteinemia (Martinelli et al. 2005). It has been further suggested that PZ deficiency does not constitute an independent risk factor for venous thromboembolism (VTE), but only increases the VTE risk with FV Leiden. Furthermore, a rare inherited coagulation disorder, hereditary combined vitamin K-dependent clotting factor deficiency (VKCFD), has been reported (Napolitano et al. 2010). This disorder results in a deficiency of the clotting factors FII, FVII, FIX, and FX and the coagulation inhibitors protein C, protein S, and PZ. VKCFD is linked with bleeding tendency with a variegated clinical picture and results from mutations of two enzymes of the vitamin K cycle: (type 1) defective γ-glutamyl carboxylase (Soute et al. 1992) or (type 2)
functional deficiency in vitamin K 2,3-epoxide reductase complex (Oldenburg et al. 2000).

**PZ in pregnancy**

Pregnancy is associated with a state of hypercoagulation linked with excessive thrombin generation, which is crucial for controlling bleeding at delivery (Kist et al. 2008, Pabingert 2008). Growing evidence implicates coagulation abnormalities in adverse pregnancy outcomes, including recurrent and non-recurrent pregnancy losses (Pabingert 2008, Gris 2009), intrauterine growth retardation, placental abruption (Alfirevic et al. 2002), intrauterine fetal death, PE, and maternal or neonatal thrombosis (Michels & Tiu 2007). This has been evidenced by the reported increases in the levels of the clotting factors FVIII, FX, and Von Willebrand factor, along with those of FVII, which increase by 200% compared with pre-pregnancy levels, and those of fibrinogen, which gradually increase till they reach 1000% (Thornton & Douglas 2010). In addition, a decreased quantity of natural anticoagulants, such as protein S and protein C, and a reduction in the overall fibrinolytic activity accompany most pregnancy complications (Thornton & Douglas 2010). Both heritable and acquired thrombophilias have been implicated in pregnancy-associated hypercoagulation (Kist et al. 2008, Pabingert 2008), which include antithrombin III, protein C, and protein S deficiencies; altered activity of procoagulant factors, in particular, those precipitated by FV Leiden; and the prothrombin G20210A mutations. On the other hand, antiphospholipid antibodies (lupus anticoagulant and anticardiolipin antibodies) are responsible for the most common acquired thrombophilias linked with adverse pregnancy outcomes.

Progressively higher PZ levels have been observed with increasing gestational age in normal pregnancies, especially among obese pregnant women (Ramsay et al. 2005), which return to normal levels at around 6–12 weeks (Thornton & Douglas 2010), and have been attributed to pregnancy-associated imbalance of fibrinolytic and hemostatic mechanisms (Kusanovic et al. 2007). PZ levels correlate with gestational age, evidenced by the progressive increases in PZ levels (20%) during the three trimesters of pregnancy, which can be attributed to the compensatory mechanisms induced by increased FXa concentrations (Erez et al. 2007), and they decline thereafter by as much as 30%, to levels below those observed in the first trimester. The increase in PZ levels becomes attenuated in patients with abnormal pregnancy outcomes, including low-birthweight babies or pregnancy-associated hypertension or diabetes (Gowri et al. 2011).

**PZ in pregnancy complications**

Insofar as PZ deficiency represents a procoagulant state and as pregnancy is linked with an aggravation of the procoagulant state that translates into adverse complications or poor outcomes, an association between altered plasma PZ concentrations and pregnancy complications has been reported. Reduced PZ levels (<1 μg/ml) have been linked with several pregnancy complications including fetal demise (Erez et al. 2009), intrauterine growth restriction (Bretelle et al. 2005), PE (Erez et al. 2009), small for gestational age (SGA) (Erez et al. 2009), HELLP syndrome (H, hemolysis; EL, elevated liver enzymes; LP, low platelet counts), which represents a severe form of PE and can be observed with or without preceding PE (Kaygusuz et al. 2011), and idiopathic recurrent miscarriage (Gris et al. 2002, AlShaikh et al. 2013).

An earlier French study has demonstrated a high prevalence of PZ deficiency in women with a first primary early (10–15 weeks of gestation) miscarriage, but not with recurrent embryonic loss (before 8 weeks of gestation), which is distinct from classical thrombophilia (Gris et al. 2002). This deficiency is unrelated to deficiencies of other vitamin K-dependent coagulation factors, including protein C and FVII, and persists despite vitamin supplementation (Gris et al. 2002). PZ deficiency has thus been proposed as a significant determinant of adverse pregnancy complication-associated thrombophilia. In light of changes in maternal–fetal circulation in the first trimester of pregnancy, the authors have suggested that PZ deficiency most probably favors a state of local thrombogenesis (Gris et al. 2002). PZ deficiency has been subsequently attributed to the presence of anti-PZ-specific IgG and IgM antibodies, the titers of which are inversely correlated with PZ concentrations in patients with recurrent fetal losses and with PZ deficiency (Gris et al. 2003). We later confirmed this in Bahraini women with idiopathic recurrent miscarriage (Sater et al. 2011).

More recent studies, including a meta-analysis (Sofi et al. 2010) and our case–control Bahraini study (AlShaikh et al. 2013), have confirmed the strong relationship between low PZ levels and adverse pregnancy complications. The meta-analysis of Sofi et al. (2010) involving 714 patients and 515 controls has demonstrated a strong association of PZ deficiency with pregnancy complications (OR (95% CI) = 3.42 (2.51–4.66)). The study of AlShaikh et al. (2013) on 282 recurrent miscarriage cases and 281 control women has also demonstrated an almost fourfold increased risk of fetal loss with PZ deficiency, which is influenced by the specific PROZ genotypes (see below). PZ deficiency has also been observed in women with the HELLP syndrome; median PZ levels in patients with the HELLP syndrome and PZ levels correlate with platelet counts and changes in liver enzyme (LDH and AST) levels, thus prompting the speculation that this may be a consequence of a preceding liver dysfunction (Kemkes-Matthes & Matthes 1995).

The lack of a relationship between PZ deficiency and pregnancy complications has also been reported by smaller studies. A high prevalence of PZ deficiency is
associated with PE, evidenced by lower maternal plasma PZ concentrations in PE women than in women with normal pregnancies (Paidas et al. 2005, Erez et al. 2007). This was in contrast to the findings of the Bretelle study in which median plasma PZ concentrations were similar for PE patients and women with uncomplicated pregnancies (Bretelle et al. 2005). This has been attributed to the small sample size (50 non-pregnant and 34 healthy pregnant control women and 61 women with complicated pregnancies) and heterogeneity in patient presentation (PE, intrauterine growth restriction, and intrauterine fetal demise) and also to differences in ethnicity (Bretelle et al. 2005). The study of Grandone et al. (2004) has also reported that PZ deficiency is not linked with unexplained fetal loss, which is due to the small sample size, low plasma PZ cut-off values (1.43 ± 0.76 μg/ml in healthy controls), and exclusion of women with known inherited (FV Leiden or FII G20210A mutations and protein C, protein S, or antithrombin deficiency) or acquired (antiphospholipid antibodies) thrombophilia. The above-mentioned studies involved lower numbers of subjects compared with the studies of Gris et al. (2002, 2003) and Sofi et al. (2010), and our study (Sater et al. 2011, AlShaikh et al. 2013), indicating study under-power. Interestingly, both the Bretelle et al. (2005) and Erez et al. (2007) studies have been reported as the main contributors to statistical heterogeneity for pregnancy complications in the meta-analysis of Sofi et al. (2010) (P for heterogeneity from 0.002 overall to 0.17 after their exclusions).

**PZ autoantibodies and adverse pregnancy outcomes**

The mechanism by which PZ deficiency leads to poor pregnancy outcomes is not clear and may be attributed to anti-PZ IgG and IgM autoantibodies (Gris et al. 2003, Sater et al. 2011) and the presence of functional mutations in the PROZ gene, in particular, in the G79A variant (Dossenbach-Glaninger et al. 2008, El-Hamid & El-Khayat 2011, AlShaikh et al. 2013). An earlier report by Gris involving 171 women with pathological pregnancies and 191 multiparous control women has demonstrated high levels of anti-PZ IgG and IgM antibodies, which are distinct from classical antiphospholipid/anticofactor antibodies, and a dose-effect relationship between anti-PZ antibody levels and poor pregnancy outcomes has been documented (Grandone et al. 2004). The association of high anti-PZ IgG and IgM autoantibody titers with poor pregnancy outcomes has been independently confirmed later in different populations (Paidas et al. 2005, Erez et al. 2009, Sater et al. 2011). Although the association between PZ levels and the presence of these autoantibodies has not been confirmed by all studies (Paidas et al. 2005), the combination of PZ deficiency and high anti-PZ autoantibody titers has been linked with an increased risk of pregnancy loss (Gris et al. 2003, Kusanovic et al. 2007).

Two small independent studies have yielded contradictory findings (Sailer et al. 2008, Erez et al. 2009). Though it did not reach statistical significance, the study of Sailer et al. (2008) has reported a trend to significance in the association of anti-PZ antibodies with adverse pregnancy outcomes. This is probably due to the low number of cases included, which resulted in adopting the 75th percentile of control subjects as the upper limit of the normal range for comparison, since the number of subjects whose antibody levels exceeded the 90th percentile of the controls was very low (Sailer et al. 2008). The study of Erez et al. (2009) involving 51 women has suggested that heightened anti-PZ antibody levels are not associated with fetal death, but rather with SGA, and that a high maternal anti-PZ IgM titer is linked with vascular placental lesions in PE patients but not in SGA neonate patients, thus prompting the conclusion that the pathological effects of anti-PZ antibodies are observed in select patients. In these studies, anti-PZ antibodies have been detected in varying titers in non-pregnant patients, thereby raising the speculation that anti-PZ antibodies constitute natural antibodies (Erez et al. 2009). Apart from the study of Gris et al. (2003), most of these studies did not address the correlation between plasma PZ levels and anti-PZ antibody titers and that pregnancy complications are observed only in patients with high titers of anti-PZ antibodies (Gris et al. 2003, Sater et al. 2011).

The mechanism by which anti-PZ autoantibodies contribute to adverse pregnancy outcomes remains to be established. Maternal plasma IgM anti-PZ autoantibody concentration > 90th percentile has been associated with vascular placental lesions in PE patients, which results in abnormal placentaion and pregnancy complications (Erez et al. 2009). Anti-PZ antibodies may also act by rapidly clearing PZ, either by enhancing immune complex formation associated with cellular or complement activation or/and by inducing the formation of inactive antibody-coated PZ molecules (Gris et al. 2003, Dorner et al. 2005). The latter mechanism is more plausible, as it has been shown to precipitate maternal hypercoagulation (Gris et al. 2003). Taken together, anti-PZ antibodies acting independently of classical antiphospholipid antibodies (lupus anticoagulant, anticardiolipin, and anti-beta2-glycoprotein I antibodies) may predict the risk of pathologic pregnancies. This has been supported by the existent dose-effect relationship between anti-PZ antibody levels and adverse pregnancy outcomes (Gris et al. 2003, Sater et al. 2011).

**PROZ/SERPINA10 (ZPI) polymorphisms in adverse pregnancy outcomes**

The wide variability in plasma PZ levels is attributed to the presence of genetic factors influencing PZ biosynthesis (Rice et al. 2001, Vasse et al. 2002), and common and rare gene variants have been reported in the PROZ
The G79A PROZ gene variant (rs3024735) has been the most investigated and linked with several coagulation disorders, including stroke (van Goor et al. 2008), thromboembolism (Nowak-Göttl et al. 2009), coronary artery disease (Le Cam-Duchez et al. 2009), and poor pregnancy outcomes (Lichy et al. 2004, AlShaikh et al. 2013). Few studies have investigated the association of PROZ gene polymorphisms with poor pregnancy outcomes, with inconclusive findings. A small Egyptian study involving 40 women with recurrent miscarriage and 30 control women has reported on the higher prevalence of the G79A minor allele in controls than in cases, suggesting a protective role of the 79A allele in recurrent miscarriage (El-Hamid & El-Khayat 2011). Similarly, an Austrian study involving 49 cases and 48 control women has reported that the 79A allele, individually and in combination with other thrombophilic risk factors (factor V Leiden and increased factor VIII activity), is associated with lower ZP concentrations and a reduced risk of early (8–12 weeks of gestation) spontaneous fetal loss (Dosensenbach-Glaninger et al. 2008).

In addition, two Greek studies involving small numbers of women with idiopathic fetal loss and controls have documented that while plasma ZP levels are significantly lower in the 79A allele carriers, the frequency of the 79A allele is similar between the cases and control women (Effraimidou et al. 2009, Topalidou et al. 2009). This suggests that low ZP levels, more than G79A, constitute a risk factor for adverse pregnancy outcomes. In contrast to these studies, we documented that the G79A minor allele was associated with an increased risk of adverse pregnancy outcomes in 287 Bahraini women with idiopathic miscarriage and 308 control women (AlShaikh et al. 2013), and both susceptible and protective PROZ haplotypes were identified (AlShaikh et al. 2013). These inconsistencies may be explained by differences in the genetic background of the studied populations, selection of cases and controls, ZP inter-individual variability, and small size of the cohorts in mainly retrospective studies.

In addition to the G79A variant, other PROZ variants have been shown to be associated with pregnancy-associated adverse effects. For example, the G-42A PROZ promoter variant has been associated with fetal losses (Grandone et al. 2008) and pulmonary embolism, but not with pregnancy-related deep venous thrombosis (Grandone et al. 2009). Furthermore, both rs3024719 (G103A) and rs3024731 (T119A) PROZ promoter variants have been associated with reduced ZP levels and an increased risk of fetal loss (AlShaikh et al. 2013). Based on the LD pattern between PROZ variants (van Goor et al. 2008, Le Cam-Duchez et al. 2009, AlShaikh et al. 2013), specific PROZ haplotypes have recently been shown to be associated with poor pregnancy outcomes (AlShaikh et al. 2013). Larger studies on different ethnic groups are needed to confirm the nature of the association of PROZ G79A and other variants with adverse pregnancy outcomes.

Few studies have investigated the link between SERPINA10 mutations and coagulation defects in humans, often with inconclusive results. ZPI deficiency resulting from mutations in the SERPINA10 gene contributes to thrombotic events (van De Water et al. 2004) and is associated with many coagulation disorders including venous thrombosis (Al-Shanqeeti et al. 2005) and atherosclerotic peripheral arterial disease (Sofi et al. 2010). Several mutations in the coding region of the SERPINA10 gene have been reported by van de Water, of which the nonsense mutations R67X and W303X create stop codons and thus lead to ZPI deficiency due to altered ZPI levels given their location within structurally important sites within the SERPINA10 gene (van De Water et al. 2004). R67X and W303X SERPINA10 variants have been associated with venous thrombembolism in New Zealander (van De Water et al. 2004) and Spanish (Corral et al. 2007) patients, but not in Italian patients (Razzari et al. 2006). Both SERPINA10 mutations were absent in Italian (Fabbro et al. 2007), Caucasian (Folsom et al. 2007), and different groups of Spanish populations (Gonzalez-Conejero et al. 2005), suggesting ethnic restriction in the distribution of these mutations (Gonzalez-Conejero et al. 2005, Fabbro et al. 2007). A lone study has reported a strong association of R67X (OR=2.66), and to a lesser extent W303X (OR=2.44), SERPINA10 variants and identified SERPINA10 haplotypes with early fetal loss, but not with embryonic miscarriages (AlShaikh et al. 2012). This extends the involvement of the genetic variants in the SERPINA10 and PROZ loci in determining the overall risk of adverse pregnancy outcomes.

**Conclusion**

The role of the ZP–ZPI complex in normal pregnancies and pregnancy complications as a systemic or a local regulator remains unclear. By regulating FXa activity, the presence of the ZP–ZPI complex may provide a local defense mechanism against vascular injury accompanying poor placentation and fetal loss. As such,
reduced PZ–ZPI activity stemming from specific anti-PZ autoantibodies and/or polymorphisms within the PROZ and SERPINA10 genes precipitates adverse pregnancy outcomes. PZ deficiency can also be acquired, and the role of contributing factors such as inflammation, obesity, smoking, hypertension, and autoimmunity in affecting plasma PZ and ZPI levels remains to be established. The apparently contradictory results reported for plasma PZ levels and the contribution of the PROZ and SERPINA10 polymorphisms can be explained by the limited number of individuals enrolled and the choice of the control groups. In conclusion, the exact role of the PZ–ZPI complex in the pathogenesis of poor pregnancy outcomes remains to be established, but cannot be dismissed, and future adequately powered studies that address the contribution of inherited and acquired risk factors are necessary.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

Funding

This review did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Acknowledgement

The authors thank Ms Parambil Puthen V K Minimol for her assistance in preparing the manuscript.

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