REPRODUCTION
REVIEW

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

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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-β2-glycoprotein I antibodies). PZ deficiency has also been reported to be constitutional, and a number of variants in the *PROZ* (PZ) gene and *SERPINA10* (*ZPI*) gene are linked with specific adverse pregnancy complications. 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.

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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 acquired and constitutional PZ–ZPI deficiency and adverse pregnancy outcomes, in particular, whether PZ deficiency could be 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).

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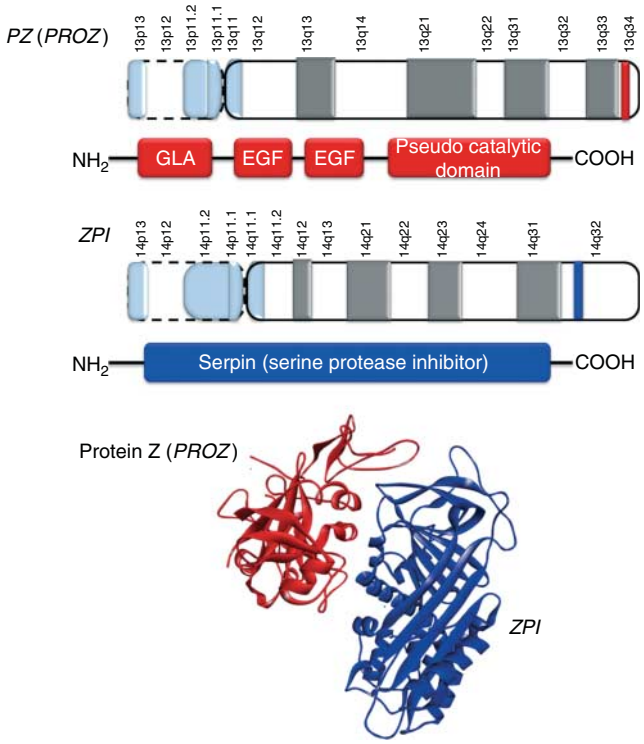

Figure 1 Organization of the *PZ* (*PROZ*) and *ZPI* genes and protein products.

Although structurally related to other coagulation serine proteases (FVIIa, FIXa, FXa, and activated protein C), PZ lacks significant proteinase 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 ($t^1/_2$ <10 s) inhibits FXa and FXIa (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* (*FVII*) 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).

PZ function

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 (Dayer *et al.* 2012), forming the FXa–ZPI–PZ complex at the phospholipid surfaces (Dayer *et al.* 2012), 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).

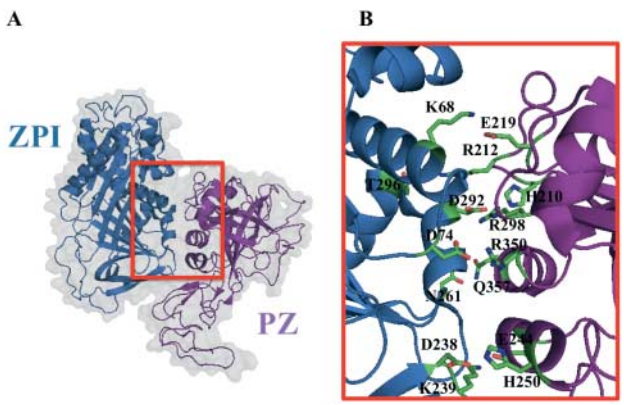

Figure 2 ZPI–PZ complex. (A) Crystal structure of the ZPI (blue)–PZ (purple) complex (PDB ID: 3H5C). (B) ZPI–PZ complex interaction domains showing amino acids interacting at the interface. Labeled interacting amino acid residues (shown as stick representations) of ZPI are K68, D74, D238, K239, N261, 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.

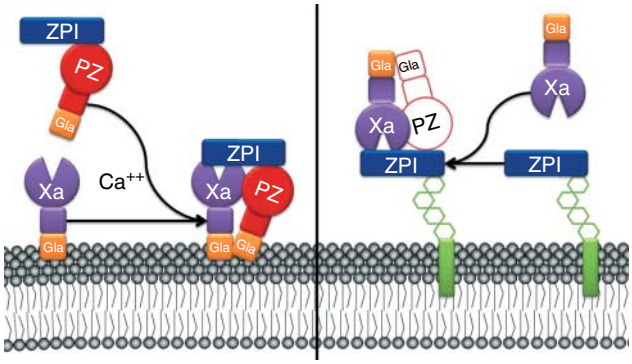

Figure 3 Two mechanisms proposed for the PZ-mediated inhibition of factor Xa by ZPI. (Left) Pre-formed circulating PZ–ZPI complex binds to factor Xa bound to phospholipid surfaces through the interaction of the Gla domain of PZ (in orange) and interacts with the Gla domain of factor Xa (in orange), (Right) ZPI is activated by glycosaminoglycans found on endothelial cell surface and as such may directly engage factor Xa and PZ.

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 inflammation (Krawiec *et al*. 2011), and a negative correlation between PZ levels and plasma interleukin 1 (IL1) and IL6 levels has been reported (Undar *et al*. 1999, Vasse *et al*. 2002). Contradictory findings have also been reported, with both increased and decreased PZ levels being observed in obese individuals, which have been attributed to the altered expression of inflammatory cytokines associated with obesity (Ramsay *et al*. 2005), presence of anti-PZ antibodies (Pardos-Gea *et al*. 2008, Sater *et al*. 2011), ethnic background of the study subjects, and possibly increased excretion of PZ.

Among the studies on PZ levels, only four studies have addressed plasma ZPI levels, but with inconclusive findings. An association between reduced ZPI levels and peripheral arterial disease (Sofi *et al*. 2010) and venous thrombosis (Al-Shanqeeti *et al*. 2005) has been documented, while no difference in ZPI levels has been reported between normal controls and antiphospholipid antibody-positive cases. A recent report by Souri *et al*. (2012) has demonstrated higher plasma ZPI concentrations (measured by in-house ELISA) in pregnant women than in non-pregnant women, which was paralleled by increased FX levels, which were suggested to contribute to the maintenance of adequate placental circulation. Whereas plasma ZPI levels did not change in non-pregnant women with a history of recurrent miscarriage, plasma PZ levels were slightly reduced, suggesting a link between recurrent miscarriage and this paradoxical unaltered ZPI–mild PZ reduction, when compared with normal pregnancies (Souri *et al*. 2012).

PZ deficiency

PZ deficiency (<1 μg/ml) is linked with a procoagulant state, 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 variegate 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 Brettele 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 placentation 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-β2-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*

locus (Rice *et al*. 2001, van Goor *et al*. 2008, Le Cam-Duchez *et al*. 2009, AlShaikh *et al*. 2013). The differential linkage disequilibrium (LD) pattern between *PROZ* variants controlling PZ production (Rice *et al*. 2001, van Goor *et al*. 2008, Le Cam-Duchez *et al*. 2009), and thus disease association (Rice *et al*. 2001, Nowak-Göttl *et al*. 2009, AlShaikh *et al*. 2013), indicates that PZ deficiency is, at least in part, constitutional (Miletich & Broze 1987). While the functional aspects of these variants have not been thoroughly assessed, some have been shown to influence plasma PZ levels, in particular G79A, A13G, and G42A; the lowest plasma PZ levels have been observed with the G/G genotype (A13G) and the A/A (G42A) and A/A (G79A) homozygous variant genotypes (Lichy *et al*. 2004).

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 PZ concentrations and a reduced risk of early (8–12 weeks of gestation) spontaneous fetal loss (Dossenbach-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 PZ 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 PZ 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, PZ 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 PZ 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 thromboembolism 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 PZ–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 PZ–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.

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