Protein Z gene variants and risk of Idiopathic Recurrent Pregnancy Loss in Saudi Arabian women

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ABSTRACT. Background: Protein Z (PZ) is a vitamin K-dependent plasma glycoprotein, it plays a key role in the physiologic inhibition of coagulation by acting as a cofactor in the inactivation of factor Xa. The relationship between PZ gene polymorphisms and pregnancy loss is controversial. To address this, we investigate the association between PZ (rs3024718, rs3024719, rs3024731, rs3024778 and rs3024772) single nucleotide polymorphisms (SNPs) and idiopathic recurrent pregnancy loss (IRPL) in in women from Tabuk region (North western region, Saudi Arabia).

Methods: A case control study, including Sixty-three women with at least three unexplained recurrent pregnancy loss (RPL) were selected and matched with seventy-eight healthy and fertile women (controls). SNP genotyping was carried out by allelic discrimination using real time PCR.

Results: The outcome was that the minor allele frequencies (MAF) are 0.23 vs 0.20, 0.24 vs 0.19, 0.02 vs 0.02, 0.26 vs 0.21, and 0.02 vs 0.01 respectively for rs3024718 A/G, rs024719 G/A, rs3024778

G/A, rs3024731 G/A, and rs3024772 A/G PZ polymorphisms. The genotype distribution was similar between women with RPL and control women (P>0.05) and none of the tested SNPs were associated with RPL under co-dominant, dominant, or recessive genetic models. The lack of association was also confirmed by haplotypes analysis showing an absence of RPL risk with the constructed haplotypes.
Conclusion: It was concluded that the studied PZ polymorphisms are not associated with the risk of recurrent pregnancy loss in the studied population**.**
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KEY WORDS: Protein Z gene; Idiopathic Recurrent Pregnancy Loss

SNP genotyping; polymorphisms

INTRODUCTION

Pregnancy loss (PL) affects up to 15% of reproducing couples (Li et al, 2002), 1% to 3% of those women have two or more consecutive pregnancy losses and are qualified as recurrent pregnancy loss (Allison and Schust, 2009). It may occur due to multiple reasons; include anatomical defects, chromosomal aberrations, endocrine factors, infections, and other immunological factors (El Hachem H et al, 2017). Approximately 50 % of recurrent pregnancy loss remain unexplained (Dranch et al, 2010). The defective maternal hemostatic response leading to utero-placental thrombosis along with hypoxia has been hypothesized to subsequently lead to adverse pregnancy outcomes like placental abruption, intrauterine growth restriction or death and preeclampsia. This may include thrombosis in decidual vessels, impairment of trophoblast invasion, villitis and placental micro-thrombi (Rai et al, 2003). An abnormality of trophoblast invasion associated with thrombotic risk factors, such as thrombophilia were described (Khong et al, 1989). Furthermore, it was described that pregnancy and delivery are associated with a disruption of the hemostatic balance favouring a hypercoagulability state with increased level of clotting factor and decreased of fibrinolytic activity (D'Uva et al, 2008; Thornton and Douglas, 2010).

Protein Z is a vitamin K-dependent plasma glycoprotein synthesized by the liver. It plays a key role in the physiologic inhibition of coagulation by acting as a cofactor in the inactivation of factor Xa. Therefore, PZ promotes the assembly of thrombin under unknown circumstances, paradoxically enhance coagulation (Yin et al, 2000, Gorski MM, 2016). Therefore, it is not surprising that the decrease of protein Z level induce pro-coagulant state and is associated with thrombotic disorder. Several findings showing an association between low and high PZ concentrations and an increased risk of thrombotic diseases such as ischemic stroke (Staton et al, 2005, Vase et al, 2001). However, until now, the association between the low levels of PZ and the risk of idiopathic RPL is the subject of debate and the results are controversial (Al-Shaikh et al, 2013). Based on the previous published data, several common and rare polymorphisms in the PZ gene were reported significantly to be associated with an altered plasma level of PZ (Lichy et al, 2004, Rice et al, 2001).

The PZ gene is located on chromosome 13 in the region 13q34 and it is highly polymorphic (Bafunno et al, 2011). The most reported polymorphisms of the PZ gene are located at the major transcription site -103 A/G (rs3024718), -119 T/A (rs3024731) -475 G/A (rs3024719) (Almawi et al, 2013). A polymorphism in intron C 42G/A, intron F 79G/A (rs3024735) are also reported in the PZ gene (Almawi et al, 2013). A nucleotide substitution of G by C in exon II of the PZ gene, resulting in the replacement of Glu-30 with a Gln residue was identified (Santacroce et al, 2006). The functional aspects of those genetic variation have not thoroughly investigated but some of them are seeming to be linked with different level of PZ secretion (Almawi et al, 2013).

In view of its capacity to reduce plasma level of PZ, a possible relationship between PZ polymorphisms and the occurrence of thrombotic conditions and idiopathic RPL were suggested (Al-Shaikh et al, 2013, Santacroce et al, 2004). Different polymorphisms are shown to be associated with high risk of idiopathic RPL via an enhanced risk of severe placental insufficiency, soon after the connection of maternal and fetal circulations (Paidias et al 2005, Gris et al, 2002).

In the present study, we conduct a case-control study to investigate the contribution of selected variants of PZ promoter (rs3024718, rs3024719, and rs3024731) and exonic (rs3024778 and rs3024772) single nucleotide polymorphisms (SNPs) on increasing idiopathic RPL risk among Saudi women.

MATERIALS AND METHODS

Patients

The present study is a case-control retrospective study. Patients (n= 63) with at least three RPL were recruited from January 2015 to January 2017 at Gynecology and obstetrical department of King Khaled hospital in Tabuk, Saudi Arabia.

Patients are women reported with three or more RPL with unknown causes. All cases presented with miscarriage caused by anatomical abnormalities, previously known systemic disease, endocrine disorders including diabetes, previous venous or arterial thrombosis or a family history of thromboembolism are excluded from this study. Chromosomal abnormalities and Rh incompatibility were performed before inclusion in the study. Patients included in this study were confirmed to be negative for, any infections which may explain the pregnancy loss like, *Toxoplasma gondii*, rubella, cytomegalovirus, herpes simplex viruses, varicella zoster virus and human immunodeficiency virus. Transvaginal ultrasound was performed to confirm spontaneous abortion.

Controls

Controls subject include 78 fertile women with at least one successful pregnancy were recruited from January 2015 to January 2017 at Gynecology and obstetrical department of King Khaled hospital in Tabuk, Saudi Arabia. Control women were matched with patients according to several risk factors (smoking, and oral contraceptive use). Controls and patients present similar distribution according to age and body mass index (BMI). An informed consent was achieved from patients and controls subject before enrolling them in the study. The study that was conduct after approval from the research ethical committee at the university of Tabuk. Blood samples were collected from cases and controls in EDTA tubes and were processed for DNA extraction and real time PCR.

Protein Z genotyping

The common PZ rs3024718 (A-475G), rs3024731 (T-119A) and rs3024719 (G-103A), (rs3024778 (G208A) and rs3024772 (G844A) polymorphisms were selected for the study. The PZ genotyping was performed using the allelic discrimination method as described (Al-Shaikh, et al 2013). The analysis was performed using real-time polymerase chain reaction (PCR), according to the procedure and instruction of the manufacturers (Applied Biosystems).

Statistical analysis

Statistical analysis was performed on SPSS software. Categorical variables were expressed as percentages of total or as mean ± SD. To determine differences in means, the Student's t test was used and Pearson $\chi 2$ or Fisher's exact test was used to assess inter-group significance. Allele frequencies were calculated, Hardy–Weinberg equilibrium was tested for each polymorphism. All analyses were conducted under additive genetic effect, using SNPStats software http://www.bioinfo.iconcologia.net/snpstats/). Linkage disequilibrium analysis was performed using HaploView 4.1 (http://www.broad.mit.edu/mpg/ HaploView), and haplotypes reconstruction was performed. Logistic regression analysis was performed to determine the odds ratios (OR) and 95 % confidence intervals (95% CI) associated with the RPL risk, taking the control women as the reference group.

The association of genotypes with RPL was conducted under codominant, dominant, and recessive models using SNPstats (http://www.gencat.cat), all P-values were two-tailed; P-value<0.05 were considered statistically significant.

RESULTS

Study subjects. The clinical characteristics of study subjects are shown in table 1. Regarding the age and BMI, patients and controls were matched, and the statistical analysis show a non-significant difference between cases and controls (p=0.93 and p= 0.23). The mean age is 29.96 ± 5.31 and 30.08 ± 7.07 respectively for patients and controls. The analysis of data show also a non-significant difference between patients and controls in term of oral contraceptive consumption (P = 0.94). The mean number of miscarriage in patients is 3.05 ± 1.44, however the mean number of live birth in the control group is 3.12 ± 1.94 and it is significantly higher than observed in patients 0.2 ±1.06 (P <0.001).

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Table 1. Clinical Characteristics of Study Participants

Parameters	Cases	Controls	p[a]
Age at inclusion in study [b]	29,96 ± 5.31	30,08 ± 7.07	0.93
Body mass index [b], kg/m²	27,16 ± 5.08	25,23 ±8.16	0.23
Obesity [c], BMI >30 kg/m2	45.1%	40.5%	0.65
Oral contraceptive [c]	5.9%	5.6%	0.94
Number of live births [b]	0.2 ± 1.06	3.12 ± 1.94	<0.001
LA	0	0	NA
Abortions [b]	3.05 ± 1.44	0.0 ± 0.0	NA

Note: Abbreviations: NA, not abdicable; SD, standard deviation.
63 cases with IRPL and 78 controls were included.
a: student t test for continuous variable, Person chi-square test for categorical variables
b: mean ± SD
c: Percent of total within each group

Association studies. The Genotypic distribution of the five polymorphisms studied shows no statistically significant evidence that the alleles frequencies deviated from Hardy-Weinberg equilibrium as shown in table 2. The prevalence of minor allele frequency (MAF) for the five polymorphisms studied were comparable between cases and controls 0.23 versus 0.20 (p=0.61), 0.24 versus 0.19 (P = 0.28), 0.02 versus 0.02 (P=0.84), 0.26 versus 0.21 (P = 0.61) and 0.02 versus 0.01 (P = 0.79) respectively for rs3024718, rs3024719, rs3024778, rs3024731 and rs3024772.

Table 2. Allelic Distribution of PZ SNPs in IRPL Cases and Control Women

Name	Localisation	Allele	Cases[b] MAF	Controls[b] MAF	χ^2	HWE	p[a]	aOR (95% CI)
rs3024718	113813853	G	0.23	0.2	0.48	0.84	0.61	1.14 (0.57- 2.32)
rs3024719	113814225	A	0.24	0.19	0.09	0.75	0.28	1.53 (0.74 - 3.21)
rs3024778	113814465	A	0.02	0.02	0.06	0.81	0.84	0.82 (0.41 – 4.95)
rs3024731	113818815	A	0.26	0.21	0.15	0.70	0.61	1.23 (0.63 – 3.42)
rs3024772	113826100	A	0.02	0.01	0.05	0.81	0.79	1.6 (0.66 – 3.61)

Note: Abbreviations: - **AOR**, adjusted odd ratio; - **CI**, confidence interval; - **IRPL**, idiopathic recurrent pregnancy loss; **HWE**, Hardy Weinberg equilibrium; - **SNP**, single nucleotide polymorphism; - χ^2, chi square. - Location on chromosome based on dbSNP, - **MAF**: Minor allele defined on controls frequency, - **P**; p value, adjusted for BMI, age and oral contraceptive.
- **a**: Person chi-square test
- **b**: frequency

We find no significant association between the five PZ polymorphisms and the risk of RPL in different mode of transmission (recessive and dominant), the results are shown in table 3.

Table 3. Distribution of PZ Genotypes and risk of IRPL

		Controls [b]	Patients [b]	P[a]	OR (95% CI)
rs3024718					
Codominant	A/A	49 (62.8%)	37 (58.7%)	0.74	1.00
	A/G	27 (34.6%)	23 (36.5%)		1.13 (0.56-2.27)
	G/G	2 (2.6%)	3 (4.8%)		1.99 (0.32-4.5)
Dominant	A/A	49 (62.8%)	37 (58.7%)	0.62	1.00
	A/G-G/G	29 (37.2%)	26 (41.3%)		1.19 (0.61-2.34)
Recessive	A/A-A/G	76 (97.4%)	60 (95.2%)	0.48	1.00
	G/G	2 (2.6%)	3 (4.8%)		1.9 (0.20-5.85)
rs3024719					
Codominant	G/G	50 (64.1%)	38 (60.3%)	0.64	1.00
	G/A	25 (32%)	21 (33.3%)		1.11 (0.59-2.26)
	A/A	3 (3.8%)	4 (6.3%)		1.75 (0.38-8.31)
Dominant	G/G	50 (64.1%)	38 (60.3%)	0.46	1.00
	G/A-A/A	28 (35.9%)	25 (39.7%)		1.56 (0.66-3.69)
Recessive	G/G-G/A	75 (96.2%)	59 (93.7%)	0.87	1.00
	A/A	3 (3.8%)	4 (6.3%)		1.69 (0.37-4.87)
rs3024731					
Codominant	T/T	50 (64.1%)	35 (55.6%)	0.59	1.00
	T/A	23 (29.5%)	23(36.5%)		1.43 (0.69-2.94)
	A/A	5 (6.4%)	5 (7.9%)		1.81 (0.37-4.74)
Dominant	T/T	50 (64.1%)	35 (55.6%)	0. 3	1.00
	T/A-A/A	28 (35.9%)	28 (44.4%)		1.43 (0.72-2.82)
Recessive	T/T-T/A	73 (93.6%)	58 (92.1%)	0.73	1.00
	A/A	5 (6.4%)	5(7.9%)		1.26 (0.37-4.48)
rs3024772					
	G/G	77 (98.7%)	61 (96.8%)		1.00
	G/A	1 (1.3%)	2 (3.2%)	0.44	-
rs3024778					
	G/G	75 (96.2%)	61 (96.8%)		1.00
	G/A	3 (3.8%)	2 (3.2%)	0.84	-

Note: Abbreviations: AOR, adjusted odd ratio; CI, confidence interval; IRPL, idiopathic recurrent pregnancy loss; χ^2, chi square.
- 63cases with IRPL and 78 controls were included.
- P value.
- **a**: Pearson chi-square test, the wild type genotype is considered as reference
- **b**: number of participant (frequency)

Also, these differences remain non-significant after adjusting for age, BMI, and oral contraceptive. The individual SNPs prevalence of the five locus (rs3024718/ rs3024719/rs3024778/rs3024731/rs3024772) of PZ haplotypes were constructed and analyzed in table 4. Eight haplotypes were commonly detected in both cases and controls. However, GGTGA haplotype shows a high prevalence in cases with IRPL (0.02) compared to those in controls (0.005) however there was no significant difference between the two groups (P=0.38). The haplotypic analysis reveals a lack of association between the different haplotype and the risk of recurrent miscarriage, and was confirmed by the absence of any block of linkage disequilibrium LD.

Table 4. Distribution of 6-Locus PZ Haplotypes in IRPL Cases and Control Women

Haplotype [a]	Case [c]	Controls [c]	P [b]	AOR (95% CI)
AGTGG	0.41	0.53	-	1 (REFERENCE)
AGAGG	0.156	0.22	0.59	1.78 (0.72 – 4.4)
AATGG	0.1	0.11	0.7	1.27 (0.31 – 7.39)
GGTGG	0.11	0.085	0.38	1.63 (0.55 – 4.85)
AAAGG	0.06	0.05	0.61	1.48 (0.34– 6.51)
GATGG	0.036	0.049	0.34	2.27 (0.43 – 6.65)
GGAGG	0.041	0.045	0.46	0.91 (0.58 – 5.23)
GGTGA	0.02	0.005	0.38	3.81 (0.78 – 4.71)

Abbreviations: - AOR, adjusted odd ratio for BMI age and oral contraceptive.; - CI, confidence interval.
- a: PZ haplotypes rs30247718/rs30247719/rs3024772/rs30247731/rs3024778.
- b: p: P value is considered as statically significant <0.05.
- c: haplotype frequencies.

DISCUSSION

RPL is a multifactorial disorder, however its pathogenesis is still controversial. The present study investigates the genetic variation of protein Z gene on RPL risk in a group of women from Saudi Arabian population. Our finding shows no significant difference between the two groups in term of allelic frequencies and genotyping distribution for all analyzed polymorphisms. The MAF of the analyzed PZ SNPs except rs3024731 were comparable to those described in HapMap CEU sample. Several previously published studies have shown that the MAF of rs3024731 appear to be lower than what was found in the Caucasian population (Nawak-Gottl et al, 2009; Santacroce et al, 2004). In this study, we found that neither of the studied SNPs are associated with RPL in Saudi Arabian women which agrees with results reported by Al-Shaikh (Al-Shaikh et al, 2013). In contrast, the rs3024719 (G-103A) and rs3024731 (T-119A) polymorphisms are significantly higher in patients than in the control group and constitute an independent risk factor for recurrent miscarriage in Bahrain population (Al-Shaikh et al, 2013). Moreover, a Grecian cohort enrolled 51 cases with recurrent miscarriage, demonstrate no association between risk of pregnancy loss and PZ polymorphisms (Tapalidou et al, 2009). Our study is in agreement with European studies have demonstrate that PZ polymorphisms was not associated with PZ deficiency and risk of preeclampsia (LeCam-Duchez et al, 2005). In addition, several studies conclude that the risk of RPL was associated with lower level of protein Z but not with the rs3024735 (G79A) variant of PZ gene which was described protective against RPL (El-Hamid et al, 2011, Dossenbach-Glaninger et al, 2008). Differential Linkage disequilibrium LD between PZ function, PZ production and diseases were investigated. It was published that ATG haplotypes is associated with increase rate of PZ levels (Nawak-Gottl et al, 2009).

The effect of PZ deficiency in the process of pregnancy was described in the early stage when the placenta starts to replace the yolk sac to supply embryo by blood (Gris et al, 2003). The wild variability of PZ level was investigated in different studies involving Arab women's and the results are controversial. An Omani study demonstrate that a PZ deficiency was observed in the first and second trimester of pregnancy, but all women had a normal pregnant outcome (Growri et al, 2011). However, Bahranian women show that the low level of since the first trimester of pregnancy loss need to be explain by other variant of PZ and protein Z inhibitor (Al-shaikh et al, 2013). According the pathogenic mechanism of PZ deficiency, described by Padias and colleagues as thrombotic disorder, is different from the classical thrombophilia pathway (Paidas et al, 2005). Insofar the PZ act in thrombotic event by limiting the effect of increased generated factor Xa, the PZ form with protein Z protease inhibitor ZPI a complex PZ_ZPI. In addition, pregnancy loss was associated with high levels of antibodies anti-PZ (Donner et al, 2005, Gris et al, 2003) without affecting the plasmatic levels of PZ. However, it is noted that low concentration of PZ is associated with patients with antiphospholipid antibodies (Forastiero et al, 2003). In view of the wide range of results observed in different populations the association between the common genetic variations within the PZ locus and the risk of recurrent pregnancy losses is controversial. Some results suggest that the effect of PZ is not related to the genetic variation but to PZ deficiency (Tapalidou et al, 2009) or/and to presence of antiphospholipid antibodies (Broze GJ Jr, 2001). It was also suggested that the PZ deficiency is often associated with lupus anticoagulant (Steffano et al, 2001, (Forastiero et al, 2003).

CONCLUSION

The lack of significant association between PZ polymorphisms and risk of recurrent pregnancy loss shown here suggest that PZ don't present an individual risk factor for RPL but may be combined with other factors and require introducing more clinical parameters. The present work faced some limitation especially the small size of patients and controls included but it has the strength for the homogeneity in their ethnic background only saudi Arabia women were included and the combination of the five PZ polymorphisms located at promoter intron and exons region. Furthermore, it will be interesting to complete this finding by an assessment of PZ levels in womens with idiopathic recurrent pregnancy loss during the three trimesters of pregnancy. It is possible that among the large number of risk factors affecting the maternal hemostasis equilibrium, knowledge of the genetic variation of PZ combined with others risk factors may help to identify other pathways that would help in better understanding of the disease.

ETHICAL APPROVAL

The current study was approved by the Research Ethics Committee at the University of Tabuk.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no conflicts of interest with respect to the research, authorship, and/or publication of this article.

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REFERENCES

Alijotase-Regi J, Ferrer-Oliveras R, Rodrigo-Anoro MJ, Farran-Codina I, et al. (2010). Anti-b2-glycoprotein-1 and anti-phosphatidylserine antibodies in women with spontaneous pregnancy loss. Fertil Steril 93(7): 2330-2336. https://doi.org/10.1016/j.fertnstert.2009.01.089

Allison JL, Schust DJ (2009). Recurrent first trimester pregnancy loss: revised definitions and novel causes. Curr Opin Endocrinol Diabetes Obes. 16(6):446-450. https://doi.org/10.1097/med.0b013e3283327fc5

Al-Shaikh FS, Sater MS, Finan RR, Racoubian E, et al. (2013). Protein Z variants associated with protein Z plasma levels and with risk of idiopathic recurrent miscarriage. Reprod Sci. 20(9):1062-1068.

https://doi.org/10.1177/1933719112473659

Almawi WY, Guarino BD, Al-Sulaiti MA, Al-Busaidi AS, et al. (2013). Endothelial nitric oxide synthase gene variants and haplotypes associated with an increased risk of idiopathic recurrent miscarriage. Hum Fertil Sep. 16(3):200-206. https://doi.org/10.3109/14647273.2013.806824

Bafunno V, Santacroce R, Margaglione M (2011).The risk of occurrence of venous thrombosis: focus on protein Z. Thromb Res. Dec;128(6):508-515. https://doi.org/10.1016/j.thromres.2011.08.007

Branch DW, Gibson M, Silver RM (2010) Clinical practice. Recurrent miscarriage. N Engl J Med 363:1740-1747.

Broze GJ Jr (2001) Protein Z-dependent regulation of coagulation. Thromb Haemost 86:8-13.
Dorner T, Hoppe B, Salama A, Pruss A, et al. (2005) Antibodies against protein Z and fetal loss: current perspectives. Clin Exp Med. 5:50-54.

https://doi.org/10.1007/s10238-005-0065-4

Dossenbach-Glaninger A, van Trotsenburg M, Helmer H, Oberkanins C, et al. (2008) Association of the protein Z intron F G79A gene polymorphism with recurrent pregnancy loss. Fertil Steril. 90 (4):1155-1160. https://doi.org/10.1016/j.fertnstert.2007.07.1376

El-Hamid SA, El-Khayat W (2011) Relationship of the Protein Z Intron F G79A and IL6 C634G gene polymorphisms with the risk of recurrent pregnancy loss in Egyptian women. J Investig Med. 59 (4):655-660.

https://doi.org/10.2310/jim.0b013e31820c9c90

El Hachem H, Crepaux V, May-Panloup P, Descamps P, et al. (2017). Recurrent pregnancy loss: current perspectives. Int J Womens Health. 17 (9):331-345.

Forastiero RR, Martinuzzo ME, Lu L, Broze GJ (2003) Autoimmune antiphospholipid antibodies impair the inhibition of activated factor X by protein Z/protein Z- dependent protease inhibitor. J Thromb Haemost 1:1764-1770. https://doi.org/10.1046/j.1538-7836.2003.00303.x

Gorski MM, Lotta LA, Pappalardo E, et al. Single nucleotide variant rs2232710 in the protein Z-dependent protease inhibitor (ZPI, SERPINA10) gene is not associated with deep vein thrombosis. PLoS One. 2016;11(3): e0151347. https://doi.org/10.1371/journal.pone.0151347

Gowri V, Mathew M, Gravell D, AlFalahi K, et al. (2011). Protein Z levels in pregnant Omani women: correlation with pregnancy outcome. J Thromb Thrombolysis. 32(4):453-458. https://doi.org/10.1007/s11239-011-0629-y

Grandone E, Favuzzi G, De Stefano V, Chinni E, et al. (2009). Protein Z g-42a variant and the risk of pregnancy-related venous thromboembolism in a cohort of Italian patients. Thromb Res. 123(6):848-850. https://doi.org/10.1016/j.thromres.2008.12.045

Gris JC, Amadio C, Mercier E, Lavigne-Lissalde G, et al. (2003). Anti-protein Z antibodies in women with pathologic pregnancies. Blood 101(12): 4850-4852. https://doi.org/10.1182/blood-2002-12-3802

Gris JC, Quéré I, Dechaud H, Mercier E, et al. (2002). High frequency of protein Z deficiency in patients with unexplained early fetal loss. Blood. 99:2606. https://doi.org/10.1182/blood.v99.7.2606

Heeb MJ, Fisher M, Paganini-Hill A (2007) Association of low protein Z levels with ischemic stroke in young women. Thromb Haemost. 97:495-496. https://doi.org/10.1160/th06-09-0539

Khong TY, De Wolf F, Robertson WB, Brosens I, et al. (1986) Inadequate maternal vascular response to placentation in pregnancies complicated by preeclampsia and by small-for-gestational age infants. Br J Obstet Gynaecol 93(10):1049-1059. https://doi.org/10.1097/00006254-198708000-00009

Le Cam-Duchez V, Verspyck E, Chretien MH, Duclos M, et al. (2005) Protein Z Polymorphisms Protein Z plasma level and Severe Preeclamspia. In: Poster in XXth Congress of the International Society on Thrombosis and Haemostasis

Li TC, Markis M, Tomsu M, Tuckerman E, Laird S (2002) Recurrent miscarriage: aetiology, management and prognosis. Hum Reprod Update. 8 (5):463-481. https://doi.org/10.1093/humupd/8.5.463

Lichy C, Kropp S, Dong-Si T, Genius J, et al. (2004) A common polymorphism of the protein Z gene is associated with protein Z plasma levels and with risk of cerebral ischemia in the young. Stroke. 35(1):40-45.

https://doi.org/10.1161/01.str.0000106909.75418.e4

McQuillan AM, Eikelboom JW, Hankey GJ, Baker R, et al. (2003) Protein Z in ischemic stroke and its etiologic subtypes. Stroke. 34:2415-2419. https://doi.org/10.1161/01.str.0000092124.52084.4b

Nowak-Gottl U, Rohlich B, Thedieck S, Huge A, et al. (2009) Association of the protein Z ATG haplotype with symptomatic nonvascular stroke or thromboembolism in white children: a family-based cohort study. Blood. 113(10): 2336-2341. https://doi.org/10.1182/blood-2008-10-181461

Paidas MJ, Ku DH, Lee MJ, Manish S, et al. (2005) Protein Z, protein S levels are lower in patients with thrombophilia and subsequent pregnancy complications. J Thromb Haemost. 3:497-501. https://doi.org/10.1111/j.1538-7836.2005.01158.x

Quack Loetscher KC, Stiller R, Roos M, Zimmermann R (2005) Protein Z in normal pregnancy. Thromb Haemost. 93:706-709. https://doi.org/10.1160/th04-08-0532

Rai R (2003). Is miscarriage a coagulopathy? Curr Opin Obstet Gynecol. 15(3):265-268. Review.

Rice GI, Futers TS, Grant PJ (2001) Identification of novel polymorphisms within the protein Z gene, haplotype distribution and linkage analysis. Thromb Haemost 85:1123.

Santacroce R, Cappucci F, Di Perna P, Sessa F, et al. (2004) Protein Z gene polymorphisms are associated with protein Z plasma levels. J Thromb Haemost 2:1197-1199. https://doi.org/10.1111/j.1538-7836.2004.00808.x

Santacroce R, Sarno M, Cappucci F, Sessa F, et al (2006) Low protein Z levels and risk of occurrence of deep vein thrombosis. J Thromb Haemost. 4:2417-2422. https://doi.org/10.1111/j.1538-7836.2006.02186.x

Sofi F, Cesari F, Abbate R, Gensini GF, et al. (2010) A meta-analysis of potential risks of low levels of protein Z for diseases related to vascular thrombosis. Thromb Haemost. 103(4):749-756. https://doi.org/10.1160/th09-09-0645

Staton J, Sayer M, Hankey GJ, Cole V, et al. (2005) Protein Z gene polymorphism,proteinZ concentrations and ischemicstroke. Stroke. 36:1123.

Steffano B, Forastiero R, Martinuzzo M, Kordich L (2001) Low plasma protein Z levels in patients with antiphospholipid antibodies. Blood Coagul Fibrinolysis. 12 (5):411-412. https://doi.org/10.1097/00001721-200107000-00012

Topalidou M, Effraimidou S, Farmakiotis D, Papadakis E, et al. (2009) Low protein Z levels, but not the intron F G79A polymorphism, are associated with unexplained pregnancy loss. Thromb Res. 124(1):24-27. https://doi.org/10.1016/j.thromres.2008.09.017

Vasse M (2008). Protein Z, a protein seeking a pathology. Thromb Haemost 100:548-556. https://doi.org/10.1160/th08-01-0024

Vasse M, Guegan-Massardier E, Borg JY, Woimant F, et al. (2001) Frequency of protein Z deficiency in patients with ischaemic stroke. Lancet. 357:933. https://doi.org/10.1016/s0140-6736(00)04218-5

Yin ZF, Huang ZF, Cui J, Fiehler R, (2000). Prothrombotic phenotype of protein Z deficiency. Proc Natl Acad Sci USA. 97:6734-6738.

https://doi.org/10.1073/pnas.120081897