COMBINED PRESENCE OF COAGULATION FACTOR XIII V34L AND PLASMINOGEN ACTIVATOR INHIBITOR 1 4G/5G GENE POLYMORPHISMS SIGNIFICANTLY CONTRIBUTE TO RECURRENT PREGNANCY LOSS IN SERBIAN POPULATION

Ivana Joksic1, Zeljko Mikovic2,3, Dejan Filimonovic2,3, Jelena Munjas4, Natasa Karadzov Orlic2,3, Amira Egic2,3, Gordana Joksic4

1Genetic laboratory department, Gynecology and Obstetrics Clinic »Narodni front«, Belgrade, Serbia
2High-risk pregnancy department, Gynecology and Obstetrics Clinic »Narodni front«, Belgrade, Serbia
3School of Medicine, University of Belgrade, Belgrade, Serbia
4Departement of Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia
5Vinca Institute of Nuclear Science, University of Belgrade, Belgrade, Serbia

Summary

Background: Recurrent pregnancy loss (RPL) is a heterogeneous condition affecting up to 5% of women of reproductive age. Inherited thrombophilia have been postulated as one of the causes of RPL. Here we examined the prevalence of nine thrombophilic gene polymorphisms among women with history of recurrent miscarriages and fertile controls.

Methods: The study included 70 women with history of at least three early pregnancy losses and 31 fertile controls with no miscarriages. We investigated mutations in genes responsible for clotting and fibrinolysis, including factor V (FV) Leiden, FV H1299R, factor II (FII) G20210A, methylene tetrahydrofolate reductase (MTHFR) C677T and A1298C, factor XIII (FXIII) V34L, plasminogen activator inhibitor-1 (PAI-1) 4G/5G and endothelial protein C receptor (EPCR) H1 and H3 haplotypes using reverse polymerase chain reaction ViennaLab cardiovascular disease StripAssays.

Results: Our results showed no significant increase in prevalence of tested polymorphisms in women with RPL.

List of abbreviations: RPL, recurrent pregnancy loss; FV, Factor V; prothrombin, FII; MTHFR, methylene tetrahydrofolate reductase; APC, activated protein C; FXIII, factor XII; EPCR, endothelial protein C receptor; PAI-1, plasminogen activator inhibitor-1; CG, control group; t-PA, tissue-type plasminogen activator; u-PA, urokinase-type plasminogen activator.
However, relative risk for PRL among women heterozygous for FXIII V34L was 2.81 times increased (OR 2.81, 95% CI 1.15–6.87, P=0.023). Haplotype analysis showed that combined presence of high-risk genotypes for FXIII and PAI-1 significantly increases risk for RPL (OR 15.98, CI 9.95% 1.11–17.46, P=0.044).

**Conclusions:** This is the first study in Serbian population that investigated prevalence of FVR2, A1298C, FXIII V34L and EPCR gene variants. Compound heterozygosity for FXIII V34L and PAI-1 4G is significant risk factor for recurrent miscarriage. Our results should be viewed in context of small case-control study, so further large prospective studies are need for confirmation of our findings.

**Keywords:** factor XIII, gene polymorphism, inherited thrombophilia, plasminogen activator inhibitor-1, recurrent pregnancy loss

**Introduction**

Recurrent pregnancy loss (RPL) is heterogeneous condition affecting up to 5% of couples (1, 2). It is defined as three or more consecutive miscarriages (3). Numerous factors such as chromosomal aberrations, endocrinological, infective and immunologic diseases, anatomic abnormalities of the uterus or hypercoagulable states can cause RPL, but in more than 40% of cases etiology will remain unclear (4). Inherited thrombophilia represents genetic predisposition for improper formation of blood clots, and it is caused by different sequence variants in genes coding for coagulation factors and enzymes included in fibrinolysis (5, 6). Proper placental formation is necessary for successful pregnancy outcome. Several studies have proposed association between adverse pregnancy outcome and inherited thrombophilia, as it is shown that they can induce placental insufficiency due to vascular thrombosis (3, 7).

Gene variants in factor V gene (FV), prothrombin (FII), methylene tetrahydrofolate reductase (MTHFR) and plasminogen activator inhibitor-1 (PAI-1) are the most extensively studied in association with RPL (3, 4, 7). FV Leiden (G1619A) abolishes cleavage site for activated protein C (APC), resulting in 10 times slower inactivation of FV (8). Risk for RPL in pregnant women heterozygous carriers of FV Leiden is increased 2-3 times (9, 10). Second gene polymorphism in FV, FVR2 (A4070G), combined with FVL, further increases risk for thrombosis (11). However, FVR2 alone does not seem to significantly increase risk for venous thrombosis (11). Prothrombin gene variant G20210A increases protein synthesis and also the odds ratio for RPL (OR 2.9) (12–14). MTHFR C677T and A1298C variants mildly diminish enzyme activity, and if present with low serum folate levels represent risk factor for hiperhomocysteinemia (15–18). High serum levels of homocysteine are associated with numerous pathologic states, including venous thrombosis (18). Indel polymorphism in promoter of PAI-1 gene determines presence of two allelic variants: with 4 or 5 guanine repeats (4G/5G), which modify gene expression (19, 20). 4G variant results in increased expression of PAI-1 and consequently diminished clot degradation, resulting in prothrombotic state (19–21).

Recently, gene polymorphisms in factor XIII (FXIII) and endothelial protein C receptor (EPCR) have also been studied in association with venous thrombosis and RPL. FXIII covalently cross-links fibrin alpha and gamma chains and plays important role in fibrinolytic system (22–24). Most commonly associated with thrombotic events is Val34Leu gene variant in FXIII (V34L) (24). Its presence leads to enhanced activation of FXIII, enhanced dimerization and polymerization of fibrin chains, which changes the structure of clot, making it more resistant to fibrinolysis (24–26). EPCR is key component of protein C anticoagulation system (27, 28). So far, four receptor haplotypes, determined by presence of 13 single nucleotide polymorphisms in linkage disequilibrium, have been described (H1 to H4) (28). H1 haplotype, tagged with minor allele G4678C, is associated with elevated levels of APC and low risk for clot formation, while H3 haplotype (A4600A) predisposes to thrombosis (27–29).

Although described gene variants and their link to adverse pregnancy outcome, including RLP, were subject of numerous studies, the results are still conflicting. The aim of the present study was to compare the frequency of FII G20210A, FVL, FVR2, MTHFR C677T and A1298C, PAI-1 4G/5G, FXIII V34L gene variants and EPCR haplotypes in series of patients with RPL with control group. This is the first study in which prevalence of FVR2, MTHFR A1298C, FXIII V34L and EPCR haplotypes was investigated in Serbian population.

**Materials and Methods**

Study was designed as prospective case control study. It was conducted at Gynecology and obstetrics Clinic »Narodni front«, Belgrade from 2014. to 2016.
Study group was comprised of 70 women experiencing 3 or more consecutive pregnancy losses. Thirty-one age-matched women with 2 or more successful pregnancies and no pregnancy losses were selected as control group. Exclusion criteria for the study were: anatomic abnormalities of uterus, acquired thrombophilia, abnormal peripheral blood karyotype, urogenital infective diseases and endocrinologic disorders. The study was conducted in accordance with Declaration of Helsinki and with approval of local Ethics committee.

Peripheral blood was taken on EDTA as anticoagulant. Genomic DNA was extracted using Thermo-Fisher Pure link kit. Nine thrombophilic gene variants (FII G20210A, FVL, FVR2, MTHFR C677T and A1298C, PAI-1 4G/5G, FXIII V34L and EPCR haplotypes H1 and H3) were simultaneously amplified in single multiplex amplification reaction (Vienna Lab StripAssay, Vienna, Austria) as described previously (30). Reverse hybridization of amplified DNA fragments to test strips was done, as well as their visualization by use of streptavidin-alkaline phosphatase conjugate and color substrates.

Statistical analysis was performed using Statistica 6.0 and SNPstats programs. Age differences in examined groups were tested by Student’s t-test. Hardy-Weinberg equilibrium was assessed by chi-square. The prevalence of gene variants in study groups was done by Fisher’s exact test. Odds ratio (OR) and 95% confidence intervals (95%CI) were calculated by using logistic regression. Haplotype frequencies and association with outcome were determined by SNPstats software. P values less than 0.05 were considered statistically significant.

## Results

Recurrent pregnancy loss (RPL) group and control group (CG) were age matched (mean age 33.2±5.4 v.s. 33.2±4.7, P=0.831). All tested gene variants were in Hardy-Weinberg equilibrium, except for MTHFR A1298C in control group (P=0.012).

Significant difference in genotype frequencies among tested groups was observed only for MTHFR A1298C (P=0.010, Table I). Although in RPL group prevalence of FV Leiden of FII G20210A gene variant was 11.4%, in control group was 0%. The remaining gene variants (FVR2, FII, MTHFR C677T, MTHFR, FXIII V34L, PAI-1 4G/5G) did not show significant difference in genotype frequencies among tested groups.

### Table I Allele frequencies (%) of analysed gene variants (FVL, FVR2, FII G20210A, MTHFR C677T and A1298C, PAI-1 4G/5G, FXIII V34L) in recurrent pregnancy loss and control group (Wt-wild type, Hz-heterozygous Ho-homozygous).

| Genotypes     | RPL N=70 |        |        | CG N=31 |        |
|---------------|----------|--------|--------|---------|--------|
|               | Wt,%     | Hz,%   | Ho,%   | Wt,%    | Hz,%   | Ho,%   |
| FV Leiden     | 88.5     | 11.4   | 0.0    | 100.0   | 0.0    | 0.0    | 0.102  |
| FVR2          | 67.1     | 32.8   | 0.0    | 70.7    | 29.3   | 0.0    | 0.818  |
| FII           | 88.5     | 11.4   | 0.0    | 100.0   | 0.0    | 0.0    | 0.102  |
| MTHFR C677T   | 50.0     | 42.8   | 7.1    | 38.7    | 58.1   | 3.2    | 0.411  |
| MTHFR         | 48.5     | 35.7   | 15.7   | 39.7    | 61.3   | 0.0    | 0.010  |
| FXIII V34L    | 42.8     | 52.8   | 4.3    | 67.7    | 32.3   | 0.0    | 0.064  |
| PAI-1 4G/5G   | 18.5     | 47.1   | 34.3   | 35.0    | 58.1   | 35.5   | 0.283  |

**Figure 1** EPCR haplotype frequencies (%) in examined groups.

**Figure 2** EPCR genotype frequencies (%) in tested groups.
Results of EPRC haplotype and genotype frequencies are shown in Figures 1 and 2, respectively.

The most frequent EPRC haplotype in RPL group is h2 (50%), and in control group h1 (50%) (Figure 1). H3 haplotype has similar frequency in both groups (8.6% vs. 8.1%, Figure 1). Most common EPRC genotype in both groups is h1h2 (25 vs. 15%), while h3h3 genotype wasn’t detected in tested subjects (Figure 2). There was no significant difference in frequency of EPRC haplotypes or genotypes in tested groups (P=0.521 and P=0.642).

Table II  Association between tested genotypes and recurrent pregnancy loss (dominant genetic model was used).

| Genotypes                        | OR (95% CI) | P    |
|----------------------------------|-------------|------|
| FV Leiden GG+GA vs. AA           | NA          | NA   |
| FVR2 AA+AG vs. GG                | 1.2 (0.48–3.03) | 0.700 |
| FII GG+GA vs. AA                 | NA          | NA   |
| MTHFR C677T CC+CT vs. TT         | 0.63 (0.27–1.49) | 0.291 |
| MTHFR A1298C AA+AG vs. CC        | 0.58 (0.24–1.39) | 0.224 |
| FXIII GG+GT vs. TT               | 2.81 (1.15–6.87) | 0.023 |
| PAI-1 5G5G+4G5G vs. 4G4G         | 1.05 (0.43–2.58) | 0.910 |
| EPCR AA+AG vs. GG                | 1.07 (0.33–3.67) | 0.900 |

OR – odds ratio; CI – confidence interval; NA – not estimated; A – adenine; G – guanine; T – thymine; C – cytosine

Table III  Association between investigated thrombophilic haplotypes and recurrent pregnancy loss.

| Haplotype | FVL | FVR2 | FII G21210A | MTHFR C677T | MTHFR A1298C | FXIII V54L | PAI-1 4G/5G | EPRC | Haplotype frequency | OR (95% CI) | P    |
|-----------|-----|------|-------------|-------------|-------------|-----------|-------------|------|---------------------|-------------|------|
| 1         | G   | A    | G           | C           | G           | G         | A           | 0.1732 | 1                   | –           | –    |
| 2         | G   | A    | G           | C           | A           | G         | G           | 0.1347 | 0.64                | 0.560       |      |
| 3         | G   | A    | G           | C           | A           | G         | GG          | 0.0999 | 0.99                | 0.991       |      |
| 4         | G   | A    | G           | C           | A           | T         | G           | 0.0873 | 13.98               | 0.044       |      |
| 5         | G   | A    | G           | T           | A           | G         | GG          | 0.0841 | 0.99                | 0.992       |      |
| 6         | G   | A    | G           | T           | A           | G         | A           | 0.0648 | 0.58                | 0.563       |      |
| 7         | G   | A    | G           | T           | A           | T         | GG          | 0.0553 | 2.21                | 0.501       |      |
| 8         | G   | A    | G           | C           | C           | T         | GG          | 0.0365 | 2.52                | 0.523       |      |
| 9         | G   | A    | G           | T           | A           | T         | G           | 0.0297 | 0.24                | 0.442       |      |
| 10        | G   | G    | G           | C           | A           | G         | GG          | 0.028  | 0.15                | 0.176       |      |

Nucleotides corresponding to» wild type» and »mutant alleles» of tested genes variants are shown in legend below

|       | FVL | FVR2 | FII G21210A | MTHFR C677T | MTHFR A1298C | FXIII V54L | PAI-1 4G/5G | EPRC |
|-------|-----|------|-------------|-------------|-------------|-----------|-------------|------|
| WT    | G   | A    | G           | G           | C           | A         | G           | GG   | A                   |
| Mut   | A   | G    | A           | T           | C           | T         | G           | G    |

OR – odds ratio; CI – confidence interval; A – adenine; G – guanine; T – thymine; C – cytosine; WT – wild type; Mut – mutant allele

Statistically significant association with recurrent pregnancy loss was determined for FXIII V34L gene variant (P=0.023, Table II). Carriers of V34L have 2.81 times higher risk for RPL (OR 2.81, 95%CI 1.15–6.87, Table II). Other examined gene variants didn’t show significant association with adverse pregnancy outcome (Table II).

Next, we examined association of thrombophilic gene haplotypes and RPL. Results are shown in Table III. Association with recurrent pregnancy loss was significant for GAGCATGA haplotype (haplotype with compound heterozygosity for FXIII V34L and PAI-1 4G, P=0.044, Table III). Combined presence of FXIII
V34L and PAI-1 4G gene variants increases the odds for recurrent pregnancy loss 13.98 times (OR 13.98, 95%CI 1.11–17.46, Table III). Other haplotypes showed no significant association with RPL (Table III).

**Discussion**

Well-balanced maternal haemostatic response is necessary for successful pregnancy outcome (31). Therefore, numerous studies suggested increased prevalence of prothrombotic mutations in women experiencing pregnancy complications such as RPL (32, 33). In this study we evaluated the influence of nine thrombophilic gene variants on recurrent pregnancy loss.

Our results show that prevalence of nine thrombophilic gene variants is not significantly increased in group of women with RPL (Table I).

Several studies found that FV Leiden and FII G20210A gene variants are major risk factor for recurrent miscarriage (34–40). Other studies, however, found weak or no association of mentioned mutations and RPL (4, 41, 42). Results of multi-centric EPCOT study have shown that risk of early miscarriage is not increased in carriers of FV Leiden mutation, and that it increases the risk for late fetal loss (41). Retrospective studies established association between FII mutation and RPL (43–45), but numerous prospective have failed to confirm such a connection (46–50). Although relative risk for pregnancy loss is 2-fold increased in FII G20210A carriers, absolute risk for adverse outcome remains low and additional risk factors are required for such complication to develop (12). We found no association between FVL, FII and RPL, which can be due to small sample size and study design itself (Table II).

Few studies have analysed association of FVR2 gene variant and RPL (51–53). They concluded that FVR2 doesn’t represent risk factor for RPL, which is in concordance with our results (51–53). Frequency of FVR2 heterozygotes in published studies ranged from 3.6–18% in controls and from 6.8–16% in women with RPL (51–53). We found higher prevalence of heterozygous carriers of FVR2 in our study group (29% and 32%, Table I), which can be explained by different ethnic background of investigated populations.

Although recent meta-analysis suggest association of MTHFR gene variants and adverse pregnancy outcomes, those results should be interpreted carefully since most of them are based on results from Asian population and no other possible causes of RPL were taken into account (54). It is well known that frequency of polymorphic alleles varies among populations of different ethnic background. We found no significant association of MTHFR C677T and A1298C and pregnancy loss (Table II), similar to other studies (51–53, 55, 56). Our result showed significant difference in A1298C allele frequencies among tested groups (P=0.010, Table I), with increased number of A1298C heterozygote carriers in control group. Relatively small CG sample size and deviation from HV equilibrium in CG for variant in question can be possible explanation for these results. Homozigosity for C667T or compound heterozygosity of C677T and A1298C, if homocysteine serum levels are normal, represent no major risk factor for adverse pregnancy outcomes (57). There is growing evidence that MTHFR testing has minimal clinical utility, thus American college of medical genetics (ACMG) recommends that it should not be ordered as a part of routine evaluation for thrombophilia (57).

Increasing evidence supports role of EPCR h3 haplotype as a risk factor for thrombotic events (58–60). H3 haplotype carriers have increased levels of soluble EPCR (sEPCR), decreased levels of functional membrane-bound EPCR, and thus reduced rate of PC activation (58–60). Meta-analysis of Dennis and coworkers (58) showed that h3 haplotype frequency among healthy subjects can differ significantly in various populations, but that it ranges form 10–51%, for h3 heterozygotes and from 0–5% for h3 homozygotes. Frequency of h3 haplotypes in our control population matches published results (16% for h3 heterozygotes and 0% for h3 homozygotes, Figure 1). Animal studies have shown that PROCR gene coding for EPCR is necessary for early embryonic development (61, 62). PROCR knock out mice show early embryonic lethality (before day 10.5). However, if such embryos are separated from extra-embryonic structures, they survive in vitro, which implies crucial role of PROCR gene in proper placental development (62). EPCR is expressed on surface of giant trophoblast cells and is in direct contact with maternal circulation. Extra-embryonic cells lacking surface EPCR are surrounded by fibrin deposits and clots, further supporting the important role of this receptor in controlling of coagulation processes on maternal-foetal interface (62). Different findings regarding EPCR haplotypes and adverse pregnancy outcome have been reported. Dendana et al. (63) showed that risk for RPL is increased in carriers of h3 haplotype. Cechery-Nouvillon et al. (64) concluded that if fetus has h3h3 genotype, risk for miscarriage is further increased. However, we found no significant association between EPCR haplotypes and recurrent miscarriage nor significant difference among haplotype frequencies in tested groups (Table II, Figure 1). Hopmeier et al. (60) suggested protective role of h1 haplotype against RPL, especially in FVL mutation carriers, while they found no significant change in relative risk for RPL in h3 haplotype carriers, and assumed that influence of EPCR haplotypes on RPL risk is small. Similar results are obtained by Kaare et al. (61), which concluded that mutations in EPCR are not considered to be major risk factor for recurrent
Prevalence of heterozygotes for FXIII V34L gene variant in our study (32% for CG and 53% RPL group, Table I) matches previously published data in European and North American populations (65, 66). Although we found no difference in frequency of FXIII V34L allele among tested groups (P=0.064, table I), risk for miscarriage in carriers of V34L is increased 2.81 times (OR 2.81, 95 %CI 1.15–6.87, P=0.023, Table II). Several studies are concordant with our results (51–53).

Frequency of PAI-1 4G/4G gene variant was not increased among women experiencing RPL compared to control, nor it was associated with increased risk for RPL (Tables I and II). Djordjevic et al. (19) reported that PAI-1 4G/4G doesn’t confer increased risk for early foetal loss. Similar results are published by various studies (51–53), although some reports suggest otherwise (67).

Interestingly, our result show that combined presence of XIII V34L and PAI-1 4G gene variants leads to substantial increase in risk for RPL (OR 13.98, 95%CI 1.11–17.46, Table II). Study by Dossenbach et al. concluded that isolated presence of FXIII V34L or PAI-1 4G variants represents no risk factor for RPL, but if present in combination it significantly increases the risk or RPL (66). These observations have a sound pathophysiological explanation. Successful placentation depends on adequate trophoblast invasion in endometrial tissue and its stabilisation by forming of fibrin links. V34L gene variant changes the structure and quality of blood clots. Accelerated fibrin formation caused by V34L presence inhibits lateral aggregation of fibrin fibers, which reduces mass/length ratio. Newly formed fibrin has finer mesh structure with thinner fibers that are more densely placed. This leads to reduced fibrinolytic activity, since t-PA and u-PA perform better on coarse fibrin mesh with larger pores (24, 66, 68). Hypofibrinolysis caused by increased PAI-1 expression in 4G variant carriers can lead to fibrin over-deposition and consequent disruption of trophoblast migration during early stages of placentation (20, 21). Our data suggest that V34L and PAI-1 4G may have additive effect by increasing fibrin resistance to degradation and reducing the activity of fibrinolytic system, thus leading to impaired placentation.

In conclusion, this is the first study in Serbian population that investigated prevalence of FVR2, A1298C, FXIII V34L and EPCR gene variants. Our data shows that compound heterozygosity for FXIII V34L and PAI-1 4G is significant risk factor for recurrent miscarriage. As polymorphisms in FXIII are currently not part of routine thrombophilia testing panel, we suggest that it should be included in diagnostic testing as it can contribute to more precise risk estimation for RPL. Although our result should be viewed in context of small case-control study, tested population was highly selected, all other known causes of pregnancy loss were previously excluded in patients. Nevertheless, further large prospective studies are need for confirmation of our findings.

Acknowledgements. This work was supported by the project funded by Ministry of Education, Science and Technological Development of the Republic of Serbia (project No. ON173046)

Conflict of interest statement
The authors stated that they have no conflicts of interest regarding the publication of this article.
Biochemical markers for prediction of hypertensive disorders of pregnancy. J Med Biochem 2019; 58: 71–82.

10. Rey E, Kahn SR, David M, Shrier I. Thrombophilic disorders and fetal loss: a meta-analysis. Lancet 2003; 361: 901–8.

11. de Visser MC, Guasch JF, Kamphuisen PW, Vos HL, Rosendaal FR, Bertina RM. The HR2 haplotype of factor V: effects on factor V levels, normalized activated protein C sensitivity ratios and the risk of venous thrombosis. Thromb Haemost 2000; 83: 577–82.

12. Kujovich JL. Prothrombin-Related Thrombophilia. 2006 Jul 25 [Updated 2014 Aug 14]. In: Pagon RA, Adam MP, Ardinger HH, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2016. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1148/

13. Rosendaal FR, Doggen CJM, Zivelin A, et al. Geographic distribution of the 20210 G to A prothrombin variant. Thromb Haemost 1998; 79: 706–8.

14. Ivanov PD, Komsa-Penkova RS, Konova EI, Kovacheva KS, Simeonova MN, Popov JD. Association of inherited thrombophilia with embryonic and postembryonic recurrent pregnancy loss. Blood Coagul Fibrinolysis 2009; 20: 134–40.

15. Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylene tetrahydrofolate reductase. Nat Genet 1995; 10: 111–3.

16. Weisberg I, Tran P, Christensen B, Sibani S, Rozen R. A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. Mol Genet Metab 1998; 64: 169–72.

17. Eldibany MM, Caprini JA. Hyperhomocysteinemia and thrombosis: an overview. Arch Pathol Lab Med 2007; 131:872–84.

18. Brown NM, Pratt VM, Buller A, et al. Detection of 677CT/1298AC+double variant+chromosomes: implications for interpretation of MTHFR genotyping results. Genet Med. 2005; 7: 278–82.

19. Djordjevic V, Gvozdenov M, Pruiner I, Kovac M, Tomic B. et al. The prevalence of PAI-1 4g/5g polymorphism in women with fetal loss - first data for a Serbian population. Journal of Medical Biochemistry 2013; 203–7.

20. Zhao L, Bracken M, DeWan A, Chen A. Association between Plasminogen Activator Inhibitor-1 Gene Polymorphisms and Recurrent Pregnancy Loss: A Systematic Review and Meta-Analysis. Am J Reprod Immun 2015; 73: 292–300.

21. Board PG, Losowsky MS, Miloszewski KJ. Factor XIII: inherited and acquired deficiency. Blood Rev 1993; 7: 229–42.

22. Mikkola H, Palotie A. Gene defects in congenital factor XIII deficiency. Semin Thromb Hemost 1996; 22: 393–8.

23. Balogh I, Szőke G, Kárpáti L, Wartiovaara U, Katona E, Komáromi I, Haramura G, Pflegler G, Mikkola H, Muszbek L. Val54Leu polymorphism of plasma factor XIII: biochemistry and epidemiology in familial thrombophilia. Blood 2000; 96(7): 2479–86.

24. Kreutz RP, Bitar A, Owens J, Desta Z, Breall JA, von der Lohe E, Sinha A, Vatta M, Nystrom P, Jin Y, Flockhart DA. Factor XIII Val54Leu polymorphism and recurrent myocardial infarction in patients with coronary artery disease. J Thromb Thrombolysis 2014; 38: 580–7.

25. Dossenbach-Glaninger A, van Trotsenburg M, Oberkanins C, Atamaniuk J. Risk for Early Pregnancy Loss by Factor XIII Val54Leu: The Impact of Fibrinogen Concentration. Journal of Clinical Laboratory Analysis 2015; 27: 444–9.

26. Morange P, Trégouët D, F Gagnon, Dennis J, Johnson C, Adediran A, de Andrade M, Heit J. The endothelial protein C receptor (PROC) Ser219Gly variant and risk of common thrombotic disorders: evidence from observational studies: a HuGE view and meta-analysis of evidence form observational studies. Blood 2012; 119: 2392–400.

27. Medina P, Navarro S, Bonet E, Martins L, Estellés A, Bertina R, Vos H, España F. Functional Analysis of Two Haplotypes of the Human Endothelial Protein C Receptor Gene. Arterioscler Thromb Vasc Biol 2014; 34: 684–90.

28. Hopmeier P, Puehringer H, van Trotsenburg M, Atamaniuk J, Oberkanins C, Dossenbach-Glaninger A. Association of endothelial protein C receptor haplotypes, factor V Leiden and recurrent first trimester pregnancy loss. Clinical Biochemistry 2008; 41: 1022–4.

29. Oberkanins C, Moritz A, de Villiers JNP, Kotze MJ, Kury F. A reverse hybridization assay for the rapid and simultaneous detection of nine HFE gene mutations. Genet Testing 2000;4: 121–4.

30. Sergi C, Al Jishi T, Walker M. Factor V Leiden mutation in women with early recurrent pregnancy loss: a meta-analysis and systematic review of the causal association. Arch Gynecol Obstet 2015; 291(3): 671–9.

31. Akdemir Y, Ayvacı H, Uludogan H. Effect of multiple thrombophilic gene mutations on uterine artery blood flow in nonpregnant recurrent pregnancy loss patients: are we searching enough? Matern Fetal Neonat Medicine 2019; 31: 1–7.

32. Barut MU, Bozkurt M, Kahraman M, et al. Thrombophilia and Recurrent Pregnancy Loss: The Enigma Continues. Med Sci Monit 2018; 24: 4288–94.

33. Rey E, Kahn SR, David M, Shrier I. Thrombophilic disorders and fetal loss: a meta-analysis. Lancet 2003; 361: 901–8.

34. Kovac M, Mitic G, Mikovic Z, Djordjevic V, Savic O, Mandic V, Rakicevic LJ, Antonijevic N, Radojkovic D. Thrombophilia in women with pregnancy-associated complications: fetal loss and pregnancy-related venous thromboembolism. Gynecol Obstet Invest 2010; 69(4): 233–8.

35. Ridker PM, Miletich JP, Buring JE, Aritory AA, Price DT, Manson JE, Hill JA. Factor V Leiden mutation as a risk factor for recurrent pregnancy loss. Ann Intern Med 1998; 128: 1000–3.
37. Yikilmaz SA, Bakanay MS, Akinci S, Alisik M, Erel Ö, Dilek İ. Thiol-disulphide homeostasis in essential thrombocytopenia patients. J Med Biochem 2019; 38: 475–80.
38. Gris JC, Quere I, Monpeyroux F, Mercier E, Ripart-Neveu S, Tailland ML, Hoffet M, Berlan J, Daures JP, Mares P. Case-control study of the frequency of thrombophilic disorders in couples with late foetal loss and no thrombotic antecedent – the Nimes Obstetricians and Haematologists Study5 (NOHA5). Thromb Haemost 1999; 81: 891–9.
39. Kupferminc MJ, Eldor A, Steinman N, Many A, Bar-Am A, Jaffa A, Fait G, Lessing JB. Increased frequency of genetic thrombophilia in women with complications of pregnancy. N Engl J Med 1999; 340: 9–13.
40. Martinelli I, Taioli E, CETin I, Marinoni A, Gerosa S, Villa MV, Bozzo M, Mannucci PM. Mutations in coagulation factors in women with unexplained late fetal loss. N Engl J Med 2000; 343: 1015–8.
41. Pabinger I, Vossen CY, Lang J, Conard J, Garcia-Dabrio MC, Miesbach W, Legnani C, Svensson P, Kaider A, Rosendaal FR. Mortality and inherited thrombophilia: results from the European Prospective Cohort on Thrombophilia. J Thromb Haemost 2012; 10(2): 217–22.
42. Peres W, Aranda F, Udry S, Lationo J, de Larranaga G. Inherited thrombophilia and pregnancy loss. Study of Argentinian cohort. Med Clin 2018. pii: S0025-7753(18)30071-X.
43. Pihusch R, Buchholz T, Lohse P, Rübsamen H, Schilders N, Hasberg U, Hiller E, Thaler CJ. Thrombophilic gene mutations and recurrent spontaneous abortion: prothrombin mutation increases the risk in the first trimester. Am J Reprod Immunol 2001; 46:124–31.
44. Raziel A, Kornberg Y, Friedler S, Schachter M, Sela BA, Ron-El R. Hypercoagulable thrombophilic defects and hyperhomocysteinemia in patients with recurrent pregnancy loss. Am J Reprod Immunol 2001; 46:124–31.
45. Reznikoff-Etiévan MF, Cayol V, Carbonne B, Robert A, Cayol V, Carbonne B, Robert A, Fait G, Lessing JB. Increased frequency of genetic thrombophilia in women with complications of pregnancy. N Engl J Med 1999; 340: 9–13.
46. Bank I, Libourel EJ, Middeldorp S, Van Pampus EC, Koopman MM, Hamulyák K, Prins MH, Van Der Meer J, Bülter HR. Prothrombin 20210A mutation: a mild risk factor for venous thromboembolism but not for arterial thrombotic disease and pregnancy-related complications in a family study. Arch Intern Med 2004; 164: 1932–7.
47. Kocher O, Cirovic C, Malynn E, Rowland CM, Bare LA, Young BA, Henslee JG, Laf‼ler TG, Huff JB, Kruskall MS, Wong G, Bauer KA. Obstetric complications in patients with hereditary thrombophilia identified using the LCx microparticle enzyme immunoassay: a controlled study of 5,000 patients. Am J Clin Pathol 2007; 127:68–75.
48. Karakantza M, Androustopoulos G, Mougiou A, Sakellariopoulos G, Kourounis G, Decavalas G. Inheritance and perinatal consequences of inherited thrombophilia in Greece. Int J Gynaecol Obstet 2008; 100: 124–9.
49. Pasquier E, Bohec C, Mottier D, Jaffuel S, Mercier B, Férec C, Collet M, De Saint Martin L. Inherited thrombophilias and unexplained pregnancy loss: an incident case-control study. J Thromb Haemost 2009; 7: 306–11.
50. Silver RM, Zhao Y, Spong CY, Sibai B, Wendel G Jr, Westrom K, Samuels P, Caritis SN, Sorokin Y, Miodownik M, O’Sullivan MJ, Conway D, Wapner RJ. Eunice Kennedy Shriver National Institute of Child Health and Human Development Maternal-Fetal Medicine Units. (NICHHD MFMU) Network. Prothrombin gene G20210A mutation and obstetric complications. Obstet Gynecol 2010; 1154–20.
51. Goodman CS, Coulam CB, Jeyendran RS, Acosta VA, Rousser R. Which thrombophilic gene mutations are risk factors for recurrent pregnancy loss? Am J Reprod Immunol 2006; 56(4): 250–6.
52. Coulam CB, Wallis D, Weinstein J, DasGupta DS, Jeyendran RS. Comparison of thrombophilic gene mutations among patients experiencing recurrent miscarriage and deep vein thrombosis. Am J Reprod Immunol 2008; 60(5): 426–31.
53. Yenicesu GI, Cetin M, Ozdemir O, Cetin A, Ozen F, Yenicesu C, Yildiz C, Kocak N. A prospective case-control study analyzes 12 thrombophilic gene mutations in Turkish couples with recurrent pregnancy loss. Am J Reprod Immunol 2010; 63(2): 126–36.
54. Rai V. Methylenetetrahydrofolate Reductase C677T Polymorphism and Recurrent Pregnancy Loss Risk in Asian Population: A Meta-analysis. Indian J Clin Biochem 2016; 31(4): 402–13.
55. Kutteh WH, Park VM, Deitche SR. Hypercoagulable state mutation analysis in white patients with early first-trimester recurrent pregnancy loss. Fertil Steril 1999; 71(6): 1048–53.
56. Varga E. Inherited thrombophilia: key points for genetic counseling. J Genet Couns 2007; 16(3): 261–77.
57. ACMG Practice Guideline: lack of evidence for MTHFR genotypes in newborn screening for congenital heart disease. Genet Med 2016; 18(2): 111–14.
58. ACCP guidelines on the diagnosis and management of thrombosis and thromboembolism in pregnancy. Chest 2014; 145(3 suppl): 64S–84S.
59. ASCO Practice Guidelines: management of patients with venous thromboembolism during pregnancy. J Clin Oncol 2014; 32(24): 2644–2654.
60. ATC guidelines on the diagnosis and management of thrombosis and thromboembolism in pregnancy. J Thromb Thrombolysis 2014; 37(3): 288–98.
61. AUC guidelines on the diagnosis and management of thrombosis and thromboembolism in pregnancy. Am J Obstet Gynecol 2014; 210(4): 440–52.
62. ACOG guidelines on the diagnosis and management of thrombosis and thromboembolism in pregnancy. Obstet Gynecol 2014; 123(1 suppl): 1–44.
63. EUGMS guidelines on the diagnosis and management of thrombosis and thromboembolism in pregnancy. J Thromb Haemost 2014; 12(3 suppl): 77–92.
64. ESC guidelines on the diagnosis and management of thrombosis and thromboembolism in pregnancy. Eur Heart J 2014; 35(37): 2620–2679.
65. ESC guidelines on the diagnosis and management of thrombosis and thromboembolism in pregnancy. Eur Heart J 2014; 35(37): 2601–2619.
66. ESC guidelines on the diagnosis and management of thrombosis and thromboembolism in pregnancy. Eur Heart J 2014; 35(37): 2581–2599.
67. ESC guidelines on the diagnosis and management of thrombosis and thromboembolism in pregnancy. Eur Heart J 2014; 35(37): 2561–2579.
68. ESC guidelines on the diagnosis and management of thrombosis and thromboembolism in pregnancy. Eur Heart J 2014; 35(37): 2541–2559.
69. ESC guidelines on the diagnosis and management of thrombosis and thromboembolism in pregnancy. Eur Heart J 2014; 35(37): 2521–2539.
70. ESC guidelines on the diagnosis and management of thrombosis and thromboembolism in pregnancy. Eur Heart J 2014; 35(37): 2501–2519.
71. ESC guidelines on the diagnosis and management of thrombosis and thromboembolism in pregnancy. Eur Heart J 2014; 35(37): 2481–2499.
72. ESC guidelines on the diagnosis and management of thrombosis and thromboembolism in pregnancy. Eur Heart J 2014; 35(37): 2461–2479.
endothelial protein C receptor genes in couples with recurrent miscarriage. Hum Reprod 2007; 22(3): 864–8.

62. Gu JM, Crawley JT, Ferrell G, Zhang F, Li W, Esmon NL, Esmon CT. Disruption of the endothelial cell protein C receptor gene in mice causes placental thrombosis and early embryonic lethality. J Biol Chem 2002; 277(45): 43335–43.

63. Dendana M, Messaoudi S, Hizem S, Jazia KB, Almawi WY, Gris JC, Mahjoub T. Endothelial protein C receptor 1651C/G polymorphism and soluble endothelial protein C receptor levels in women with idiopathic recurrent miscarriage. Blood Coagul Fibrinolysis 2012; 23(1): 30–4.

64. Cochery-Nouvellon E, Chauleur C, Demattei C, Mercier E, Fabbro-Peray P, Marès P, Mismetti P, Lissalde-Lavigne G, Gris JC. The A6936G polymorphism of the endothelial protein C receptor gene is associated with the risk of unexplained foetal loss in Mediterranean European couples. Thromb Haemost 2009; 102(4): 656–67.

65. Coulam CB, Jeyendran RS, Fishel LA, Roussev R. Multiple thrombophilic gene mutations rather than specific gene mutations are risk factors for recurrent miscarriage. Am J Reprod Immunol 2006; 55(5): 360–8.

66. Dossenbach-Glaninger A, van Trotsenburg M, Dossenbach M, Oberkanins C, Moritz A, Krugluger W, Huber J, Hopmeier P. Plasminogen activator inhibitor 1 4G/5G polymorphism and coagulation factor XIII Val34Leu polymorphism: impaired fibrinolysis and early pregnancy loss. Clin Chem 2003; 49(7): 1081–6.

67. Buchholz T, Lohse P, Rogenhofer N, Kosian E, Pfusch R, Thaler CJ. Polymorphisms in the ACE and PAI-1 genes are associated with recurrent spontaneous miscarriages. Hum Reprod 2003; 18(11): 2473–7.

68. Ariëns RA, Philippou H, Nagaswami C, Weisel JW, Lane DA, Grant PJ. The factor XIII V54L polymorphism accelerates thrombin activation of factor XIII and affects cross-linked fibrin structure. Blood 2000; 96(3): 988–95.

Received: March 14, 2019
Accepted: May 6, 2019