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Association of *VEGFA*, factor V and prothrombin gene polymorphisms with early pregnancy loss

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Abstract

Background: Many studies conducted to assess the associations between the gene polymorphisms of factor V, prothrombin, and vascular endothelial growth factor gene A and recurrent early pregnancy loss (REPL) have shown conflicting findings. **The aim of the study:** We designed this study and selected the most common polymorphisms that have been analyzed before, *VEGFA −2578C/A (rs699947*), *VEGFA 936C/T* (*rs3025039*), *FVL G1691A (rs6025),* and prothrombin *FII G20210A (rs1799963*) to be the candidate genetic polymorphisms for analysis of their association with idiopathic early pregnancy loss in Russian women. **Materials and methods:** 100 women with idiopathic early pregnancy loss were enrolled and classified into two subgroups: sporadic early pregnancy loss (SEPL), consisting of 50 women, and recurrent early pregnancy loss (REPL), consisting of 50 women. The control group included 56 women with full-term babies. Genotyping was performed using commercially available kits (Syntol, Russia) for Real time-PCR method. Genotype and allele distributions in studied groups were compared using the chi-square test and Fisher's exact test. The tests and calculation of Odds ratio with 95% confidence intervals (CIs) were conducted employing the statistical software SPSS, version 22. **Results:** The heterozygous genotype (*CA*) for *VEGFA rs699947* was significantly associated with REPL. Findings have shown that women carrying the heterozygous genotype had a higher REPL risk (OR 9.04, 95% CI 4.33-18.7). No significant associations with SEPL or REPL were found for the other studied polymorphisms. **Conclusion:** Our findings suggest that heterozygosity for *VEGFA rs699947* gene polymorphism may play a role in predisposition to idiopathic early pregnancy loss and can be a genetic risk factor for recurrent early miscarriage in Russian women.

Keywords: recurrent pregnancy loss; VEGFA; factor V; prothrombin; gene polymorphisms

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Introduction. Early pregnancy loss (EPL), defined as the spontaneous failure of a pregnancy within the first 12 weeks of gestation, is an important reproductive problem happening roughly in 10% of all clinically recognized pregnancies and 80% of all pregnancy loss cases [1]. Such a loss is called sporadic early pregnancy loss (SEPL) when it occurs once in nonconsecutive rounds of gestations, affecting 25-50% of all reproductive-age women, but when it happens two or more times called recurrent early pregnancy loss (REPL), affecting 2-5% of couples seeking pregnancy [1, 2]. Until now, a variety of causative factors have been explained to be implicated in pregnancy abortion, including uterine anomalies, endocrine and autoimmune disorders, acquired and hereditary thrombophilias, in addition to nutritional and lifestyle factors, but only 50% of EPL causes could be identified [2, 3].

To achieve a successful pregnancy, it requires from placenta to develop and function properly. The placenta may lose its perfusion function as a result of formation of microthrombi in the placental vascular bed, causing serious complications such as abruption of placenta, fetal growth restriction, and pregnancy loss. Thrombophilia is a predisposition to the development of thrombosis caused by inherited and acquired defects in clotting process. Inherited thrombophilia occurs due to various disorders of blood coagulation, encompassing the mutation of factor V (FV), the prothrombin (FII), and shortage of the plasma anticoagulants, such as antithrombin, protein S, and protein C. Within these abnormalities, Factor V Leiden (*FVL G1691A*) and *FII G20210A* are comparatively common, whereas the others are infrequent. The involvement of inherited thrombophilia in early abortion and vascular disorders during pregnancy has been screened in several studies that have found an association between inherited thrombophilia and recurrent early abortion [4], while the other has not [5], therefore the findings appear to be inconsistent.

To ensure the progressive development of gestation needs that the implanting embryo must induce the capillaries formation through the angiogenesis process for providing its blood supply. The best-reported inducer of angiogenesis is the vascular endothelial growth factor (VEGFA). VEGFA is an efficient proangiogenic glycoprotein exerting a proliferative effect on endothelial cells residing in pre-existing blood vessels and therefore induces fetal-maternal blood vessel formation in the uterus [6]. During early pregnancy, VEGFA is secreted from the ovarian follicles and embryonic trophoblast; it plays a vital role in the maturation of oocytes, the proliferation of trophoblast, the increase of vascular permeability and endothelial cell proliferation resulting in successful embryo implantation as well as in maintaining growth and development of embryo through the establishment of normal chorionic villous vascularization [7].

Many polymorphisms have been revealed to influence the activity and expression of the *VEGFA* gene. Some studies have not found any association between REPL and *VEGFA -2578 C/A*, *VEGFA 936C/T* polymorphisms [8], while others found that *VEGFA -2578C/A* and *VEGFA 936C/T* were associated [9, 10]. Recently, a study found that the *VEGFA 936C/T* was associated with REPL whereas the *VEGFA -2578C/A* was not [11], however, another study performed on Russian women found that *VEGFA -2578C/A* was associated with increased risk of first-trimester pregnancy loss [12].

The inconsistencies in all aforementioned genetic studies' findings of the implication of gene polymorphisms of VEGFA, factor V, and prothrombin in the pathogenesis of early abortion led to trouble obtaining a correct assessment of their association with REPL.

The aim of the study. We designed this study and selected the most common *VEGFA*

polymorphisms that have been analyzed before, *VEGFA −2578C/A (rs699947*), *VEGFA 936C/T* (*rs3025039*), in addition to such clotting factors as Factor V (*FVL G1691A rs6025*) and prothrombin (*FII G20210A rs17*99963) to be the candidate genetic polymorphisms for an analysis of their association with idiopathic early pregnancy loss in Russian women.

Materials and methods. *Participants and samples.* We conducted a case-control study to analyze *VEGFA* gene polymorphisms (*VEGFA −2578C/A rs699947*, *VEGFA 936C/T rs3025039*, Factor V Leiden (*FVL G1691A rs6025),* and prothrombin (*FII G20210A rs1799963*) to reassess whether these polymorphisms are maternal risk factors for EPL and REPL in Russian women from Central Russia. All women subjected to this study gave informed consent for participation. The approval to conduct this study was given by the Ethics Committee of the Institute of Medicine of RUDN University. The study was conducted on DNA samples from 100 women who suffered from early pregnancy loss (EPL) that happened within the first 12 weeks of pregnancy with an average age of 31.5±4.9 years. The case group was classified into two groups: one group called SEPL referring to women with a single non-successive pregnancy loss, included 50 participants, and the second group called REPL referring to women with two or more consecutive pregnancy losses, consisted of 50 participants that involved women with primary REPL, those who had not ever had full-term pregnancy and women with secondary REPL, those who had at least one viable fetus prior to miscarriages. The etiological agent of miscarriage could not be diagnosed by classical criteria for early miscarriage evaluation. All early abortion cases experienced chronic diseases were not included in the study. 56 age-matched healthy women 29.2±3.5 years were enrolled as a control group, they had normal pregnancies and no past history of previous miscarriage or any other reproductive problems.

DNA extraction and genotyping of SNPs. Genomic DNA was extracted from peripheral blood samples using a standard procedure of a commercially available kit (Syntol, Russia). Extracted DNA was stored at −20 °C. Genotyping for *VEGFA rs699947*, *VEGFA rs3025039*, *FVL rs6025*, and *FII rs1799963* was performed using commercially available kits for Real time-PCR (Syntol, Russia).

Statistical analysis. Allele and genotype frequencies for studied polymorphisms in cases and controls were computed. Genotype and allele distributions in studied groups were compared using the chi-square test (X^2). Fisher's exact test was used when the frequencies were less than 5%. The tests and calculation of Odds ratio with 95% confidence intervals (CIs) were conducted employing the statistical software SPSS, version 22. P-values ≤ 0.05 were considered statistically significant.

Results and discussion. Genotype and allele frequencies for studied polymorphisms are shown in table.

The findings of the study have not recorded any significant differences in the distributions of the wild genotype (*GG*) and the heterozygous genotype (*GA*) for Factor V Leiden (FVL) *rs6025* polymorphism between all the studied groups. Similarly, no significant differences were found for *FII rs1799963* polymorphism. The minor homozygous genotype (*AA*) for both polymorphisms was not detected in any of the studied groups.

A significant difference in the genotype frequencies for *VEGFA rs699947* polymorphism was shown in women with REPL (*CC* 16.0%, *CA* 72.0%, *AA* 12.0%, P<0.05) when compared with those in healthy women (*CC* 30.4%, *CA* 46.4%, *AA* 23.2%). What is striking in the result that the incidence of the heterozygous genotype was significantly higher, while the wild and minor homozygous genotypes were less frequent among women with recurrent early pregnancy loss than those in healthy women. The findings have revealed that the heterozygous genotype was highly associated with the increased risk of recurrent early miscarriage in the carrier women (OR 9.04, 95% CI 4.33-18.7). No significant differences were found in the distribution of different genotypes and alleles for *VEGFA rs3025039* polymorphism in all studied patient groups when compared with the control group.

Table

Genotype and allele frequencies (%) for *FVL rs6025, FII rs1799963, VEGFA rs3025039,* and *VEGFA rs699947* gene polymorphisms in studied groups

Genotypes and alleles	Control (n=56)	EPL (n=100)	SEPL (n=50)	REPL (n=50)
VEGFA rs699947				
CC	30.4	27.0	38.0	16.0*
CA	46.4	56.0	40.0	72.0*
AA	23.2	17.0	22.0	12.0*
C	53.6	55.0	58.0	52.0
A	46.4	45.0	42.0	48.0
VEGFA rs3025039				
CC	64.3	69.0	64.0	74.0
CT	28.6	26.0	30.0	22.0
TT	7.1	5.0	6.0	4.0
C	78.6	82.0	79.0	85.0
T	21.4	18.0	21.0	15.0
FII rs1799963				
GG	92.9	94.0	92.0	96.0
GA	7.1	6.0	8.0	4.0
AA	0.0	0.0	0.0	0.0
G	96.45	97.0	96.0	98.0
A	3.55	3.0	4.0	2.0
FVL rs6025				
GG	98.2	100	100	100
GA	1.8	0.0	0.0	0.0
AA	0.0	0.0	0.0	0.0
G	99.1	100	100	100
A	0.9	0.0	0.0	0.0

Note: *- p<0.05 in comparison with control

To maintain the growth and development of a fetus, a new blood vessel plexus should be formed in the chorionic villous. VEGFA is a survival factor for endothelial cells and plays also a crucial role in inducing growth of the maternal and fetal blood vessels in the uterus during placenta formation as well as supports embryo development via angiogenesis and vasculogenesis [7]. Several studies have reported that functional polymorphisms of the *VEGFA* gene may have a part in the pathogenesis of REPL. It has been found that *VEGFA −2578C/A (rs699947*) and *936C/T (rs3025039*) polymorphisms have an adjusting influence on the level of the *VEGF* gene expression, where noticed the occurrence of *VEGFA 936T (rs3025039)* in the 3′-untranslated region (3′UTR) or *VEGFA –2578A (rs699947)* in the promoter region of *VEGFA* gene results in the lowered expression of *VEGFA* gene and as a result reduced production of the corresponding protein [8].

The current study results have shown that frequencies of homozygotes for *VEGFA –2578C/A (rs699947*) were significantly lower, while heterozygotes were significantly more frequent in women with recurrent pregnancy loss than those in women of the control group (P-value 0.029). It was found in a study that the occurrence of *A* allele at *–2578-* position is associated with a decreased level of VEGF mRNA expression and as a result *C*A genotype at this site may lead to slightly decreased VEGF protein concentration. Accordingly, VEGFA protein level produced in heterozygous becomes not sufficient for complete angiogenesis and may lead to inadequate fetoplacental vascularization and gestation failure [12]. This explanation may present a clarification for the increased heterozygous genotype level in REPL women whereas can-

not interpret the increased *AA* genotype level in healthy women. Based on the genetic belief that REPL is a polygenic problem and there must be various gene defects that contribute to its onset, we can suppose that the higher occurrence of *AA* genotype among healthy women might not be enough to cause recurrent miscarriages in the absence of the other integrative risk agents. Further, we recommend analyzing the *VEGFA rs699947* polymorphism in a larger sample size.

This study findings support those published by an author who conducted a study on Russian women, where reported that the –2578 heterozygote (*CA*) frequency was significantly higher whereas the homozygous genotype (*AA*) was lower in REPL women compared to healthy women [12]. Likewise, another study found a deficiency in the –2578 *AA* genotype incidence among women with REPL comparing with that in healthy women [9].

The *FVL rs6025* (*G1691A*) and *FII rs1799963* (*G20210A*) polymorphisms are the most common abnormalities in *FV* and *FII* genes, where *FII rs1799963* arises due to the substitution of *G* with *A* at position *20210* in the 3'- untranslated region of the prothrombin gene causing an elevation in plasma prothrombin concentration by 20% – 50% and *FVL rs6025* is a missense mutation resulting in the production of a malfunctioning protein, which resists inhibiting effect by active protein C [4]. Our findings did not show any association between these polymorphisms and any of the early pregnancy loss types, which coincide with another study findings that did not reveal the association, too [5]. Thus, the results indicate that the studied patients suffering from idiopathic miscarriages did not have the most common genetic variants leading to hereditary thrombophilia.

Conclusion. Our findings suggest that heterozygosity for *VEGFA rs699947* gene polymorphism may play a role in predisposition to idiopathic early pregnancy loss and can be a genetic risk factor for recurrent early miscarriage in Russian women.

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Conflict of interests

The authors have no conflict of interest to declare.

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