

Screening the Women with Habitual Abortions for Factor II G20210A and Factor V G1691A Mutations in Turkish Population

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OBJECTIVE: To study the Factor II (prothrombin) G20210A and Factor V G1691A (FV-Leiden) mutations among women with habitual abortions.

STUDY DESIGN: Thirty women who had 3 or more abortions were included in this study. Molecular analysis of both prothrombin G20210A and factor V Leiden G1691A was performed using PCR and RFLP.

RESULTS: The ratios of FV heterozygote and homozygote patients were found as 16.7% and 3.3% respectively. Besides in 3.3% of patients Prothrombin G20210A GA heterozygote genotype was established and none of the patients showed FII homozygote genotype

CONCLUSION: Our results indicates the importance of molecular screening the women with a history of 3 or more habitual abortions for these mutations.

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Key Words: Habitual abortion, Prothrombin G20210A, Factor V Leiden G1691A mutation, RFLP (Restriction Fragment Length Polymorphism)

Habitual abortions (defined as ≥ 3 spontaneous consecutive pregnancy losses) affect 1%-2% of women of reproductive age.¹ The origin is multifactorial, and the established risk factors include chromosomal aberrations, anatomical malformations, hormonal imbalance, and phospholipid antibodies. In recent years, interest has focused on inherited abnormalities of the coagulation system, associated with an increased predisposition to thromboembolic complications and also recurrent abortions.^{2,3}

An inherited defect in anticoagulation known as resistance to activated protein C has been described and this mutation, factor V Leiden (FVL), is associated with 3 to 5-fold increased risks for first episodes and recurrent episodes of venous thromboembolism.⁴ This mutation is due to a single G-A nucleotide transition in exon 10 of the FV gene that results in the replacement of arginine at amino acid position 506 with glutamine.⁵ This mutation is found in Caucasians with a varying prevalence of 1-13%, depending on geographic site and ethnicity. Heterozygotes for FVL have an approximate 8-fold increased relative risk for the development of venous thrombosis, and homozygotes are estimated to have an approximately 90-fold increased relative risk.^{5,6}

Another single G-A transition, was reported at position 20210 of the prothrombin (FII) gene, occurring in the 3' un-

translated region of the gene just before the site of polyadenylation. FII G20210A mutation is present in approximately 2% of healthy Caucasians, and in 6% of patients with a first episode of venous thrombosis. FII G20210A mutation increases the relative risk of venous thrombosis by approximately 3-fold.⁵

In this study, we investigated Factor II G20210A and Factor V G1691A mutations among the patients referred with the complaints of habitual abortions.

Materials and Methods

Subjects

Thirty woman referred to Genetics Department with the complaints of habitual abortions were included in this study. All of the patients had 2 or more abortions in their previous pregnancies. Molecular analysis of both prothrombin G20210A and factor V Leiden G1691A was performed using PCR and Restriction Fragment Length Polymorphism (RFLP).

Factor II (prothrombin) G20210A and Factor V G1691A (FV-Leiden) mutations

Factor V primers included: the sense strand primer, 5'-TCAGGCAGGAACAACACCAT-3'; and the antisense strand primer, 5'-GGTACTTCAAGGACAAAATACCTGTAAAAGCT-3'; and prothrombin primers included the sense strand primer, 5'-GCACAGACGGCTGTTCTCTT-3'; and the antisense primer, 5'-ATAGCACTGGGAGCATTGAAGC-3'. DNA was amplified in a thermal cycling reaction consisting of a 5 min enzyme activation at 95°C, followed by 35 cycles of 1 min at 94°C, 1 min at 55°C, 1 min at 72°C, and a final extension time for 5 min at 72°C. The amplified products (241 bp for Factor V Leiden and 345 bp for F II 20210) was then digested with HindIII overnight. After restriction digested products were resolved by electro-

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phoresis on a 3% agarose gel and stained with ethidium bromide for analysis.^{7,8}

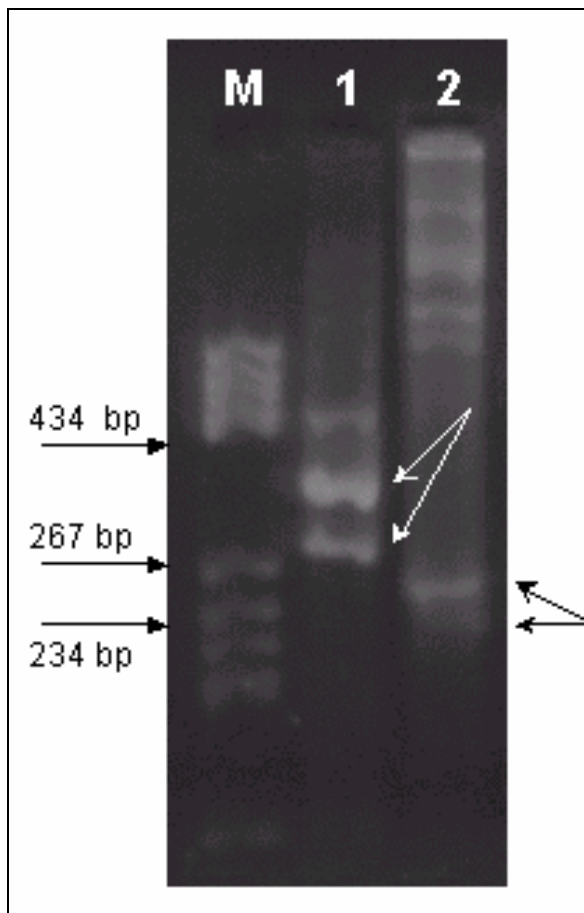


Figure I. Ethidium bromide-stained 3% agarose gel PCR analysis for genotypes of factor V Leiden and of prothrombin G20210: M: 8-587 DNA marker, 1: Prothrombin 20210 heterozygote patient (345-322 bp; marked by white arrows), 2: Factor V Leiden heterozygote patient (241-210 bp; marked by black arrows).

Results

Molecular analysis of 30 woman patients revealed 5 Factor V Leiden 1691 GA heterozygote patients, 1 Factor V Leiden 1691 AA homozygote patient and 1 patient carrying Prothrombin G20210A GA heterozygote genotype (Figure I). The ratios of FV heterozygote and homozygote patients were found as 16.7% and 3.3% respectively. Besides in 3.3% of patients Prothrombin G20210A GA heterozygote genotype was established and none of the patients showed FII homozygote genotype (Table 1).

Table 1. The genotypes of 30 women for Factor II G20210A and Factor V G1691A mutations.

Mutations	No of patients	Ratio (%)
FII G20210A GA heterozygote	1	3.3
FII G20210A GA homozygote	0	0
FV 1691 GA heterozygote	5	16.7
FV 691 AA homozygote	1	3.3

Discussion

FVL carriership is associated with a 4-4.9-fold increase in the risk of habitual abortion. Women carrying the FII variant allele present a 3.5-4-fold increase in the risk of fetal loss. It has been reported that both FVL and FII G20210A are genetic factors that increase susceptibility to fetal loss and add further to the notion that thrombophilia plays a role in the pathophysiology of habitual abortion.^{3,9}

Maternal venous thromboembolism leading to recurrent fetal loss is multi-factorial and involves the participation of a number of acquired risk factors, including advanced age (>40 years of age), obesity, hyperemesis, pre-eclampsia, infection, or systemic disease, and inherited risk factors, including FVL, FII G20210A, and MTHFR C677T and A1298T mutations.¹⁰ First-trimester abortions are caused by implantation failures rather than by thrombotic tendencies.¹¹

The prevalence of FII G20210A polymorphism does not differ significantly between women with two and three or more fetal losses, while it confers a significant risk for primary miscarriages.¹²

FVL can be found in nearly 50% of women with gestational thrombosis.² The association between FVL and late recurrent fetal loss is stronger than for early recurrent fetal loss. The association between FVL and recurrent fetal loss is stronger if other potential causes of fetal loss are excluded. Late non-recurrent fetal loss is associated with FVL, FII G20210A mutation, and protein S deficiency.¹³ It has been demonstrated that the FII polymorphism was significantly more prevalent in women with second trimester loss, intrauterine growth retardation, and abruptio placentae.^{3,12}

The positive association between FVL and FII G20210A mutations and recurrent miscarriages prompts the suggestion of screening for both mutations before or early in the pregnancy before instituting anticoagulant therapy, especially in women with family history of venous thrombosis, oral contraceptive use, and/or pregnancy thrombosis-associated complications.¹⁰

In our study our results revealed that FV mutations are more common than FII mutations in women with habitual abortions. Although our study group is limited, our results of FV mutation indicates the importance of molecular screening the women with a history of 3 or more habitual abortions for these mutations. Besides if it is possible a specimen from the abortus material has also to be studied for these mutations in order to give a perspective to the clinician from the mother and fetus. This will also reflect light on the effect of these mutations for fetus, separate from the mother.

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