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The Effect of Factor-XI (rs3756008) Polymorphism on Recurrent Pregnancy Loss in Iranian Azeri Women

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Abstract

Background: Recurrent pregnancy loss (RPL) is a heterogeneous condition consisting of three or more consecutive abortions before 20th week of gestation. Despite extensive research on the causative effects of RPL, approximately 50% - 60% of RPLs are still idiopathic. Although an increasing number of prospective studies have been conducted with sufficient power to investigate the association between various thrombophilias and RPL, some controversy still remains toward screening for thrombophilia in women with RPL.

Objectives: The aim of this study was to investigate the association between recurrent pregnancy loss and polymorphism in factor-XI (A > T) gene in Iranian Azeri women.

Methods: This is an association study with a case-control design. The study participants consisted of 320 women with RPL from Iranian Azeri origin. The control group comprised 320 age- and ethnically-matched healthy women in the reproductive age. Genomic DNA was extracted from the whole blood and genotyping was performed using PCR amplification followed by restriction fragment length polymorphism (RFLP) analysis.

Results: The genotype frequencies in the case group were 54/68% AA, 45/31% AT, and 0/9% TT, while the frequencies in the control group were 52/18% AA, 47/32% AT, and 0/6% TT. No significant association was found between factor XI rs3756008 polymorphism and RPL (P > 0.05).

Conclusions: There is no significant association between factor XI rs3756008 (A > T) polymorphism and RPL among Iranian Azeri women.

Keywords: FXI, Polymorphism, Recurrent Pregnancy Loss

1. Background

Recurrent pregnancy loss (RPL) is an event of two or more successive pregnancy losses in the first trimester that comprises up to 5% of clinically pregnancy losses (1). RPL may occur due to genetic, hormonal, metabolic, uterine, infectious, immune and thrombophilic factors (2). According to these heterogeneous causes, the appropriate evaluation of patients is necessary to explore the pathophysiological mechanisms; thereby, the cases experiencing RPL would receive a better treatment (3). Thrombophilic disorder is one of the most common complications of pregnancy that affects approximately 1% to 2% of women (4).

Thrombophilia is identified as a major cause of RPL after chromosomal abnormalities with a rate of up to 40%, especially in the first half of pregnancy (5). Thrombophilia is a disorder of coagulation which increases the risk of thrombosis (blood clots in blood vessels) (6).

Coagulation FXI was discovered nearly 50 years ago; its intricate role in hemostasis and thrombosis was not unveiled until the past decade. It is well confirmed that FXI is a plasma serine protease zymogen with a key function in bridging the initiation and amplification of blood coagulation in vivo (7, 8). Coagulation factor XI (FXI) is essential for normal function of the intrinsic pathway of blood coagulation (9). Genetic variants in the FXI gene are risk factors for venous thrombosis among both Whites and Blacks (1).

Factor XI is synthesized in the liver. FXI deficiency is uncommon and presents as an autosomal recessive or acquired disorder. It is commonly asymptomatic. Many studies have previously suggested an association between factor XI (FXI) deficiency and autoantibodies to FXI and recurrent pregnancy loses (10). It can activate factor XI, which subsequently activates factor IX (previous intrinsic pathway); however, this is its marginal function. The transfor-

mation of the plasminogen to plasmin by factor XII and initiation of fibrinolysis is of greater importance. In the human body, the processes of coagulation and fibrinolysis are constantly in a dynamic balance; so, factor XI leakage can increase the risk of thrombosis (11).

There is a double risk of thrombosis as 10% in the population with high levels of FXI compared to the rest without significantly high levels of this factor. There is also a correlation between the risk of venous thrombosis and polymorphisms in the location 4q35.2, where there exists a gene for coagulation FXI (12, 13).

Glueck CJ et al. (2010) study addressed the activity of FXI as a possible cause for miscarriage. Their results indicated that high FXI activity is independently associated with RPL and it should be measured in women with RPL (14)

In this study, the effect of factor-XI rs3756008 polymorphism on recurrent pregnancy loss syndrome was investigated by using RFLP-PCR method among Iranian Azeri women.

2. Methods

This case-control study was conducted to determine the association between factor XI rs3756008 (A > T) polymorphism and recurrent pregnancy loss. A total of 320 patients and 320 healthy controls referring to the teaching hospitals affiliated to Tabriz University of Medical Sciences between 2011 and 2013 took part in this retrospective case-control study.

The subjects were women aged 20 - 35 years who had experienced at least three consecutive abortions before 20th week of gestation. The patients' karyotypes and the structure of uterine were normal; no infection-related miscarriages were detected; and any other identifiable cause of abortion was not figured out. Thus, the events were classified as unexplained pregnancy loss. The age matched control subjects were selected from healthy fertile women with at least two live births and no history of pregnancy loss. In order to prevent the epidemiological bias, all recruited subjects had a same ethnicity and belonged to Iranian Azeri origin. All participants were informed about the study and signed a consent form.

In DNA extraction stage, blood samples (5 mL) from antecubital vein were collected into tubes containing EDTA as anticoagulant. Genomic DNA was extracted using the proteinase K method. Nanodrop instrument was employed to determine the quality and quantity of each DNA sample followed by electrophoresis on 1% agarose gel (Merck Germany) to confirm the results. The extracted DNA samples were stored at -20°C until analysis.

DNA samples were amplified and investigated for polymorphisms in factor XI rs3756008 (A > T) using polymerase chain reaction (PCR) and primers (MWG Biotech): 1 cycle of initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation (94°C for 1 minute), annealing (50°C for 45 seconds), extension (72°C for 45 seconds) and a final extension at 72°C for 5 minutes. In total, 25μL PCR reaction volumes were used (Forward Primer 1μL; Reverse Primer 1 μL; 10X Buffer 2.5 μL; dNTP 10 mM 0.5 μL; Mg 50 mM 0.95 μL; Taq DNA polymerase 10X0 18 μL; DNA 3 μL). The PCR products were electrophoresed on 1.5% agarose gel stained by ethidium bromide. Following amplification reaction, the PCR products were digested through restriction fragment length polymorphism (RFLP) analysis using the appropriate restriction endonucleases. After being digested by MluCI enzyme (Sina Gene), the PCR product (194 bp) remains intact if A allele is present and yields two fragments (131 bp and 63 bp) if the polymorphic T allele is present. Then, the samples were incubated at 37°C for 5 and 15 minutes, respectively. Electrophoresis of the digested products was performed on 3% agarose gel stained by ethidium bromide. The size of bands was estimated by using a molecular weight marker. A gel documentation instrument was used to visualize the bands of PCR and digested products of RFLP analysis.

The data were analyzed using the statistical package SPSS (version 17). They were expressed as mean $\pm$ standard deviation for quantitative variables and number and percentage for qualitative values. $P < 0.05$ was considered statistically significant.

3. Results

320 patients with at least three unexplained RPLs (mean 5, range 3 - 7) and 320 age- and ethnically-matched controls who had at least two successful deliveries from Iranian Azeri origin were screened for the Factor-XI (rs3756008) polymorphism.

The genotype frequencies of Factor-XI (rs3756008) variant in the case group were 54/68% AA, 45/31% AT, and 0/9% TT, while the frequencies in the control group were 52/18% AA, 47/32% AT, and 0/6% TT (Table 3). Based on the statistical analysis, no significant difference was found between patients and controls concerning Factor-XI (rs3756008) genotype frequencies. The allele frequencies for Factor-XI (rs3756008) polymorphism were also deliberated based on groups of subjects (Table 3). Accordingly, there were no meaningful differences in the prevalence of Factor-XI (rs3756008) variants between women with RPL and their healthy controls. Finally, it was assumed that Factor-XI (rs3756008) polymorphism is not related to RPL. Also, genotype frequencies of the two investigated polymorphisms were in Hardy-Weinberg equilibrium (HWE).

Table 1. Primer Sequences Used for Detection of Gene Polymorphisms

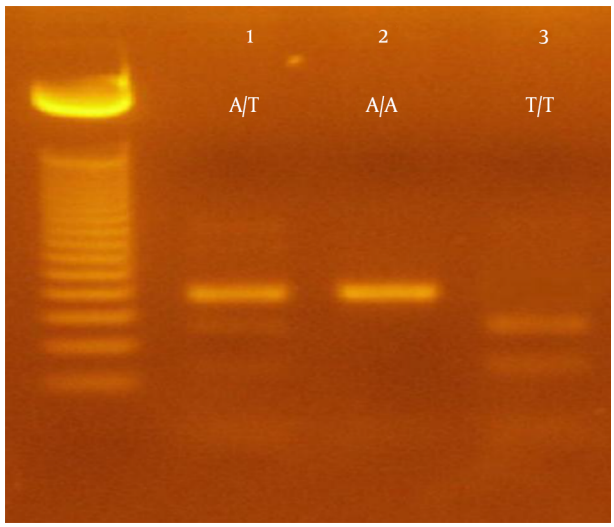
Polymorphism	Primer Sequence	Melting Temperature	Size of Amplified Product	Reference
FXI rs3756008 (A > T)	5'-TTTGGTTTTCCAGTGAAGCA-3'	48	194	16
	5'-GTGCCAAGAATGGCTTTCA-3'	47		

Table 2. Demographic Variables in Patient and Control Groups

Variable	Patient Group	Control Group	P Value
Age			0.15
20 - 25 years	26 cases (8%)	35 cases (11%)	
26 - 30 years	192 cases (60%)	105 cases (35%)	
31 - 35 years	102 cases (32%)	180 cases (56%)	
BMI (Kg/m^2)	25.16 ± 3.16	23.44 ± 3.10	0.02
Education			0.14
Under diploma and diploma	224 (70%)	160 (50%)	
High educated	96 (30%)	160 (50%)	
Systolic BP	114.12 ± 8.88	112.89 ± 8.32	0.32
Diastolic BP	73 ± 8.09	74.6 ± 7.4	0.40

Abbreviation: BMI: body mass index.

Figure 1. Gel Picture Showing RFLP Fragments for SNP rs3756008



PCR product (194 bp) remains intact if A allele is present and yields two fragments (131 bp and 63 bp) in case of T allele.

4. Discussion

This study examined the polymorphism in Factor XI rs3756008 in 320 Iranian Azeri women with RPL in comparison with 320 healthy Iranian Azeri women. The present study was not significantly associated with RPL and Factor XI polymorphism. In this study, RPL was defined as three or more miscarriages.

Despite extensive research to explain the causative effects of recurrent pregnancy loss (RPL), about 50% - 60% of RPLs are still idiopathic. Despite the increasing number of prospective studies with sufficient power to investigate the association between various thrombophilias and RPL, some controversy still remains regarding screening for thrombophilia in women with RPL. Therefore, routine screening is not cost-effective and justified. On the other hand, clinicians need guidelines for screening.

The AA genotype was less prevalent in control women compared to RPL patients, though this difference was not significant. The AT genotype was more frequent in the control women than RPL patients but this difference also was not significant. Also, the TT genotype was less frequent in the control women compared to RPL patients but this difference also was not significant, as observed in Table 3.

rs3756008 (A > T) allele frequencies showed that there is no significant difference between the RPL patients and the controls. The frequency of the major A allele was higher in RPL patients than controls and also, the T allele was more prevalent in RPL patients than controls, as observed in Table 3. Therefore, factor II G20210A polymorphism seems not to be associated with RPL in the investigated popula-

Table 3. Factor-XI Genotype and Allele Frequency

Genotype	Patient Group (320)		Control Group (320)		P Value	OR (95% CI)
	Percent	Cases	Percent	Cases		
Co-dominant						
AA	54.68	172	52.18	167	ref	
AT	45.31	145	47.32	151	0.691	0.931 (0.682 - 1.273)
TT	0.9	3	0.6	2	0.1	1.455 (0.240 - 8.849)
Dominant						
AA	53.75	172	52.19	167	ref	
AT + TT	46.25	148	47.11	153	0.751	0.938 (0.688 - 1.280)
Recessive						
TT	0.93	3	0.6	2	ref	1
AA + AT	99.09	317	99.4	318	1	0.664 (0.11 - 4)
Over dominant						
AT	45.31	145	47.18	151	ref	1
AA + TT	54.68	175	52.82	169	0.692	1.08 (0.709 - 1.268)
A normal	76.40	489	75.78	485	ref	1
T minor	23.59	151	24.21	155	0.89	1/085 (0/596 - 1/975)

tion.

So far, several studies have demonstrated women with thrombophilia have higher risk for RPL, second-trimester miscarriage and other complications of pregnancy (15, 16). Hereditary thrombophilia is increasingly recognized as an important risk factor of RPL. This inheritance of body to inappropriate coagulant formation is induced by genetic make-up of the individuals. Genetic variation in terms of polymorphisms in the genes has a role in the coagulation process and is proposed as a risk factor for RPL (2).

Factor XI rs3756008 (A > T) polymorphism showed no significant difference between the RPL patients and the controls women. J. Sokol et al. demonstrated the associated activity of coagulation factor XI in patients with RPL. While several studies have indicated that this kind of polymorphism has no association with RPL, Parand et al. showed an association between XI rs3756008 and RPL (17-20).

However, we have not found any evidence demonstrating the association of this polymorphism with RPL. This contradictory result may be due to racial, ethnic, and regional differences in studied patients.

Contradictory results in different populations suggest that the role of rs1799963 and rs3756008 polymorphisms in patients with RPL possibly is affected by increased amount of prothrombin in the diet, and geographical and racial diversity (21). Further study is necessary to explain the role of these polymorphisms in recurrent abortion syndrome, and the present study was an attempt to achieve this purpose in Iranian Azeri population.

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Footnotes

Authors' Contribution: Seyed Ali Rahmani and Alireza Isazadeh designed the study concept, analyzed data and prepared the manuscript; Saba Hajazimian, Nazila Moghtaran and Nazila Fathi Maroufi conducted experimental studies and drafted the manuscript; Milad Mohammadoo-Khorasani involved in drafting the manuscript and final revision of the manuscript.

Conflicts of Interest: The authors declare that they have no conflicts of interest

References

1. Austin H, De Staercke C, Lally C, Bezemer ID, Rosendaal FR, Hooper WC. New gene variants associated with venous thrombosis: a replication study in White and Black Americans. *J Thromb Haemost.* 2011;**9**(3):489–95. doi: 10.1111/j.1538-7836.2011.04185.x. [PubMed: 21232005].

2. Rull K, Nagirnaja L, Laan M. Genetics of recurrent miscarriage: challenges, current knowledge, future directions. *Front Genet.* 2012;**3**:34. doi: 10.3389/fgene.2012.00034. [PubMed: 22457663].
3. Coulam CB, Clark DA, Beer AE, Kutteh WH, Silver R, Kwak J, et al. Current clinical options for diagnosis and treatment of recurrent spontaneous abortion. Clinical Guidelines Recommendation Committee for Diagnosis and Treatment of Recurrent Spontaneous Abortion. *Am J Reprod Immunol.* 1997;**38**(2):57–74. [PubMed: 9272202].
4. Clifford K, Rai R, Watson H, Regan L. An informative protocol for the investigation of recurrent miscarriage: preliminary experience of 500 consecutive cases. *Hum Reprod.* 1994;**9**(7):1328–32. [PubMed: 7962442].
5. Brenner B, Sarig G, Weiner Z, Younis J, Blumenfeld Z, Lanir N. Thrombophilic polymorphisms are common in women with fetal loss without apparent cause. *Thromb Haemost.* 1999;**82**(1):6–9. [PubMed: 10456445].
6. Heit JA. Thrombophilia: common questions on laboratory assessment and management. *Hematology Am Soc Hematol Educ Program.* 2007:127–35. doi: 10.1182/asheducation-2007.1.127. [PubMed: 18024620].
7. Kravtsov DV, Matafonov A, Tucker EI, Sun MF, Walsh PN, Gruber A, et al. Factor XI contributes to thrombin generation in the absence of factor XII. *Blood.* 2009;**114**(2):452–8. doi: 10.1182/blood-2009-02-203604. [PubMed: 19351955].
8. He R, Chen D, He S. Factor XI: hemostasis, thrombosis, and antithrombosis. *Thromb Res.* 2012;**129**(5):541–50. doi: 10.1016/j.thromres.2011.11.051. [PubMed: 22197449].
9. Kong M, Kim Y, Lee C. Functional investigation of a venous thromboembolism GWAS signal in a promoter region of coagulation factor XI gene. *Mol Biol Rep.* 2014;**41**(4):2015–9. doi: 10.1007/s11033-014-3049-1. [PubMed: 24420855].
10. Ozgu-Erdinc AS, Togrul C, Aktulay A, Buyukkagnici U, Yapar Eyi EG, Erkaya S. Factor XII (Hageman) levels in women with recurrent pregnancy loss. *J Pregnancy.* 2014;**2014**:459192. doi: 10.1155/2014/459192. [PubMed: 25436153].
11. Rygal P, Kuc A. Perioperative management of cardiac surgery patients with factor XII deficiency - two case reports. *Anaesthesiol Intensive Ther.* 2012;**44**(4):217–20. [PubMed: 23348490].
12. Koster T, Rosendaal FR, de Ronde H, Briet E, Vandenbroucke JP, Bertina RM. Venous thrombosis due to poor anticoagulant response to activated protein C: Leiden Thrombophilia Study. *Lancet.* 1993;**342**(8886-8887):1503–6. [PubMed: 7902898].
13. Pomp ER, Lenselink AM, Rosendaal FR, Doggen CJ. Pregnancy, the postpartum period and prothrombotic defects: risk of venous thrombosis in the MEGA study. *J Thromb Haemost.* 2008;**6**(4):632–7. doi: 10.1111/j.1538-7836.2008.02921.x. [PubMed: 18248600].
14. Glueck CJ, Pranikoff J, Khan N, Riaz K, Chavan K, Raj P, et al. High factor XI, recurrent pregnancy loss, enoxaparin. *Fertil Steril.* 2010;**94**(7):2828–31. doi: 10.1016/j.fertnstert.2009.12.084. [PubMed: 21094629].
15. Sarig G, Younis JS, Hoffman R, Lanir N, Blumenfeld Z, Brenner B. Thrombophilia is common in women with idiopathic pregnancy loss and is associated with late pregnancy wastage. *Fertil Steril.* 2002;**77**(2):342–7. [PubMed: 11821094].
16. Wolf CE, Haubelt H, Pauer HU, Hinney B, Krome-Cesar C, Legler TJ, et al. Recurrent pregnancy loss and its relation to FV Leiden, FII G20210A and polymorphisms of plasminogen activator and plasminogen activator inhibitor. *Pathophysiol Haemost Thromb.* 2003;**33**(3):134–7. doi: 10.1159/000077821. [PubMed: 15170393].
17. Ashour MJ, Sharif FA. The relationship between gene polymorphisms of coagulation factors II, V and XI and risk of recurrent pregnancy loss in Palestine. *Int J Med Med Sci.* 2015;**3**(3):88–93.
18. Dahm AE, Bezemer ID, Bergrem A, Jacobsen AF, Jacobsen EM, Skretting G, et al. Candidate gene polymorphisms and the risk for pregnancy-related venous thrombosis. *Br J Haematol.* 2012;**157**(6):753–61. doi: 10.1111/j.1365-2141.2012.09121.x. [PubMed: 22533697].
19. Sokol J, Biringer K, Skerenova M, Stasko J, Kubisz P. Activity of coagulation factor XI in patients with spontaneous miscarriage: the presence of risk alleles. *J Obstet Gynaecol.* 2015;**35**(6):621–4. doi: 10.3109/01443615.2014.991284. [PubMed: 25517908].
20. Parand A, Zolghadri J, Nezam M, Afrasiabi A, Haghpanah S, Karimi M. Inherited thrombophilia and recurrent pregnancy loss. *Iran Red Crescent Med J.* 2013;**15**(12):13708. doi: 10.5812/ircmj.13708. [PubMed: 24693393].
21. Rey E, Kahn SR, David M, Shrier I. Thrombophilic disorders and fetal loss: a meta-analysis. *Lancet.* 2003;**361**(9361):901–8. doi: 10.1016/S0140-6736(03)12771-7. [PubMed: 12648968].