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Effects of coagulation factor XIII (Val34Leu) 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 two or more consecutive abortions occurring before 20 weeks of gestation. One of the clotting factor genes encodes factor XIII (*FXIII*), which is involved in fibrin formation. The most common polymorphism in the *FXIII* genes is the conversion of G to T in exon 2 (val34leu) of the *FXIIIA* gene, which leads to the substitution of valine with leucine. The objective of this study was to investigate the association between RPL and *FXIII* val34leu polymorphisms in a sample population of Iranian Azeri women.

Methods: A prospective case-control study was performed on a cohort of 310 RPL patients and 290 healthy controls. DNA was extracted from the whole blood and fragments of the Val34Leu polymorphism were amplified by polymerase chain reaction (PCR), followed by DNA sequencing. Genotyping was performed using the Sequenom MassArray system.

Results: The genotype frequencies of *FXIII* in the case group were 60.64% GG, 34.83% GT, and 4.41% TT, whereas the frequencies in the control group were 58.96% GG, 36.5% GT, and 4.48% TT. T allele frequencies in the case and control groups were 78.06% and 21.93%, respectively, and G allele frequencies were 77.24% and 22.75%, respectively.

Conclusions: No significant association was observed between the Val34Leu polymorphism and RPL among Iranian Azeri women.

Keywords: FXIII; polymorphism; recurrent pregnancy loss; Val34Leu.

Introduction

Recurrent pregnancy loss (RPL) is a multifactorial event consisting of two or more consecutive abortions occurring before 20 weeks of gestation [1]. RPL is a serious reproductive issue affecting 1%–5% of mature women [2]. The causes vary greatly and include genetic, anatomical, chromosomal and endocrinological factors. Environmental factors such as exposure to ethylene oxide and lead have also been considered as possible etiologies of RPL [3]. In some cases, RPL arises from immunological problems [4] and mutations in coagulation factors [5]. However, the etiology is unknown in approximately 40%–50% of patients [1].

Thrombophilia is a disease that increases the potential for blood clots. A woman is at a higher risk of developing venous thrombophilia during pregnancy, owing to the various physiological changes involved [6]. However, there is a balance between the mother's coagulation system and fibrinolysis that prevents fibrin deposition in the placental vessels and the spaces between the tufted capillaries, thus stabilizing the clotting system [7]. Nevertheless, women with thrombophilia disorders have an increased risk of developing venous thromboembolism during pregnancy as well as other vascular complications such as preeclampsia and abortion [8]. A common cause of genetic thrombophilia is a deficiency in clotting factor genes. Clotting factor XIII (*FXIII*), which is involved in fibrin formation, acts as an adhesive protein and is important for

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implantation and connection of the cytotrophoblast to the endometrial wall. Defects in this protein can cause separation of the placenta from the uterus [9]. The most common polymorphism in *FXIII* genes is the conversion of G to T in exon 2 (val34leu) of the *FXIIIA* gene, which leads to the substitution of valine with leucine [10]. The val34leu polymorphism in exon 2 can have an anti-fibrinolytic effect on fibrin primary connections [11].

The present study was performed to determine the association between the *FXIIIA* val34leu (rs5985) polymorphism and RPL in an Iranian Azeri population.

Materials and methods

In total, 310 women with RPL and 290 healthy women from an Iranian Azeri population were investigated. The women with RPL, ages ranging from 20 to 35 years, all had at least three successive miscarriages before 20 weeks of gestation. The control group comprised women with at least two successful deliveries.

Blood samples were collected into tubes with ethylenediaminetetraacetic acid (EDTA). The proteinase K method was used for DNA extraction, the quality and quantity of which were determined using the NanoDrop (nanodrop1000 thermo scientific, USA) instrument and electrophoresis on 1% agarose gel. The Val34Leu polymorphisms were detected (192bp) by polymerase chain reaction (PCR). Thirty-two cycles were performed in 10 µL reaction volume containing 15 ng DNA, 2.5 mM $MgCl_2$, 0.2 mM dNTP, 10 pmol of each primer (forward: 5′CATGCCTTTTCTGTTGTCTTC3′; reverse: 5′TACCTTGCAGGTTGACGCCCCGGGGCACTA3′), and 0.25 U DNA Taq polymerase (Sigma, USA). The cycles were as follows: 30 s at 94 °C for denaturation, 30 s at 62 °C for primer annealing, and 1 min at 72 °C for extension. Genotyping was performed by a sequencing method. The DNA sample for sequencing was obtained by incubation of the PCR products with 0.1 U alkaline phosphatase and 0.5 U exonuclease I for 45 min at 37 °C, and then heat inactivation for 15 min at 85 °C. The PCR products were then sequenced using the ABI Prism BigDye Terminator Cycle Sequencing Kit (version 3.1). Finally, the obtained sequences were analyzed using ABI PRISM model 3100 DNA Sequencer to specify their genotypes. Val34Leu polymorphisms were measured using the Sequenom MassARRAY iPLEX Platform. Initially, a locus-specific PCR was performed, followed by single-base extension using mass modified di-deoxy-nucleotide terminators of an oligonucleotide primer that immediately anneals upstream of the polymorphic site of interest. Using matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry, recognized the SNP allele.

A χ^2-test (SPSS software version 17) was used to analyze differences in the *FXIII* genotype distribution and allele frequency between the case and control groups. The T-test was used to analyze the demographic variables. A p-value <0.05 was considered statistically significant.

Results

Demographic variables were compared between the case and control groups (Table 1). In our study, no significant differences in age, education, or systolic and diastolic blood pressure were observed between patients and healthy women ($p > 0.05$); whereas, the BMI (kg/m^2) in the patient group was significantly higher than that in the control group ($p < 0.05$).

The genotype frequencies of the *FXIII* polymorphism in the case group were 60.64% GG, 34.83% GT, and 4.41% TT, whereas those in the control group were 58.96% GG, 36.5% GT, and 4.48% TT (Table 2). T allele frequencies in the case and control groups were 78.06% and 21.93%, respectively; G allele frequencies were 77.24% and 22.75%.

In the case and control groups, 108 (34.83%) and 106 (36.55%) women, respectively, were heterozygous for the Val34Leu polymorphism ($p > 0.05$); 14 (4.41%) and 13 (4.48%) women were homozygous (TT) ($p > 0.05$).

Discussion

The association between coagulation gene polymorphisms [12, 13], immunological gene polymorphisms

Table 1: Demographic variables of women in the patient and control groups.

Demographic variable	Patient group	Control group	p-Value
Age			
20–25 years	26 cases (8.3%)	34 cases (11.7%)	0.15
26–30 years	184 cases (59.3%)	98 cases (33.8%)	
31–35 years	100 cases (32.4%)	158 cases (54.5%)	
BMI, kg/m^2	25.66 ± 3.16	23.14 ± 3.10	0.02
Education			
Under diploma and diploma	218 (70.3%)	149 (66.8%)	0.18
Higher diploma	92 (29.7%)	141 (33.2%)	
Systolic BP	114.12 ± 8.88	111.89 ± 8.32	0.34
Diastolic BP	73 ± 8.09	74.6 ± 7.4	0.40

Statistical significance: $p < 0.05$; BMI, body mass index; BP, blood pressure.

Table 2: Genotype and allele frequencies of VAL34leu polymorphisms in the patient and control groups.

Genotype	Patient group (310)		Control group (290)		p-Value	OR (95% CI)
	Percent, %	Cases	Percent, %	Cases		
GG	60.64	188	58.96	171	0.88	1
GT	34.83	108	36.55	106	0.89	1
TT	4.41	14	4.48	13	1.00	1.501 (0.139–6.133)
T normal	78.06	484	77.24	448	0.86	1.498 (0.509–1.503)
G minor	21.93	136	22.75	132	0.91	0.988 (0.742–1.233)

Statistical significance: $p < 0.05$; OR, odds ratio; CI, confidence interval.

[14], chromosomal abnormalities, etc. [3] has been extensively investigated. Val34Leu is one of the most common polymorphisms in factor XIII deficiency associated with different diseases such as intracranial hemorrhage, myocardial infarction, and thrombosis [15, 16]. Several studies have been performed in Iran and abroad on the Val34Leu *FXIII* gene polymorphisms. Some have proposed this polymorphism to be a risk factor for RPL, while others disregarded the relationship between this polymorphism and RPL. Considering the effect of the *FXIII* gene on pregnancy outcomes and the reported association between different polymorphisms and RPL occurrence, we focused on Val34Leu polymorphisms here; however, no association was ascertained in Iranian Azeri women.

Elmahgoub et al. [17] suggested that the *FXIII* Val-34Leu polymorphism was positively associated with RPL and that carriers of the heterozygous (Val/Leu) and homozygous (Leu/Leu) factor XIII polymorphism might be at an increased risk of RPL. Li et al. [18] in reported that the *FXIII* Val34Leu polymorphism has a significant relationship with RPL in the dominant model and the co-dominant model (Val/Val vs. Val/Leu). The results showed that the *FXIII* Val34Leu polymorphism may reduce RPL risk [18]. Torabi et al. [19] also found no link between the IG103T polymorphism and RPL in a population in Tehran. In a study by Bagheri et al. [20] on this polymorphism between a patient and control group from a population in Urmia, the difference was not significant. The results of the present study were in agreement with the Torabi et al. and Bagheri et al. studies. Therefore, Val34Leu polymorphisms were probably not involved in the pathogenesis of RPL in an Iranian population of Azeri origin and did not affect normal pregnancy.

In some studies, the differences in BMI were reported to be significant between the case and control groups, in contrast to other studies that reported no significant relation. Our study however reported a significant association between case and control groups; this result concurred with that of two different studies [21, 22], but Liu et al. [23] and Yue et al. [24] were reported contrary results.

Such varying results might be due to the presence of other involved genes [25], geographic differences [26], sample size and selection bias [27], presence of more than one predisposing factors [28], ethnic heterogeneity [29] and different environmental factors [30]. Determining the exact association between gene polymorphisms and RPL will provide us with a better understanding of the condition and enable us to detect women who are at risk of pregnancy loss. Furthermore, the identification of gene variants would help to improve treatment strategies. However, it is essential that these studies and designs be replicated in larger-sized populations from different ethnic and origins.

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References

1. Gupta R, Prakash S, Parveen F, Agrawal S. Association of CTLA-4 and TNF-α polymorphism with recurrent miscarriage among North Indian women. Cytokine 2012;60:456–62.
2. Meka A, Reddy BM. Recurrent spontaneous abortions: an overview of genetic and non-genetic backgrounds. Int J Hum Genet 2006;6:109.

3. Calderwood CJ. Thromboembolism and thrombophilia in pregnancy. Curr Obstet Gynaecol 2006;16:321–6.
4. Porter TF, LaCoursiere Y, Scott JR. Immunotherapy for recurrent miscarriage. Cochrane Database Syst Rev 2006:CD000112.
5. Yu X-W, Li X, Ren Y-H, Li X-C. Tumour necrosis factor-α receptor 1 polymorphisms and serum soluble TNFR1 in early spontaneous miscarriage. Cell Biol Int 2007;31:1396–9.
6. Battinelli EM, Marshall A, Connors JM. The role of thrombophilia in pregnancy. Thrombosis 2013; Article ID 516420, 9.
7. Buchholz T, Thaler CJ. Inherited thrombophilia: impact on human reproduction. Am J Reprod Immunol 2003;50:20–32.
8. Abbate R, Sofi F, Gensini F, Fatini C, Sticchi E, Fedi S. Thrombophilias as risk factors for disorders of pregnancy and fetal damage. Pathophysiol Haemost Thromb 2003;32:318–21.
9. Pasquier E, De Saint Martin L, Kohler H, Schröder V. Factor XIII plasma levels in women with unexplained recurrent pregnancy loss. J Thromb Haemostasis 2012;10:723–5.
10. Naderi M, Dorgalaleh A, Tabibian S, Alizadeh S, Eshghi P, Solaimani G. Current understanding in diagnosis and management of factor XIII deficiency. Iran J Pediatr Hematol Oncol 2013;3:164.
11. Kohler H-P. Role of blood coagulation factor XIII in vascular diseases. Swiss Med Wkly 2001;131:31–4.
12. Isazadeh A, Hajazimian S, Rahmani SA, Mohammadoo-Khorasani M, Samanmanesh S, Karimkhanilouei S. The effects of factor II (rs1799963) polymorphism on recurrent pregnancy loss in Iranian Azeri women. Riv Ital Med Lab 2017;13:37–40.
13. Isazadeh A, Hajazimian S, Rahmani SA, Mohammadoo-Khorasani M, Moghtaran N, Maroufi NF. The effect of factor-XI (rs3756008) polymorphism on recurrent pregnancy loss in Iranian Azeri women. Gene Cell Tissue 2017;4:e43717.
14. Jassem RM, Shani WS, Loisel DA, Sharief M, Billstrand C, Ober C. HLA-G polymorphisms and soluble HLA-G protein levels in women with recurrent pregnancy loss from Basrah province in Iraq. Hum Immunol 2012;73:811–7.
15. Naderi M, Dorgalaleh A, Alizadeh S, Kazemi A, Dargahi H, Tabibian S, et al. Assessment of relationship between CNS bleeding in factor XIII deficiency and thrombin-activatable fibrinolysis inhibitor polymorphism. Arak Medical University Journal 2013;16:83–90.
16. Dorgalaleh A, Alizadeh S, Tabibian S, Bamedi T, Karimi M. Molecular analysis of the largest group of patients with factor XIII deficiency in southeast of Iran. Blood 2013;122:4780.
17. Elmahgoub IR, Afify RA, Aal AA, El-Sherbiny WS. Prevalence of coagulation factor XIII and plasminogen activator inhibitor-1 gene polymorphisms among Egyptian women suffering from unexplained primary recurrent miscarriage. J Reprod Immunol 2014;103:18–22.
18. Li J, Wu H, Chen Y, Wu H, Xu H, Li L. Genetic association between FXIII and β-fibrinogen genes and women with recurrent spontaneous abortion: a meta-analysis. J Assist Reprod Gen 2015;32:817–25.
19. Torabi R, Zarei S, Zeraati H, Zarnani AH, Akhondi MM, Hadavi R, et al. Combination of thrombophilic gene polymorphisms as a cause of increased the risk of recurrent pregnancy loss. J Reprod Infertil 2012;13:89–94.
20. Bagheri M, Rad IA, Omrani MD, Nanbaksh F. The Val34Leu genetic variation in the A subunit of coagulation factor XIII in recurrent spontaneous abortion. Sys Biol Reprod Med 2011;57:261–4.
21. Yue J, Tong Y, Zhou J, Liu Q, Yang J. Genetic variant in interleukin-18 is associated with idiopathic recurrent miscarriage in Chinese Han population. Int J Mol Sci 2015;16:4180–9.
22. Bohiltea CL, Radoi VE. Interleukin-6 and interleukin-10 gene polymorphisms and recurrent pregnancy loss in Romanian population. Int J Reprod BioMed 2014;12:617–22.
23. Liu R-X, Wang Y, Wen L-H. Relationship between cytokine gene polymorphisms and recurrent spontaneous abortion. Int J Clin Exp Med 2015;8:9786.
24. Yue J, Tong Y, Xie L, Ma T, Yang J. Genetic variant in IL-33 is associated with idiopathic recurrent miscarriage in Chinese Han population. Sci Rep 2016;6:23806–13.
25. Rossi M, Sharkey AM, Viganò P, Fiore G, Furlong R, Florio P, et al. Identification of genes regulated by interleukin-1β in human endometrial stromal cells. Reproduction 2005;130:721–9.
26. Hefler LA, Tempfer CB, Bashford MT, Unfried G, Zeillinger R, Schneeberger C, et al. Polymorphisms of the angiotensinogen gene, the endothelial nitric oxide synthase gene, and the interleukin-1β gene promoter in women with idiopathic recurrent miscarriage. Mol Hum Reprod 2002;8:95–100.
27. Traina É, Daher S, Moron AF, Sun SY, Franchim CS, Mattar R. Polymorphisms in VEGF, progesterone receptor and IL-1 receptor genes in women with recurrent spontaneous abortion. J Reprod Immunol 2011;88:53–7.
28. Saijo Y, Sata F, Yamada H, Kondo T, Kato EH, Kishi R. Single nucleotide polymorphisms in the promoter region of the interleukin-6 gene and the risk of recurrent pregnancy loss in Japanese women. Fertil Steril 2004;81:374–8.
29. Zhou L, Cai J, Liu G, Wei Y, Tang H. Associations between interleukin-1 gene polymorphisms and coronary heart disease risk: a meta-analysis. PLoS One 2012;7:e45641.
30. Barton PT, Gerber S, Skupski DW, Witkin SS. Interleukin-1 receptor antagonist gene polymorphism, vaginal interleukin-1 receptor antagonist concentrations, and vaginal Ureaplasma urealyticum colonization in pregnant women. Infect Immun 2003;71:271–4.