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REPRODUCTIVE MEDICINE

Factor V Leiden G1691A and factor II G20210A point mutations and pregnancy in North-West of Iran

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Abstract
Purpose The roles of several hereditary predispositions for venous thromboembolism have been evaluated in women with habitual abortion. We studied the prevalence of FV Leiden G1691A and FII G20210A mutations in women with habitual abortion and healthy controls.
Methods 60 unrelated fertile females, as controls, and 70 unrelated women with at least three consecutive pregnancy losses entered at the present study. MAS-PCR was carried out for detection of FV Leiden G1691A and FII G20210A mutations.
Results FV Leiden G1691A mutation was not found in the studied cases and controls, that is, all of the cases and the controls had normal FV Leiden 1691GG genotype. FII 20210AA genotype was not found in any of patients or controls. 2.5% of alleles (3 out of 120 chromosomes) in controls and 15.714% of alleles (22 out of 140 chromosomes) in cases had FII 20210A mutation. The FII G20210A allele frequency was 0.157 in cases and 0.025 in controls. Regarding FII G20210A mutation, the distribution of GG, GA and AA genotypes were 48 (68.57%), 22 (31.43%) and 0 (0%) in the cases and 95 (95%), 5 (5%) and 0 (0%) in the controls, respectively. Significant differences in both FII G20210A alleles and FII G20210A genotypes frequencies were observed in the cases versus the controls.
Conclusion FII G20210A mutation is significantly associated with habitual abortion.

Keywords Factor V Leiden · Coagulation factor II · Pregnancy loss

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Introduction

Habitual abortion (HA) is defined as at least three consecutive spontaneous pregnancy losses that occur before 20 weeks' gestation [1]. HA is known as a heterogeneous disease and the etiology of HA remains unexplained, however, the role of anticoagulants in the prevention of HA cannot be ruled out [1, 2]. Hereditary and acquired risk factors play important roles in thromboembolism which can result some problems during pregnancy [3]. Interaction of genetic background and environmental factors could result in venous thromboembolism [4]. Factor V Leiden (FV Leiden) and prothrombin gene mutations are common hereditary risk factors for venous thromboembolism [4, 5]. Results of Coulam et al. [6] implied that multiple thrombophilic gene mutations rather than specific gene mutations have been associated with recurrent miscarriage [6]. In most of the cases, transition of G to A at nucleotide position 1691 of the factor V gene leads to production of a defective factor V (Factor V Leiden) that is associated with resistance to activated protein C [7], and also transition of G to A at nucleotide position 20210 of the 3′ un-translated region of the prothrombin gene associated with high levels of plasma prothrombin [8]. FV Leiden and factor II (FII) G20210A mutations are associated with increased production of

thrombin and risk of venous thrombosis [9]. Hypercoagulation state predispose individuals to complications in pregnancy, e.g. pre-eclampsia and HA [10, 11]. It has been demonstrated that FV Leiden G1691A and FII G20210A mutations are independent risk factors for venous thromboembolism [12]. The results of several investigations show that FV Leiden G1691A and FII G20210A mutations are associated with unexplained recurrent miscarriages [13–22]. But, some others found no association [23–26]. The present case–control study was conducted to evaluate the FV Leiden G1691A and FII G20210A mutations in women with a history of three or more consecutive pregnancy losses and healthy controls.

Methods

Ethics approval was obtained from Urmia University of Medical Sciences. This case–control study was carried out in Motahari Hospital (Urmia, Iran), an Obstetrics and Gynecology referral hospital, from March 2008 through September 2010. 60 healthy fertile unrelated females (controls) with age range of 20–38 years (mean 29.41 ± 5.01 years) and 70 unrelated women with a history of three or more consecutive pregnancy losses (cases) with age range of 18–40 years (mean 27.62 ± 5.37 years) were entered at study. Considering the age and BMI, there was no significant difference between cases and controls (P value >0.05). Controls had a history of two or more successful live births. All cases had a history of three or more consecutive spontaneous pregnancy losses that occurred before 20 weeks' gestation. Participants with a history of any known systemic diseases, cardiovascular diseases, urogenital tract disorders, immunological and endocrine abnormalities, and other confounding factors such as obesity, thyroid disease, diabetic mellitus, lupus, abnormal karyotype, and induced abortions were excluded from the study [12, 21, 27]. Before blood sampling, informed written consent has been taken from all participants. Genomic DNA was extracted from 3–5 ml of EDTA anti-coagulated blood by salting out method as described previously [28]. Multiplex allele specific polymerase chain reaction (MAS-PCR) amplification was performed using sequence-specific primers for detecting of FV Leiden G1691A and FII G20210A mutation. MAS-PCR assay and sequences of normal/mutant primers described by Ranguelov et al. [29] [29]. DNA was amplified using normal specific or mutant specific primers as forward primer and a common reverse primer. The sequences of primers were as follows: FV Leiden G1691A common primer: 5′-gga cta ctt gac aat tac tgt tct ctt g-3′, FV Leiden G1691A wiled type primer:5′-gca gat ccc tgg aca gac g-3′ and FV Leiden G1691A mutant type primer: 5′-gca gat ccc tgg aca gac a-3′; FII G20210A common primer: 5′-tct aga aac agt tgc ctg gca g-3′,FII G20210A Wiled type primer: 5′-gca ctg gga gca ttg agg atc-3′, FII G20210A mutant type primer: 5′-gca ctg gga gca ttg agg att-3′ [14]. A 25 µl PCR reaction was carried out containing 1 µl of DNA, 10X PCR buffer (Gene Fanavaran, Tehran-Iran), 2.5 mmol/L MgCl2, 200 M each of dNTPs, 0.2 µl Super Taq (Gene Fanavaran, Tehran-Iran), and 1 pmol/each reverse and forward primers. The PCR Thermal profile was: initial denaturation at 95°C for 10 min and then 10 cycles were carried out by denaturation at 94°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 1 min; then 25 cycles were carried out by denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 1 min [29]. The PCR reactions followed by extension at 72°C for 7 min. Amplified PCR fragments from MAS-PCR were analyzed under UV transilluminator after electrophoresis on 2% agarose gel containing ethidium bromide. FV Leiden G1691A (R506Q, FV-Leiden) and FII G20210A mutations give a PCR product of 150 and 340 bp, respectively.

Statistical Analysis

The frequencies of FV Leiden G1691A (R506Q, FV-Leiden) and FII G20210A mutant and normal alleles, as well as homozygote and heterozygote genotypes were obtained via direct counting. Hardy–Weinberg equilibrium was examined in cases and controls by Chi-square test with Yate's correction. All frequencies were compared between cases and controls using a 2 × 2 contingency table with Fisher's exact test. χ^2 and P value, the odds ratio (OR), and 95% confidence interval (CI) were calculated through the Epi Info version 6 package and Microsoft Excel 2003. A two-tailed P value of <0.05 was accepted as significant.

Results

Figure 1 shows the frequencies of FII 20210G/A alleles and genotypes in cases and controls. We have studied 260 chromosomes (60 unrelated-females and 70 cases) from Azeri Turkish origin for the presence or absence of FV Leiden G1691A and FII G20210A mutations. FV Leiden G1691A mutation was not found in the studied cases and controls. FII 20210AA genotype was also not found in any case of patients or controls group. 2.5% of alleles (3 out of 120 chromosomes) in controls and 15.714% of alleles (22 out of 140 chromosomes) in cases had FII 20210A mutation. The FII G20210A allele frequency was 0.157 in cases and 0.025 in controls. Regarding FII G20210A mutation, the distribution of GG, GA and AA genotypes were 48 (68.57%), 22 (31.43%) and 0(0%) in cases and 95 (95%), 5 (5%) and 0

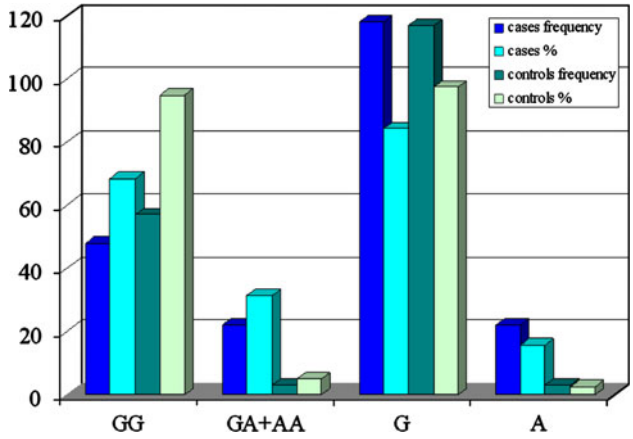

Fig. 1 Frequencies of FII 20210G/A alleles and genotypes in cases and controls

(0%) in controls, respectively. In the present study, FII G20210A allele distributions of the cases ($\chi^2 = 2.433 < 3.84$, P value $= 0.296 > 0.05$) and controls ($\chi^2 = 0.039 < 3.84$, P value $= 0.980 > 0.05$) were in agreement with the expected distribution (Hardy–Weinberg equilibrium) by the Chi-square test with 2 degree of freedom. Significant differences in both FII G20210A alleles and FII G20210A genotypes frequencies were observed in cases versus controls (see Table 1). But this does not fit to FV Leiden G1691A alleles and genotypes. Figure 2 is a representative image of the gels.

Discussion

Venous thrombosis is defined as a multifactorial disease and results from compound interaction of inherited abnormalities of blood coagulation process and acquired risk factors [30]. This is the first study that investigated the FV Leiden G1691A and FII G20210A alleles and genotype distributions in the Iranian Azeri Turkish females with habitual abortion. The findings of the present study showed that the FV Leiden mutant allele "FV Leiden 1691A" was not found in any of our cases and controls. Our results failed to determine any association between FV Leiden G1691A mutation and HA. This finding is in agreement with several studies [23–26, 31], but are inconsistence with some others [13–22]. In the present study, 2.5% of alleles in the controls and 15.7% of alleles in the cases had FII 20210A mutation which implies that the FII G20210A allele was significantly higher among the cases (2.5 vs. 15.7%). Our finding is consistence with many studies [14, 16, 20, 21], but it is inconsistent with Behjati et al. [32] study. The high prevalence of FII 20210A mutation in the studied population indicates that screening for FII 20210A mutation should be considered for detection of high risk patients. The limitation of the present study is that cases and controls are not precisely matched considering number of contributors. Therefore, studies of cases and healthy controls in a cohort of large and matched sample size are necessary to analyze more details in the studied population and management of high risk carriers.

Table 1 FII 20210G/A alleles and genotypes frequencies in cases and controls

FII 20210G/A allele/genotype	Cases F (%F)	Controls F (%F)	OR (95% CI)	Uncorrected:Yates-corrected	
				χ^2	P value
GG	48 (68.571)	57 (95)	0.11 (0.03–0.44)	14.53	0.0001
GA + AA	22 (31.429)	3 (5)	8.71 (2.27–39.08)	12.88	0.0003
G	118 (84.286)	117 (97.5)	0.14 (0.03–0.50)	12.98	0.0003
A	22 (15.714)	3 (2.5)	7.27 (1.99–31.41)	11.51	0.0006

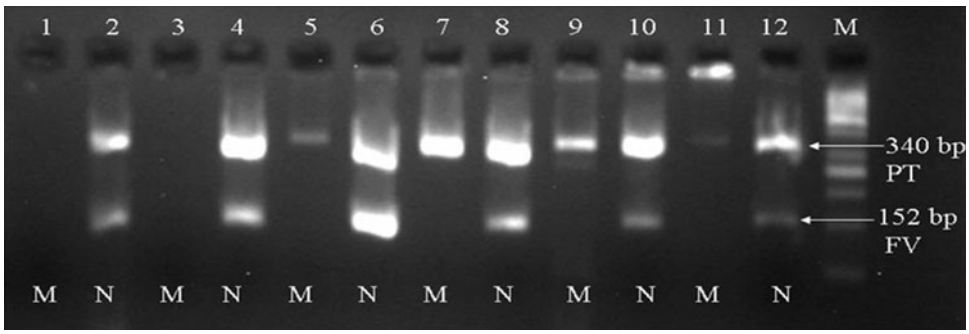

Fig. 2 Detection of FV Leiden G1691A and FII G20210A mutations by MAS-PCR. FV Leiden G1691A mutation results in a PCR product of 150 bp and FII G20210A mutation produces a 340 bp fragment. Two PCR reactions have been carried out for each sample, first lane with mutant allele, and the second lane with normal allele. PCR reactions of six samples have been shown in the figure. [e.g., *lanes 1 and 2* are from a person without mutations with two normal bands, and *lanes 5 and 6* are from a person who is heterozygote for FII (PT), and normal for FV]. *Lane M* DNA ladder (50 bp)

Conclusion

FII G20210A mutation is significantly associated with HA in tested population.

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Conflict of interest I declare that there is no conflict of interest with any others but the authors, and also the article has not been sent to any journals at the same time.

References

1. De Santis M, Cavaliere AF, Straface G, Di Gianantonio E, Caruso A (2006) Inherited and acquired thrombophilia: pregnancy oucome and treatment. Reprod Toxicol 22(2):227–233
2. Reznikoff-Etievant MF, Cayol V, Zou GM, Abuaf N, Robert A, Johanet C, Milliez J (1999) Habitual abortions in 678 healthy patients: investigation and prevention. Hum Reprod 14(8):2106–2109
3. Adelberg AM, Kuller JA (2002) Thrombophilias and recurrent miscarriage. Obstet Gynecol Surv 57(10):703–709
4. Baglin C, Brown K, Luddington R, Baglin T (1998) Risk of recurrent venous thromboembolism in patients with the factor V Leiden (FVR506Q) mutation: effect of warfarin and prediction by precipitating factors. East Anglian Thrombophilia Study Group. Br J Haematol 100(4):764–768
5. Tosetto A, Rodeghiero F, Martinelli I, De Stefano V, Missiaglia E, Chiusolo P, Mannucci PM (1998) Additional genetic risk factors for venous thromboembolism in carriers of the factor V Leiden mutation. Br J Haematol 103(3):871–876
6. Coulam CB, Jeyendran RS, Fishel LA, Roussev R (2006) Multiple thrombophilic gene mutations rather than specific gene mutations are risk factors for recurrent miscarriage. Am J Reprod Immunol 55(5):360–368
7. Bertina RM, Koeleman BPC, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, van der Velden PA, Reitsman PH (1994) Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 369:64–67
8. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM (1996) A common genetic variation in the 3′-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 88:3698–3703
9. Dahlbäck B (1997) Resistance to activated protein C as risk factor for thrombosis; molecular mechanism, laboratory investigation and clinical management. Semin Haematol 34:217–234
10. Alfirevic Z, Roberts D, Martlew V (2002) How strong is the association between maternal thrombophilia and adverse pregnancy outcome? A systematic review. Eur J Obstet Gynecol Reprod Biol 101:6–14
11. Rigó J Jr, Nagy B, Fintor L, Tanyi J, Beke A, Karádi I, Papp Z (2000) Maternal and neonatal outcome of preeclamptic pregnancies: the potential roles of factor V Leiden mutation and 5, 10 methylenetetrahydrofolate reductase. Hypertens Pregnancy 19(2):163–172
12. Finan RR, Tamim H, Ameen G, Sharida HE, Rashid M, Almawi WY (2002) Prevalence of factor V G1691A (factor V Leiden) and prothrombin G20210A gene mutations in a recurrent miscarriage population. Am J Hematol 71(4):300–305
13. Grandone E, Margaglione M, Colaizzo D, d'Addedda M, Cappucci G, Vecchione G, Scianname N, Pavone G, Di Minno G (1997) Factor V Leiden is associated with repeated and recurrent unexplained fetal losses. Thromb Haemost 77:822–824
14. Reznikoff-Etiévan MF, Cayol V, Carbonne B, Robert A, Coulet F, Milliez J (2001) Factor V Leiden and G20210A prothrombin mutations are risk factors for very early recurrent miscarriage. BJOG 108(12):1251–1254
15. Murphy RP, Donoghue C, Nallen RJ, D'Mello M, Regan C, Whitehead AS, Fitzgerald DJ (2000) Prospective evaluation of the risk conferred by factor V Leiden and thermolabile methylenetetrahydrofolate reductase polymorphisms in pregnancy. Arterioscler Thromb Vasc Biol 20:266–270
16. Souza SS, Ferriani RA, Pontes AG, Zago MA, Franco RF (1999) Factor V leiden and factor II G20210A mutations in patients with recurrent abortion. Hum Reprod 14(10):2448–2450
17. Foka ZJ, Lambropoulos AF, Saravelos H, Karas GB, Karavida A, Agorastos T, Zournatzi V, Makris PE, Bontis J, Kotsis A (2000) Factor V Leiden and prothrombin G20210A mutations, but not methylenetetrahydrofolate reductase C677T, are associated with recurrent miscarriages. Hum Reprod 15:458–462
18. Wramsby ML, Sten-Linder M, Bremme K (2000) Primary habitual abortions are associated with high frequency of factor V Leiden mutation. Fertil Steril 74(5):987–991
19. Glueck CJ, Wang P, Goldenberg N, Sieve L (2004) Pregnancy loss, polycystic ovary syndrome, thrombophilia, hypofibrinolysis, enoxaparin, metformin. Clin Appl Thromb Hemost 10:323–334
20. Sottilotta G, Oriana V, Latella C, Luise F, Piromalli A, Ramirez F, Mammì C, Santoro R, Iannaccaro P, Muleo G, Lombardo VT (2006) Genetic prothrombotic risk factors in women with unexplained pregnancy loss. Thromb Res 117(6):681–684
21. Mahjoub T, Mtiraoui N, Tamim H, Hizem S, Finan RR, Nsiri B, Almawi WY (2005) Association between adverse pregnancy outcomes and maternal factor V G1691A (Leiden) and prothrombin G20210A genotypes in women with a history of recurrent idiopathic miscarriages. Am J Hematol 80(1):12–19
22. Brenner B, Sarig G, Weiner Z, Younis J, Blumenfeld Z, Lanir N (1999) Thrombophilic polymorphisms are common in women with fetal loss without apparent cause. Thromb Haemost 82:6–9
23. Zahed LF, Rayes RF, Mahfouz RA, Taher AT, Maarouf HH, Nassar AH (2006) Prevalence of factor V Leiden, prothrombin and methylene tetrahydrofolate reductase mutations in women with adverse pregnancy outcomes in Lebanon. Am J Obstet Gynecol 195(4):1114–1118
24. Hashimoto K, Shizusawa Y, Shimoya K, Ohashi K, Shimizu T, Azuma C, Murata Y (1999) The factor V Leiden mutation in Japanese couples with recurrent spontaneous abortion. Hum Reprod 14(7):1872–1874
25. Sotiriadis A, Vartholomatos G, Pavlou M, Kolaitis N, Dova L, Stefos T, Paraskevaidis E, Kalantaridou SN (2007) Combined thrombophilic mutations in women with unexplained recurrent miscarriage. Am J Reprod Immunol 57(2):133–141
26. Altintas A, Pasa S, Akdeniz N, Cil T, Yurt M, Ayyildiz O, Batun S, Isi H (2007) Factor V Leiden and G20210A prothrombin mutations in patients with recurrent pregnancy loss: data from the southeast of Turkey. Ann Hematol 86(10):727–731
27. Bagheri M, Abdi Rad I, Omrani MD, Nanbaksh F (2010) Polymorphisms of the angiotensin converting enzyme gene in Iranian Azeri Turkish women with unexplained recurrent pregnancy loss. Hum Fertil (Camb) 13(2):79–82
28. Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucl Acids Res 16:1215
29. Ranguelov RD, Rosenthal N, Bromley C, Vasef MA (2002) Detection of factor V leiden and prothrombin gene mutations in patients who died with thrombotic events. Arch Pathol Lab Med 126(10):1193–1196

30. Rosendaal FR (1999) Venous thrombosis: a multicausal disease. Lancet 353:1167–1173
31. Yamada H, Sata F, Saijo Y, Kishi R, Minakami H (2005) Genetic factors in fetal growth restriction and miscarriage. Semin Thromb Hemost 31(3):334–345
32. Behjati R, Modarressi MH, Jeddi-Tehrani M, Dokoohaki P, Ghasemi J, Zarnani AH, Aarabi M, Memariani T, Ghaffari M, Akhondi MA (2006) Thrombophilic mutations in Iranian patients with infertility and recurrent spontaneous abortion. Ann Hematol 85(4):268–271