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Research Paper

Association of Two MMP-8 Gene Polymorphisms With Recurrent Pregnancy Loss in Iranian Women

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ABSTRACT

Background: It has been reported that less than 5% of women experience recurrent pregnancy loss (RPL). different matrix metalloproteinases (MMPs) have proteolysis function with a main role in the stable development of the fetus.

Objective: This study aims to assess the associations between two single nucleotide polymorphisms (rs2509013 C>T and rs11225395 G>A) of MMP-8 gene and RPL among 130 Iranian women with a history of RPL and 130 controls.

Methods: Genotyping of the MMP-8 gene was done for the two polymorphisms by using Sanger sequencing method.

Results: High frequency of AA genotype (OR: 2.5, 95%CI:1.02-4.1, P<0.01) and A allele (OR:1.95, 95% CI:0.95-3.1, P<0.001) of rs11225395 G>A polymorphism in patients compared to controls. This high frequency was also reported in the haplotypes and combined genotypes of polymorphism.

Conclusion: The MMP-8 gene may be involved in RPL risk and is a potential biomarker for RPL susceptibility.

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1. Introduction

The loss of a clinical pregnancy prior to 20 weeks of gestational age is defined as recurrent pregnancy loss (RPL) or abortion which is experienced by 2-5% of couples. According to the European Society of Human Reproduction and Embryology, it refers to the loss of two or more consecutive pregnancies [1]. Although 30-40% of RPL cases are unexplained and related to unknown genetic reasons, the involved factors include thrombosis, balanced chromosomal instability, endocrine system dysfunction, antiphospholipid syndrome, immunological problems, environmental factors, maternal age, and anatomic abnormalities [1].

Nowadays, the study of genetic factors which involved in RPL is very important. One of the genetic factors is matrix metalloproteinases (MMPs). They have proteolysis function and play important roles in degradation and remodeling of extracellular matrix (ECM). In pregnancy, MMPs have main role in implantation of embryo, trophoblastic invasion, and placentation. MMPs are a family of proteolysis family and MMP-8 is a member of this family. The MMP comprises a large group of zinc-dependent endopeptidases including collagenases (MMP1, MMP8 and MMP13), stromelysins (MMP3, MMP7 and MMP10) and gelatinases (MMP2 and MMP9) [2, 3]. MMP-8 is a collagenase and its function is degradation of type I collagen, which normally provides tensile strength to the ECM of the cervix, uterus, and fetal membranes [3].

The role of MMPs proteins in pathogenesis of different disease is already known [4, 5]. The level of MMP-8 in cervical fluid of pregnant women changes based on the pregnancy time. Studies have shown that high expression of MMP-8 in amniotic fluid is associated with an increased risk of spontaneous preterm delivery [6, 7]. On the other hand, some Single Nucleotide Polymorphisms (SNPs) in MMP-8 genes have been associated with pathogenesis of different diseases, including pre-term labor [8-11].

Park et al. reported the association of polymorphisms of MMP-8 gene with RPL among Korean women [12]. In this regard, and given the evidence about implication of SNPs in MMP-8 gene expression and susceptibility to various diseases, this study aims to evaluate the association of two polymorphisms of MMP-8 gene with RPL for the first time in Iranian women.

2. Material and Methods

Subjects and sampling

In this study, 130 women with a history of RPL (experiencing at least two consecutive pregnancy losses before 20 weeks of gestation) and 130 controls participated. The exclusion criteria were as follow: history of smoking and alcohol consumption in patients and their partners. Inclusion criteria were pregnancy loss due to anatomic, hormonal, chromosomal aberration, infectious, autoimmune and thrombotic disease of women. Samples were recruited from the infertility clinic of the obstetrics and gynecology center in Kosar Hospital, Qazvin, IRAN between 2018 and 2019. The control group included women with no history of RPL and with at least one normal pregnancy.

Genotyping

We used the whole blood of samples for genomic DNA extraction using a thermo scientific kit (No. 69504, Thermo Fisher Scientific Inc., Germany). Genotyping of two SNPs of MMP-8 (rs2509013 C>T and rs11225395 G>A) were determined by sequencing method. The primer pairs for MMP-8 rs2509013 polymorphism were as following: Forward= 5'-GATTATCATATCTGGTAAAAACAAAATATC-3, and Reverse= 5'- ATATTCTGTATTGGTGTTAAATCGGC-TA-3'. For rs11225395 polymorphism, the primer pairs were as: Forward= 5'-CTGTTGAAGGCCTAGAGCTGCT-GCTCC-3', Reverse=5'-GATCTTCTCTTCAAACTCT ACCC-3'. Our PCR condition was set as following: primary denaturation at 95ºC for 5 minutes, 40 cycles of denaturation at 95ºC for 2 minutes, annealing at 60ºC for 1 minute, extension at 70ºC for 1 minute, and a final extension at 70ºC for 5 minutes. After PCR procedure, we sequenced PCR products by Sanger sequencing method using ABI 3730XL Capillary Sequencer. A normal sequence of Methylenetetrahydrofolate Reductase (MTHFR) was obtained from NCBI website (http://www.ncbi.nlm.nih.gov), which was assembled by using Chromas software, v. 2.4.

Statistical analysis

The results were analyzed in GraphPad PRISM v. 8.4 software. Differences in the genotype and haplotype frequencies between the patients and controls were compared using multivariate logistic regression analysis. The data were presented using mean and standard deviation (for continuous variables) or percentage (for categorical variables). The Hardy-Weinberg equilibrium (HWE) P-values of genotype frequencies were estimated using the Chi-square test to identify deviations from the HWE. The significance level was set at 0.05.

Table 1. Demographic information of participants (N=130)

Characteristics	Mean±SD		P
	Control	Patients	
Age (y)	30.2±1.20	31.2±0.98	
BMI (Kg/m^2)	22.3±0.85	21.8±1.1	
History of RPL (n)	-	3.01±0.75	<0.001
Average gestational weeks	39.01±0.98	8.1±0.75	
RPL at <10 weeks (%)	-	91.2	

RPL: Recurrent Pregnancy Loss; BMI: Body Mass Index.

3. Results

The clinical characteristics of patients and controls are presented in Table 1. Age and Body Mass Index (BMI) were not significantly different between patients and controls, but there was a significant difference between the groups in gestational age (P<0.001). Data analysis results showed that the frequency of CT and TT genotypes in rs2509013 polymorphism were not significantly different in RPL patients compared to controls (P=0.25 and 0.12). The frequency of C and T alleles was not significantly different between patients and controls (P=0.32 and 0.28) (Table 2). Regarding the rs11225395 polymorphism, we observed a significant difference only in AA genotype frequency in patients compared to controls (P<0.01). The GA genotype frequency was not significantly different between the study groups (P=0.12), while the A allele frequency was significantly higher in patients than in controls (P<0.001) (Table 2).

To evaluate whether the SNPs interactions with the same chromosome had synergistic effects on the RPL risk, we performed haplotype analysis on two SNPs (Table 3). Based on the results, the most haplotypes that had the A allele of MMP-8 rs11225395 G>A were associated with a decreased RPL risk compared to the reference haplotype (C-G). Moreover, some haplotypes with A allele of MMP-8 rs11225395 G>A were associated with the RPL risk.

The analysis of different combined genotypes of the two polymorphisms of the MMP-8 gene showed that the two combinations with the AA genotype of rs11225395 were associated with the RPL risk; these genotypes were CT/AA (Adjusted odds ratio=2.6, 95% CI:1.2-5.9, P=0.025) and TT/AA (Adjusted odds ratio=1.5, 95% CI: 0.83-2.7, P<0.01) (Table 4).

Table 2. Genotypes of MMP-8 gene polymorphisms in the study groups

Polymorphism	Genotypes	No. (%)		AOR (95% CI)	P
		Controls	Patients		
Rs2509013	CC	66(50.7)	58(44.6)	1(Ref)	
	CT	5(3.8)	6(4.6)	0.9(0.53-1.7)	0.25
	TT	59(45.3)	66(50.7)	1.5(0.41-5.6)	0.12
	C allele	137(52.6)	122(46.9)	1(Ref)	0.32
	T allele	123(47.4)	138(53.1)	1.2(0.68-2.3)	0.28
Rs11225395	GG	57(43.8)	50(38.4)	1(Ref)	
	GA	50(38.4)	40(30.7)	1.5(0.83-2.7)	0.12
	AA	23(17.8)	46(35.3)	2.5(1.02-4.1)	<0.01
	G allele	164(63)	140(54.0)	1(Ref)	
	A allele	73(27)	132(50.7)	1.95(0.95-3.1)	<0.001

OR: Odds Ratio; AOR: Adjusted Odds Ratio; OR was adjusted by the age of participants.

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Table 3. Haplotypes of MMP-8 gene polymorphisms in the study groups (n=260)

Haplotypes	No. (%)		OR (95% CI)	P
	Controls	Patients		
C-G	160(61.5)	120(46.1)	1.45(0.92-2.13)	0.042
C-A	10(3.8)	24(9.23)	1.19(0.82-0.88)	0.02
T-G	75(28.8)	69(26.5)	0.88(0.77-1.2)	0.4
T-A	15(5.76)	47(18.07)	2.1(1.17-3.3)	0.035

OR: Odds Ratio.

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4. Discussion

Recurrent miscarriage is a complex process where various factors such as the anatomy of female reproductive organs, their physiology, function, and genetic factors are involved [13]. The proper interaction between maternal decidua cells and the embryo is essential for the functional fetal–maternal interface. The ECM play an important role in these process [14]. Re-modelling of the ECM enabled by the enzymes MMPs. The MMP-8 gene is located on chromosome 11q22.2. It is used as one of the biomarkers of preterm delivery, because high concentrations of MMP-8 in the cervical fluid are associated with spontaneous preterm delivery [6].

One of the reasons of high expression of MMP-8 is the presence of some polymorphisms in different regions of this gene. These polymorphisms have an effect on the expression rate of this gene. There was different polymorphisms in MMP-8 gene. To our knowledge, the is the first study that evaluated the association of MMP-8 gene polymorphisms with RPL among Iranian women. In this regard, we evaluated two polymorphisms rs2509013 C>T (intron variant), MMP-8 rs11225395 G>A (promoter variant) in this gene. Our results showed the significant association of A allele of rs11225395 G>A polymorphism with RPL.

Various studies have been conducted on the relationship between different polymorphisms of the MMP family genes and RPL. For example, Li et al. showed that the polymorphisms of rs243865 in MMP2 gene and rs3918242 in MMP9 gene were significantly associated with the risk of RPL in Chinese women [15]. Pereza et al. reported that MMP2 gene-735 C/T and MMP9 gene-1562 C/T polymorphisms might be associated with an increased risk of idiopathic RPL in women [16]. Behforouz et al. showed that MMP-3 gene rs35068180 polymorphism may modulate the RPL risk in Iranian women [17].

Table 4. Combined genotypes of MMP-8 gene polymorphisms in the study groups

Combined Genotypes	Controls (n=130), No.	Patients (n=130), No.	AOR (95% CI)	P
CC/GG	48	36	1.13 (0.32-2.13)	0.42
CC/GA	28	30	1.09 (0.52-2.18)	0.27
CC/AA	12	10	0.8 (0. 37-1.9)	0.4
CT/GG	16	12	1.6 (0.47-5.3)	0.3
CT/GA	4	1	1.7(0.95-2.7)	0.09
CT/AA	10	22	2.6(1.2-5.9)	0.025
TT/GG	4	3	0.95(0.52-1.7)	0.39
TT/GA	4	1	1.5 (0.83-2.7)	0.095
TT/AA	4	15	1.5 (0.83-2.7)	<0.01

AOR: Adjusted Odds Ratio.

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There is no evidence to suggest that MMP-2 gene (rs243865, rs2285053) and MMP 9 gene (rs3918242, rs17576) polymorphisms are associated with the RPL risk. About the MMP-8 gene, so far only one study has been conducted in the Korean population by Park et al. whose results are against our results. They showed that only the intron variant (rs2509013 C>T), but not the promoter region variant (rs1122539 G>A), was associated with the decreased risk of idiopathic RPL [12]. This discrepancy is because of the adaptation of various genotypes in different races and ethnicities [18]. In two studies by Näkki and Han on different populations, no association between rs2509013 C>T polymorphism and osteoarthritis was observed [19, 20]. However, there are different reports from promoter region variant, rs1122539, indicating its association with various diseases, such as preeclampsia and arterial disease [10]. Pradhan-Palikhe showed the over expression of MMP-8 gene with rs1122539 polymorphism in serum samples of patients with arterial disease [21]. Since increased expression of MMP-8 gene has been reported in the serum and amniotic samples of women with recurrent miscarriage and our study showed the significant association of rs1122539 polymorphism with RPL, it seems that this polymorphism in the promoter region can increases the MMP-8 gene expression.

5. Conclusion

In conclusion, the AA genotype of MMP-8 rs11225395 G>A is associated with RPL risk among the Iranian women. This association is maintained in the haplotype and genotype combination tests. The future studies are recommended to examine the associations between these polymorphisms with MMP-8 expression rate and the serum levels of homocysteine and uric acid to correlate these factors to the pathogenesis of RPL.

Ethical Considerations

Compliance with ethical guidelines

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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References

[1] Rai R, Regan L. Recurrent miscarriage. Lancet. 2006; 368(9535):601-11. [PMID]

[2] Page-McCaw A, Ewald AJ, Werb Z. Matrix metalloproteinases and the regulation of tissue remodelling. Nat Rev Mol Cell Biol. 2007; 8(3):221-33. [DOI:10.1038/nrm2125] [PMID] [PMCID]

[3] Rahkonen L, Rutanen EM, Nuutila M, Sainio S, Sorsa T, Paavonen J. Matrix metalloproteinase-8 in cervical fluid in early and mid pregnancy: Relation to spontaneous preterm delivery. Prenat Diagn. 2010; 30(11):1079-85. [DOI:10.1002/pd.2614] [PMID]

[4] Nagase H, Visse R, Murphy G. Structure and function of matrix metalloproteinases and TIMPs. Cardiovasc Res. 2006; 69(3):562-73. [DOI:10.1016/j.cardiores.2005.12.002] [PMID]

[5] Shiomi T, Lemaître V, D'Armiento J, Okada Y. Matrix metalloproteinases, a disintegrin and metalloproteinases, and a disintegrin and metalloproteinases with thrombospondin motifs in non-neoplastic diseases. Pathol Int. 2010; 60(7):477-96. [DOI:10.1111/j.1440-1827.2010.02547.x] [PMID] [PMCID]

[6] Białkowska K, Marciniak W, Muszyńska M, Baszuk P, Gupta S, Jaworska-Bieniek K, et al. Polymorphisms in MMP-1, MMP-2, MMP-7, MMP-13 and MT2A do not contribute to breast, lung and colon cancer risk in polish population. Hered Cancer Clin Pract. 2020; 18:16. [DOI:10.1186/s13053-020-00147-w] [PMID] [PMCID]

[7] Rahkonen L, Rutanen EM, Unkila-Kallio L, Nuutila M, Nieminen P, Sorsa T, et al. Factors affecting matrix metalloproteinase-8 levels in the vaginal and cervical fluids in the first and second trimester of pregnancy. Hum Reprod. 2009; 24(11):2693-702. [DOI:10.1093/humrep/dep284] [PMID]

[8] Anumba DO, El Gelany S, Elliott SL, Li TC. Circulating levels of matrix proteases and their inhibitors in pregnant women with and without a history of recurrent pregnancy loss. Reprod Biol Endocrinol. 2010; 8:62. [DOI:10.1186/1477-7827-8-62] [PMID] [PMCID]

[9] An F, Du J, Cao Y, Shi J, Guo Y, Jin T, et al. MMP8 polymorphism is associated with susceptibility to osteonecrosis of the femoral head in a Chinese Han population. Oncotarget. 2017; 8(13):21561-6. [DOI:10.18632/oncotarget.15371] [PMID] [PMCID]

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Inflammatory Diseases
(Previous Title: The Journal of Qazvin University of Medical Sciences)

[10] Rahimi Z, Zangeneh M, Rezaeyan A, Shakiba E, Rahimi Z. MMP-8 C-799T and MMP-8 C+17G polymorphisms in mild and severe preeclampsia: Association between MMP-8 C-799T with susceptibility to severe preeclampsia. Clin Exp Hypertens. 2018; 40(2):175-8. [PMID]

[11] Palavalli LH, Prickett TD, Wunderlich JR, Wei X, Burrell AS, Porter-Gill P, et al. Analysis of the matrix metalloproteinase family reveals that MMP8 is often mutated in melanoma. Nat Genet. 2009; 41(5):518-20. [DOI:10.1038/ng.340] [PMID] [PMCID]

[12] Park HS, Ko KH, Kim JO, An HJ, Kim YR, Kim JH, et al. Association Study between the Polymorphisms of Matrix Metalloproteinase (MMP) Genes and Idiopathic Recurrent Pregnancy Loss. Genes (Basel). 2019; 10(5):347. [DOI:10.3390/genes10050347] [PMID] [PMCID]

[13] Ford HB, Schust DJ. Recurrent pregnancy loss: Etiology, diagnosis, and therapy. Rev Obstet Gynecol. 2009; 2(2):76-83. [PMID] [PMCID]

[14] White CA, Robb L, Salamonsen LA. Uterine extracellular matrix components are altered during defective decidualization in interleukin-11 receptor alpha deficient mice. Reprod Biol Endocrinol. 2004; 2:76. [DOI:10.1186/1477-7827-2-76] [PMID] [PMCID]

[15] Li L, Liu J, Qin S, Li R. The association of polymorphisms in promoter region of MMP2 and MMP9 with recurrent spontaneous abortion risk in Chinese population. Medicine (Baltimore). 2018; 97(40):e12561. [DOI:10.1097/MD.0000000000012561] [PMID] [PMCID]

[16] Pereza N, Ostojić S, Volk M, Kapović M, Peterlin B. Matrix metalloproteinases 1, 2, 3 and 9 functional single-nucleotide polymorphisms in idiopathic recurrent spontaneous abortion. Reprod Biomed Online. 2012; 24(5):567-75. [DOI:10.1016/j.rbmo.2012.01.008] [PMID]

[17] Behforouz A, Dastgheib SA, Abbasi H, Karimi-Zarchi M, Javaheri A, Hadadan A, et al. Association of MMP-2, MMP-3, and MMP-9 Polymorphisms with Susceptibility to Recurrent Pregnancy Loss. Fetal Pediatr Pathol. 2021; 40(5):378-86. [DOI:10.1080/15513815.2019.1710879] [PMID]

[18] Varela MA, Amos W. Heterogeneous distribution of SNPs in the human genome: Microsatellites as predictors of nucleotide diversity and divergence. Genomics. 2010; 95(3):151-9. [DOI:10.1016/j.ygeno.2009.12.003] [PMID]

[19] Näkki A, Videman T, Kujala UM, Suhonen M, Männikkö M, Peltonen L, et al. Candidate gene association study of magnetic resonance imaging-based hip osteoarthritis (OA): Evidence for COL9A2 gene as a common predisposing factor for hip OA and lumbar disc degeneration. J Rheumatol. 2011; 38(4):747-52. [DOI:10.3899/jrheum.100080] [PMID]

[20] Han C, Ren Y, Kong L, Eerdun T, Bai L. Associations of polymorphisms in MMP-7 and MMP-8 with osteoarthritis risk in a Han Chinese population. Int J Clin Exp Med. 2018; 11(5):5129-34. https://e-century.us/files/ijcem/11/5/ijcem0067194.pdf

[21] Pradhan-Palikhe P, Pussinen PJ, Vikatmaa P, Palikhe A, Kivimäki AS, Lepäntalo M, et al. Single nucleotide polymorphism -799C/T in matrix metalloproteinase-8 promoter region in arterial disease. Innate Immun. 2012; 18(3):511-7. [DOI:10.1177/1753425911423852] [PMID]