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The association of polymorphisms in promoter region of *MMP2* and *MMP9* with recurrent spontaneous abortion risk in Chinese population

Li Li, PhD[a], Jia Liu, PhD[a], Shuang Qin, PhD[b], Ruiman Li, PhD[a,*]

Abstract

This study aimed to reveal the genetic association between polymorphisms in promoter region of matrix metalloproteinase 2 (*MMP2*) and matrix metalloproteinase 9 (*MMP9*) and the risk of recurrent spontaneous abortion (RSA) in Chinese population.

A total of 129 RSA patients and 116 relative controls were selected and the genotyping of polymorphism was conducted by polymerase chain reaction with sequencing. Genotype distribution of polymorphism in the control group was tested the status of Hardy–Weinberg equilibrium and then, genotype frequencies were compared between the case and control groups by chi-squared test. Odds ratio (OR) with the corresponding 95% confidence interval (95% CI) was computed to express the risk of RSA caused by polymorphism. Moreover, the linkage disequilibrium of polymorphisms in *MMP2* was analyzed by Haploview software.

CT genotype and T allele of rs243865 in *MMP2* were significantly associated with the increased susceptibility to RSA in Chinese population (CT vs. CC: OR = 1.926, 95% CI = 1.101–3.368; T vs. C: OR = 1.751, 95% CI = 1.146–2.676). Similarly, CT genotype carriers of rs3918242 in *MMP9* were obviously more in RSA patients than that of the controls (P = .037), which indicated it was associated with the risk of RSA occurrence (OR = 1.760, 95% CI = 1.034–2.995). So was T allele in RSA development (OR = 1.595, 95% CI = 1.061–2.398). Haplotypes C-T and T-C were also the risk factors of RSA (OR = 1.673, 95% CI = 1.103–2.536; OR = 2.171, 95% CI = 1.372–2.436).

MMP2 rs243865 and *MMP9* rs3918242 polymorphisms are significantly associated with the risk of RSA in Chinese population.

Abbreviations: 95% CI = 95% confidence interval, BMI = body mass index, ECM = extracellular matrix, For. = forward, HWE = Hardy–Weinberg equilibrium, MMPs = matrix metalloproteinases, OR = odds ratio, PCR = polymerase chain reaction, RSA = recurrent spontaneous abortion, SNP = single nucleotide polymorphism.

Keywords: haplotype, *MMP2*, *MMP9*, polymorphism, recurrent spontaneous abortion

1. Introduction

Recurrent spontaneous abortion (RSA) is a pregnancy complication characterized by 2 or more consecutive spontaneous abortions before 20 weeks of pregnancy[1] and affects 1% to 3% of fertile couples.[2] According to the previous reports, RSA is attributed to multiple factors, containing infection, endocrine, anatomical and immune factors, genetic and external environmental factors.[3] But approximately 50% of RSA cases still remain unexplained.[4] Certainly, genetic background is widely paid attention by scholars and a number of genes are discovered to be associated with RSA development, such as *LEPR*, *MTHFR*, and factor V genes.[5,6] Recently, with the development of molecular techniques, various genetic variants have been confirmed to be associated with RSA occurrence risk.

Matrix metalloproteinases (MMPs) are a Ca^{2+}–Zn^{2+}-dependent endopeptidases and play an important role in cell proliferation, migration, apoptosis, and angiogenesis.[7] They participate in the degradation of the extracellular matrix (ECM), which is a key event in all processes of normal reproduction in humans.[8,9] The expression and proper activation of MMPs in decidua and extravillous trophoblast are very important for human early pregnancy.[10] A study based on whole genomic sequencing and bioinformatics analysis demonstrated that mutations in *MMP9* showed close association with RSA.[11]*MMP2* and *MMP9* belong to gelatinases, a subgroup of MMPs, which are involved in various physiological and pathological progresses.[12]*MMP2* is usually secreted by endothelial cells, interstitial cells, macrophages, T cells, eosnophils, and neutrophils, and *MMP9* is mainly synthesized and secreted by inflammatory cells.[13]*MMP2* and *MMP9* play a crucial role in human endometrial stomal cells terminally differentiating into decidual cells and are found in decidual tissues throughout gestation.[14] The dysregulation of *MMP2* and *MMP9* could lead to excessive endometrial matrix degradation, thus contributing to RSA.[15]*MMP2* and *MMP9* enzymes are, respectively, encoded by *MMP2* and *MMP9* genes, which contain multiple single nucleotide polymorphisms (SNPs).[16,17] Some SNPs in promoter

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Li Li, Jia Liu, and Shuang Qin have contributed equally to this work.

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[a] Department of Obstetrics and Gynecology, First Affiliate Hospital of Jinan University, [b] Department of Obstetrics, Guangzhou Women and Children's Medical Center, Guangzhou, China.

[*] Correspondence: Ruiman Li, Department of Obstetrics and Gynecology, First Affiliate Hospital of Jinan University, Guangzhou 510000, China (e-mail: sdfgswed@126.com).

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region play an important role in disease development.[18,19] Moreover, the relationship between variants in *MMP2* and *MMP9* genes and RSA risk has been reported in several published articles. A case–control study carried out among Slovenian population demonstrated that *MMP2* and *MMP9* polymorphisms were significantly associated with RSA risk.[10] However, the genetic association of *MMP2* and *MMP9* polymorphisms with RSA has been rarely reported in Chinese population.

In the present study, we explored the association of *MMP2* and *MMP9* polymorphisms in promoter region with the risk of RSA development in Chinese Han population, and rs243865, rs2285053 in *MMP2* and rs3918242 in *MMP9* were selected.

2. Materials and methods

2.1. Subjects

In this study, a case–control design was adopted which included 129 RSA cases and 116 corresponding controls as the case and control groups, respectively. RSA cases were inpatients and outpatients in Obstetric and Gynecologic Department of First Affiliate Hospital of Jinan University during from January 2014 to May 2016, with the age range of 19 to 46 years old. The included criteria of the cases were as following description: a couple with 2 or more times spontaneous abortion; the couple without abnormal karyotype or thrombotic diseases. RSA patients would be excluded which were caused by endocrine factors, infection, abnormal anatomy, and autoantibody. The corresponding controls were also from Obstetric and Gynecologic Department of the same hospital with no <1 normal pregnancy without complications in the age of 17 to 43 years. Women with the history of spontaneous abortion and preterm were excluded. The controls were frequency-matched with the cases in age. This research was reviewed and supported by the Ethics Committee of First Affiliate Hospital of Jinan University and was also in accordance with ethics rules of Declaration of Helsinki. The objective of this study was informed every subject and written consents were signed by subjects before collecting sample.

The basic characteristics of subjects were also investigated and recorded by trained professional doctor, including age, body mass index (BMI), live birth, number of abortions, and family history of RSA.t

2.2. DNA extraction

Two milliliters peripheral venous blood was collected from every participant in the early morning and put into blood collection tube with ethylenediaminetetraacetic acid, stored at −80°C. Blood genomic DNA was extracted by TIANamp Genomic DNA Kit according to the manufacturer's instruction. And then they were placed in −20°C refrigerator for standby application.

2.3. Genotyping

The genotyping of *MMP2* rs243865, rs2285053 and *MMP9* rs3918242 polymorphisms was conducted by polymerase chain reaction (PCR) and sequencing. First, PCR primers were designed by Primer Premier 5.0 software and synthesized in Sangon Biotech (Sangon, Shanghai, China). PCR primer sequences of polymorphisms were listed in Table 1. PCR system was a volume of 25.0 μL solution, consisted of 12.5 μL 2× PCR Master Mix, 1.0 μL of forward and reverse primers, 20 ng genomic DNA, and added ddH_2O to the final volume. PCR was performed according to the following procedure: 95°C predenaturation 5 minutes, followed by 30 cycles of 95°C denaturation 45 seconds, the specific temperature (Table 1) for annealing 30 seconds, 72°C extension 30 seconds, and 72°C for the final extension 7 minutes. The quality of PCR products was tested by 1.0% agarose gel electrophoresis.

Table 1

PCR primer sequences of *MMP2* and *MMP9* polymorphisms in promoter region.

		Primer sequences	Annealing temperature, °C
MMP2			
rs243865	For.	5′-ATTCTTTCAGCCCCTGACCT-3′	62
	Rev.	5′-CCTGTGACAACCGTCTCTGA-3′	
rs2285053	For.	5′-ATAGGGTAAACCTCCCCACATT-3′	65
	Rev.	5′-GGTAAAATGAGGCTGAGACCTG-3′	
MMP9			
rs3918242	For.	5′-GCCTGGCACATAGTAGGCCC-3′	59
	Rev.	5′-CTTCCTAGCCAGCCGGCATC-3′	

For. = forward, MMP = matrix metalloproteinase, PCR = polymerase chain reaction, Rev. = reverse.

Then the eligible PCR amplification products were sequenced in Shanghai Sangon Biotech Co., Ltd for determining the genotype of every polymorphism in the case and control groups.

2.4. Statistical analysis

First, the genotype frequencies of polymorphism were obtained via direct counting and genotype distribution of every polymorphism in *MMP2* and *MMP9* was checked whether was consistent with Hardy–Weinberg equilibrium (HWE) by chi-squared test. Then genotypes of polymorphism were compared for frequency difference between the case and control groups by chi-squared test, too. The relative risk of RSA caused by genetic variants in promoter region of *MMP2* and *MMP9* was expressed by calculating odds ratio (OR) and the corresponding 95% confidence interval (95% CI). Data processing was conducted by PASW Statics 18.0 software. $P < .05$ was defined as the statistically significant difference. The linkage disequilibrium of polymorphisms in *MMP2* was analyzed by Haploview software.

3. Results

3.1. The characteristics analysis of subjects

The basic characteristics of participants in the case and control groups are shown in Table 2. The average age of RSA cases and

Table 2

The basic characteristics of RSA patients and the controls.

	RSA patients (n = 129)	Controls (n = 116)	*P*
Age/year			
Mean age	28.14 ± 4.53	27.38 ± 4.16	.516
Age range	19–46	17–43	
Body mass index, kg/m^2	24.37 ± 3.28	22.35 ± 2.65	.012
Live birth	None	1.87 ± 0.75	
No. of abortion/%			
2	31/24.03	None	
≥3	98/75.97		
Family history			
Yes	42/32.56	21/18.10	.010
No	87/67.44	95/81.90	

RAS = recurrent spontaneous abortion.

Table 3

The genotype distribution of *MMP2* and *MMP9* polymorphisms between the case and control groups.

	RSA patients (n = 129/%)	Controls (n = 116/%)	*P*	OR (95% CI)	P_{HWE}
MMP2					
rs243865					.088
CC	67/51.94	79/68.10	—	Ref.	
CT	49/37.98	30/25.86	.021	1.926 (1.101–3.368)	
TT	13/10.08	7/6.04	.109	2.190 (0.826–5.804)	
C	183/70.93	188/81.03	—	Ref.	
T	75/29.07	44/18.97	.009	1.751 (1.146–2.676)	
rs2285053					.133
CC	59/45.74	62/53.45	—	Ref.	
CT	52/40.31	41/35.34	.299	1.333 (0.775–2.293)	
TT	18/13.95	13/11.21	.355	1.455 (0.655–3.230)	
C	170/65.89	165/71.12	—	Ref.	
T	88/34.11	67/28.88	.214	1.275 (0.869–1.870)	
MMP9					
rs3918242					.831
CC	60/46.51	71/61.21	—	Ref.	
CT	58/44.96	39/33.62	.037	1.760 (1.034–2.995)	
TT	11/8.53	6/5.17	.142	2.169 (0.757–6.215)	
C	178/68.99	181/78.02	—	Ref.	
T	80/31.01	51/21.98	.024	1.595 (1.061–2.398)	

CI = confidence interval, HWE = Hardy–Weinberg equilibrium, MMP = matrix metalloproteinase, OR = odds ratio, RAS = recurrent spontaneous abortion, Ref. = reference.

the controls was 28.14 ± 4.53 and 27.38 ± 4.13 years, respectively. The 2 groups did not show significant difference in age ($P = .516$). However, BMI of RSA patients was obviously higher than that of the controls (24.37 ± 3.28 and 22.35 ± 2.65, $P = .012$). The live birth of the controls was 1.87 ± 0.75. More than 75% of RSA patients suffered from 3 or more times spontaneous abortions. People with family history of RSA were easily subject to RSA, compared with people without family history ($P = .010$).

3.2. The association analysis of polymorphisms in MMP2 and MMP9 with the risk of RSA development

The genotype frequencies of polymorphisms in *MMP2* and *MMP9* were compared between the case and control groups, and the results are summarized in Table 3. For rs243865 polymorphism in *MMP2*, heterozygous genotype CT showed a significantly higher frequency in RSA patients (37.98%) than that in the controls (25.86%), compared with CC genotype (51.94% and 68.10%, $P = .021$), which indicated that the carriage of CT genotype in *MMP2* rs246865 polymorphism significantly increased the risk of RSA (OR = 1.926, 95% CI = 1.101–3.368). Similarly, T allele of rs243865 was also associated with the obviously elevated risk of RSA (OR = 1.751, 95% CI = 1.146–2.676). But another polymorphism in *MMP2*, rs2285053 was not significantly associated with RSA occurrence risk in our study population in genotype or allele.

CT genotype of rs3918242 in promoter region of *MMP9* was detected at significantly more frequency in the case group than the control group ($P = .037$). CT genotype was a risk factor for RSA development (OR = 1.760, 95% CI = 1.034–2.995), but not the homozygous genotype TT. Allele T and C frequencies of rs3918242 in the case and control groups were 31.01%, 21.98% and 68.99%, 78.02%, respectively, the significant distribution difference was found ($P = .024$). C allele obviously increased individual susceptibility to RSA (OR = 1.595, 95% CI = 1.061–2.398).

In addition, the genotype distribution of each polymorphism in control group was consistent with HWE ($P = .088$, .133, and .831, respectively), suggesting that our study population was a typical Mendelian population.

3.3. The role of haplotype between polymorphisms in RSA occurrence

The strong linkage disequilibrium between rs243865 and rs2285053 in *MMP2* was observed, and 3 haplotypes were recorded, C-C, C-T, and T-C. The haplotype frequencies in the case and control groups are shown in Table 4. Both of C-T and T-C haplotypes were significantly correlated with the elevated risk

Table 4

The haplotype analysis of polymorphisms in *MMP2*.

	Haplotype/%			
rs243865–rs2285053	RSA patients	Controls	*P*	OR (95% CI)
C–C	95/36.82	121/52.15	—	Ref.
C–T	88/34.11	67/28.88	.015	1.673 (1.103–2.536)
T–C	75/29.07	44/18.97	.001	2.171 (1.372–2.436)

CI = confidence interval, MMP = matrix metalloproteinase, OR = odds ratio, RAS = recurrent spontaneous abortion, Ref. = reference.

of RSA, compared with haplotype C-C (OR = 1.673, 95% CI = 1.103–2.536; OR = 2.171, 95% CI = 1.372–2.436).

4. Discussion

With the development of sequencing technique, whole genomic sequence becomes an effective tool to identify the potentially unknown genetic loci for the human disease. However, the whole genomic sequence may only provide a rude genetic association of the genetic loci with disease, and the results should be verified by a further case–control study. Additionally, the whole genomic sequence is with high cost, and the bioinformatic analysis is difficult. For the known genetic loci, whole genomic sequence may be unnecessary. Based on the published articles, we hypothesized that variants in *MMP2* and *MMP9* genes might be correlated with risk of RSA. Therefore, a case–control study was designed to investigate the impact of polymorphisms in promoter region of *MMP2* and *MMP9* on RSA occurrence risk in Chinese Han population. First of all, the analysis results of basic characteristics of participants between the case and control groups showed that the age was frequency-matched between the 2 groups and BMI is a influence factor of RSA, that is, the higher the BMI, the greater the risk suffering from RSA. The result is consistent with the study of Sugiura-Ogasawara.[20] Family history was also an important risk factor of RSA. Exceeded 3 quarters of RSA cases had experienced 3 or more times spontaneous abortions. Therefore, environmental factors are also the important influence factors of RSA. Agenor and Bhattacharya[21] report that smoking, alcohol consumption, maternal age, and pre-existing medical conditions are all the risk factors of reproduction miscarriage.

For genetic factors, in the present study, *MMP2* rs243865 polymorphisms in genotype distribution was significantly different between RSA patients and the controls and the carriage CT genotype in rs243865 obviously increased the risk of individuals subject to RSA, compared with people with CC genotype and T allele of rs243865 was also correlated to the significantly elevated risk of RSA, but in the study of Pereza et al, rs243865 (−1306C/T) was not associated with RSA occurrence in Slovenian population.[10] In our study, rs2285053 in *MMP2* was not an independent risk factor of RSA, but it is found to significantly increase individual susceptibility to RSA in Slovenian population. We also found that both of the genotype and allele distribution of rs3918242 in *MMP9* between RSA patients and the controls were obviously different, indicating that it was obviously associated with the occurrence of RSA. Pereza et al also obtained the similar results with ours, but Singh et al think that rs3918242 (−1562C/T) is not associated with recurrent early pregnancy loss risk in Indian population.[22] The inconsistent results may be caused by different ethnicity, sample size, and the influence of environmental factors. In addition, the strong linkage disequilibrium was found between rs243865 and rs2285053 in *MMP2*, and haplotypes C-T, T-C were showed the significantly increased risk of RSA occurrence. The results obtained in our study might be helpful for early screening of RSA. The results could guide to confirm the pregnant women who had high risk of RSA. Early medical care and treatments are necessary for those individuals to prevent RSA.

MMPs play an important role in reproductive tract and are secreted by connective tissue cells located on stroma. Singh et al report dysregulation of ECM caused by MMPs alteration may be an key event to lead to RSA in infected women and MMPs/TIMPs dysregulation result in excessive degradation of endometrial matrix to affect pregnancy, then leading to RSA.[15] The study of Choi et al show that low expression of angiogenesis-related genes have the influence on RSA development, including MMPs.[23] Jokimaa had verified the important role of MMPs in recurrent miscarriages as early as 2002.[24]*MMP2* and *MMP9* are the important work members of subclass in MMPs and they may be altered the expression by SNPs in some diseases.

The common SNPs in *MMP2* and *MMP9* are mostly located on promoter region and are widely studied in multiple diseases. Rs243865 (−1306C>T) is a mutation in promoter region of *MMP2* and it is found to be possibly associated with the high transcription level and affect enzyme activity.[24] Meanwhile, the mutant genotype of rs243865 can significantly down regulate the expression of MMP2 protein in women, compared with CC genotype.[25] Multiple diseases have been identified to be correlated with rs243865, such as cervical cancer,[26] ankylosing spondylitis.[27] Rs2285053 (−753C>T) is also located on the promoter region of *MMP2* and it is also associated with several diseases, such as gallbladder, nasopharyngeal carcinoma.[28,29] In addition, it exists the linkage disequilibrium with rs243865 which is an important risk factor of diseases.[29]*MMP9* rs3918242 (−1562C/T) polymorphism in promoter region is also the risk factor of some diseases, such as pre-eclampsia in pregnant women,[30] coronary artery disease.[31]

In conclusion, *MMP2* rs243865 and *MMP9* rs3918242 polymorphisms are significantly correlated with the risk of RSA development in Chinese Han population and haplotype is also non-negligible risk of disease. Some limitations in this study should be paid attention, including 1 population, small sample size, and only independent polymorphism analysis. In addition, some other MMP family members might also have the capacity to influence individual susceptibility to RSA. For example, the whole genomic sequencing study constructed by Quintero-Ronderos et al suggested that *MMP10* mutation was also associated with risk of RSA.[11] However, due to the shorter study period, the genetic effects of other *MMPs*' members on RSA risk had not been explored in our study. Therefore, more well-designed studies with large sample size are needed to explain the genetic association of *MMPs* genetic polymorphisms with RSA susceptibility.

Author contributions

Conceptualization: Li Li, Jia Liu, Shuang Qin.
Data curation: Li Li, Jia Liu, Shuang Qin.
Formal analysis: Li Li, Jia Liu, Shuang Qin.
Funding acquisition: Li Li, Jia Liu, Shuang Qin, Ruiman Li.
Investigation: Li Li, Jia Liu, Shuang Qin, Ruiman Li.
Methodology: Li Li, Jia Liu, Shuang Qin, Ruiman Li.
Project administration: Li Li, Jia Liu, Shuang Qin, Ruiman Li.
Resources: Li Li, Jia Liu, Shuang Qin, Ruiman Li.
Software: Li Li, Jia Liu, Shuang Qin.
Supervision: Li Li, Jia Liu, Shuang Qin.
Validation: Li Li, Jia Liu, Shuang Qin.
Visualization: Li Li, Jia Liu, Shuang Qin.
Writing – original draft: Li Li, Jia Liu, Shuang Qin.
Writing – review & editing: Li Li, Jia Liu, Shuang Qin.

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