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

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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 Haplovie 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 = 1.372–3.436).

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\textsuperscript{[1]} and affects 1% to 3% of fertile couples.\textsuperscript{[2]} According to the previous reports, RSA is attributed to multiple factors, containing infection, endocrine, anatomical and immune factors, genetic and external environmental factors.\textsuperscript{[3]} But approximately 50% of RSA cases still remain unexplained.\textsuperscript{[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.\textsuperscript{[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\textsuperscript{2+}–Zn\textsuperscript{2+}-dependent endopeptidases and play an important role in cell proliferation, migration, apoptosis, and angiogenesis.\textsuperscript{[7]} They participate in the degradation of the extracellular matrix (ECM), which is a key event in all processes of normal reproduction in humans.\textsuperscript{[8,9]} The expression and proper activation of MMPs in decidua and extravillous trophoblast are very important for human early pregnancy.\textsuperscript{[10]} A study based on whole genomic sequencing and bioinformatics analysis demonstrated that mutations in MMP9 showed close association with RSA.\textsuperscript{[11]} MMP2 and MMP9 belong to gelatinases, a subgroup of MMPs, which are involved in various physiological and pathological progresses.\textsuperscript{[12]} MMP2 is usually secreted by endothelial cells, interstitial cells, macrophages, T cells, eosinophils, and neutrophils, and MMP9 is mainly synthesized and secreted by inflammatory cells.\textsuperscript{[13]} MMP2 and MMP9 play a crucial role in human endometrial stromal cells terminal differentiating into decidual cells and are found in decidual tissues throughout gestation.\textsuperscript{[14]} The dysregulation of MMP2 and MMP9 could lead to excessive endometrial matrix degradation, thus contributing to RSA.\textsuperscript{[15]} MMP2 and MMP9 enzymes are, respectively, encoded by MMP2 and MMP9 genes, which contain multiple single nucleotide polymorphisms (SNPs).\textsuperscript{[16,17]} Some SNPs in promoter
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.

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 ddH₂O 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.

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 Haplovaw 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 control groups were 28.14±4.53 and 27.38±4.16 years, respectively. However, there was no statistically significant difference in age between the case and control groups. The frequency of live birth in RSA cases and control groups was 0.75% and 27.38% respectively. The frequency of live birth was also not statistically significant difference between the case and control groups.

\[\begin{array}{c|c|c}
\text{Age} / \text{year} & \text{RSA patients} / n = 129 & \text{Controls} / n = 116 & P \\
\hline
\text{Mean age} & 28.14 \pm 4.53 & 27.38 \pm 4.16 & .516 \\
\text{Age range} & 19–46 & 17–43 & \\
\text{Body mass index, kg/m}^2 & 24.37 \pm 3.28 & 22.35 \pm 2.65 & .012 \\
\text{Live birth} & \text{None} & 1.87 \pm 0.75 & \\
\text{No. of abortion} & \geq 2 & 31/24.03 & \text{None} \\
\text{≥3} & 98/75.97 & & \\
\text{Family history} & \text{Yes} & 42/23.56 & 21/18.10 & .010 \\
\text{No} & 87/67.44 & 95/81.90 & \\
\end{array}\]

\(\text{RS} = \text{recurrent spontaneous abortion}\)

### Table 1

| Primer sequences | Annealing temperature, °C |
|------------------|-------------------------|
| MMP2             |                         |
| rs243865 For.      | 5’-ATTCTTTCGCCCCGGAT-3’ |
| rs243865 Rev.      | 5’-CTCTTACGCACCTGGGCT-3’ |
| rs2285053 For.      | 5’-ATAGGATAAATGTGGGAG-3’ |
| rs2285053 Rev.      | 5’-GTAACAAATGGTGGGAG-3’ |
| MMP9             |                          |
| rs3918242 For.       | 5’-GCCTGCCCATAGTGGCC-3’ |
| rs3918242 Rev.       | 5’-CTTCTTGCGACGCGGAT-3’ |

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

### 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/m²     | 24.37 ± 3.28         | 22.35 ± 2.65  | .012 |
| Live birth                 | None                | 1.87 ± 0.75   | |
| No. of abortion            | ≥2                   | 31/24.03       | |
| ≥3                         | 98/75.97            |               | |
| Family history             | Yes                  | 42/23.56       | .010 |
| No                         | 87/67.44            | 95/81.90       | |

\(\text{RS} = \text{recurrent spontaneous abortion}\)
The genotype frequencies of polymorphisms in MMP2 and MMP9 with the risk of RSA development

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

| Polymorphism | RSA patients (n=129%) | Controls (n=116%) | P  | OR (95% CI) | P_{HWE} |
|--------------|-----------------------|-------------------|----|-------------|---------|
| rs243865     |                       |                   |    |             |         |
| CC           | 67/51.94              | 79/68.10          | —  | —           | .088    |
| CT           | 49/37.98              | 30/25.86          | .021| 1.026 (1.101–3.368) |         |
| TT           | 13/10.80              | 7/6.04            | .109| 2.190 (0.826–5.804)  |         |
| C            | 183/70.93            | 188/81.03         | —  | —           | .088    |
| T            | 17/13.72             | 44/18.97          | .009| 1.751 (1.146–2.676)  |         |
| rs2285053    |                       |                   |    |             |         |
| CC           | 59/45.74             | 62/53.45          | —  | —           | .133    |
| 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         | —  | —           | .088    |
| T            | 88/34.11             | 67/28.88          | .214| 1.275 (0.869–1.870)  |         |
| rs3918242    |                       |                   |    |             |         |
| CC           | 60/46.51             | 71/61.21          | —  | —           | .831    |
| 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            | 181/71.22         | —  | —           | .088    |
| T            | 80/31.01             | 51/28.78          | .024| 1.595 (1.001–2.398)  |         |

Table 4

The haplotype analysis of polymorphisms in MMP2.

| Haplotype | RSA patients | Controls | P     | OR (95% CI) |
|-----------|--------------|----------|-------|-------------|
| C-C       | 95/36.82     | 121/52.15| —     | —           |
| C-T       | 88/34.11     | 67/28.88 | .015  | 1.673 (1.103–2.336) |
| T-C       | 75/29.07     | 44/18.97 | .001  | 2.171 (1.372–2.436) |

The 2 groups did not show significant difference in age (P = .316). 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
of RSA, compared with haplotype C-C (OR = 1.673, 95% CI = 1.103–2.356; 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 Sugiuara-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] Jokima had verified the important role of MMPs in recurrent miscarriages as early as 2002. [23] MMP2 and MMP9 are the important work members of subclass in MMPs and they may be altered the expression by SNPs in some diseases. [10]

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 mutation 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. [22] MMP9 rs3918242 (−1562C>T) polymorphism in promoter region is also the risk factor of some diseases, such as pre-eclampsia in pregnant women, [30] and 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.

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References

[1] Abdi-Shayan S, Monfaredan A, Moradi Z, et al. Association of CD46 IVS1–1724 C>G single nucleotide polymorphism in Iranian women with unexplained recurrent spontaneous abortion (URSA). Iran J Allergy Asthma Immunol 2016;15:303–8.
[2] Li L, Dou L, Leung PC, et al. Chinese herbal medicines for unexplained recurrent miscarriage. Cochrane Database Syst Rev 2016;CD010568.

[3] Goncalves RO, Fraga LR, Santos WV, et al. Association between the thrombophilic polymorphisms MTHFR C677T, Factor V Leiden, and prothrombin G20210A and recurrent miscarriage in Brazilian women. Genet Mol Res 2016;15.

[4] Quintero-Ronderos P, Mercier E, Fukuda M, et al. Novel genes and thrombophilic polymorphisms MTHFR C677T, Factor V Leiden, and recurrent pregnancy loss. Int J Reprod Biomed (Yazd) 2016;14:329–34.

[5] Abdul-Muneer PM, Pfi, Smith SD, Choudhury RH, Matos P, et al. Changes in vascular receptor genes is a risk factor for idiopathic recurrent spontaneous abortion. Croat Med J 2016;57:566–71.

[6] Abul-Muneer PM, Phister BJ, Haorah J, et al. Role of matrix metalloproteinases in the pathogenesis of traumatic brain injury. Mol Neurobiol 2016;53:6106–23.

[7] Cathcart JM, Banach A, Liu A, et al. Interleukin-6 increases matrix metalloproteinase-14 (MMP-14) levels via down-regulation of p53 to drive cancer progression. Oncotarget 2016;7:61107–20.

[8] Smith SD, Choudhury RH, Matsos P, et al. Changes in vascular extracellular matrix composition during decidual spiral arteriole remodeling in early human pregnancy. Histol Histopathol 2016;31:537–71.

[9] Perezn Ostojc S, Volk M, et al. Matrix metalloproteinases 1, 2, 3 and 9 functional single-nucleotide polymorphisms in idiopathic recurrent spontaneous abortion. Reprod Biomed Online 2012;24:567–73.

[10] Quintero-Ronderos P, Mercier E, Fukuda M, et al. Novel genes and mutations in patients affected by recurrent pregnancy loss. PLoS ONE 2017;12:e0186149.

[11] Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res 2003;92:827–39.

[12] Medeiros NI, Fares RC, Franco EP, et al. Differential expression of matrix metalloproteinases 2, 9 and cytokines by neutrophils and monocytes in the clinical forms of Chagas disease. PLoS Negl Trop Dis 2017;11:e005284.

[13] Niu R, Okamoto T, Iwase K, et al. Quantitative analysis of matrix metalloproteinase-2 and -9, and their tissue inhibitors-1 and -2 in human placenta throughout gestation. Life Sci 2000;66:1127–37.

[14] Singh N, Prasad P, Das B, et al. Involvement of matrix metalloproteinases and their inhibitors in endometrial extracellular matrix turnover in Chlamydia trachomatis-infected recurrent spontaneous aborters. Pathog Dis 2017.

[15] Chen YC, Wu YR, Mesri M, et al. Associations of matrix metalloproteinase-9 and tissue inhibitory factor-1 polymorphisms with Parkinson disease in Taiwan. Medicine 2016;95:e2672.

[16] Shi Y, Zhang J, Tan C, et al. Matrix metalloproteinase-2 polymorphisms and incident coronary artery disease: a meta-analysis. Medicine 2015;94:e824.

[17] Hao Y, Tian S, Sun M, et al. Association between matrix metalloproteinase gene polymorphisms and development of ischemic stroke. Int J Clin Exp Pathol 2015;8:1647–52.

[18] Zhang L, Xi RX, Zhang ZZ. Matrix metalloproteinase variants associated with risk and clinical outcome of esophageal cancer. Genet Mol Res 2015;14:4616–24.

[19] Sugiuira-Ogasawara M. Recurrent pregnancy loss and obesity. Best Pract Res Clin Obstet Gynaecol 2015;29:489–97.

[20] Agnonor A, Bhattacharyya S. Infertility and miscarriage: common pathways in manifestation and management. Womens Health (Lond) 2015;11:527–41.

[21] Singh K, Nair RR, Khanam A. Functional SNP -1562C>T in the promoter region of MMP9 and recurrent early pregnancy loss. Reprod Biomed Online 2012;24:61–5.