SPANDIDOS PUBLICATIONS

BIOMEDICAL REPORTS 3: 874-878, 2015

Association between nm23 gene polymorphisms and the risk of endometriosis

XI HUANG[1], WEI ZHAO[2], YAN LI[1] and SHAN KANG[2]

Departments of [1]Molecular Biology, and [2]Obstetrics and Gynecology, The Fourth Hospital of Hebei Medical University, Shijiazhuang, Hebei 050011, P.R. China

Received July 10, 2015; Accepted August 4, 2015

DOI: 10.3892/br.2015.511

Abstract. The first tumor metastasis-suppressor gene, nm23, may have an important role in the pathogenesis of endometriosis (EM). The present study aimed to evaluate whether nm23 gene polymorphisms are correlated with the risk of the development of EM in North Chinese women, as a preliminary study. The case-control study was conducted with 379 EM patients and 384 unrelated healthy controls. Genotyping of two polymorphisms within the nm23 gene promoter region (rs16949649 T/C and rs2302254 C/T) were performed using polymerase chain reaction-restriction fragment length polymorphism. The data showed that the rs16949649 and rs2302254 polymorphisms within the nm23 gene were not associated with the risk of developing EM. There were no statistical differences in the distribution of nm23 genotypes between patients with EM and the control group (P=0.490 and P=0.440, respectively). For the rs16949649 T/C, compared with the C/T + T/T genotype, the C/C genotype did not increase the risk of EM [odds ratio (OR)=0.81; 95% confidence interval (CI), 0.57-1.17]. For the rs2302254 C/T, compared with the C/T + C/C genotype, the T/T genotype did not increase the risk of EM (OR=1.46; 95% CI, 0.81-2.64). In conclusion, the findings in the present pilot study suggest that nm23 polymorphisms do not contribute to EM susceptibility. However, more studies in larger populations are required to confirm these results.

Introduction

As a common, benign, chronic gynecological disease, endometriosis (EM) is characterized by the presence of endometrial gland and stroma outside the uterine cavity, affecting 6-10% of women of reproductive age (1,2). EM causes several problems, such as infertility, dysmenorrhea and pelvic pain, which seriously effect patient health and quality of life (3,4). Although numerous studies have been undertaken to identify the pathogenesis of EM, the mechanisms involved in the growth of ectopic endometrium remain unclear. A large body of evidence suggests that genetic factors have important roles in the development and progression of EM (5,6), however, the genes responsible for these progresses have not been defined. EM is known to be a benign disease, which can be characterized by invasion and atypical growth of endometrial cells at the ectopic sites, and it may occur at distant sites, such as tumor metastasis. A few of the tumor invasion and metastasis-related genes are believed to contribute to the occurrence and continuation of this disease (7-10).

The nm23 gene, also known as *NME1*, was first isolated as a metastasis-suppressor gene on the basis of its reduced expression in highly metastatic murine K-1735 melanoma cell lines (11). Following this, ≥9 human nm23 genes have been discovered to date (12-15). The majority of studies have found that the reduced expression of the nm23 gene at the mRNA and protein level is correlated with characteristics of aggressive cancer, such as invasiveness and metastasis, in a variety of tumor types (16). In ovarian carcinoma, low levels of nm23-H1 expression were associated with lymph node metastasis (17). Additionally, the expression of the nm23 gene was decreased in the eutopic and ectopic endometrial stromal cells (ESCs) from women with EM (18). Therefore, the aberrant expression of the nm23 gene may have an important role in EM.

Two single-nucleotide polymorphisms (SNPs), rs16949649 and rs2302254, were in the 5'-promoter region of the nm23 gene. *In vitro* biochemical analyses showed that minor alleles in rs2302254 and rs3760468, which is in strong linkage disequilibrium with rs16949646, altered the nuclear proteins binding capacity and reduced nm23 gene promoter activity (19). However, the potential association of these nm23 gene polymorphisms with EM is unclear. We hypothesized that women with different genotypes in the nm23 gene polymorphisms may have different incidences of EM. Therefore, a case-control analysis was used to investigate the genetic influence of the rs16949649 and rs2302254 polymorphisms on the susceptibility of EM in North Chinese women.

Materials and methods

Subjects. All the subjects were women of the Han ethnicity in North China and written informed consent was provided for

Correspondence to: Professor Shan Kang, Department of Obstetrics and Gynecology, The Fourth Hospital of Hebei Medical University, 12 Jiankang Road, Shijiazhuang, Hebei 050011, P.R. China
E-mail: ksjq62cn@sina.com

Key words: endometriosis, nm23 gene, risk, single-nucleotide polymorphism, susceptibility

participation in the study. A case-control study was conducted, including 379 patients ranging in age from 19 to 51 years old [mean ± standard deviation (SD), 35.85±7.64 years] with EM between December 2001 and October 2009 at the Hebei Medical University Fourth Hospital (Shijiazhuang, Hebei, China). All the patients, who had not received treatment with hormones, were diagnosed by laparoscopy and histological examination, and the severity of the disease was staged according to the revised American Fertility Society classification (1985). All the patients had their disease classified as stage III-IV. General characteristics for all the patients were recorded in detail in the medical chart.

A group of control subjects, consisting of 384 women without any malignant disease and EM ranging in age from 18 to 51 years old (mean ± SD, 36.04±8.53), was randomly selected from a routine health survey in the same hospital. There were no significant differences in age, menarche age, gravidity and parity between the 2 groups (all P>0.05) (Table I), so no adjustments were carried out. The Ethics Committee of Hebei Obstetrics and Gynecology Institute approved the study.

DNA extraction. A total of 5 ml of venous blood from each subject was drawn in vacutainer tubes containing EDTA and was stored at 4˚C. Genomic DNA was extracted within 1 week by proteinase K (Merck KGaA, Darmstadt, Germany) digestion, followed by a salting out procedure according to the method reported by Miller *et al* (20).

Genotyping. Genotyping was determined by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Briefly, the PCR reaction was performed in a total 20 μl volume containing 100 ng of DNA template, 2.0 μl 10X PCR buffer, 0.4 μl of 10 mmol/l deoxynucleotide triphosphates, 1 unit of Taq DNA polymerase (Tiangen Biotech Co., Ltd, Beijing, China) and 200 nM of each primer. The PCR conditions for the rs16949649 and rs2302254 polymorphisms were 94˚C for 5 min, followed by 35 cycles of 45 sec at 94˚C, 45 sec at 63˚C and 45 sec at 72˚C, with a final elongation at 72˚C for 7 min to allow for the complete extension of all the PCR fragments. Accordingly, a 354-base pair (bp) PCR amplification fragment was generated using the primers: rs16949649T/C forward, 5'-CGGCTCCTGATTCCATTTTTG TAC-3' and reverse, 5'-GCTTCTGGGAGGGATGGGAGT ATA-3'; and a 263-bp amplification fragment using the primers: rs2302254 C/T forward, 5'-CGCGAACGAAGG AAGTGAGTCA-3' and reverse, 5'-GCCGCCAGCACCCGA AAC-3'.

A 5.5 μl aliquot of every PCR product was subjected to digestion overnight at 37˚C in a 10 μl reaction volume containing restriction enzymes as follows: 5 units of *Hif*I (Sangon Biotechnology Co., Ltd., Shanghai, China) for the rs16949649 T/C polymorphisms and 5 units of *Ban*II (Sangon Biotechnology Co., Ltd.) for the rs2302254 C/T polymorphisms. After the overnight digestion, the products for the SNPs were separated by a 4% agarose gel that was stained with ethidium bromide. For rs16949649 T/C polymorphisms, the homozygous C/C was represented by DNA bands of 65, 126 and 163 bp, and the homozygous T/T was identified by DNA bands of 163 and 191 bp, whereas the heterozygous CT exhibited a combination of all the above bands (65, 126, 163 and 191 bp). The rs2302254 C/C genotype yielded fragments of 88 and 175 bp, however, the T/T genotype was not cleaved at the mutated site and revealed a fragment of 263 bp, whereas the heterozygous CT exhibited a combination of all the above bands (88, 175 and 263 bp). The primers, length of PCR products, restriction enzymes and fragment length are summarized in Table II.

Table I. Characteristics of endometriosis (EM) patients and controls in a North Chinese population.

Groups	Age, years	Menarche age, years	Gravidity	Parity
Control, n=384	36.04±8.53	14.15±1.55	1.58±1.22	0.73±0.63
EM, n=379	35.85±7.64	14.16±1.51	1.63±1.33	0.78±0.65
P-value[a]	0.66	0.81	0.34	0.65

Data are mean ± standard deviation. [a]Student's t-test vs. controls. EM, endometriosis.

For a negative control, distilled water was used instead of DNA in the reaction system for each panel of PCR. The PCR of 15% of the samples were run in duplicate for quality control, with a reproducibility of 100%.

Statistical analysis. All the data analyses were carried out using SPSS 13.0 statistical software (SPSS, Inc., Chicago, IL, USA). Comparison of observed and expected genotype frequencies were obtained by Hardy-Weinberg equilibrium (HWE) and were evaluated by the χ^2 test. Two-sided contingency tables using the χ^2 test were performed to compare the allele and genotype distributions of rs16949649 T/C and rs2302254 C/T polymorphisms in the study groups. Comparisons of age, menarche age, gravidity and parity in the study groups were carried out using the Student's t-test and data are presented as mean ± SD. Odds ratio (OR) and respective 95% confidence intervals (CI) were calculated using an unconditional logistic regression model. P<0.05 was considered to indicate a statistically significant difference for all statistical analyses.

Results

Patient characteristics. The mean age of EM patients and controls was 35 years (range, 19-51 years) and 36 years (range, 18-51 years), respectively. There was no significant difference in age distribution between the cases and controls (P>0.05). All 379 cases and 384 controls were successfully genotyped using the PCR-RFLP method. The frequency distributions of the nm23 gene rs16949649 T/C and rs2302254 C/T genotypes in the control groups did not significantly deviate from that expected for HWE (χ^2=2.80, 0.84; and P=0.094, 0.36, respectively).

Association of the nm23 gene rs16949649 T/C and rs2302254 C/T polymorphisms with the risk of EM. For the rs16949649 T/C polymorphism, the frequencies of the T and C alleles were 58.0 and 42.0% in the cases, and 61.1 and 38.9%

Table II. PCR conditions for the nm23 restriction fragment length polymorphisms.

Polymorphisms	Primers	Product length	Restriction enzyme	Fragment length
rs16949649 T/C	F: 5'-CGGCTCCTGATTCCATTTTTGTAC-3' R: 5'-GCTTCTGGGAGGGATGGGAGTATA-3'	354 bp	*Hinf*I	163+126+65 bp (C) 191+163 bp (T)
rs2302254 C/T	F: 5'-CGCGAACGAAGGAAGTGAGTCA-3' R: 5'-GCCGCCAGCACCCGAAAC-3'	263 bp	*Ban*II	175+88 bp (C) 263 bp (T)

PCR, polymerase chain reaction; F, forward; R, reverse; bp, base pairs.

Table III. Genotype and allele frequency distribution for the two nm23 gene polymorphisms in the cases and controls

Groups	Controls, no. (%), n=384	Cases, no. (%), n=379	P-value
rs16949649 T/C genotype			
C/C	66 (17.2)	77 (20.3)	0.490
C/T	167 (43.5)	164 (43.3)	
T/T	151 (39.3)	138 (36.4)	
Allele			
C	299 (38.9)	318 (42.0)	0.229
T	469 (61.1)	440 (58.0)	
rs2302254 C/T genotype			
C/C	215 (56.0)	217 (57.3)	0.440
C/T	140 (36.4)	142 (37.4)	
T/T	29 (7.6)	20 (5.3)	
Allele			
C	570 (74.2)	576 (76.0)	0.424
T	198 (25.8)	182 (24.0)	

Table IV. Association of the 2 polymorphisms with the risk of developing endometriosis.

Genotypes	Controls, no. (%)	Cases, no. (%)	OR (95% CI)
rs16949649 T/C			
C/C	66 (17.2)	77 (20.3)	
C/T + T/T	318 (82.8)	302 (79.7)	0.81 (0.57-1.17)
rs2302254 C/T			
T/T	29 (7.6)	20 (5.3)	
C/T + C/C	355 (92.4)	359 (94.7)	1.46 (0.81-2.64)

OR, odds ratio; CI, confidence interval.

in the controls, respectively. There was no significant difference between the 2 groups (P=0.229) (Table III). Genotype frequencies of T/T, T/C and C/C in the cases were 36.4, 43.3 and 20.3%, and 39.3, 43.5 and 17.2% in the controls, respectively. There was no significant difference between the two groups (P=0.490) (Table III). Compared with the C/T + T/T genotype, the C/C genotype did not increase the risk of EM (OR=0.81; 95% CI, 0.57-1.17) (Table IV).

For the rs2302254 C/T polymorphism, the frequencies of the C and T alleles were 76.0 and 24.0% in the cases, and 74.2 and 25.8% in the controls, respectively. There was no significant difference between the 2 groups (P=0.424) (Table III). Genotype frequencies of C/C, C/T and T/T in the cases were 57.3, 37.5 and 5.3%, and 56.0, 36.5 and 7.6% in the controls, respectively. There was no significant difference between the 2 groups (P=0.440) (Table III). Compared with the C/T + C/C genotype, the T/T genotype did not increase the risk of EM (OR=1.46; 95% CI, 0.81-2.64) (Table IV).

Discussion

Although previous studies have shown that aberrant expression of nm23 may have a role in the development of EM, there is no study examining the association between the genetic variations in the nm23 gene and the risk of EM. In the present study, whether the SNPs in the nm23 gene would affect the risk of EM development in women was investigated. The results revealed that the rs16949649 T/C and rs2302254 C/T polymorphisms of the nm23 gene promoter may not be correlated with the risk of EM in North Chinese women. To the best of our knowledge, this is the first study to explore the association between polymorphisms in the nm23 gene and the risk of EM.

Thus far, the pathogenesis of EM remains unclear, however, invasive mechanisms have been implicated in the development of this disease (21,22). The first tumor metastasis-suppressor gene, nm23, may be involved in the development of EM. Thus far, the expression of the nm23 gene in EM has been reported in 4 studies (7,9,18,23). Li and Kong (9) showed that the protein and mRNA expression levels of nm23-H1 in specimens of EM were significantly lower than those in normal endometrium using immunohistochemical surfactant protein and reverse transcription-PCR. Two recent studies (18,23) demonstrated that the expression of the nm23 gene was decreased in the eutopic and ESCs from women with EM. By contrast, Schneider *et al* (7) found that the nm23 gene was overexpressed in numerous cases of women with EM. Therefore, the aberrant expression of the nm23 protein may have a role in the development of EM.

The aberrant expression of the nm23 protein has been reported in a variety of tumor types and EM; however, the understanding of the regulating mechanisms remains limited.

As SNPs in the nm23 gene promoter may influence its binding with transcription factors and affect promoter activity as well as gene transcription, different SNPs may have different impacts on the expression of the gene and subsequently protein. Ouatas *et al* (24) reported that three regions are involved in the differential expression levels of the nm23-H1 promoter fragment among a panel of human breast carcinoma cell lines. The deletion of the 544-bp *Avr*II fragment, which contains the SNP rs16949649, resulted in a 20% increase in nm23 promoter activity in MCF7 breast cancer cell lines, and deletion of the 195-bp *Nhe*I-*Xba*I fragment, which contains the SNP rs2302254, abolished nm23 gene promoter activity. In addition, an *in vitro* functional analysis in MDA-MB-231 and MCF7 breast cancer cells showed that the rs2302254 minor allele reduced NME1 promoter activity by ~20% in the MCF7 cells, and the minor allele in the rs3760648, an SNP in close proximity to and in strong LD with rs16949649, reduces NME1 promoter activity by 18% compared to the major allele in both cell types (19). Taken together, these studies suggested that rs16949649 and rs2302254 polymorphisms in the nm23 gene promoter may be functional polymorphisms. Therefore, the present study focused on these SNPs in the 5'-promoter region of nm23 gene.

Certain studies have reported the potential association between the rs16949649 and rs2302254 polymorphisms in the nm23 gene promoter region and the risk of certain solid tumors. Qu *et al* (19) reported that patients carrying the C allele in rs16949649 were associated with higher breast cancer-specific mortality [hazard ratio (HR)=1.4; 95% CI, 1.1-1.9] as compared with patients carrying the wild-type allele. SNP rs2302254 was also associated with breast cancer prognosis, and the association was statistically significant for the risk of breast cancer relapse, metastasis and fatality (HR=1.3; 95% CI, 1.0-1.6). Wang *et al* (25) showed that women with heterozygous genotypes TC in rs16949649 or CT in rs2302254 exhibited a higher risk of developing endometrial cancer compared with women with wild-type or homozygous genotypes. Feng *et al* (26) also concluded that Taiwan women with the polymorphic heterozygotes TC in rs16949649 of the nm23 gene had the tendency to develop cervical neoplasia when compared with their homozygous counterparts (P=0.058).

Additionally, it was reported that silencing of the nm23 gene in ESCs stimulated angiogenesis through promoting the secretion of vascular endothelial growth factor (VEGF) of endometrial stromal cells (23). The VEGF gene has a critical role in angiogenesis (27,28), which is crucial to stimulate ESC proliferation, adhesion and invasion. Several studies have investigated the association of certain polymorphisms in the VEGF gene with the susceptibility to EM (29-31), however, no study has focused on the association of the nm23 gene functional polymorphisms with the risk of the development of EM.

The present study may have certain limitations due to the study design. As it was a hospital-based study, case and control subjects were recruited from the hospital, and selection bias may occur. Owing to the limitation of the study, further larger population-based studies would be warranted to confirm these findings. In addition, only two SNPs in the nm23 gene promoter region were explored, however, there were numerous other SNPs in the nm23 gene without investigation. In the future, more studies regarding the association between other SNPs in the nm23 gene and the risk of EM are required.

In conclusion, this is the first study to investigate the potential connection between the polymorphisms in the nm23 gene promoter with the risk of developing EM in North Chinese women. The study results suggested that the rs16949649 T/C and rs2302254 C/T polymorphisms of the nm23 gene promoter may not be associated with the risk of the development of EM. Therefore, these two nm23 gene polymorphisms did not contribute to EM susceptibility.

References

1. Ballard K, Lowton K and Wright J: What's the delay? A qualitative study of women's experiences of reaching a diagnosis of endometriosis. Fertil Steril 86: 1296-1301, 2006.
2. Greene R, Stratton P, Cleary SD, Ballweg ML and Sinaii N: Diagnostic experience among 4,334 women reporting surgically diagnosed endometriosis. Fertil Steril 91: 32-39, 2009.
3. Goldman MB and Cramer DW: The epidemiology of endometriosis. Prog Clin Biol Res 323: 15-31, 1990.
4. Treloar SA, Do KA, O'Connor VM, O'Connor DT, Yeo MA and Martin NG: Predictors of hysterectomy: An Australian study. Am J Obstet Gynecol 180: 945-954, 1999a.
5. Simpson JL, Bischoff FZ, Kamat A, Buster JE and Carson SA: Genetics of endometriosis. Obstet Gynecol Clin North Am 30: 21-40, 2003.
6. Zondervan KT, Cardon LR and Kennedy SH: The genetic basis of endometriosis. Curr Opin Obstet Gynecol 13: 309-314, 2001.
7. Schneider J, Jimenez E, Rodriguez F and del Tanago JG: c-*myc*, c-*erb*-B2, nm23 and p53 expression in human endometriosis. Oncol Rep 5: 49-52, 1998.
8. Niu HY and Wang DB: Expression and significance of KISS-1 and MMP-9 gene in endometriosis model of nude mice. J Chin Med Univ 33: 487-489, 2004.
9. Li TC and Kong LN: Expression of nm23-H1 gene in endometriosis. Chin J Pathophysiology 23: 116-119, 2007.
10. Kang S, Zhao XW, Wang N, Chen SC, Zhou RM and Li Y: Association of polymorphisms of the MMP-2 and TIMP-2 genes with the risk of endometriosis in North Chinese women. Fertil Steril 90: 2023-2029, 2008.
11. Steeg PS, Bevilacqua G, Pozzatti R, Liotta LA and Sobel ME: Altered expression of NM23, a gene associated with low tumor metastatic potential, during adenovirus 2 Ela inhibition of experimental metastasis. Cancer Res 48: 6550-6554, 1988.
12. Steeg PS: Metastasis-suppressors alter the signal transduction of cancer cells. Nat Rev Cancer 3: 55-63, 2003.
13. Lacombe ML, Milon L, Munier A, Mehus JG and Lambeth DO: The human Nm23/nucleoside diphosphate kinases. J Bioenerg Biomembr 32: 247-258, 2000.
14. Ouatas T, Salerno M, Palmieri D and Steeg PS: Basic and translational advances in cancer metastasis: Nm23. J Bioenerg Biomembr 35: 73-79, 2003.
15. Crawford RM, Treharne KJ, Best OG, Muimo R, Riemen CE and Mehta A: A novel physical and functional association between nucleoside diphosphate kinase A and AMP-activated protein kinase alpha1 in liver and lung. Biochem J 392: 201-209, 2005.
16. Steeg PS: Tumor metastasis: Mechanistic insights and clinical challenges. Nat Med 12: 895-904, 2006.
17. Mandai M, Konishi I, Koshiyama M, Mori T, Arao S, Tashiro H, Okamura H, Nomura H, Hiai H and Fukumoto M: Expression of metastasis-related nm23-H1 and nm23-H2 genes in ovarian carcinomas: Correlation with clinicopathology, EGFR, c-erbB-2, and c-erbB-3 genes, and sex steroid receptor expression. Cancer Res 54: 1825-1830, 1994.
18. Li MQ, Shao J, Meng YH, Mei J, Wang Y, Li H, Zhang L, Chang KK, Wang XQ, Zhu XY, *et al*: NME1 suppression promotes growth, adhesion and implantation of endometrial stromal cells via Akt and MAPK/Erk1/2 signal pathways in the endometriotic milieu. Hum Reprod 28: 2822-2831, 2013.
19. Qu S, Long J, Cai Q, Shu XO, Cai H, Gao YT and Zheng W: Genetic polymorphisms of metastasis-suppressor gene NME1 and breast cancer survival. Clin Cancer Res 14: 4787-4793, 2008.
20. Miller SA, Dykes DD and Polesky HF: A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16: 1215, 1988.

21. Spuijbroek MD, Dunselman GA, Menheere PP and Evers JL: Early endometriosis invades the extracellular matrix. Fertil Steril 58: 929-933, 1992.
22. Zeitvogel A, Baumann R and Starzinski-Powitz A: Identification of an invasive, N-cadherin-expressing epithelial cell type in endometriosis using a new cell culture model. Am J Pathol 159: 1839-1852, 2001.
23. Chang KK, Liu LB, Jin LP, Meng YH, Shao J, Wang Y, Mei J, Li MQ and Li DJ: NME1 suppression of endometrial stromal cells promotes angiogenesis in the endometriotic milieu via stimulating the secretion of IL-8 and VEGF. Int J Clin Exp Pathol 6: 2030-2038, 2013.
24. Ouatas T, Clare SE, Hartsough MT, De La Rosa A and Steeg PS: MMTV-associated transcription factor binding sites increase nm23-H1 metastasis-suppressor gene expression in human breast carcinoma cell lines. Clin Exp Metastasis 19: 35-42, 2002.
25. Wang PH, Yi YC, Tsai HT, Tee YT, Ko JL, Han CP, Liu YF, Lin LY and Yang SF: Significant association of genetic polymorphism of human nonmetastatic clone 23 type 1 gene with an increased risk of endometrial cancer. Gynecol Oncol 119: 70-75, 2010.
26. Feng CY, Wang PH, Tsai HT, Tee YT, Ko JL, Chen SC, Lin CY, Han CP, Yang JS, Liu YF, *et al*: Polymorphisms of human nonmetastatic clone 23 type 1 gene and neoplastic lesions of uterine cervix. Reprod Sci 17: 886-893, 2010.
27. Ferrara N and Davis-Smyth T: The biology of vascular endothelial growth factor. Endocr Rev 18: 4-25, 1997.
28. Ferrara N, Gerber HP and LeCouter J: The biology of VEGF and its receptors. Nat Med 9: 669-676, 2003.
29. Fang F, Gong L, Wang X and Zhang L: The association between vascular endothelial growth factor (VEGF) +405G>C genetic polymorphism and endometriosis. Exp Biol Med (Maywood): Jan 7, 2015 (Epub ahead of print).
30. Kang S, Zhao J, Liu Q, Zhou R, Wang N and Li Y: Vascular endothelial growth factor gene polymorphisms are associated with the risk of developing adenomyosis. Environ Mol Mutagen 50: 361-366, 2009.
31. Liu Q, Li Y, Zhao J, Sun DL, Duan YN, Wang N, Zhou RM and Kang S: Association of polymorphisms -1154G/A and -2578C/A in the vascular endothelial growth factor gene with decreased risk of endometriosis in Chinese women. Hum Reprod 24: 2660-2666, 2009.