Involvement of vascular endothelial growth factor -460 C/T, +405 G/C and +936 C/T polymorphisms in the development of endometriosis

MALGORZATA SZCZEPAŃSKA[1], ADRIANNA MOSTOWSKA[2], PRZEMYSLAW WIRSTLEIN[1,2], JANA SKRZYPCZAK[1] and PAWEŁ P. JAGODZIŃSKI[2]

[1]Department of Obstetrics, Gynecology and Gynecological Oncology, Division of Reproduction; [2]Department of Biochemistry and Molecular Biology, Poznan University of Medical Sciences, Poznań 60-781, Poland

Received October 21, 2014; Accepted November 11, 2014

DOI: 10.3892/br.2014.409

Abstract. There are inconsistent data on the contribution of vascular endothelial growth factor (*VEGF*) -460 C/T (rs833061), +405 G/C (rs2010963) and +936 C/T (rs3025039) single-nucleotide polymorphisms (SNPs) to endometriosis in different ethnicities. Therefore, using high-resolution melting curve analysis, the present study examined the distribution of these SNPs in females with endometriosis-related infertility and a control group. None of the three *VEGF* SNPs were associated with endometriosis-related infertility in the dominant and recessive models. The lowest P-values of the trend were observed for the *VEGF* +936 C/T (rs3025039) SNP in endometriosis-related infertility (P_{trend}=0.149). Similarly, haplotype analyses of *VEGF* SNPs did not demonstrate any SNP combination as a risk for endometriosis-related infertility, and the lowest overall P-values, P=0.141 and P_{corr}=0.395, were observed for a haplotype (TGT) of the above SNPs. Taken together, these results did not demonstrate the contribution of *VEGF* C/T, +405 G/C and +936 C/T SNPs to endometriosis-related infertility.

Introduction

Endometriosis is common disorder of the female reproductive organs that is attributed to the existence of functional endometrial tissue outside the uterine cavity, most frequently within the pelvic or abdominal cavity (1). This disorder develops in 3-10% of females of the reproductive age and can be responsible for infertility in 30-50% of females with this condition (1-3). The development and progression of endometriosis may be supported by the abnormal expression of genes, such as those encoding immune components, proteins regulating estrogen and progestin activity, cell growth factors and angiogenic proteins (4-9).

The vascular endothelial growth factor (VEGF) (10) is primarily involved in angiogenesis and is associated with various mechanisms encompassing action on endothelial cells, such as proliferation, survival and migration (11). VEGF was first discovered as a specific mitogen of endothelial cell (12), although it is biosynthesized by various cells, including keratinocytes macrophages, platelets, mesangial cells in the kidney and malignant cells (11,13-16). As VEGF-induced angiogenesis is an integral step in the pathogenesis of endometriosis, the survival of endometrial implants is primarily dependent on a sufficient supply of blood (17-19). The early growth of endometrial implants is characterized by a pink-red appearance resulting from its increased vascular density (20,21). Furthermore, peritoneal fluid from females with endometriotic lesions shows increased levels of different angiogenic growth compounds and decreased concentrations of anti-angiogenic factors (22).

Although the association of various *VEGF* polymorphisms with the development of endometriosis in various ethnicities has been evaluated, the data are inconsistent between different studies (23-29). Therefore, the present study aimed to investigate the distribution of *VEGF* -460 C/T (rs833061), +405 G/C (rs2010963) and +936 C/T (rs3025039) single-nucleotide polymorphisms (SNPs) in females with endometriosis-related infertility and a control group.

Materials and methods

Study subjects. Peripheral blood samples from females with endometriosis and control females were collected from the Gynecologic and Obstetrical University Hospital, Division of Reproduction at Poznan University of Medical Sciences, Poland. The studied females included two groups: 154 were included in the infertile endometriosis group and 385 were used as the fertile control group (Table I). The stage of endometriosis was assessed according to the revised classification of the American Society for Reproductive Medicine (30). All the included patients with endometriosis and the controls had

Correspondence to: Dr Paweł P. Jagodzinski, Department of Biochemistry and Molecular Biology, Poznań University of Medical Sciences, 6 Święcickiego Street, Poznań 60-781, Poland
E-mail: pjagodzi@am.poznan.pl

Key words: polymorphisms, endometriosis, infertility, vascular endothelial growth factor

Table I. Clinical characteristics of females with endometriosis and the controls.

Characteristic	Endometriosis	Controls
No.	154	385
Age, years[a]	32 (21-42)	32 (20-40)
Parity	NA	1 (1-4)[a]
Duration of infertility, years[a]	4 (1-8)	NA
rASRM, stage	I (n=83) II (n=71)	NA

[a]Median (range). NA, not applicable; rASRM, revised American Society for Reproductive Medicine classification (30).

a laparoscopic and histologically-confirmed diagnosis of endometriosis. The fertile females assigned to the control group exhibited chronic pelvic pain without any pelvic abnormalities determined by laparoscopy and were diagnosed as having varicose veins in the pelvic floor but no signs of past or present inflammation. The inclusion and exclusion criteria for the patients with endometriosis and the fertile control females were previously described in detail (31). The patients and controls were matched for age and were all Caucasians of Polish descent (Table I). Written informed consent was obtained from all the participating individuals. The study procedures were approved by the local Ethical Committees of Poznan University of Medical Sciences and were carried out in accordance with the code of ethics of the Declaration of Helsinki.

VEGF polymorphism evaluation. Genomic DNA was isolated from peripheral blood leukocytes by salt extraction. SNPs for genotyping were selected based on previous case-control studies (23-29). DNA samples were genotyped for three SNPs, -460 C/T (rs833061), +405 G/C (rs2010963) and +936 C/T (rs3025039), in *VEGF*. The genotyping was performed by a high-resolution melting curve analysis using the LightCycler 480 system (Roche Diagnostics, Mannheim, Germany) (Table II). The genotyping quality was evaluated by repeated genotyping of 10% randomly selected samples.

Statistical analysis. For each SNP, the Hardy-Weinberg equilibrium (HWE) was assessed by Pearson's goodness-of-fit χ^2 statistic. Differences in the allele and genotype frequencies between the cases and controls were computed using Fisher's exact test. The SNPs were studied for associations with endometriosis using the Cochran-Armitage trend test. The odds ratio (OR) and associated 95% confidence intervals (95% CI) were also assessed. The data were analyzed under recessive and dominant inheritance models. Pair-wise linkage disequilibrium (LD) between the selected SNPs was computed as D' and r^2 values using HaploView 4.0 software (http://www.broadinstitute.org//scientific-community/software). HaploView 4.0 software was also used for a haplotype analysis. Significant P-values were corrected using the 1,000-fold permutation test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Association between the VEGF SNPs and endometriosis-related infertility. The frequency of all the studied genotypes did not exhibit divergence from HWE between the studied groups ($P>0.05$). The number of genotypes, OR and 95% CI calculations for the three *VEGF* SNPs are listed in Table III. The lowest P-values of the trend test were observed for the *VEGF* +936 C/T (rs3025039) SNP with regards to endometriosis-related infertility ($P_{trend}=0.149$) (Table III). However, none of the three *VEGF* SNPs were associated with endometriosis-related infertility according to the dominant and recessive models (Table III). In addition, haplotype analyses of the *VEGF* SNPs did not reveal any SNP combination as a risk factor for endometriosis-related infertility (Table IV); the lowest overall P-values, $P=0.141$ and $P_{corr}=0.395$, were observed for a haplotype (TGT) of these SNPs (Table IV). The *VEGF* SNPs were in weak pairwise LD. The D' and r^2 values, as calculated from the control samples, had ranges of 0.007-0.964 (Table V).

Table II. Characteristics of the polymorphisms genotyped in the *VEGF* gene.

SNP	rs no.	Localization	SNP function	Alleles[a]	MAF[b]	Primers for PCR amplification (5'-3')	PCR product length, bp	Ann. temp., ˚C	Melt. temp., ˚C
-460 C/T	rs833061	chr6:43737486	nearGene-5	C/T	0.49	F: TCTTCGAGAGTGAGGACGTG R: ATTGGAATCCTGGAGTGACC	108	61	80-95
+405 G/C	rs2010963	chr6:43738350	UTR-5	C/G	0.30	F: GCTCCAGAGAGAAGTCGAGGA R: CACCCCCAAAAGCAGGTC	107	61	80-95
+936 C/T	rs3025039	chr6:43752536	UTR-3	C/T	0.12	F: CACACCATCACCATCGACA R: GCTCGGTGATTTAGCAGCA	191	61	80-95

[a]According to the Single Nucleotide Polymorphism database (dbSNP). Underlining denotes the minor allele in the control samples. [b]MAF from 1000 Genomes project for EUR samples. All the polymorphisms were genotyped using high-resolution melting analysis. *VEGF*, vascular endothelial growth factor; SNP, single-nucleotide polymorphism; MAF, minor allele frequency; PCR, polymerase chain reaction; ann., annealing; temp, temperature; melt., melting.

Table III. Association of the polymorphic variants of the *VEGF* gene with the risk of endometriosis.

SNP	rs no.	Alleles	Genotypes cases[a]	Genotypes controls[a]	P_t	P_g	P_a	$OR_{dominant}$ (95% CI)[b]	P-value	$OR_{recessive}$ (95% CI)[c]	P-value
-460 C/T	rs833061	C/T	48/68/38	96/197/92	0.422	0.256	0.418	0.734 (0.486-1.107)	0.140	1.043 (0.675-1.612)	0.849
+405 G/C	rs2010963	C/G	84/60/10	200/155/29	0.556	0.839	0.558	0.906 (0.622-1.318)	0.605	0.850 (0.404-1.790)	0.669
+936 C/T	rs3025039	C/T	116/33/4	262/114/8	0.149	0.160	0.154	0.685 (0.447-1.051)	0.082	1.262 (0.374-4.254)	0.749[d]

Underlining denotes the minor allele in the control samples. [a]Order of genotypes: DD/Dd/dd (d is the minor allele in the control samples). [b]Dominant model: dd+Dd vs. DD (d is the minor allele). [c]Recessive model: dd vs. Dd+DD (d is the minor allele). [d]Fisher's exact test. *VEGF*, vascular endothelial growth factor; SNP, single-nucleotide polymorphism; P_t, P_{trend} value; P_g, $P_{genotypic}$ value; P_a, $P_{allelic}$ value; OR, odds ratio; 95% CI, 95% confidence interval.

Table IV. Haplotype analysis of the polymorphisms genotyped in the *VEGF* gene.

Polymorphisms	Haplotypes	Frequency	Case/control ratios	χ^2	P-value	P_{corr} value[a]
rs833061_rs2010963	CG	0.507	0.525/0.500	0.562	0.454	0.815
	TC	0.267	0.252/0.273	0.483	0.487	0.844
	TG	0.220	0.215/0.222	0.062	0.804	1.000
rs2010963_rs3025039	GC	0.634	0.662/0.623	1.408	0.235	0.529
	CC	0.207	0.204/0.208	0.018	0.894	0.999
	GT	0.093	0.079/0.099	1.107	0.293	0.626
	CT	0.066	0.056/0.070	0.727	0.394	0.750
rs833061_rs2010963_rs3025039	CGC	0.435	0.457/0.426	0.854	0.356	0.890
	TCC	0.201	0.196/0.203	0.053	0.818	1.000
	TGC	0.199	0.205/0.197	0.081	0.776	1.000
	CGT	0.072	0.068/0.074	0.107	0.744	1.000
	TCT	0.066	0.056/0.071	0.792	0.373	0.901
	TGT	0.021	0.011/0.025	2.171	0.141	0.395

[a]P-value calculated using permutation test and a total of 1,000 permutations. *VEGF*, vascular endothelial growth factor.

Table V. Linkage disequilibrium between the markers of the *VEGF* gene in the control samples.

Genotype	rs833061	rs2010963	rs3025039
rs833061	-	0.964	0.179
rs2010963	0.365	-	0.182
rs3025039	0.007	0.017	-

D' above diagonal; r^2 below diagonal. *VEGF*, vascular endothelial growth factor.

Discussion

There are certain studies that report the increased production of VEGF in females with endometriosis, and increased VEGF levels have been demonstrated in the peritoneal tissue and blood plasma and peritoneal fluid of females with endometriosis (32-34). In addition to these observations, an *in vitro* study revealed that in the presence of peritoneal fluid from endometriotic females, endometrial cell cultures produce higher VEGF-A protein levels compared to the cultures from controls (35). There are also studies indicating an association between the VEGF levels in endometriosis and infertility. Lee and Ho (36) reported that VEGF in endometriotic females significantly inhibits sperm motility, acrosome reaction and sperm-oocyte interaction, which may result in endometriosis-associated subfertility/infertility.

There are also several animal model studies suggesting a role of VEGF overproduction, as well as anti-VEGF treatment in the regression of endometrial lesions.

Vascular density and VEGF levels are also significantly increased in endometrial implants compared to eutopic endometrium in an experimental rat model of ectopic peritoneal endometriosis (37). Furthermore, a murine endometriosis implant model showed that VEGF-C is increased in the endometrium and promotes the development of experimental endometriosis (38). However, inhibitors of aromatase and tumor necrosis factor, and treatment with resveratrol and anti-VEGF monoclonal antibodies resulted in reduced VEGF levels, which were linked to the regression of endometriotic implants in a rat model of endometriosis (39-41). Recently, a role for *miR-199a-5p* in endometriosis development has been indicated in ectopic endometrial mesenchymal stem cells and targeting the 3'-untranslated region (UTR) of *VEGF-A* mRNA by *miR-199a-5p* in an animal model led to a decrease in the size of endometriotic lesions *in vivo* (42).

Altogether, these studies suggest that polymorphisms in the *VEGF* gene potentially modulate its expression and support the development of endometriotic lesions. However, in the present study, there was no association of *VEGF* polymorphism -460 C/T (rs833061), +405 G/C (rs2010963), +936 C/T (rs3025039) or SNP haplotypes with endometriosis in the presence of infertility.

Thus far, there are reports of no association of *VEGF* -460 C/T in Northern Iran and of *VEGF* +405 G/C in samples from all Iranian populations (23,24). Additionally, no contribution of the +936 C/T SNP with endometriosis in Korean females has been observed (25). However, the +405 G/C *VEGF* polymorphism has been associated with a higher susceptibility of endometriosis in Northern Iran, Turkish, South Indian, Italian and Korean females (23,26-29,43). Bhanoori *et al* (44) demonstrated that the -460T/+405C haplotype of *VEGF* was less frequently identified in females with endometriosis compared to the controls, and *VEGF* -460 T/T homozygotes and the T allele are associated with a higher risk of endometriosis in Chinese females. There are also studies that demonstrate the association of the *VEGF* +936 C/T polymorphism in Caucasian and Japanese females with endometriosis (45,46). In addition, several meta-analyses have demonstrated that the *VEGF* +936 C/T SNP can predispose to endometriosis (47-49).

A contribution of the *VEGF* -2578 A/C SNP to endometriosis was also observed in the Estonian population, as well as *VEGF* -460/-1154/-2578 TGC, CAA, TAA and TAC haplotypes to endometriosis in North Chinese females (50,51).

There are certain studies evaluating the functional role of -460 C/T, +405 G/C and +936 C/T SNPS on *VEGF* expression. The +405 G/C SNP in the 5'-UTR exhibits a strong effect on the production of the VEGF protein (52,53). Distinct SNPs located in the 5'-UTR may account for the binding of different transcription factors in modulating *VEGF* transcription levels (54). Watson *et al* (54) demonstrated a dose-dependent effect of the +405 G allele, whereby the highest VEGF protein biosynthesis was observed for the GG genotype, an intermediate level for GC and the lowest for CC. In addition to this finding, Stevens *et al* (55) reported increased promoter activity and *VEGF* expression for the -460C/+405G haplotype compared to the -460T/+405C haplotype. The study by Renner *et al* (56) observed that the +936 C/T SNP, which is situated in the 3'-UTR, is linked to VEGF production and blood plasma levels.

Despite the contribution of the *VEGF* -460 C/T, +405 G/C and +936 C/T SNPs to the development of endometriosis in several ethnicities, the present genetic investigation failed to confirm these selected SNPs as a risk factor or endometriosis. However, as the study was conducted using a relatively small sample, it should be replicated in larger groups from different populations.

Acknowledgements

The present study was supported by the Poznan University of Medical Sciences (grant no. 502-01-01124182-07474).

References

1. Brawn J, Morotti M, Zondervan KT, Becker CM and Vincent K: Central changes associated with chronic pelvic pain and endometriosis. Hum Reprod Update 20: 737-747, 2014.
2. Ulukus M, Cakmak H and Arici A: The role of endometrium in endometriosis. J Soc Gynecol Investig 13: 467-476, 2006.
3. de Ziegler D, Borghese B and Chapron C: Endometriosis and infertility: pathophysiology and management. Lancet 376: 730-738, 2010.
4. Tseng JF, Ryan IP, Milam TD, Murai JT, Schriock ED, Landers DV and Taylor RN: Interleukin-6 secretion in vitro is up-regulated in ectopic and eutopic endometrial stromal cells from women with endometriosis. J Clin Endocrinol Metab 81: 1118-1122, 1996.
5. Noble LS, Simpson ER, Johns A and Bulun SE: Aromatase expression in endometriosis. J Clin Endocrinol Metab 81: 174-179, 1996.
6. Nishida M, Nasu K, Ueda T, Fukuda J, Takai N and Miyakawa I: Endometriotic cells are resistant to interferon-gamma-induced cell growth inhibition and apoptosis: a possible mechanism involved in the pathogenesis of endometriosis. Mol Hum Reprod 11: 29-34, 2005.
7. Zeitoun K, Takayama K, Sasano H, Suzuki T, Moghrabi N, Andersson S, Johns A, *et al*: Deficient 17beta-hydroxysteroid dehydrogenase type 2 expression in endometriosis: failure to metabolize 17beta-estradiol. J Clin Endocrinol Metab 83: 4474-4480, 1998.
8. Nishida M, Nasu K, Fukuda J, Kawano Y, Narahara H and Miyakawa I: Down-regulation of interleukin-1 receptor type 1 expression causes the dysregulated expression of CXC chemokines in endometriotic stromal cells: a possible mechanism for the altered immunological functions in endometriosis. J Clin Endocrinol Metab 89: 5094-5100, 2004.
9. Taylor HS, Bagot C, Kardana A, Olive D and Arici A: HOX gene expression is altered in the endometrium of women with endometriosis. Hum Reprod 14: 1328-1331, 1999.
10. Healy DL, Rogers PA, Hii L and Wingfield M: Angiogenesis: a new theory for endometriosis. Hum Reprod Update 4: 736-740, 1998.
11. Ferrara N: Vascular endothelial growth factor: basic science and clinical progress. Endocr Rev 25: 581-611, 2004.
12. Ferrara N, Houck K, Jakeman L and Leung DW: Molecular and biological properties of the vascular endothelial growth factor family of proteins. Endocr Rev 13: 18-32, 1992.
13. Sunderkotter C, Steinbrink K, Goebeler M, Bhardwaj R and Sorg C: Macrophages and angiogenesis. J Leukoc Biol 55: 410-422, 1994.
14. Verheul HM, Hoekman K, Luykx-de Bakker S, Eekman CA, Folman CC, Broxterman HJ and Pinedo HM: Platelet: transporter of vascular endothelial growth factor. Clin Cancer Res 3: 2187-2190, 1997.
15. Frank S, Hubner G, Breier G, Longaker MT, Greenhalgh DG and Werner S: Regulation of vascular endothelial growth factor expression in cultured keratinocytes. Implications for normal and impaired wound healing. J Biol Chem 270: 12607-12613, 1995.
16. Iijima K, Yoshikawa N, Connolly DT and Nakamura H: Human mesangial cells and peripheral blood mononuclear cells produce vascular permeability factor. Kidney Int 44: 959-966, 1993.
17. Groothuis PG, Nap AW, Winterhager E and Grümmer R: Vascular development in endometriosis. Angiogenesis 8: 147-156, 2005.
18. May K and Becker CM: Endometriosis and angiogenesis. Minerva Ginecol 60: 245-254, 2008.
19. Taylor RN, Lebovic DI and Mueller MD: Angiogenic factors in endometriosis. Ann NY Acad Sci 955: 89-100, 2002.
20. McLaren J: Vascular endothelial growth factor and endometriotic angiogenesis. Hum Reprod Update 6: 45-55, 2000.
21. Laschke MW, Schwender C, Vollmar B and Menger MD: Genistein does not affect vascularization and blood perfusion of endometriotic lesions and ovarian follicles in dorsal skinfold chambers of Syrian golden hamsters. Reprod Sci 17: 568-577, 2010.
22. Laschke MW and Menger MD: In vitro and in vivo approaches to study angiogenesis in the pathophysiology and therapy of endometriosis. Hum Reprod Update 13: 331-342, 2007.
23. Emamifar B, Salehi Z, Mehrafza M and Mashayekhi F: The vascular endothelial growth factor (VEGF) polymorphisms and the risk of endometriosis in northern Iran. Gynecol Endocrinol 28: 447-450, 2012.
24. Toktam M, Kioomars SN, Kourosh K, Adel S, Behrokh MM, Mohhamad Mehdi A and Hamid Reza KK: Association of vascular endothelial growth factor (VEGF) +405 g>c polymorphism with endometriosis in an Iranian population. J Reprod Infertil 11: 33-37, 2010.
25. Kim JG, Kim JY, Jee BC, Suh CS, Kim SH and Choi YM: Association between endometriosis and polymorphisms in endostatin and vascular endothelial growth factor and their serum levels in Korean women. Fertil Steril 89: 243-245, 2008.

26. Attar R, Agachan B, Kuran SB, Toptas B, Eraltan IY, Attar E and Isbir T: Genetic variants of vascular endothelial growth factor and risk for the development of endometriosis. In Vivo 24: 297-301, 2010.
27. Altinkaya SO, Ugur M, Ceylaner G, Ozat M, Gungor T and Ceylaner S: Vascular endothelial growth factor +405 C/G polymorphism is highly associated with an increased risk of endometriosis in Turkish women. Arch Gynecol Obstet 283: 267-272, 2011.
28. Vanaja MC, Rozati R, Nassaruddin K and Vishnupriya S: Association of VEGF +405G>C polymorphism with endometriosis. Front Biosci (Elite Ed) 5: 748-754, 2013.
29. Kim SH, Choi YM, Choung SH, Jun JK, Kim JG and Moon SY: Vascular endothelial growth factor gene +405 C/G polymorphism is associated with susceptibility to advanced stage endometriosis. Hum Reprod 20: 2904-2908, 2005.
30. No authors listed: Revised American Society for Reproductive Medicine classification of endometriosis: 1996. Fertil Steril 67: 817-821, 1997.
31. Szczepańska M, Wirstlein P, Skrzypczak J and Jagodziński PP: Polymorphic variants of CYP17 and CYP19A and risk of infertility in endometriosis. Acta Obstet Gynecol Scand 92: 1188-1193, 2013.
32. Szubert M, Suzin J, Duechler M, Szuławska A, Czyż M and Kowalczyk-Amico K: Evaluation of selected angiogenic and inflammatory markers in endometriosis before and after danazol treatment. Reprod Fertil Dev 26: 414-420, 2014.
33. Pupo-Nogueira A, de Oliveira RM, Petta CA, Podgaec S, Dias JA Jr and Abrao MS: Vascular endothelial growth factor concentrations in the serum and peritoneal fluid of women with endometriosis. Int J Gynaecol Obstet 99: 33-37, 2007.
34. Cho S, Choi YS, Jeon YE, Im KJ, Choi YM, Yim SY, Kim H, Seo SK and Lee BS: Expression of vascular endothelial growth factor (VEGF) and its soluble receptor-1 in endometriosis. Microvasc Res 83: 237-242, 2012.
35. Braza-Boïls A, Gilabert-Estellés J, Ramón LA, Gilabert J, Marí-Alexandre J, Chirivella M, España F and Estellés A: Peritoneal fluid reduces angiogenesis-related microRNA expression in cell cultures of endometrial and endometriotic tissues from women with endometriosis. PLoS One 8: e62370, 2013.
36. Lee TC and Ho HC: Effects of prostaglandin E2 and vascular endothelial growth factor on sperm might lead to endometriosis-associated infertility. Fertil Steril 95: 360-362, 2011.
37. Machado DE, Berardo PT, Palmero CY and Nasciutti LE: Higher expression of vascular endothelial growth factor (VEGF) and its receptor VEGFR-2 (Flk-1) and metalloproteinase-9 (MMP-9) in a rat model of peritoneal endometriosis is similar to cancer diseases. J Exp Clin Cancer Res 29: 4, 2010.
38. Xu H, Zhang T, Man GC, May KE, *et al*: Vascular endothelial growth factor C is increased in endometrium and promotes endothelial functions, vascular permeability and angiogenesis and growth of endometriosis. Angiogenesis 16: 541-551, 2013.
39. Ceyhan ST, Onguru O, Fidan U, Ide T, Yaman H, Kilic S and Baser I: Comparison of aromatase inhibitor (letrozole) and immunomodulators (infliximab and etanercept) on the regression of endometriotic implants in a rat model. Eur J Obstet Gynecol Reprod Biol 154: 100-104, 2011.
40. Sevket O, Sevket A, Buyukpinarbasili N, Molla T, Kilic G, Ates S and Dansuk R: The effects of ranibizumab on surgically induced endometriosis in a rat model: a preliminary study. Reprod Sci 20: 1224-1229, 2013.
41. Ergenoğlu AM, Yeniel AÖ, Erbaş O, Aktuğ H, Yildirim N, Ulukuş M and Taskiran D: Regression of endometrial implants by resveratrol in an experimentally induced endometriosis model in rats. Reprod Sci 20: 1230-1236, 2013.
42. Hsu CY, Hsieh TH, Tsai CF, Tsai HP, Chen HS, Chang Y, Chuang HY, Lee JN, Hsu YL and Tsai EM: miRNA-199a-5p regulates VEGFA in endometrial mesenchymal stem cells and contributes to the pathogenesis of endometriosis. J Pathol 232: 330-343, 2014.
43. Gentilini D, Somigliana E, Vigano P, Vignali M, Busacca M and Di Blasio AM: The vascular endothelial growth factor +405G>C polymorphism in endometriosis. Hum Reprod 23: 211-215, 2008.
44. Bhanoori M, Arvind Babu K, Pavankumar Reddy NG, Lakshmi Rao K, Zondervan K, Deenadayal M, Kennedy S and Shivaji S: The vascular endothelial growth factor (VEGF) +405G>C 5'-untranslated region polymorphism and increased risk of endometriosis in South Indian women: a case control study. Hum Reprod 20: 1844-1849, 2005.
45. Cosín R, Gilabert-Estellés J, Ramón LA, España F, Gilabert J, Romeu A and Estellés A: Vascular endothelial growth factor polymorphisms (-460C/T, +405G/C, and 936C/T) and endometriosis: their influence on vascular endothelial growth factor expression. Fertil Steril 92: 1214-1220, 2009.
46. Ikuhashi Y, Yoshida S, Kennedy S, Zondervan K, Takemura N, Deguchi M, Ohara N and Maruo T: Vascular endothelial growth factor +936 C/T polymorphism is associated with an increased risk of endometriosis in a Japanese population. Acta Obstet Gynecol Scand 86: 1352-1358, 2007.
47. Li YZ, Wang LJ, Li X, Li SL, Wang JL, Wu ZH, Gong L and Zhang XD: Vascular endothelial growth factor gene polymorphisms contribute to the risk of endometriosis: an updated systematic review and meta-analysis of 14 case-control studies. Genet Mol Res 12: 1035-1044, 2013.
48. Xu S, Wu W, Sun H, *et al*: Association of the vascular endothelial growth factor gene polymorphisms (-460C/T, +405G/C and +936T/C) with endometriosis: a meta-analysis. Ann Hum Genet 76: 464-471, 2012.
49. Liang S, Huang Y and Fan Y: Vascular endothelial growth factor gene polymorphisms and endometriosis risk: a meta-analysis. Arch Gynecol Obstet 286: 139-146, 2012.
50. Lamp M, Saare M, Laisk T, Karro H, Kadastik U, Metspalu A, Peters M and Salumets A: Genetic variations in vascular endothelial growth factor but not in angiotensin I-converting enzyme genes are associated with endometriosis in Estonian women. Eur J Obstet Gynecol Reprod Biol 153: 85-89, 2010.
51. 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.
52. Brogan IJ, Khan N, Isaac K, Hutchinson JA, Pravica V and Hutchinson IV: Novel polymorphisms in the promoter and 5' UTR regions of the human vascular endothelial growth factor gene. Hum Immunol 60: 1245-1249, 1999.
53. Watson CJ, Webb NJ, Bottomley MJ and Brenchley PE: Identification of polymorphisms within the vascular endothelial growth factor (VEGF) gene: correlation with variation in VEGF protein production. Cytokine 12: 1232-1235, 2000.
54. Akiri G, Nahari D, Finkelstein Y, Le SY, Elroy-Stein O and Levi BZ: Regulation of vascular endothelial growth factor (VEGF) expression is mediated by internal initiation of translation and alternative initiation of transcription. Oncogene 17: 227-236, 1998.
55. Stevens A, Soden J, Brenchley PE, Ralph S and Ray DW: Haplotype analysis of the polymorphic human vascular endothelial growth factor gene promoter. Cancer Res 63: 812-816, 2003.
56. Renner W, Kotschan S, Hoffmann C, Obermayer-Pietsch B and Pilger E: A common 936 C/T mutation in the gene for vascular endothelial growth factor is associated with vascular endothelial growth factor plasma levels. J Vasc Res 37: 443-448, 2000.