Association study of vascular endothelial growth factor gene polymorphisms in endometrial carcinomas in a Japanese population

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Summary

Objective: Vascular endothelial growth factor (VEGF) is one of the most potent endothelial cell mitogens and plays a critical role in angiogenesis of endometrial carcinomas. Several studies have demonstrated positive associations between VEGF gene polymorphisms and several carcinomas. In this study we investigated whether VEGF gene polymorphisms are associated with endometrial carcinomas in a Japanese population. *Methods:* The allele frequencies and genotype distributions of VEGF -460 C/T, +405 G/C, and +936 C/T polymorphisms were examined in 105 endometrial carcinomas and 179 controls using PCR-RFLP analysis. An association of these polymorphisms with three-year disease-free survival was evaluated using the Kaplan-Meier method. *Results:* No significant differences in the allele frequencies and genotype distributions of VEGF -460 C/T (p = 0.54, 0.90), +405 G/C (p = 0.31, 0.17), and +936 C/T polymorphisms (p = 0.46, 0.24) were observed between endometrial carcinoma patients and controls. There were no significant differences in the frequencies of haplotype -460 T/+405 C between patients and controls. Futhermore, VEGF -460 C/T, +405 G/C, and +936 C/T polymorphisms were not associated with three-year disease-free survival of endometrial carcinoma patients. *Conclusions:* Although limited by sample size, our study did not demonstrated any evidence that VEGF -460 C/T, +405 G/C, and +936 C/T polymorphisms are associated with an increased risk of endometrial carcinomas in Japanese women.

Key words: Endometrial carcinoma; Gene polymorphism; Polymerase chain reaction; Restriction fragment length polymorphism; Vascular endothelial growth factor.

Introduction

Endometrial carcinoma is one of the most common gynecologic malignancies and the age-standardized incidence rate is rising in Japan, possibly due to changing lifestyle and dietary patterns. Prolonged unopposed estrogen stimulation such as the use of tamoxifen, late menopause, and obesity have been identified as major risk factors for endometrial carcinomas. Recent studies show a relationship between the hereditary syndrome such as the Lynch syndrome and endometrial carcinomas, and demonstrate that a genetic factor is another risk factor of endometrial carcinomas [1, 2].

Angiogenesis is an important step in the development of cancer and is necessary for primary tumor growth, invasiveness, and metastasis. Increased tumor vascularization and expression of angiogenic factors are associated with advanced tumor stage and poor prognosis in various cancers. Vascular endothelial growth factor (VEGF), the most potent endothelial cell mitogen, has been shown to play a critical role in tumor angiogenesis [3]. VEGF binds to two kinds of VEGF receptor tyrosine kinases on endothelial cells and activates intracellular signal transduction pathways, leading to the promotion of angiogenesis and vascular permeability [3].

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VEGF has been shown to be up-regulated at mRNA and protein levels in a variety of carcinomas [6-12]. Elevated serum or intratumoral VEGF levels were associated with advanced stage disease and poor prognosis for several cancers, including breast [3], renal [4, 5], lung [6], endometrial [7, 8], and prostate carcinomas [9].

Serum levels of VEGF were significantly increased with the advanced FIGO stage of endometrial carcinomas [7]. Yokoyama *et al*. demonstrated that VEGF is a useful biomarker to predict myometrial invasion and lymph node metastasis in endometrial carcinoma [8]. These results suggest that VEGF may contribute to the pathogenesis of endometrial carcinomas.

Several studies have recently demonstrated positive associations between VEGF gene polymorphisms and several diseases. Among more than 30 single nucleotide polymorphisms (SNPs) located on the VEGF gene, three SNPs such as -460 C/T in the promoter region, +405 G/C in the 5'-untranslated region, and +936 C/T in the 3'-untranslated region were reported to be implicated in VEGF production and the risk for several diseases [3]. The -460 C/T polymorphism was associated with prostate cancer [10] and oral cancer [11], whereas the VEGF +405 G/C polymorphism was associated with endometriosis [12]. The VEGF +936 C/T polymorphism was associated with preeclampsia [13] and breast cancer [14].

Polymorphisms in VEGF have been extensively studied as possible factors influencing susceptibility to numerous

cancers [3, 10, 11, 14, 15]. The frequency of the VEGF -460 T/T genotype was shown to be significantly increased in patients with oral and prostate cancers compared to controls [10, 11], and that of the VEGF +405 C allele was significantly increased in prostate cancer with high histological grade [15]. The frequency of the VEGF +936 T allele was significantly lower in breast cancer patients compared to controls [3, 14].

However, it remains unknown whether VEGF gene polymorphisms are associated with endometrial carcinomas. In this study we investigated the possible associations between the VEGF -460 C/T, +405 G/C, and +936 C/T polymorphisms and endometrial carcinomas in a Japanese population by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis.

Materials and Methods

Subjects

The design of this study was approved by the Medical Ethics Review Committee of Kobe University Graduate School of Medicine. Written informed consent was obtained from all women involved in this study. The patient group consisted of 105 unrelated Japanese women with surgically confirmed endometrial carcinoma based on operative records and histological findings. All the patients had surgically confirmed Stage I to IV diseases according to the Federation of Gynecology and Obstetrics (FIGO) classification [16]. The age of patients with endometrial carcinoma ranged from 36 to 80 yrs with the mean age of 57.1 ± 11.0 yrs. Patients were excluded from the study if the operative records were unavailable or if there was any doubt about the diagnosis. Women who were non-Japanese (ethnicity/race) were excluded. Age-matched controls with a mean age of 56.6 ± 10.9 yrs consisted of 179 healthy Japanese women who had participated in a routine cancer detection program for gynecologic cancers at our hospital. They had no history or suggestive clinical evidence of endometrial pathology (Table 1). Patients were followed-up for cancer recurrence and mortality. Of the total 105 patients, 50 (47.6%) patients could be followed-up via in-person contact. Among them, four patients have died to date, and the remaining 46 participants are still living.

Genotyping

Genomic DNA was extracted from EDTA anticoagulated whole blood using the Wizard DNA Purification Kit (Promega, Madison, WI, USA). The –460, +405, and +936 polymorphisms in the VEGF gene were determined by using PCR-RFLP analysis.

Genotyping for VEGF -460 C/T polymorphism was performed by the forward primer 5'-TACGTGCGGACAGGGCCTGA-3' and the reverse primer 5'-TACGTGCGGAGGGCCTGA-3', followed by digestion with the restriction enzyme BstUI. Genotyping for VEGF +405 G/C polymorphism was performed by the forward primer 5'-AATTATTTTTGCTTGCCATT-3' and the reverse primer 5'-GTCTGTCTGTCTGTCTGTCCGTCA-3', followed by digestion with the restriction enzyme BsmFI. VEGF +936 C/T polymorphism was performed by the forward primer 5'-AAGGAAGAGGAGACTCTGCGC-3' and the reverse primer 5'-TATGTGGGTGGGTGTGTCTACAGG-3', followed by digestion with the restriction enzyme NlaIII. The conditions for the genotyping were as follows: PCR in a 20 µl reaction mixture containing 20 ng of genomic DNA, 10 pmol of each primer, 250 µM of dNTPs and 1.0 unit of Taq gold DNA polymerase. The concentration of $MgCl_2$ varied between the PCR reactions for the different polymorphisms with 1.5 mM for VEGF -460 C/T polymorphism and VEGF +405 G/C polymorphism, and 2.0 mM for VEGF +936 C/T polymorphism. The PCR was conducted with ABI 9700 thermocycler (PE Applied Biosystem, Foster City, CA, USA) by using the following thermal profiles: an initial denaturing cycle of 94°C for 1 min, 32 cycles of denaturing at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min, and a final cycle of 72°C for 5 min for VEGF -460 C/T polymorphism; an initial denaturing cycle of 96°C for 12 min, 35 cycles of denaturing at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min, and a final cycle of 72°C for 5 min for VEGF +405 G/C polymorphism; an initial denaturing cycle of 96°C for 12 min, 35 cycles of denaturing at 94°C for 40 s, annealing at 64°C for 1 min, and extension at 72°C for 40 s, and a final cycle of 72°C for 5 min for VEGF +936 C/T polymorphism. Digestions with the appropriate restriction enzyme were performed according to the manufacturer's instructions (New England Biolabs, Beverly, MA, USA) at 60°C for 24 h in the case of VEGF -460 C/T polymorphism, 65°C for 24 h in the case of VEGF +405 G/C polymorphism, and 37°C for 24 h in the case of VEGF +936 C/T polymorphism. DNA fragments were subjected to electrophoresis in a 2% agarose gel for the VEGF +405 G/C and +936 C/T and a 4% gel for the VEGF -460 C/T. Gel was stained with ethidium bromide (0.1 µg/ml) and visualized by ultraviolet illumination.

Statistical analysis

Genotype distributions were examined for the significant departure from the Hardy-Weinberg equilibrium by a goodness-of-fit (chi-square) test. Chi square analysis was used to examine differences in the proportions of genotype of three polymorphisms between patients and controls. Fisher's exact test was applied when appropriate. Odds Ratios (OR) and 95% confidence intervals (CIs) were used to compare categorical variables; $p < 0.05$ was considered statistically significant.

Cases were divided into two subgroups consisting of women with Stage I-II and Stage III-IV disease, endometrioid and non-endometrioid cancers, and the allele frequencies and genotype distributions of the VEGF polymorphisms in the subgroups were analyzed separately. Haplotype frequencies and standardized disequilibrium coefficient (D') were evaluated using the program Haploview (available at http://www.broad.mit.edu/mpg/haploview/tutorial/php); $p < 0.05$ was considered significant.

Three-year disease-free survival rates were evaluated using the Kaplan-Meier method and the differences in survival across

Table 1. — *Characteristics of the Japanese endometrial carcinoma samples at diagnosis*.

Characteristics	Endometrial carcinoma patients (n = 105)
Age at diagnosis (yrs)	57.1 ± 11.0
Histological type	
endometrioid	89 (84.8%)
non-endometrioid	16 (15.2%)
Stage at diagnosis	
I	62 (59.0%)
II	23 (21.9%)
III	19 (18.1%)
IV	1 (1.0%)

Table 2. — *Distribution of VEGG -460 C/T polymorphism in endometrial carcinomas and controls.*

Disease	VEGF -460 genotype (%)				Allele (%)		
	T/T	C/T	C/C	p value	T	C	p value
Endometrial carcinomas (n = 105)	54 (51.4)	42 (40.0)	9 (8.6)	p = 0.54[a]	150 (71.4)	60 (28.6)	p = 0.90[a]
Histological types							
endometrioid (n = 89)	46 (51.7)	36 (40.5)	7 (7.8)	p = 0.67[a]	128 (71.9)	50 (28.1)	p = 0.82[a]
non-endometriodi (n = 16)	8 (50.0)	6 (37.5)	2 (12.5)	p = 0.57[a]	22 (68.8)	10 (31.2)	p = 0.79[a]
Stage							
I-II (n = 85)	46 (54.1)	31 (36.5)	8 (9.4)	p = 0.29[a]	123 (72.4)	47 (27.6)	p = 0.74[a]
III-IV (n = 20)	8 (40.0)	11 (55.0)	1 (5.0)	p = 0.74[a]	27 (67.5)	13 (32.5)	p = 0.65[a]
Controls (n = 179)	86 (48.0)	82 (45.8)	11 (6.2)		254 (71.0)	104 (29.0)	

[a]versus controls.

Table 3. — *Distribution of VEGG +405 G/C polymorphism in endometrial carcinomas and controls.*

Disease	VEGF +405 genotype (%)				Allele (%)		
	G/G	G/C	C/C	p value	G	C	p value
Endometrial carcinomas (n = 105)	25 (23.8)	52 (49.5)	28 (26.7)	p = 0.31[a]	102 (48.6)	108 (51.4)	p = 0.17[a]
Histological types							
endometrioid (n = 89)	20 (22.5)	43 (48.3)	26 (29.2)	p = 0.22[a]	83 (46.6)	95 (53.4)	p = 0.09[a]
non-endometriodi (n = 16)	5 (31.2)	9 (56.3)	2 (12.5)	p = 0.53[a]	19 (59.4)	13 (40.6)	p = 0.59[a]
Stage							
I-II (n = 85)	20 (23.5)	44 (51.8)	21 (24.7)	p = 0.32[a]	84 (49.4)	86 (50.6)	p = 0.28[a]
III-IV (n = 20)	5 (25.0)	8 (40.0)	7 (35.0)	p = 0.51[a]	22 (55.0)	18 (45.0)	p = 0.26[a]
Controls (n = 179)	58 (32.4)	79 (44.1)	42 (23.5)		195 (54.5)	163 (45.5)	

[a]versus controls.

Table 4. — *Distribution of VEGG +936 C/T polymorphism in endometrial carcinomas and controls.*

Disease	VEGF +936 genotype (%)				Allele (%)		
	C/C	C/T	T/T	p value	C	T	p value
Endometrial carcinomas (n = 105)	59 (56.2)	39 (37.1)	7 (6.70)	p = 0.46[a]	157 (74.8)	53 (25.2)	p = 0.24[a]
Histological types							
endometrioid (n = 89)	51 (57.3)	32 (36.0)	6 (6.70)	p = 0.60[a]	134 (75.3)	44 (24.7)	p = 0.32[a]
non-endometriodi (n = 16)	8 (50.0)	7 (43.8)	1 (6.20)	p = 0.54[a]	23 (71.9)	9 (28.1)	p = 0.34[a]
Stage							
I-II (n = 85)	47 (55.3)	31 (36.5)	7 (8.20)	p = 0.39[a]	125 (73.5)	45 (26.5)	p = 0.16[a]
III-IV (n = 20)	12 (60.0)	8 (40.0)	0 (0.00)	p = 0.44[a]	32 (80.0)	8 (20.0)	p = 0.89[a]
Controls (n = 179)	114 (63.7)	55 (30.7)	10 (5.60)		283 (79.1)	75 (20.9)	

[a]versus controls.

different genotypes were assessed using the log rank test. The end point for disease-free survival was cancer recurrence/metastasis or death related to endometrial carcinoma. The disease-free survival period was calculated as the time from initial diagnosis to the end points of the study, censoring at the date of last contact.

Results

Genotyping of the VEGF -460 C/T, +405 G/C, and +936 C/T polymorphisms was successful in all 105 cases and 179 controls. The breakdown for stages of endometrial carcinoma are shown in Table 1.

The genotype distributions were all in Hardy-Weinberg equilibrium in both cases and controls. The genotype distributions and allele frequencies of the VEGF -460 C/T, +405 G/C, and +936 C/T polymorphisms in endometrial carcinoma patients and controls are shown in Tables 2, 3, and 4. The allele frequencies among control individuals with these polymorphisms were comparable to those of controls in other published studies using individuals from the Japanese population [19, 23]. The -460 allele frequencies of our controls were significantly different from those reported in UK (p = 0.0000005) [17] and Indian populations (p = 0.0000005) [18]. Of the VEGF +405 G/C polymorphism, the allele frequency in Indian controls is significantly different from those of Korean (p = 0.0001) [12] and Japanese (p = 0.0000002) [19]. There were no significant differences in the genotype distributions and allele frequencies of the VEGF-460 C/T, +405 G/C, and +936 C/T polymorphisms between endometrial carcinoma patients and the controls. Furthermore, the stratification by histologic types and staging failed to identify statistically significant differences between endometrial carcinoma patients and the controls (Tables 2, 3, and 4).

There was strong linkage disequilibrium between the -460 T and +405 C alleles (D' = 0.91), but not between -460 and +936 or between +405 and +936. Haplotype analysis was therefore conducted between -460 C/T and +405G/C polymorphisms alone. The frequencies of -460/+405 haplotype T/C, T/G, C/G, and C/C were 49.6%, 21.4%, 27.2%, and 1.8% in endometrial carcinoma cases and 44.4%, 26.6%, 27.9%, and 1.1% in

Table 5. — *Haplotype frequencies of VEGG -460 C/T and +405 G/C polymorphism in endometrial carcinomas and controls.*

Haplotypes -460	+405	Haplotype frequencies (%)[a] Endometrial carcinoma	Controls	Odd Ratios	95% CI
T	C	49.6	44.4	1	–
T	G	21.4	26.6	0.70	0.38-1.29
C	G	27.2	27.9	0.88	0.50-1.57
C	C	1.8	1.1	1.52	0.21-11.0

[a]Observed haplotype frequencies were estimated by the Expectation-Maximization method using a Haploview program.

control subjects, respectively (Table 5). No significant difference was noted in the frequencies of haplotype -460 T/+405 C between cases and controls.

The possible associations between the genotypes in three VEGF polymorphisms and three-year disease-free survival were evaluated in 55 patients using the Kaplan Meier method. During the study period, seven patients recurred. The Kaplan Meier survival curves showed no associations between VEGF -460 C/T, +405 G/C, and +936 C/T polymorphisms and disease-free survival in endometrial carcinoma, respectively ($p = 0.58$, $p = 0.14$, and $p = 0.69$).

Discussion

In this study we investigated the possible associations between endometrial carcinomas and VEGF -460 C/T, +405 G/C and +936 C/T polymorphisms in a Japanese population. We could not find any associations between these polymorphisms and endometrial carcinomas. We performed separate analyses on histological types and stratification by the FIGO stage, which failed to show any significant differences. In addition, we evaluated the associations of these polymorphisms with three-year disease-free survival of the patients, but could not find any associations between these polymorphisms and disease-free survival. To the best of our knowledge, this study appears to be the first report to demonstrate no associations between VEGF -460 C/T, +405 G/C, and +936 C/T polymorphisms and endometrial carcinomas in a Japanese population.

Ku *et al*. investigated the possible association of VEGF -460 C/T polymorphism and oral cancer by PCR-RFLP analysis in a Taiwanese population, and reported that VEGF -460 T/T genotype frequency in patients was significantly increased compared to the controls [11]. Chen *et al*. also reported that VEGF -460 T/T genotype frequency in patients with prostate cancer was significantly increased compared to the controls in the same population [10]. These studies suggest that VEGF -460 T allele may be a risk factor for the development and progression of various cancers. The difference in the results between previous data and ours might be due to the ethnic variation because disease susceptibility is dependent on both the prevalence of polymorphism in the population and exposure to environmental factors. In the VEGF -460 C/T polymorphism, the allele frequency in our Japanese controls is similar with that of the Korean population, but is significantly different from that of Indian ($p < 0.01$) and UK populations ($p < 0.01$). Therefore, our results may not be directly applicable to other populations including Indian and UK populations.

Several studies have demonstrated positive associations between VEGF +405 G/C polymorphism and diseases such as breast cancer [20], showing that the frequency of VEGF +405 C allele was increased in patients compared to controls. Awata *et al*. showed that fasting serum VEGF levels were higher in a normal Japanese population with VEGF +405 C/C genotype [21], suggesting that VEGF +405 G/C polymorphism is associated with VEGF synthesis. Taken together, VEGF +405 C allele may be involved in the pathogenesis of several diseases, although it still remains unclear how polymorphisms in the untranslated region of the VEGF gene influence its protein production [18, 21, 22]. In contrast, no associations have been noted between VEGF +405 G/C polymorphism and diseases such as preeclampsia [13]. Our results coincide with the results of Han *et al*., Seo *et al*., and Papazoglou *et al*., which could not find a positive association between this polymorphism and diseases.

There are several reports which have shown positive associations between VEGF +936 C/T polymorphism who and the progression or aggressiveness of tumors. Krippl *et al*. reported that the carriers of VEGF +936 T allele were at a decreased risk for breast cancer and that carriers of +936 T allele showed significantly lower VEGF plasma levels [14]. On the other hand, no associations were reported between VEGF +936 C/T polymorphism and diseases such as renal cell carcinoma in a Japanese population [23] and breast cancer in Polish and German populations [20]. We could not find any associations between endometrial carcinomas and this polymorphism in the population studied.

Lu *et al*. investigated the possible associations between these three polymorphisms and breast cancer patients in a Chinese population [3]. They found strong linkage disequilibrium between the VEGF -460 C/T and +405 G/C polymorphisms ($D' = 0.94$) and an association of the VEGF -460 C and +405 G haplotype with poorer survival of breast cancer patients, but failed to identify an association of VEGF +936 C/T polymorphism with overall survival or disease-free survival [3]. In this study, we found a similar strong linkage disequilibrium between the -460 T and +405 C alleles ($D' = 0.91$), but the haplotype analysis did not reveal a positive association between endometrial carcinoma patients and controls. We also evaluated the association of VEGF -460 C/T, +405 G/C and +936 C/T polymorphisms with the three-year disease-free survival of endometrial carcinoma patients, but could not find any clear associations between these polymorphisms and the disease-free survival of this cancer.

In conclusion, this study appears to be the first description to demonstrate no associations between endometrial cancer and the VEGF -460 C/T, +405 G/C, and +936 C/T polymorphisms, suggesting that these two polymorphisms are unlikely to be involved in the pathogenesis of

endometrial cancer in a Japanese population. Although this model is biologically plausible, we recognize that our conclusions are based on relatively small numbers and will require verification from additional independent studies because the sample sizes are not sufficient to conclude the differences as non-significant (the power level of < 80%).

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References

[1] Soliman P.T., Broaddus R.R., Schmeler K.M., Daniels M.S., Gonzalez D., Slomovitz B.M. *et al*.: "Women with synchronous primary cancers of the endometrium and ovary: do they have Lynch syndrome?". *J. Clin. Oncol*., 2005, *23,* 9344.

[2] Pistorius S., Kruger S., Hohl R., Plaschke J., Distler W., Saeger H. D. *et al*.: "Occult endometrial cancer and decision making for prophylactic hysterectomy in hereditary nonpolyposis colorectal cancer patients". *Gynecol. Oncol*., 2006, *102*, 189.

[3] Lu H., Shu X. O., Cui Y., Kataoka N., Wen W., Cai O. *et al*.: "Association of genetic polymorphisms in the VEGF gene with breast cancer survival". *Cancer Res*., 2005, *65*, 5015.

[4] Jacobsen J., Rasmuson T., Grankvist K., Liungberg B.: "Vascular endothelial growth factor as prognostic factor in renal cell carcinoma". *J. Urol*., 2000, *163*, 343.

[5] Sato K., Tsuchiya N., Sasaki R., Shimoda N., Satoh S., Ogawa O. *et al*.: "Increased serum levels of vascular endothelial growth factor in patients with renal cell cancer". *Jpn. J. Cancer Res*., 1999, *90,* 874.

[6] Takizawa H., Kondo K., Fujino H., Kenzaki K., Miyoshi T., Sakiyama S. *et al*.: "The balance of VEGF-C and VEGFR-3 mRNA is a predictor of lymph node metastasis in non-small cell lung cancer". *Br. J. Cancer,* 2006, *3*, 75.

[7] Mazurek A., Pierzynski P., Kuc P., Kopinski P., Terlikowski S., Niklinska W. *et al*.: "Evaluation of angiogenesis, p-53 tissue protein expression and serum VEGF in patients with endometrial cancer". *Neoplasma,* 2004, *51*, 193.

[8] Yokoyama Y., Charnock-Jones D.S., Licence D., Yanaihara A., Hastings J.M., Holland C.M. *et al*.: "Expression of vascular endothelial growth factor (VEGF)-D and its receptor, VEGF receptor 3, as a prognostic factor in endometrial carcinoma". *Clin. Cancer Res*., 2003, *9,* 1361.

[9] Wong S. Y., Haack H., Crowlev D., Barry M., Bronson R. T., Hvnes R. O.: "Tumor secreted vascular endothelial growth factor C is necessary for prostate cancer lymphangiogenesis, but lymphangiogenesis is unnecessary for lymph node metastasis". *Cancer Res*., 2005, *65*, 9789.

[10] Lin C.C., Wu H.C., Tsai F.J., Chen H.Y., Chen W.C.: "Vascular endothelial growth factor gene -460 C/T polymorphism is a biomarker for prostate cancer". *Urology,* 2003, *62,* 374.

[11] Ku K.T., Wan L., Peng H.C., Tsai M.H., Tsai C.H., Tsai F.J.: "Vascular endothelial growth factor gene -460 C/T polymorphism is a biomarker for oral cancer". *Oral Oncol.,* 2005, *41*, 497.

[12] Kim S.H., Choi Y.M., Choung S.H., Jun J.K., Kim J.G., Moon S.Y.: "Vascular endothelial growth factor gene +405 C/G polymorphism is associated with susceptibility to advanced stage endometriosis". *Hum. Reprod*., 2005, *20*, 2904.

[13] Papazoglou D., Galazios G., Koukourakis M.I,, Panagopoulos I., Kontomanolis E.N., Papatheodorou K. *et al*.: "Vascular endothelial growth factor gene polymorphisms and preeclampsia". *Mol. Hum. Reprod*., 2004, *10,* 321.

[14] Krippl P., Langsenlehner U., Renner W., Yazdani-Biuki B., Wolf G., Wascher T.C. *et al*.: "A common 936 C/T gene polymorphism of vascular endothelial growth factor is associated with decreased breast cancer risk". *Int. J. Cancer,* 2003, *10*, 468.

[15] Sfar S., Hassen E., Saad H., Mosbah F., Chouchane L.: "Association of VEGF genetic polymorphisms with prostate carcinoma risk and clinical outcome". *Cytokine*, 2006, *35*, 21.

[16] Mazurek A., Pierzynski P., Kuc P., Kopinski P., Terlikowski S., Niklinska W. *et al*.: "Evaluation of angiogenesis, p-53 tissue protein expression and serum VEGF in patients with endometrial cancer". *Neoplasma,* 2004, *51,* 193.

[17] Summers A.M., Coupes B.M., Brennan M.F., Ralph S.A., Short C.D., Brenchlev P.E.: "VEGF -460 genotype plays an important role in progression to chronic kidney disease stage 5". *Nephrol. Dial. Transplant,* 2005, *20,* 2427.

[18] Bhanoori M., Arvind Babu K., Pavankumar Reddy N.G., Lakshmi Rao K., Zondervan K., Deenadayal M. *et al*.: "The vascular endothelial growth factor (VEGF) +405 G > C 5'-untranslated region polymorphism and increased risk of endometriosis in South Indian women: a case control study". *Hum. Reprod*., 2005, *20,* 1844.

[19] Kariyazono H., Ohno T., Khajoee V., Ihara K., Kusuhara K., Kinukawa N. *et al*.: "Association of vascular endothelial growth factor (VEGF) and VEGF receptor gene polymorphisms with coronary artery lesions of Kawasaki disease". *Pediatr. Res*., 2004, *56,* 953.

[20] Rueda B., Lopez-Nevot M.A., Lopez-Diaz M.J., Garcia-Porrua C., Martin J., Gonzalez-Gay M.A.: "A functional variant of vascular endothelial growth factor is associated with severe ischemic complications in giant cell arteritis". *J. Rheumatol*., 2005, *32,* 1737.

[21] Boiardi L., Casali B., Nicoli D., Farnetti E., Chen O., Macchioni P. *et al*.: "Vascular endothelial growth factor gene polymorphisms in giant cell arteritis". *J. Rheumatol*., 2003, *30,* 2160.

[22] Watson C.J., Webb N.J., Bottomlev M.J., Brenchlev P.E.: "Identification of polymorphisms within the vascular endothelial growth factor (VEGF) gene: correlation with variation in VEGF protein production". *Cytokine,* 2000, *12,* 1232.

[23] Abe A., Sato K., Habuchi T., Wang L., Li Z., Tsuchiya N. *et al*.: "Single nucleotide polymorphisms in the 3' untranslated region of vascular endothelial growth factor gene in Japanese population with or without renal cell carcinoma". *Tohoku J. Exp. Med*., 2002, *198,* 181.

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