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*Original Article*

# Role of *VEGF* -2578C/A, +936C/T and -460T/C genetic polymorphisms in the risk of ovarian cancer in Chinese women

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**Abstract:** In the present study, we carried out a hospital-based case-control study to investigate the association between *VEGF* genetic polymorphisms (-2578C/A rs699947, +936C/T rs3025039 and -460T/C rs833061) and risk of developing ovarian cancer. The case-control study comprised 163 patients with ovarian cancer and 276 controls were recruited into our study between January 2013 and January 2015. The *VEGF* -2578C/A, +936C/T and -460T/C polymorphisms was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). We found statistically significant differences between ovarian cancer patients and control subjects in terms of *VEGF* -2578C/A ($\chi^2$=15.83, P<0.001). Using unconditional logistic regression analysis, the C/A and A/A genotypes of *VEGF* -2578C/A increased the risk of ovarian cancer when compared to the C/C genotype, and the adjusted Ors (95% CI) were 1.92 (1.22-3.02) and 3.70 (1.45-9.94), respectively. Moreover, the A allele of *VEGF* -2578C/A was associated with an increased risk towards ovarian cancer compared with the C allele (OR=2.03, 95% CI=1.43-2.88). In conclusion, we suggests that *VEGF*-2578C/AC polymorphism was associated with the risk of developing ovarian cancer in a Chinese population.

**Keywords:** *VEGF*, -2578C/A, +936C/T, -460T/C, ovarian cancer, Chinese population

## Introduction

Ovarian cancer is one of the major causes of cancer-related death in females worldwide, largely resulting from the growth, metastasis and invasion of the cancer cells [1]. It is estimated that the morbidity of ovarian cancer is about $4.1/10^5$ and the mortality is about $1.9/10^5$ in Chinese population according to the international Agency for Research on Cancer investigation in 2012 (IARC, 2012). The pathogenesis of ovarian cancer is not clear and not well understood. The development of ovarian cancer is involved in many environmental and lifestyle factors, such as age, early menarche, late menopause, unbearing, high fact dietary andindustrial talcum powder and asbestos exposure as well as long-term use of hormone supplement therapy [2, 3]. Previous study has reported that about 15% patients with ovarian cancer present familial aggregation, which suggested that genetic factors may contribute to the development of ovarian cancer [4]. Numerous studies have been performed to elucidate the molecular mechanisms of ovarian cancer, such as Kras gene 3'-UTR gene, Insulin-like growth factor I gene, hypoxia-inducible factor-1a gene, kinesin-like factor 1B gene and Nuclear factor-κB gene [5-9].

Vascular endothelial growth factor (*VEGF*) is a one of the key initiators and an critical pro-angiogenic growth factor, and it is one of the most potent endothelial cell mitogens and plays an important role in regulating egress of plasma proteins and cells that directly and indirectly stimulate angiogenesis [10, 11]. The *VEGF* gene is located on chromosome 6p12-p21 with eight exons and seven introns, and has a 14-kb coding region. Single-nucleotide polymorphisms (SNPs) are the most common sequence variations occurring in the human genome. SNPs in *VEGF* gene may affect the property of the respective *VEGF*. Three common SNPs were observed in the promoter region of *VEGF*, including-2578C/A (rs699947), +936C/T

**Table 1.** The primer sequences and restriction enzymes for *VEGF* -2578C/A, +936C/T and -460T/C

| *VEGF* | Primers (5'-3') | Restriction enzymes | Product sizes |
|---|---|---|---|
| -2578C/A | Forward: GGATGGGGCTGACTAGGTAAGC | BglII | AA: 202 bp and 122 bp |
| | | | CA: 324 bp, 202 bp and 122 bp |
| | Reverse: AGCCCCCTTTTCCTCCAAC | | CC: 324 bp |
| +936C/T | Forward: AAGGAAGAGGAGACTCTGCGC | Hsp92II | TT: 122 bp and 86 bp |
| | Reverse: TATGTGGGTGGGTGTGTCTACAG | | CT: 198 bp, 122 bp and 86 bp |
| | | | CC: 198 bp |
| -460T/C | Forward: TGAATGGAGCGAGCAGCGTCT | Bsh1236I | TT: 175 bp |
| | Reverse: CGTGCGGACAGGGCCTGAGA | | CT: 155 bp, 175 bp and 20 bp |
| | | | CC: 155 bp and 20 bp |

(rs3025039) and -460T/C (rs833061). It is reported that the three SNPs play a critical role in protein synthesis of *VEGF* [12, 13]. Previous studies have reported that the *VEGF* genetic polymorphisms contribute to the risk of developing cancers [14-19]. Currently, only one previous study reported the relationship between *VEGF* genetic polymorphisms and susceptibility to ovarian cancer in Chinese population [20]. In the present study, we carried out a hospital-based case-control study to investigate the relationship between *VEGF* genetic polymorphisms (-2578C/A rs699947, +936C/T rs3025039 and -460T/C rs833061) and risk of developing ovarian cancer.

## Materials and methods

### *Subjects*

The case-control study comprised 163 patients with ovarian cancer and 276 controls, hence a total of 439 subjects were recruited in the study. The patients with ovarian cancer were obtained from the Affiliated Tumor Hospital of Zhengzhou University between January 2013 and January 2015. Newly diagnosed primary ovarian cancer cases were included in the study with no age, histological and TNM restrictions. The exclusion criteria was to exclude those subjects who had a history of acute or chronic infection diseases, end-stage of liver and kidney diseases and other malignant tumors.

The control subjects were recruited from the hospital, these were the individuals who came for normal health check-ups from the Affiliated Tumor Hospital of Zhengzhou University between January 2013 and January 2015. Individuals with any malignant tumors were excluded as controls.

The demographic and clinical characteristics of study subjects were collected using medical records or structured questionnaires. A detailed questionnaire was filled for each patients and control subjects by a trained interviewer. All the information regarding the epidemiological factors and the smoking habits ofthe subjects was obtained with the help of a questionnaire form filled by a trained personnel. Inorder to obtain a clear idea about the total smoking exposure, pack-years were obtained using thisequation: [(cigarettes per day/20) × number of years smoked]. The clinical details of thepatients such as histology, TNM staging and other important information was collected from the medical records of the patients from the hospital.

The mean ages of patients with ovarian cancer and control subjects were 52.52±8.56 and 51.70±9.15 years, respectively. The BMI were 25.10±2.85 and 23.61±3.14 kg/m$^2$ for ovarian cancer patients and control subjects, respectively. Each study subject signed an inform consent before recruitment into this study. The performance of our study was approved by the ethics committee of the Affiliated Tumor Hospital of Zhengzhou University.

### *DNA extraction and genotyping*

Each study subject was asked to provide five mL peripheral blood, and the DNA was extracted from the blood samples using QIAamp DNA Blood Mini Kit (QIAGEN, USA) following the manufacturer's recommendation. The *VEGF* -2578C/A, +936C/T and -460T/C polymorphisms was determined by polymerase chain re-

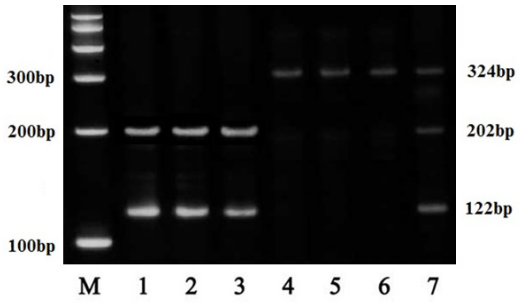


**Figure 1.** Genotypes for *VEGF* -2578C/A polymorphism. 1-3 lanes: AA genotype; 4-6 lanes: CA genotype: 7 lane: CC genotype.

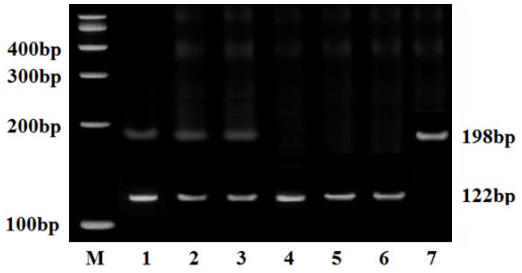


**Figure 2.** Genotypes for *VEGF* +936C/T polymorphisms. 1-3 lanes: CT genotype; 4-6 lanes: TT genotype: 7 lane: CC genotype.

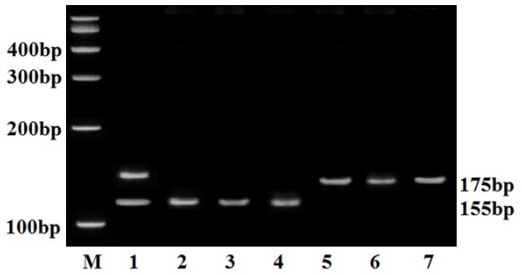


**Figure 3.** Genotypes for *VEGF* -460T/C polymorphisms. 1 lane: CT genotype; 2-4 lanes: CC genotype: 5-7 lane: TT genotype.

action-restriction fragment length polymorphism (PCR-RFLP). The primer sequences and restriction enzymes for *VEGF* -2578C/A, +936C/T and -460T/C were shown in **Table 1**. The PCR reaction was performed in 15 μl of reaction mixture containing 1× PCR buffer, 1.5 μl (25 mmol/L) $MgCl_2$, with 0.25 μl (10 pmol/μl) of both primers, 0.3 μl (10 mmol/L) of each dNTP's, 0.25 μl (5 u/ul) Taq polymerase and approximately 1.0 μl DNA. The PCR conditions included an initial melting step of 94°C for 5 min and followed by 30 cycles of 94°C for 45 s, variable annealing temperature for 30 s and 72°C for 45 s for all the four polymorphic sites. PCR amplification was checked by using 1.5% agarose gel electrophoresis (**Figures 1-3**).

*Statistical analysis*

The statistical variation within the demographic and clinical variables of the two study groups was done usingthe chi-square tests ($\chi^2$ test) for the categorical data and student *t* test for continuous variables. The statistical difference between the allele and genotype frequencies of patients and controls were carried out using Pearson's Chi-square test. Genotypic frequencies in control subjects for each SNP were tested for departure from Hardy-Weinberg equilibrium (HWE) using an exact Chi-square test. To evaluate the correlation of ovarian cancer susceptibility and *VEGF* -2578C/A, +936C/T and -460T/C polymorphisms, the unconditional logistic regression analysis was taken to calculate adjusted Odds Ratio (OR) with their 95% Confidence Intervals (CI) with adjustment for possible confounders. Statistical analyses were performed using SPSS 16.0 statistical packages (SPSS, Chicago, IL). All *P* values in this study were two-sided.

## Results

The demographic and clinical variables of the two study groups are shown in **Table 2**. Using Chi-square test or student t test, the ovarian cancer patients were comparable with the control subjects in respect of mean age (*t*=0.93, P=0.18), menopausal status ($\chi^2$=0.87, P=0.35), tobacco smoking ($\chi^2$=1.45, P=0.23), alcohol consumption ($\chi^2$=0.56, P=0.46), and using of hormone replacement therapy ($\chi^2$=2.76, P=0.10). However, significant statistical differences were observed between ovarian cancer patients and control subjects regarding family history of cancers in the first relatives ($\chi^2$=7.11, P=0.008) and use of hormone replacement therapy ($\chi^2$=4.97, P<0.001).

The genotypic frequencies distribution of *VEGF* -2578C/A, +936C/T and -460T/C genes aregiven in **Table 3**. We found statistically significant differences between ovarian cancer patients and control subjects in terms of *VEGF* -2578C/A ($\chi^2$=15.83, P<0.001). However, there were no statistical differences between the two study groups with respect to *VEGF* +936C/T ($\chi^2$=0.87, P=0.65) and -460T/C ($\chi^2$=0.27, P=0.87) geno-

**Table 2.** Characteristics of ovarian cancer patients and control subjects

| Variables | Patients N=163 | % | Controls N=276 | % | $\chi^2$ test or t test | *P* value |
|---|---|---|---|---|---|---|
| Mean age, years | 52.52±8.56 | | 51.70±9.15 | | 0.93 | 0.18 |
| Menopausal status | | | | | | |
| Pre-menopausal | 60 | 36.81 | 114 | 41.30 | | |
| Post-menopausal | 103 | 63.19 | 162 | 58.70 | 0.87 | 0.35 |
| Tobacco smoking | | | | | | |
| No | 143 | 87.73 | 252 | 91.30 | | |
| Yes | 20 | 12.27 | 24 | 8.70 | 1.45 | 0.23 |
| Alcohol consumption | | | | | | |
| No | 125 | 76.69 | 220 | 79.71 | | |
| Yes | 38 | 23.31 | 56 | 20.29 | 0.56 | 0.46 |
| Family history of cancers in the first relatives | | | | | | |
| No | 152 | 93.25 | 271 | 98.19 | | |
| Yes | 11 | 6.75 | 5 | 1.81 | 7.11 | 0.008 |
| Use of hormone replacement therapy | | | | | | |
| No | 153 | 93.87 | 269 | 97.46 | | |
| Yes | 10 | 6.13 | 8 | 2.90 | 2.76 | 0.10 |
| Body mass index, kg/m$^2$ | | 25.10±2.85 | | 23.61±3.14 | 4.97 | <0.001 |
| TNM stage | | | | | | |
| I-II | 76 | 46.63 | | | | |
| III-IV | 87 | 53.37 | | | | |
| Histopathology | | | | | | |
| Serous | 94 | 57.67 | | | | |
| Mucinous | 22 | 13.50 | | | | |
| Clear cell | 12 | 7.36 | | | | |
| Endometrioid | 24 | 14.72 | | | | |
| Others | 11 | 6.75 | | | | |

typic distributions. The genotypic distributions of the three SNPs did not deviate from the HWE in patients (*P* values were 0.21, 0.28 and 0.74, respectively) and controls (*P* values were 0.26, 0.62 and 0.82, respectively). Moreover, the MAFs of *VEGF* -2578C/A, +936C/T and -460T/C genes were similar with those in the databases from National Center of Biotechnology Information (https://www.ncbi.nlm.nih.gov/snp/).

The correlation between *VEGF* -2578C/A, +936C/T and -460T/C genetic polymorphisms and risk of ovarian cancer were presented in **Table 4**. The wide type genotype of the three SNPs were taken as the reference group. Using unconditional logistic regression analysis, the C/A and A/A genotypes of *VEGF* -2578C/A increased the risk of ovarian cancer when compared to the C/C genotype, and the adjusted Ors (95% CI) were 1.92 (1.22-3.02) and 3.70 (1.45-9.94), respectively. Moreover, the A allele of *VEGF* -2578C/A was associated with an increased risk towards ovarian cancer compared with the C allele (OR=2.03, 95% CI=1.43-2.88). However, no significant association was found between *VEGF* +936C/T and -460T/C genetic polymorphisms and development of ovarian cancer by logistic regression analysis.

## Discussion

*VEGF* is one of the most important factors that is involved in the activation of tumor-related angiogenesis [21, 22]. Previous studies have reported that the *VEGF* plasma levels are associated with tumor growth and pathogenesis of ovarian cancer patients [23, 24]. Single nucleotide polymorphisms (SNP) refer to the alteration of a single nucleotide base, by insertion, deletion, or replacement, and thus influences the expression and function of protein

**Table 3.** Genotypic distributions of *VEGF* -2578C/A, +936C/T and -460T/C genes

| *VEGF* | Patients | % | Controls | % | $\chi^2$ test | *P* value | HWE *P* value (patients) | HWE *P* value (controls) | MAF (controls) |
|---|---|---|---|---|---|---|---|---|---|
| -2578C/A | | | | | | | | | |
| C/C | 90 | 55.22 | 200 | 72.46 | | | | | |
| C/A | 58 | 35.58 | 67 | 24.28 | | | | | |
| A/A | 15 | 9.20 | 9 | 3.26 | 15.83 | <0.001 | 0.21 | 0.26 | 0.1540 |
| +936C/T | | | | | | | | | |
| C/C | 109 | 66.87 | 192 | 69.57 | | | | | |
| C/T | 46 | 28.22 | 75 | 27.17 | | | | | |
| T/T | 8 | 4.91 | 9 | 3.26 | 0.87 | 0.65 | 0.28 | 0.62 | 0.1685 |
| -460T/C | | | | | | | | | |
| T/T | 87 | 53.37 | 153 | 55.44 | | | | | |
| C/T | 63 | 38.65 | 104 | 37.68 | | | | | |
| C/C | 13 | 7.98 | 19 | 6.88 | 0.27 | 0.87 | 0.74 | 0.82 | 0.2572 |

**Table 4.** Relationship between *VEGF* -2578C/A, +936C/T and -460T/C genetic polymorphisms and risk of ovarian cancer

| *VEGF* | Patients | % | Controls | % | Adjusted OR (95% CI)[1] | *P* value |
|---|---|---|---|---|---|---|
| -2578C/A | | | | | | |
| C/C | 90 | 55.22 | 200 | 72.46 | 1.0 (Ref.) | - |
| C/A | 58 | 35.58 | 67 | 24.28 | 1.92 (1.22-3.02) | 0.003 |
| A/A | 15 | 9.2 | 9 | 3.26 | 3.70 (1.45-9.94) | 0.002 |
| Allele | | | | | | |
| C | 238 | 73.01 | 467 | 84.6 | 1.0 (Ref.) | - |
| A | 88 | 26.99 | 85 | 15.4 | 2.03 (1.43-2.88) | <0.001 |
| +936C/T | | | | | | |
| C/C | 109 | 66.87 | 192 | 69.57 | 1.0 (Ref.) | - |
| C/T | 46 | 28.22 | 75 | 27.17 | 1.08 (0.68-1.71) | 0.73 |
| T/T | 8 | 4.91 | 9 | 3.26 | 1.57 (0.51-4.71) | 0.37 |
| Allele | | | | | | |
| C | 264 | 80.98 | 459 | 83.155 | 1.0 (Ref.) | - |
| T | 62 | 19.02 | 93 | 16.845 | 1.16 (0.80-1.68) | 0.42 |
| -460T/C | | | | | | |
| T/T | 87 | 53.37 | 153 | 55.44 | 1.0 (Ref.) | - |
| C/T | 63 | 38.65 | 104 | 37.68 | 1.07 (0.69-1.64) | 0.76 |
| C/C | 13 | 7.98 | 19 | 6.88 | 1.20 (0.52-2.71) | 0.63 |
| Allele | | | | | | |
| T | 237 | 72.695 | 410 | 74.28 | 1.0 (Ref.) | - |
| C | 89 | 27.305 | 142 | 25.72 | 1.08 (0.78-1.49) | 0.61 |

[1]Adjusted for age, family history of cancer in the first relatives and use of hormone replacement therapy.

[25]. Amino acid substitutions that occur at promoter important regions in the *VEGF* might influence the expression of this protein. In previous experimental studies have indicated that genetic variability of *VEGF* could influence the activity, function and expression of this protein [26, 27]. In the present study, we investigate whether the *VEGF* -2578C/A, +936C/T and -460T/C polymorphic sites of *VEGF* gene could have an impact on ovarian cancer in Chinese population. Our study indicated that the C/A and A/A genotypes of -2578C/A were associated to increase the risk of ovarian cancer in Chinese population regardless of potential confounding factors.

Angogenesis is a necessary factor for the growth of microscopic cancers into larger tumors, and it have an important role in the pathogenesis of ovarian cancer [20, 28]. It is reported that the *VEGF* protein is an critical factors of angiogenesis, and it is a key step for tumor development and progression of ovarian cancers [29]. Considering the polymorphism -2578C/A in *VEGF*, the C to A allele change led to the reduction of *VEGF* gene transcription, which may influence the expression of several viral and cellular genes [30, 31]. Previous study have reported that C allele of

*VEGF*-2578C/A showed an elevated gene transcription activity of 1.95-fold compared to the A allele [32]. Another study revealed that the C/A genotype of *VEGF* was associated with the high expression of this protein, while the C/C genotype was correlated with the low expression [33].

Previous studies have reported the association between *VEGF* -2578C/A genetic polymorphisms and development of several kinds of cancers, such as hepatocellular carcinoma, cervical cancer, breast cancer, lung cancer, osteosarcoma, renal cell carcinomas [16, 18, 34-37]. Machado et al. carried out a case-control study in Portuguese Chinese, and reported that *VEGF* -2578C/A genetic polymorphisms was associated with an increased risk of hepatocellular carcinoma [37]. Zidi et al. conducted a case-control study with 86 cervical cancer patients and 124 controls in Tunisian population, and demonstrated that *VEGF* -2578C/A and -1154G/A genetic variation may contribute to the development of cervical cancer [36]. Kapahi et al. performed a study and comprised of 204 breast cancer patients and 204 controls, and reported that the *VEGF* -2578C/A and -460T/C genetic polymorphisms are correlated with the risk of developing breast cancer [35]. Zhao et al. performed a study with 176 osteosarcoma and 176 controls in a Chinese population, and suggested that *VEGF* -2578C/A and -460T/C genetic polymorphisms may be correlated with an elevated risk of osteosarcoma [38]. Xian et al. indicated that the *VEGF* -2578C/A and -936C/T genetic polymorphisms may contribute to the etiology of renal cell carcinomas in a Chinese population. However, Liu et al. carried out a study with 414 patients with primary lung cancer and 338 healthy controls in a Chinese population, and reported that no relationship existed between *VEGF* 460T/C, -1154G/A and -2578C/A genetic polymorphisms and development of lung cancer [34].

For the association between *VEGF* genetic polymorphisms and development of ovarian cancer, only three previous studies have reported their relationship, but the results are inconclusive [20, 28, 39]. Li et al. carried out a case-control study with 303 epithelial ovarian cancer patients and 303 healthy controls in China, but theyrevealed that the *VEGF* -2578C/A, +936C/T and -460T/C genetic polymorphisms did not contribute to the susceptibility to epithelial ovarian cancer [20]. Rinck-Junior et al. conducted a study with 131 ovarian cancer patients and 137 controls in Brazilian population, and demonstrated that *VEGF* +936C/T contribute to the development of epithelial ovarian cancer [28]. Knoac et al. carried out a study with 47 ovarian cancer patients and 106 healthy control in Turkey, and reported that the *VEGF* +936C/T and -460T/C polymorphisms could not contribute to the development of ovarian cancers [39]. In our study, we observed that the C/A and A/A genotypes of -2578C/A contribute to the susceptibility of ovarian cancer, and part of our results were in line with previous studies. The discrepancies of these studies may be caused by differences in ethnicity, selection of controls, and sample sizes.

Two limitations should be taken into consideration in this study. First, the study subjects were collected from only one hospital in China, and this study design may result in selection bias. Second, the sample size is relatively small, and the small sample size could cause low statistical power to determine the differences between the patients and controls. Therefore, further studies with more ethnicities and larger sample sizes are greatly required to evaluate the association of *VEGF* gene polymorphisms and development of ovarian cancer.

In conclusion, our study suggests that *VEGF*-2578C/AC polymorphism was associated with risk of developing ovarian cancer. Further investigations and studies with much larger sample size are required to confirm the relationship between *VEGF*-2578C/A, +936C/T and -460T/C gene polymorphisms and risk of ovarian cancer.

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## Disclosure of conflict of interest

None.

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