# Gender-specific Association of the *VEGF* –2578C>A Polymorphism in Korean Patients with Colon Cancer

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**Abstract**. *Background: Angiogenesis is involved in the development of cancer, promoting tumor growth, invasiveness and metastasis. Vascular endothelial growth factor (VEGF) is a potent angiogenic factor. The present case control study was carried out, to determine whether there is an association between the VEGF –2578C>A polymorphism and colon cancer. Patients and Methods: DNA samples taken from 246 patients with colon cancer and 203 healthy controls were amplified by polymerase chain reaction for VEGF –2578C>A polymorphism. Results: Genotype frequencies of the VEGF –2578C>A polymorphism were not significantly different between patient and control groups. However, when the data were stratified by gender, the frequency of the –2578CA+AA (A allele-bearing) genotype was marginally significant different with protective effect for colon cancer in women (odds ratio, OR, 0.60; 95% confidence interval, CI, 0.36-0.99; p=0.056). The –2578CA+AA genotype was also associated with reduced risk in patients with proximal colon cancer (OR, 0.55; 95% CI, 0.31-0.97; p=0.049). This association also remained in women with proximal colon cancer. Conclusion: Although the VEGF –2578C>A polymorphism had no influence on susceptibility to colon cancer, some genotypes showed a significant difference between the case and control groups when the data were stratified by gender and the original location of tumor, suggesting that the VEGF –2578C>A polymorphism, at least in Koreans, is a genetic determinant of colon cancer risk.*

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Tumor-induced angiogenesis is promoted by secreted or activated angiogenic factors that stimulate endothelial migration, proliferation and capillary morphogenesis. Ultimately, this angiogenesis can lead to cancer progression and metastasis. As a potent angiogenic factor, vascular endothelial growth factor (VEGF) and its potential role in this process has attracted considerable interest. VEGF is involved in endothelial cell proliferation and increase of capillary permeability (1, 2). Thus, it could be a specific mitogen involved in tumor angiogenesis. Overexpression of VEGF has been observed in a variety of tumor tissues, including colon tumors (3, 4) and, in physiological and pathological studies, VEGF overexpression is associated with the risk of poor survival and advanced stage of several types of cancers (5, 6).

*VEGF* polymorphisms have been associated with the risk for several types of cancer and other diseases with a putative angiogenic basis (5, 7-13). At least 30 single-nucleotide polymorphisms (SNP) in this gene have been described in the literature (14-16). Transition C to A at nucleotide position –2578 relative to translation start site were observed (14). Interestingly, individuals with the A allele at position –2578 also had an insertion of 18 nucleotides, whereas CC homozygotes did not contain this insertion. The *–2578A* allele has been associated with decreased VEGF expression (17).

Despite the association studies, the effect of *VEGF* polymorphisms on the risk of colon cancer has not been reported yet. Since VEGF is significant in the angiogenesis of various types of tumors, it is reasonable to hypothesize that VEGF is a good candidate for determining the risk of developing colon cancer. To test this hypothesis, we investigated the possible association between the –2578C>A polymorphism in the *VEGF* gene promoter and patients with colon cancer.

## Patients and Methods

*Study participants.* A total of 246 patients (mean age±SD, 59.33±13.27 years) with colon cancer diagnosed at Bundang CHA Hospital, Pochon CHA University from July 1999 to June 2004

were enrolled in this study. Of the colon cancer patients, there were 128 men (aged 58.71±12.55 years; range, 18 to 81 years ) and 118 women (aged 60.61±13.96 years; range, 23 to 89 years). Sixty-nine consecutive patients (aged 57.91±14.00 years; range, 18 to 80 years) with a cancerous distal colon (*i.e.*, descending and sigmoid colon) and 161 consecutive patients (aged 60.16±12.99 years; range, 24 to 89 years) with a cancerous proximal colon (*i.e.*, from the caecum to the splenic flexure) underwent primary surgery. Tumors were typed as adenocarcinomas or mucinous adenocarcinomas according to criteria established by WHO (18). The control group consisted of 203 individuals (aged 46.57±16.54 years; range, 24 to 85 years) who were randomly selected following health screening to exclude those with a history of thrombotic diseases or cancer. The study was approved by the Institutional Review Board (IRB) of Pochon CHA University, South Korea.

*VEGF genotyping.* DNA analysis total genomic DNA was prepared from leukocytes after lysis of red blood cells. The area spanning the *VEGF* –2578C>A polymorphic site was amplified by polymerase chain reaction (PCR) from genomic DNA using previously described primers and reaction conditions (10). The polymorphism was identified following digestion of amplified DNA with the endonuclease MvaI.

*Data analysis*. Allele and genotype frequencies between the case and control groups were compared using the $\chi^2$ test. Odds ratios (ORs) and 95% confidence interval (95% CI) were used as a measure of the strength of the association between the *VEGF* genotypes and colon cancer. Stratification analysis was used to study subgroups of age and gender. The statistical analysis was performed with GraphPad Prism 4.0 (GraphPad Software, Inc., San Diego, CA, USA).

## Results

Table I presents the comparison of genotype frequencies of the *VEGF* –2578C>A polymorphism according to the study group as a whole gender and age between the case and control groups. The genotype distributions for the *VEGF* –2578C>A polymorphism did not deviate significantly from the Hardy-Weinberg equilibrium in either group. The genotype frequencies were not significantly different between the control and case groups. However, the frequency of the –2578CA+AA genotype in patients was marginally associated with reduced risk for colon cancer in women (OR, 0.60; 95% CI, 0.36-0.99; $p=0.056$) when stratified by gender.

When the data were stratified by the original location of the tumor, frequency of the -2578CA+AA genotype was also protective against colon cancer in patients with proximal colon cancer (OR, 0.55; 95% CI, 0.31-0.97; $p=0.049$) (Table II). In the women with proximal colon cancer, the –2578AA and –2578CA+AA genotypes were associated with reduced risk for colon cancer (OR, 0.10; 95% CI, 0.01-1.67; $p=0.033$, and OR, 0.36; 95% CI, 0.16-0.80; $p=0.015$, respectively). For distal colon cancer, however, there was no significant difference in the genotype frequencies between the two groups (Table III).

Table I. *Comparison of genotype frequencies of the VEGF –2578C>A polymorphism.*

| | Controls (%) | Cases (%) | OR (95% CI) | *p* |
|---|---|---|---|---|
| CC | 106 (52.2) | 149 (60.6) | 1.0 (-) | - |
| CA | 82 (40.4) | 83 (33.7) | 0.72 (0.49-1.07) | 0.109 |
| AA | 15 (7.4) | 14 (5.7) | 0.66 (0.31-1.43) | 0.326 |
| CA+AA | 97 (47.8) | 97 (39.4) | 0.71 (0.49-1.04) | 0.085 |
| Male | | | | |
| CC | 41 (57.7) | 76 (59.4) | 1.0 (-) | - |
| CA | 27 (38.0) | 43 (33.6) | 0.86 (0.47-1.59) | 0.641 |
| AA | 3 (4.2) | 9 (7.0) | 1.62 (0.41-6.31) | 0.750 |
| CA+AA | 30 (42.3) | 52 (40.6) | 0.94 (0.52-1.68) | 0.881 |
| Female | | | | |
| CC | 65 (49.2) | 73 (61.9) | 1.0 (-) | - |
| CA | 55 (41.7) | 40 (33.9) | 0.65 (0.38-1.10) | 0.112 |
| AA | 12 (16.4) | 5 (4.2) | 0.37 (0.12-1.11) | 0.077 |
| CA+AA | 67 (50.8) | 45 (38.1) | 0.60 (0.36-0.99) | 0.056 |
| Age≥55years | | | | |
| CC | 43 (58.9) | 108 (62.4) | 1.0 (-) | - |
| CA | 28 (38.4) | 58 (33.5) | 0.82 (0.46-1.46) | 0.556 |
| AA | 2 (2.7) | 7 (4.0) | 1.39 (0.28-6.98) | 1.000 |
| CA+AA | 30 (41.1) | 65 (37.6) | 0.86 (0.49-1.51) | 0.668 |
| Age<55years | | | | |
| CC | 59 (48.0) | 41 (56.2) | 1.0 (-) | - |
| CA | 53 (43.1) | 25 (34.2) | 0.68 (0.36-1.26) | 0.274 |
| AA | 11 (8.9) | 7 (9.6) | 0.92 (0.33-2.56) | 1.000 |
| CA+AA | 64 (52.0) | 32 (43.8) | 0.72 (0.40-1.29) | 0.302 |

Align values within columns.

## Discussion

Polymorphisms of the *VEGF* gene have been associated with risk for several types of cancer, although the results from various populations were not always consistent. For example, the *VEGF* –1154AA genotype has been associated with reduced prostate cancer risk and less advanced melanomas (12, 13). The *936T* allele has been also associated with reduced risk for breast cancer (6, 19). The *–460C* or *405G* allele has been associated with reduced overall survival in patients with breast cancer, whereas the *–460T/405C/936C* haplotype has been related to increased breast cancer survival in a Chinese case control study (6). The –2578C>A polymorphism has been also reported the association with recurrent pregnancy loss (10), pre-eclampsia (20), Alzheimer's disease (9, 21) and breast cancer (22, 23).

Table II. *Comparison of genotype frequencies of the VEGF –2578C>A polymorphism in patients with the proximal type of colon cancer.*

| Proximal type | Controls (%) | Cases (%) | OR (95% CI) | *p* |
|---|---|---|---|---|
| CC | 106 (52.2) | 46 (66.7) | 1.0 (-) | - |
| CA | 82 (40.4) | 20 (29.0) | 0.56 (0.31-1.02) | 0.060 |
| AA | 15 (7.4) | 3 (4.3) | 0.46 (0.13-1.67) | 0.282 |
| CA+AA | 97 (47.8) | 23 (33.3) | 0.55 (0.31-0.97) | 0.049 |
| Male | | | | |
| CC | 41 (57.7) | 19 (59.4) | 1.0 (-) | - |
| CA | 27 (38.0) | 10 (31.3) | 0.80 (0.32-1.98) | 0.656 |
| AA | 3 (4.2) | 3 (9.4) | 2.16 (0.40-11.7) | 0.392 |
| CA+AA | 30 (42.3) | 13 (40.6) | 0.94 (0.40-2.18) | 1.000 |
| Female | | | | |
| CC | 65 (49.2) | 27 (73.0) | 1.0 (-) | - |
| CA | 55 (41.7) | 10 (27.0) | 0.44 (0.19-0.98) | 0.056 |
| AA | 12 (16.4) | 0 (0.0) | 0.10 (0.005-1.67) | 0.033 |
| CA+AA | 67 (50.8) | 10 (27.0) | 0.36 (0.16-0.80) | 0.015 |
| Age≥55 years | | | | |
| CC | 43 (58.9) | 29 (65.9) | 1.0 (-) | - |
| CA | 28 (38.4) | 14 (31.8) | 0.74 (0.33-1.64) | 0.549 |
| AA | 2 (2.7) | 1 (2.3) | 0.74 (0.06-8.56) | 1.000 |
| CA+AA | 30 (41.1) | 15 (34.1) | 0.74 (0.34-1.62) | 0.557 |
| Age<55 years | | | | |
| CC | 59 (48.0) | 17 (68.0) | 1.0 (-) | - |
| CA | 53 (43.1) | 6 (24.0) | 0.39 (0.14-1.07) | 0.069 |
| AA | 11 (8.9) | 2 (8.0) | 0.63 (0.13-3.13) | 0.727 |
| CA+AA | 64 (52.0) | 8 (32.0) | 0.43 (0.17-1.08) | 0.081 |

Table III. *Comparison of genotype frequencies of the VEGF –2578C>A polymorphism in patients with the distal type of colon cancer.*

| Distal type | Controls (%) | Cases (%) | OR (95% CI) | *p* |
|---|---|---|---|---|
| CC | 106 (52.2) | 93 (57.8) | 1.0 (-) | - |
| CA | 82 (40.4) | 58 (36.0) | 0.81 (0.52-1.25) | 0.375 |
| AA | 15 (7.4) | 10 (6.2) | 0.76 (0.33-1.77) | 0.671 |
| CA+AA | 97 (47.8) | 68 (42.2) | 0.80 (0.53-1.21) | 0.340 |
| Male | | | | |
| CC | 41 (57.7) | 50 (58.8) | 1.0 (-) | - |
| CA | 27 (38.0) | 30 (35.3) | 0.91 (0.47-1.77) | 0.866 |
| AA | 3 (4.2) | 5 (5.9) | 1.37 (0.31-6.07) | 0.730 |
| CA+AA | 30 (42.3) | 35 (41.2) | 0.96 (0.50-1.81) | 1.000 |
| Female | | | | |
| CC | 65 (49.2) | 43 (56.6) | 1.0 (-) | - |
| CA | 55 (41.7) | 28 (36.8) | 0.77 (0.42-1.40) | 0.451 |
| AA | 12 (16.4) | 5 (6.6) | 0.63 (0.21-1.92) | 0.593 |
| CA+AA | 67 (50.8) | 33 (43.4) | 0.74 (0.42-1.31) | 0.318 |
| Age≥55 years | | | | |
| CC | 43 (58.9) | 73 (61.9) | 1.0 (-) | - |
| CA | 28 (38.4) | 39 (33.1) | 0.82 (0.44-1.52) | 0.533 |
| AA | 2 (2.7) | 6 (5.1) | 1.77 (0.34-9.15) | 0.710 |
| CA+AA | 30 (41.1) | 45 (38.1) | 0.88 (0.49-1.60) | 0.761 |
| Age<55 years | | | | |
| CC | 59 (48.0) | 20 (46.5) | 1.0 (-) | - |
| CA | 53 (43.1) | 19 (44.2) | 1.06 (0.51-2.19) | 1.000 |
| AA | 11 (8.9) | 4 (9.3) | 1.07 (0.31-3.75) | 1.000 |
| CA+AA | 64 (52.0) | 23 (53.5) | 1.06 (0.53-2.13) | 1.000 |

Based on the involvement of VEGF in the risk of advanced stage cancer through tumor growth and metastasis of several types of cancer, including colon cancer, we evaluated the relationship between the *VEGF* –2578C>A polymorphism and colon cancer in a Korean patient case control study. In the present study, the –2578CA+AA genotype was marginally associated with reduced risk for colon cancer in women when stratified by gender. The –2578AA and –2578CA+AA genotypes were also protective in proximal colon tumor. These trends remained in women with proximal colon cancer even when the data were stratified by gender. Occasionally the population admixture may cause false positive associations.

However, Koreans have traditionally had a very low rate of interracial marriages, leading to a very homogeneous population. Therefore, the sample selection bias in our data is minimal. To confirm our results, more studies covering different racial or ethnic groups of homogeneous population are required. We next looked at the individual alleles' genotype ratio among different populations from the literature. For *VEGF –2578A* allele, its allele ratio was 0.378 to 0.504 in Caucasians, 0.28 in Chinese and 0.276 in Koreans, which showed that the allele ratio was similar between Asian populations (9, 10, 14, 21-24). So there was a racial difference in the frequency of *–2578A* allele (Table IV).

Overexpression of *VEGF* mRNA has been described in several kinds of tumor tissues, including colon cancer. Inter-individual variations in plasma VEGF levels have also been reported (25). In this study, proximal colon cancer in women is associated with some genotypes of the *VEGF* –2578C>A polymorphism. That is, the association was gender-specific, suggesting that VEGF production according to *VEGF* –2578C>A genotype might differ between men and women. In addition, the association was also specific for the original location of the colon tumor, indicating that the VEGF production depends on the location of the colon

Table IV. *VEGF –2578C>A genotypes and allele frequencies among world populations studied previously.*

| Population | | Genotype | | | Allele frequencies | | Refs |
|---|---|---|---|---|---|---|---|
| | n | CC (%) | CA (%) | AA (%) | C | A | |
| Polish* | 423 | 106 (25.1) | 207 (48.9) | 110 (26.0) | 0.496 | 0.504 | 22 |
| American* | 495 | 129 (26.1) | 236 (47.7) | 130 (26.3) | 0.499 | 0.501 | 23 |
| French | 664 | 182 (27.4) | 324 (48.8) | 158 (23.8) | 0.518 | 0.482 | 21 |
| British | 74 | 24 (32.4) | 34 (46.0) | 16 (21.6) | 0.554 | 0.446 | 14 |
| Italian | 347 | 114 (32.9) | 182 (52.5) | 51 (14.7) | 0.591 | 0.409 | 9 |
| Greek* | 82 | 27 (32.9) | 34 (41.5) | 21 (25.6) | 0.622 | 0.378 | 10 |
| Korean | 203 | 106 (52.2) | 82 (40.4) | 15 (7.4) | 0.724 | 0.276 | - |

*Female sample only. Refs.: references.

tumor. Although we could not estimate VEGF levels, VEGF expression in proximal colon tumors could be lowed in combination with low VEGF production in women. Association studies between patients with breast cancer and VEGF levels have been performed in other populations. For example, VEGF levels are increased in breast cancer patients with the 405GG (or –634GG) genotype (26). In addition, the *936T* allele is associated with lower VEGF levels in healthy people (27, 28). Another possibility is that the *VEGF* –2578C>A polymorphism influences promoter activity, since the –2578C>A polymorphic site is located in a potential binding site for the Myeloid Zinc Finger-1 transcription factor. For example, Stevens *et al.* (25) reported that the *–460C/405G* haplotype had increased *VEGF* promoter activity.

The *–2578A* allele has been associated with decreased VEGF expression (17). As expect, *–2578A* allele-bearing genotypes were protective effect in women and proximal type of colon cancer. Therefore, determining whether *VEGF* polymorphisms can be used as risk factors for colon cancer would be an interesting topic for further studies. On the other hand, VEGF inhibitors in clinical use such as bevacizumab or sorafenib, data on *VEGF* polymorphisms in colon cancer could be have predictive information (29, 30).

In summary, this is the first report on the significant association of *VEGF* –2578C>A polymorphism in colon cancer patients. The *VEGF* –2578C>A polymorphism, at least in Koreans, is a genetic determinant for the reduced risk of proximal colon cancer in women. To obtain more evidence on associations between *VEGF* polymorphisms and colon cancer, population studies in other ethnicities are required. Furthermore, the biochemical mechanisms of the *VEGF* –2578C>A polymorphism effects on VEGF levels should be elucidated.

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