Gender-specific Association between Polymorphism of *Vascular Endothelial Growth Factor* (*VEGF* 936 C>T) Gene and Colon Cancer in Korea

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Abstract. *Background: Angiogenesis is an essential process in the development, growth and metastasis of malignant tumors such as colon cancer. Vascular endothelial growth factor (VEGF) is a potent angiogenic factor. A case control study was carried out to determine whether there is an association between the VEGF 936C>T polymorphism and colon cancer. Patients and Methods: DNA samples taken from 262 colon cancer patients and 229 healthy controls were amplified by polymerase chain reaction for the VEGF 936C>T polymorphism. Results: Genotype frequencies of the VEGF 936C>T polymorphism were significantly different between patient and control groups (CT+TT, odds ratio(OR): 1.524, 95% confidence interval (CI): 1.033-2.249). When stratified by gender and age, the frequencies of the T allele-bearing genotypes significantly increased risk for colon cancer in women and patients younger than 55 years (in women, OR: 1.996, 95% CI: 1.151-3.464 and in <55 years, OR: 4.156, 95% CI: 1.885-9.163). In addition, this association remained in most cases with distal and proximal colon cancer. Conclusion: Our study suggests that the VEGF 936C>T polymorphism might be a genetic determinant for colon cancer, at least in Koreans.*

Angiogenesis, the formation of new capillaries from existing blood vessels, is essential for the growth of a solid tumor (1). Many studies have shown that malignant tumors depend on angiogenesis for their growth and metastasis (2). It is generally assumed that microvessel formation around a tumor is stimulated by various angiogenic factors secreted by the tumor cells (3, 4). Among them, vascular endothelial growth factor (VEGF) is considered one of the strongest promoters of angiogenesis in colon cancer (2). A significant correlation between the microvessel count and VEGF expression in tumor cells has also been demonstrated (5). Aside from the induction of tumor angiogenesis, VEGF has several additional functions that serve to enhance tumor progression; these functions include enhancing the permeability of tumor vessels (6), inducing serine protease and inhibiting either apoptosis of endothelial cells (7) or maturation of dendritic cells (8).

Many studies have reported a correlation between VEGF expression and several malignancies, including breast (5), gastrointestinal (9), urinary tract (10) and ovarian (11) tumors. *VEGF* polymorphisms have been associated with the risk for several kinds of cancer and other diseases with a putative angiogenic basis (12). At least 30 single-nucleotide polymorphisms (SNP) in this gene have been described in the literature (13). One of these, the 936C>T polymorphism in the 3'-untranslated region (3'-UTR) of the *VEGF* gene, was shown to affect VEGF plasma levels, and carriers of the *VEGF 936T* allele had significantly reduced VEGF plasma levels (14).

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 possible associations between genetic variation at the 936C>T polymorphic site in the 3'-UTR of the *VEGF* gene in patients who have had colon cancer compared to healthy individuals.

Patients and Methods

Study participants. A total of 262 patients (mean age±SD, 60.23±13.16 years) with colon cancer diagnosed at Bundang CHA Hospital, Pochon CHA University, from July 1999 to June 2004 were enrolled in this study. Among the colon cancer patients, there were 136 men (age 59.23±12.53 years; range, 18 to 84 years) and 126 women (age 61.32±13.79 years; range, 23 to 95 years). Seventy-two consecutive patients (age 58.99±14.00 years; range, 18

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to 84 years) with a cancerous proximal colon (*i.e.* descending and sigmoid colon) and 189 consecutive patients (age 60.68±12.87 years; range, 24 to 95 years) with a cancerous distal 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 (15). The control group consisted of 229 individuals (age 59.57±11.80 years; range, 31 to 91 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. Three milliliters of fasting venous blood samples were obtained before surgery. Genomic DNA was extracted from peripheral blood lymphocytes by proteinase K digestion and phenol/chloroform extraction. Samples were frozen at –20˚C until further analysis. The *VEGF* 936C>T genotype was determined using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. The PCR primers used to detect the *VEGF* 936C>T polymorphism were 5'-AGG AAG AGG GAC TCT GCG CAG AGC-3' (forward) and 5'-TAA ATG TAT GTA TGT GGG TGG GTG TGT CTA CAG G-3' (reverse). The PCR product was digested overnight with the appropriate restriction enzymes (New England BioLabs, Beverly, MA, USA). The restriction enzyme for *VEGF* 936C>T genotyping was *Nla*III. The *VEGF 936T* allele was cut into two fragments of 122 and 86 base pairs, whereas the *VEGF 936C* allele remained uncut with a length of 208 base pairs.

Data analysis. Cases and controls were compared using Student's *t*-test for continuous variables and the χ^2 test for categorical variables. Odds ratios (ORs) and 95% confidence intervals (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

The baseline characteristics of colon cancer patients and of controls are shown in Table I. Fasting plasma folate levels were significantly lower in colon cancer patients than that in controls. Table II presents the comparison of genotype frequencies of the *VEGF* 936C>T polymorphism between the case and control groups according to the study group as a whole, gender and age. The genotype frequencies of *VEGF* 936C>T polymorphism in colon cancer patients and controls conformed to the Hardy-Weinberg equilibrium (p=0.302). The distribution of CT+TT genotypes of the *VEGF* 936C>T polymorphism was significantly different between the control and case groups (OR, 1.524; 95% CI, 1.033-2.249; p=0.0398). The frequencies of the 936CT genotype and the 936CT+TT genotypes in patients were associated with increased risk for colon cancer in females (p=0.0458 and p=0.0141) when stratified by gender.

When the data were stratified by age, the association remained in patients less than 55 years old (p=0.0013 and p=0.0003 for CT and CT+TT genotypes, respectively) (Table III). Interestingly, significant differences in individuals less than 55 years old remained in patients with proximal colon cancer when the data were stratified by the original location of the tumor (p=0.0367 and p=0.0058 for CT and CT+TT genotypes, respectively).

Moreover, in females with distal colon cancer, the 936CT and 936CT+TT genotypes were associated with increased risk for colon cancer (p=0.0267 and p=0.0099, respectively) (Table IV). When the data were stratified by age, the association also remained in individuals less than 55 years old with distal colon cancer (p=0.0025 and p=0.0014 for CT, CT+TT genotypes, respectively).

Table I. *Baseline characteristics in colon cancer patients and healthy controls.*

	Controls (n=229)	Colon cancer (n=262)	*p*-value
Male (%)	112 (48.9)	136 (51.9)	0.5273
Age (years, mean±SD)	59.57±11.80	60.23±13.16	0.5607
tHcy* (μmol/L, mean±SD)	9.695±3.997	10.690±5.719	0.2005
Folate (nmol/L)	9.930±6.646	5.720±3.427	0.0007

*Total plasma homocysteine.

Discussion

Functional polymorphisms, which affect the regulation of gene expression, can contribute to differences between individuals in susceptibility to and severity of a disease. The effect may be seen with one polymorphism alone or in combination with other polymorphisms. Several studies have shown that polymorphisms in the promoter as well as in the 5'- and 3'-untranslated regions of the *VEGF* gene are associated with the production of the VEGF protein (13, 16, 17). For example, the less common *T* allele of the *VEGF* 936C>T polymorphism in the 3'-untranslated region of the *VEGF* gene has been reported to correlate with lower VEGF plasma levels and a reduced risk of breast cancer (18, 19). In contrast, in a Japanese study, no relationship was found between this polymorphism and VEGF serum levels (12). The *VEGF* 936C>T polymorphism has also been reported to associate with colon squamous cell carcinoma (20), pre-eclampsia (21), and primary lung cancer (22). The contribution of this *VEGF* 936C>T polymorphism to oncogenesis has been investigated in several types of cancer but never in colon cancer, until now, to our knowledge (17, 23).

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* 936C>T polymorphism and colon cancer in a Korean patient case control study. In the present study, the *VEGF 936T* allele was associated with increased risk for colon cancer in females

Table II. *Comparison of genotype frequencies of the VEGF 936C>T polymorphism in colon cancer patients and healthy controls.*

	Controls (%)	Cases (%)	OR (95% CI)	*p*-value
Overall				
CC	169 (73.8)	170 (64.9)	1.0 (-)	-
CT	57 (24.9)	83 (31.7)	1.448 (0.972-2.157)	0.0710
TT	3 (1.3)	9 (3.4)	2.982 (0.793-11.21)	0.1400
CT+TT	60 (26.2)	92 (35.1)	1.524 (1.033-2.249)	0.0398
T allele frequency	0.138	0.193		
Male				
CC	81 (72.3)	94 (69.1)	1.0 (-)	-
CT	30 (26.8)	41 (30.1)	1.178 (0.675-2.055)	0.5754
TT	1 (0.9)	1 (0.7)	0.862 (0.053-14.01)	1.0000
CT+TT	31 (27.7)	42 (30.9)	1.167 (0.673-2.026)	0.6747
T allele frequency	0.143	0.158		
Female				
CC	88 (75.2)	76 (60.3)	1.0 (-)	-
CT	27 (23.1)	42 (33.3)	1.801 (1.016-3.194)	0.0458
TT	2 (1.7)	8 (6.3)	4.632 (0.954-22.49)	0.0512
CT+TT	29 (24.8)	50 (39.7)	1.996 (1.151-3.464)	0.0141
T allele frequency	0.132	0.230		
≥55 years old				
CC	109 (69.0)	128 (68.1)	1.0 (-)	-
CT	46 (29.1)	54 (28.7)	1.000 (0.625-1.598)	1.0000
TT	3 (1.9)	6 (3.2)	1.703 (0.416-6.973)	0.5158
CT+TT	49 (31.0)	60 (31.9)	1.043 (0.661-1.645)	0.9077
T allele frequency	0.165	0.176		
<55 years old				
CC	60 (84.5)	42 (56.8)	1.0 (-)	-
CT	11 (15.5)	29 (39.2)	3.766 (1.695-8.369)	0.0013
TT	0 (0.0)	3 (4.1)	9.965 (0.501-198.1)	0.0757
CT+TT	11 (15.5)	32 (43.2)	4.156 (1.885-9.163)	0.0003
T allele frequency	0.077	0.236		

and in patients less than 55 years old when stratified by gender and age, respectively. Genotypes with the *T* allele were also indicative of increased risk in these less than 55 years old with proximal colon cancer. These trends remained in females and in these less than 55 years old with distal colon cancer when stratified by gender and age.

Surprisingly, our results strongly indicate that the *T* allele is linked to an increased risk for colon cancer, despite being associated with lower circulating levels of VEGF (24, 25). This finding was observed when the data were stratified by the original location of the tumor. It may therefore be assumed that high VEGF levels are not a prerequisite for colon cancer susceptibility. In the case of gliomas, low-expression *VEGF* genotypes coexisted with high VEGF levels in patients, but not in healthy controls (26). High VEGF expression was attributed to independent cancer and tumor stroma production (26). The findings of this study clearly demonstrate that the low-expression *T* allele is associated with an increased colon cancer risk; however, the underlying mechanism might not involve angiogenesis, but rather other VEGF-related functions such as thrombosis. As previously mentioned, our results are in accordance with a significant increase in the *T* allele frequency reported in cancer patients with thrombotic complications compared to healthy controls and non-thrombotic cancer patients (27). This association was reported in many cases, while the 936TT genotype was associated with larger tumors and the presence of metastases (28). In conclusion, this study clearly implicates the low-expression *T* allele with an increased risk of colon cancer.

We were interested in studying *VEGF* 936C>T polymorphisms in the Korean population because this population has a relatively homogeneous ethnic origin in contrast to the more heterogeneous characteristics of the ethnic groups examined in previous studies. From the literature, the *VEGF 936T* allele frequency was 0.138 in Koreans and 0.157 in Japanese, indicating that the allele ratio was similar in the two Asian populations (18). Still, there was a racial difference in the frequency of the *T* allele (Table V).

There is now compelling evidence that VEGF production is controlled by polymorphisms within the *VEGF* genes (12, 18). These functional polymorphisms may result in altered transcription factor recognition sites, which subsequently affect transcriptional activation and alter protein production (18). As with many environmental risks, the relative risk or

Table III. *Comparison of genotype frequencies of the VEGF 936C>T polymorphism in patients with the proximal type of colon cancer and healthy controls.*

	Controls (%)	Cases (%)	OR (95% CI)	*p*-value
Overall				
CC	169 (73.8)	46 (63.9)	1.0 (-)	-
CT	57 (24.9)	23 (31.9)	1.482 (0.827-2.658)	0.2159
TT	3 (1.3)	3 (4.2)	3.674 (0.717-18.82)	0.1245
CT+TT	60 (26.2)	26 (36.1)	1.592 (0.906-2.798)	0.1342
T allele frequency	0.138	0.201		
Male				
CC	81 (72.3)	20 (60.6)	1.0 (-)	-
CT	30 (26.8)	13 (39.4)	1.755 (0.777-3.963)	0.1966
TT	1 (0.9)	0 (0.0)	1.325 (0.052-33.76)	1.0000
CT+TT	31 (27.7)	13 (39.4)	1.698 (0.754-3.825)	0.2042
T allele frequency	0.143	0.197		
Female				
CC	88 (75.2)	26 (66.7)	1.0 (-)	-
CT	27 (23.1)	10 (25.6)	1.254 (0.537-2.925)	0.6584
TT	2 (1.7)	3 (7.7)	5.077 (0.804-32.04)	0.0925
CT+TT	29 (24.8)	13 (33.3)	1.517 (0.691-3.334)	0.3044
T allele frequency	0.132	0.205		
≥55 years old				
CC	109 (69.0)	32 (68.1)	1.0 (-)	-
CT	46 (29.1)	14 (29.8)	1.037 (0.506-2.122)	1.0000
TT	3 (1.9)	1 (2.1)	1.135 (0.114-11.30)	1.0000
CT+TT	49 (31.0)	15 (31.9)	1.043 (0.518-2.100)	1.0000
T allele frequency	0.165	0.170		
<55 years old				
CC	60 (84.5)	14 (56.0)	1.0 (-)	-
CT	11 (15.5)	9 (36.0)	3.506 (1.220-10.08)	0.0367
TT	0 (0.0)	2 (8.0)	20.86 (0.949-458.8)	0.0421
CT+TT	11 (15.5)	11 (44.0)	4.286 (1.548-11.87)	0.0058
T allele frequency	0.077	0.260		

OR might be small because of genetic variation. Haplotype analysis, which is currently the focus of intense genetic research efforts, will enable more specific risk estimates than single locus analyses because it should reduce the dimension of association tests and increase statistical power (29). Identification of associations between candidate genes and disease will be among the main objectives in the development of individual-based medicine. Prior to this study, we demonstrated a significant association of *VEGF* –2578C>A polymorphism in colon cancer patients. The *VEGF* –2578C>A polymorphism is a genetic determinant for the reduced risk of proximal colon cancer in women (30). Based on prior study, future studies of other *VEGF* sequence variants and on their biological functions are also needed to understand the role of *VEGF* polymorphisms and haplotypes in determining the risk of colon cancer. Moreover, since genetic polymorphisms often vary between ethnic groups, further studies are needed to clarify the association between *VEGF* polymorphisms and colon cancer in diverse ethnic populations.

In conclusion, our findings indicate that the *VEGF* 936C>T polymorphism maybe useful as an indicator of susceptibility to colon cancer. Further large-scale genetic studies, including haplotype analyses, may be needed to improve statistical power and to investigate the functional relevance of *VEGF* polymorphisms.

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Table IV. *Comparison of genotype frequencies of the VEGF 936C>T polymorphism in patients with the distal type of colon cancer and healthy controls.*

	Controls (%)	Cases (%)	OR (95% CI)	*p*-value
Overall				
CC	169 (73.8)	123 (65.1)	1.0 (-)	-
CT	57 (24.9)	60 (31.7)	1.446 (0.940-2.225)	0.0996
TT	3 (1.3)	6 (3.2)	2.748 (0.674-11.21)	0.1789
CT+TT	60 (26.2)	66 (34.9)	1.511 (0.993-2.300)	0.0549
T allele frequency	0.138	0.190		
Male				
CC	81 (72.3)	73 (71.6)	1.0 (-)	-
CT	30 (26.8)	28 (27.5)	1.036 (0.566-1.896)	1.0000
TT	1 (0.9)	1 (1.0)	1.110 (0.068-18.07)	1.0000
CT+TT	31 (27.7)	29 (28.4)	1.038 (0.571-1.886)	1.0000
T allele frequency	0.143	0.147		
Female				
CC	88 (75.2)	50 (57.5)	1.0 (-)	-
CT	27 (23.1)	32 (36.8)	2.086 (1.123-3.873)	0.0267
TT	2 (1.7)	5 (5.7)	4.400 (0.823-23.53)	0.1049
CT+TT	29 (24.8)	37 (42.5)	2.246 (1.236-4.081)	0.0099
T allele frequency	0.132	0.241		
≥55 years old				
CC	109 (69.0)	95 (67.9)	1.0 (-)	-
CT	46 (29.1)	40 (28.6)	0.998 (0.602-1.654)	1.0000
TT	3 (1.9)	5 (3.6)	1.912 (0.445-8.217)	0.4799
CT+TT	49 (31.0)	45 (32.1)	1.054 (0.646-1.719)	0.9007
T allele frequency	0.165	0.179		
<55 years old				
CC	60 (84.5)	28 (57.1)	1.0 (-)	-
CT	11 (15.5)	20 (40.8)	3.896 (1.646-9.225)	0.0025
TT	0 (0.0)	1 (2.0)	6.368 (0.251-161.4)	0.3258
CT+TT	11 (15.5)	21 (42.9)	4.091 (1.737-9.634)	0.0014
T allele frequency	0.077	0.224		

Table V. *VEGF 936C>T genotypes and allele frequencies among world populations studied previously.*

Population		Genotype			Allele frequency		Reference
	n	CC (%)	CT (%)	TT (%)	C	T	
Turkish	120	120 (96.0)	5 (4.0)	0 (0.0)	0.980	0.020	33
German	187	156 (83.4)	28 (15.0)	3 (1.6)	0.909	0.091	34
American	479	353 (73.7)	118 (24.6)	8 (1.7)	0.860	0.140	23
Greek	153	111 (72.5)	39 (25.5)	3 (2.0)	0.853	0.147	20
Japanese	102	70 (68.6)	32 (31.4)	0 (0.0)	0.843	0.157	18
Austrian	119	85 (71.4)	30 (25.2)	4 (3.4)	0.840	0.160	19
Polish*	422	297 (70.4)	114 (27.0)	11 (2.0)	0.839	0.161	31
Canadian	146	100 (68.5)	43 (29.5)	3 (2.0)	0.813	0.167	32
Korean	229	169 (73.8)	57 (24.9)	3 (1.3)	0.862	0.138	present study

*Female sample only.

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