Original Article

A case-control study about the association between vascular endothelial growth inhibitor gene polymorphisms and breast cancer risk in female patients in Northeast China

Shaoli Han[1], Lei Liu[1,2], Fengyan Xu[1], Shuang Chen[1], Weiguang Yuan[1,3], Zhenkun Fu[1], Dalin Li[4], Dianjun Li[1,3]

[1]Department of Immunology; [2]College of Bioinformatics Science and Technology; [3]Institute of Cancer Prevention and Treatment, Harbin Medical University, Harbin 150081, China; [4]Department of Surgery, the Third Affiliated Hospital of Harbin Medical University, Harbin 150081, China

Contributions: (I) Conception and design: D Li, D Li; (II) Administrative support: None; (III) Provision of study materials or patients: S Han; (IV) Collection and assembly of data: S Han, S Chen, W Yuan, Z Fu; (V) Data analysis and interpretation: S Han, F Xu; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Dalin Li. Department of Surgery, the Third Affiliated Hospital of Harbin Medical University, Harbin 150081, China. Email: lidalin1975@163.com; Dianjun Li. Department of Immunology, Institute of Cancer Prevention and Treatment, Harbin Medical University, Harbin 150081, China. Email: dianjunli@163.com.

Abstract

Objective: The inhibition of the neovascularization in tumors is a potential therapeutic target of cancer. Vascular endothelial growth inhibitor (VEGI) is a member of the TNF superfamily which has the ability to suppress the formation of new vessels in tumors. In order to study the association between *VEGI* gene polymorphisms and breast cancer risk, a case-control study was conducted in Chinese Han women in Northeast China.

Methods: Our study involved 708 female breast cancer patients and 685 healthy volunteers. Four SNPs of *VEGI* gene were analyzed through the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The association between *VEGI* gene polymorphisms and breast cancer risk was analyzed in our study. The relation between *VEGI* gene variants and clinical features of breast cancer including lymph node (LN) metastasis, estrogen receptor (ER), progestrogen receptor (PR), tumor protein 53 (p53), human epidermal growth factor receptor 2 (Her-2) and triple negative (ER-/PR-/Her-2-) status was analyzed as well.

Results: We found that the CT genotype and T allele of rs6478106 were more frequent in patients than in controls. There was also a statistical difference in the distribution of $C_{rs6478106}G_{rs4263839}$ haplotype between patients and controls. In addition, SNP rs6478106 and rs4979462 were related with the Her-2 status.

Conclusions: Our results suggest that *VEGI* gene variants may be related to the breast cancer risk and the clinical features of breast cancer in Chinese Han women in Northeast China.

Keywords: Vascular endothelial growth inhibitor (VEGI); breast cancer; single nucleotide polymorphisms (SNPs)

Submitted Jun 08, 2015. Accepted for publication Oct 09, 2015.

doi: 10.21147/j.issn.1000-9604.2016.04.07

View this article at: http://dx.doi.org/10.21147/j.issn.1000-9604.2016.04.07

Introduction

Breast cancer is one of the most frequent malignant tumors worldwide and the second leading cause of cancer death in women (1). Research showed that significant percentage of breast cancer patients experienced a delayed treatment because of their misconceptions about breast cancer (2). As cases of breast cancer are increasing year by year, a better understanding of the causes leading to breast cancer

is necessary. Although, there are many possible factors contributing to the cause, development, and prognosis of breast cancer (3), genetic factors have an extremely important influence on the risk of breast cancer (4). In addition, many studies have shown that the SNPs in some genes can affect the susceptibility to breast cancer (5,6). As is well known, the endothelium plays an important role in maintaining vascular homeostasis. The endothelial cells proliferate, migrate and interact with other cells such as stromal cells to form new capillaries during the cancer angiogenesis. The new blood vessels are extremely important for the growth of cancer cells. Therefore, to a certain extent, the inhibition of endothelial cell proliferation can suppress the angiopoiesis of cancer cells. However, some studies have indicated that VEGI, also known as TNFSF15 or TNF ligand-related molecule, can inhibit the proliferation of endothelial cells and exert an anti-angiogenic effect on the endothelial cells (7). VEGI-192, an isoform of VEGI, has been reported to be able to eliminate the endothelial cells in tumors and suppress the development of tumors (8). VEGI always acts as a co-stimulator to induce T cell proliferation and cytokine secretion (9,10).

Many studies have shown that VEGI is related to various diseases including bowel disease (11), lung cancer (12), prostate cancer (13), and breast cancer (14). Except for its ability to inhibit the endothelial cells, VEGI could influence the development of diseases through participating in various pathways. Research results indicated that VEGI plays an essential role in activating the transcription factor κB and caspase-3, leading to PARP cleavage (15). Moreover, VEGI was also involved in immune response by inducing the secretion of GM-CSF and IFN-γ (10). In cancers or wounds, *VEGI* gene expression also decreased at the inflammation and angiogenesis sites (16). Other studies have demonstrated that VEGI is mediated by DR3 to inhibit the growth and migration of tumor cells (17). Studies that involved cell cycle suggested that VEGI maintained early G1 arrest in the G0/G1 cells and induced the programmed death in the endothelial cell cycle (7).

VEGI is a member of the TNF superfamily firstly discovered in 1999. It is mainly produced by vessel endothelial cells and also expressed on antigen-presenting cells and lymphocytes such as T cells and dendritic cells. *VEGI* maps to human chromosome 9q32 and contains 4 exons and 3 introns. *VEGI* gene polymorphisms have exhibited a connection with certain inflammatory and autoimmune diseases, such as Crohn's Disease (18), inflammatory bowel disease (19), and psoriatic arthritis (20). However, the association between *VEGI* gene polymorphisms and breast cancer in Chinese people has never been studied. The purpose of this paper is to discuss the association of *VEGI* gene polymorphism with breast cancer in northeast China. We selected four SNPs located at the *VEGI* gene (rs4263839, rs6478106, rs4979462, rs7848647) that had been reported before and examined whether these SNPs are associated with the development of breast cancer in Chinese Han women.

Materials and methods

Blood sample preparation

In total, 708 patients and 685 healthy volunteers were involved in our research. The cases and controls are all females and age matched (cases, 50.02±9.80 years old; controls, 49.32±9.56 years old). We used Chi-squared test and independent-samples T test to detect the difference between the ages of cases and controls and P>0.05. Blood samples of breast cancer patients were obtained from the Third Affiliated Hospital of Harbin Medical University. Diagnostic indicators including tumor size, human epidermal growth factor receptor 2 (Her-2), p53, estrogen receptor (ER), progestrogen receptor (PR), and lymph node (LN) metastasis were all collected from the patients' medical records. The ER and PR positivity was defined by a 10% positively staining of nuclei. The p53 positive status was defined as p53>25% in the cell staining. The IHC scores of 3+ or 2+ were considered positive for Her-2 (0, negative; 1+, <25%; 2+, 25-50%; 3+, >50%). Blood samples of healthy controls were collected from neighborhood volunteers without a history of cancer or autoimmune diseases. The clinical features of patients with breast cancer were shown in *Table 1*.

Ethics statement

This study was conducted at the department of immunology in Harbin Medical University. The patients and healthy volunteers were not genetically related. Before recruitment, a written informed consent was signed by each participant and the study was approved by the institutional ethical review board. The ethics approval was obtained from Harbin Medical University.

DNA extraction from blood samples

Three-milliliter blood samples were taken from the Third

Table 1 Clinical features of breast cancer patients

Features	Cases No. (%)
Tumor type	
Infiltrating ductal carcinoma	555 (78.39)
Intraductal carcinoma	99 (13.98)
Infiltrating lobular carcinoma	19 (2.68)
Mucinous carcinoma	12 (1.69)
Others	23 (3.25)
Lymph node metastasis	
Positive	261 (36.86)
Negative	391 (55.23)
Unknown	56 (7.91)
ER	
Positive	395 (55.79)
Negative	225 (31.78)
Unknown	88 (12.43)
PR	
Positive	405 (57.20)
Negative	213 (30.08)
Unknown	90 (12.71)
Her-2	
Positive	137 (19.35)
Negative	482 (68.08)
Unknown	89 (12.57)
p53	
Positive	81 (11.44)
Negative	529 (74.72)
Unknown	98 (13.84)
IHC type	
ER or PR (+)/Her-2 (–)	380 (53.67)
ER or PR (+)/Her-2 (+)	86 (12.15)
ER (–)/PR (–)/Her-2 (–)	102 (14.41)
ER (–)/PR (–)/Her (+)	50 (7.06)
Unknown	90 (12.71)

ER, estrogen receptor; PR, progesterone receptor; Her-2, human epidermal growth factor receptor 2; p53, tumor protein 53; IHC, immunohistochemistry.

Affiliated Hospital to the laboratory, the blood samples were mixed with anticoagulant and stored at –20 ℃. The lymphocytes were obtained through centrifugation. We used the Universal Genomic DNA Extraction Kit Ver. 3.0 (TaKaRa, Japan) to extract DNA according to the manufacturer's protocol.

Genotyping

Genotyping was conducted using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. The primers were designed by using Gene Runner software, and the primer sequences of each SNP were: rs6478106 (F: 5'-ACTTCATCCACTTCTCCTC-3', R: 5'-AGACGTTCTGACTACTATTCC-3'), rs4264839 (F: 5'-GGACCCTGATTGCTACATG-3', R: 5'-GTTACAGACCAGGGAGGATC-3'), rs4979462 (F: 5'-AAGGGCTCTCAGACATCATC-3', R: 5'-TCAAAGCATAGACACCACAAG-3'), rs7848647 (F: 5'-ACAGAGGAGCTAGGAAGATG-3', R: 5'-TCCTGGCTCTACCACTTG-3'). All the primers were produced by Invitrogen Company. The PCR reaction mixture contained 0.4 μg DNA, 2.5 mM dNTP mix (TaKaRa, Japan), 2.5 μL 10× PCR reaction buffer including 10 mM Tris-HCl, 50 nM KCl and 2.5 nM $MgCL_2$ (TaKaRa, Japan), 4 μM primers (Invitrogen, China), 2.5 U Taq DNA polymerase (TaKaRa, Japan), and added sterile double distilled water to a final volume of 25 μL. The PCR program consisted of an initial melting step at 94 ℃ for 15 minutes, 35 cycles of 30 seconds at 94 ℃, 30 seconds at annealing temperatures, 1 minute at 72 ℃, and a terminal step at 72 ℃ for 5 minutes. The annealing temperatures for each SNP were rs6478106 (56.0 ℃), rs4263839 (57.0 ℃), rs4979462 (56.5 ℃), and rs7848647 (57.2 ℃). The PCR products contained the SNP sites and were examined in 2% agarose gel electrophoresis. The RFLP was conducted in a final volume of 10 μL, including 5 μL PCR products, 1× NEB buffer (50 Mm, Tris-HCl, 100 mM NaCl, 10 mM $MgCl_2$, 1 mM dithiothreitol), and 0.25 μL restriction enzyme (NEB, UK). The reaction mix was incubated in water bath according to the optimal temperature for each restriction enzyme for 4-6 hours, and the digested fragments were separated by 2% (rs4263839, rs4979462, rs7848647) or 3% (rs6478106) agarose gel electrophoresis as the difference in length among the restricted fragments was smaller than other three SNPs. The restriction enzymes for each SNP were as follows: rs6478106 (*Eco*53KI), rs4263839 (*Apo*I), rs4979462 (*Msc*I), and rs7848647 (*Cvi*QI). The digested fragments were 105 bp and 151 bp with T allele and 256 bp with C allele in rs6478106, 132 bp and 213 bp with A allele and 345 bp with G allele in rs4263839, 101 bp and 306 bp with C allele and 407 bp with T allele in rs4979462, 120 bp and 296 bp with C allele, and 416 bp with T allele in rs7848647. The results of PCR-RFLP products for each SNP is shown in the *Figures S1-S8*.

Direct sequencing of random samples was conducted to verify the accuracy of the genotyping results. About 10% of our samples were tested by direct sequencing and the results were in accord with our PCR-RFLP analysis results.

Statistical analysis

We tested the genotype frequencies of these SNPs for Hardy Weinberg equilibrium (HWE) among healthy controls. The genotype frequencies in the breast cancer cases and healthy controls were analyzed in different genetic models (codominant model, dominant model, and recessive model) using the chi-squared test or Fisher's test. In the codominant model, the major allele homozygotes were used as the reference group, and the heterozygotes and minor allele homozygotes were compared to the reference group, respectively. The dominant model was the combination of minor homozygotes and heterozygotes compared to the major allele homozygotes. In the recessive model, the minor homozygotes were compared to the combination of heterozygotes and major allele homozygotes. The P value, estimated odds ratios (ORs) and 95% confidence intervals (95% CIs) were all calculated using the statistical software SPSS version 18.0 and Haploview 4.1 (http://www.broad.mit.edu/mpg/haploview/) was used to tag all the common haplotypes and their frequencies in the breast cancer cases and healthy controls. Haplotypes were constructed based on D' values using our own data. The linkage disequilibrium (LD) was constructed by D' and r^2. The statistical significance was set at $P<0.05$, and 10,000 permutations were run to evaluate the P values using Haploview 4.1 to verify the correctness of the significance.

Results

VEGI gene polymorphisms with the risk of breast cancer

We have chosen four SNPs at *VEGI* gene to test the association between *VEGI* gene polymorphisms and breast cancer risk. The genotype frequencies of these four SNPs are shown in *Table 2*. The distributions of genotypes of these four SNPs we selected were in Hardy Weinberg equilibrium in healthy control group. Based on the data, in rs6478106, compared to the CC genotype, the CT genotype was related to an increased risk in breast cancer (P=0.001, OR=1.438, 95% CI, 1.153-1.793). There was also a significant difference in genotype distribution in rs6478106 under dominant model (P=0.001, OR=1.437, 95% CI, 1.161-1.777). Furthermore, compared with the C allele, the T allele of rs6478106 also increased the risk of breast cancer (*Table 3*). This result indicated that rs6478106 may have a strong association with breast cancer risk.

Haplotypes of the SNPs in the VEGI gene in cases and controls

The association between the haplotype and breast cancer risk was analyzed using Haploview 4.1 version software. Two blocks were constructed based on the solid spin of LD method. Block 1 contained rs6478106 and rs4263839, and there were four haplotypes in this block ($C_{rs6478106}A_{rs4263839}$, $T_{rs6478106}G_{rs4263839}$, $C_{rs6478106}G_{rs4263839}$ and $T_{rs6478106}A_{rs4263839}$). We found that the $C_{rs6478106}G_{rs4263839}$ haplotype got a higher frequency in breast cancer patients (P=0.0136). The $T_{rs6478106}G_{rs4263839}$ haplotype and $T_{rs6478106}A_{rs4263839}$ haplotype had lower frequencies in cases (P=0.0244; P=0.0184). However, after correcting the P value for multiple testing, significant differences were only found for the $C_{rs6478106}G_{rs4263839}$ haplotype (P=0.0488). Both rs4979462 and rs7848647 belonged to block2 and constructed four haplotypes ($C_{rs4979462}T_{rs7848647}$, $T_{rs4979462}C_{rs7848647}$, $C_{rs4979462}C_{rs7848647}$ and $T_{rs4979462}T_{rs7848647}$), and they were not associated with the risk of breast cancer (*Table 4*).

Clinical features and VEGI gene polymorphisms

The association between *VEGI* gene polymorphisms and the clinical features of breast cancer including ER, PR, p53, Her-2, LN metastasis and triple negative breast cancer (TNBC) status were also analyzed in our study. We found an association only between *VEGI* gene polymorphisms and Her-2 status. In comparison with the CC genotype, patients with the TT genotype of rs6478106 may exhibit increased expression of Her-2 (P=0.004, OR=2.522, 95% CI, 1.320-4.818), and this association was also significant in the recessive model (P=0.004, OR=2.397, 95% CI, 1.302-4.411). Moreover, compared to the CC genotype of rs4979462, the TT genotype had higher frequencies in Her-2 positive patients (P=0.002, OR=2.835, 95% CI, 1.455-5.523), and in the recessive model, the distribution of the genotype of rs4979462 was also associated with Her-2 expression (P=0.001, OR=2.835, 95% CI, 1.489-3.397). The T allele of rs6478106 and the T allele of rs4979462 appeared more frequently in Her-2 positive cases (P=0.0356, P=0.0354), but after correcting the P value for multiple testing, no significant difference was found.

Table 2 The genotype frequencies of *VEGI* gene polymorphisms in cases and controls

SNP ID	Genotype	Case No. (%)	Control No. (%)	OR (95% CI)	P value
rs6478106	CC	306 (44.22)	361 (53.24)	Reference	
	CT	334 (48.27)	274 (40.41)	1.438 (1.153-1.793)	0.001
	TT	52 (7.51)	43 (6.34)	1.427 (0.926-2.197)	0.105
	Dominant			1.437 (1.161-1.777)	0.001
	Recessive			1.200 (0.789-1.824)	0.393
rs4263839	GG	194 (27.60)	201 (29.65)	Reference	
	GA	381 (54.20)	344 (50.74)	1.148 (0.898-1.467)	0.271
	AA	128 (18.21)	133 (19.62)	0.997 (0.729-1.363)	0.986
	Dominant			1.106 (0.875-1.396)	0.399
	Recessive			0.912 (0.697-1.194)	0.504
rs4979462	CC	374 (54.05)	325 (48.73)	Reference	
	CT	272 (39.31)	287 (43.03)	0.824 (0.659-1.029)	0.087
	TT	46 (6.65)	55 (8.25)	0.727 (0.478-1.105)	0.134
	Dominant			0.808 (0.653-1.000)	0.050
	Recessive			0.792 (0.527-1.190)	0.261
rs7848647	TT	168 (24.17)	149 (22.37)	Reference	
	TC	373 (53.67)	355 (53.30)	0.932 (0.716-1.214)	0.601
	CC	154 (22.16)	162 (24.32)	0.843 (0.617-1.152)	0.283
	Dominant			0.904 (0.703-1.163)	0.432
	Recessive			0.886 (0.688-1.139)	0.344

The data was analyzed with the SPSS18.0 software. VEGI, vascular endothelial growth inhibitor.

Table 3 The allele frequencies of *VEGI* gene polymorphisms in cases and controls

SNP ID	Allele	Cases No. (%)	Controls No. (%)	OR (95% CI)	P value
rs6478106	C	946 (68.35)	996 (73.45)	Reference	
	T	438 (31.65)	360 (26.55)	1.281 (1.086-1.511)	0.003*
rs4263839	G	769 (54.69)	746 (55.01)	Reference	
	A	637 (45.31)	610 (44.99)	1.013 (0.872-1.177)	0.866
rs4979462	C	1,020 (73.70)	937 (70.24)	Reference	
	T	364 (26.30)	397 (29.76)	0.842 (0.712-0.996)	0.045#
rs7848647	T	709 (51.01)	653 (49.02)	Reference	
	C	681 (48.99)	679 (50.98)	0.924 (0.795-1.074)	0.301

P*=0.0086 after correcting the P value for multiple testing by Haploview program using 10,000 permutations. P#>0.05 after multiple testing by Haploview program using 10,000 permutations. VEGI, vascular endothelial growth inhibitor.

The positive results are shown in *Table 5* and others were in *Tables S1-S5*.

Clinical features and haplotypes of VEGI SNPs

The analysis results of the association between the haplotypes and the clinical features did not show a significant difference (*Tables S6-S11*).

Discussion

The growth and development of tumors rely on many

Table 4 The haplotype frequencies of *VEGI* gene in cases and controls

Haplotype		Frequency	Cases	Controls	P value
rs6478106	rs4263839				
C	A	0.437	0.440	0.433	0.6877
T	G	0.276	0.257	0.295	0.0244[&]
C	G	0.272	0.293	0.251	0.0136*
T	A	0.016	0.010	0.021	0.0184[&&]
rs4979462	rs7848647				
C	T	0.473	0.462	0.484	0.2482
T	C	0.251	0.266	0.237	0.0721
C	C	0.248	0.242	0.254	0.4802
T	T	0.028	0.030	0.026	0.5384

P*=0.0448 after correcting the P value for multiple testing by Haploview program using 10,000 permutations. P[&], P[&&]>0.05 after correcting the P value for multiple testing by Haploview program using 10,000 permutations. VEGI, vascular endothelial growth inhibitor.

Table 5 Genotype and allele frequencies of *VEGI* gene polymorphisms and Her-2 status

SNP ID	Genotype	Her-2 (+) No. (%)	Her-2 (–) No. (%)	OR (95% CI)	P value
rs6478106	CC	54 (40.00)	215 (45.84)	Reference	
	CT	62 (45.93)	224 (47.76)	1.102 (0.731-1.661)	0.642
	TT	19 (14.07)	30 (6.40)	2.522 (1.320-4.818)	0.004
	Dominant			1.270 (0.860-1.874)	0.229
	Recessive			2.397 (1.302-4.411)	0.004
	Allelic			1.355 (1.020-1.799)	0.036[^]
rs4979462	CC	68 (50.00)	257 (54.68)	Reference	
	CT	50 (36.76)	189 (40.21)	1.000 (0.663-1.507)	0.999
	TT	18 (13.24)	24 (5.11)	2.835 (1.455-5.523)	0.002
	Dominant			1.207 (0.823-1.768)	0.335
	Recessive			2.835 (1.489-5.397)	0.001
	Allelic			1.371 (1.021-1.842)	0.035[^^]

P[^]>0.05, P[^^]>0.05 after multiple testing by Haploview program using 10,000 permutations. VEGI, vascular endothelial growth inhibitor. Her-2, human epidermal growth factor receptor 2.

factors, and the growth of new vessels in tumors is extremely important. The nutrients and oxygen that tumor cells need are transported by blood vessels, and the spread of cancer cells also depends on blood vessels. Therefore, the suppression of vessel formation can act as a potential therapeutic target in cancers. In recent research, VEGI has been identified as an inhibitory protein that inhibits the growth of vascular endothelial cells in cancers (21). VEGI is mediated by DR3 and modulates neovascularization and inflammation (22,23). VEGF receptor 1 could also be regulated by VEGI to inhibit the angiogenesis (24). Decreased expression of VEGI can promote tumor development (25). Recent studies showed that tumor vasculature could be suppressed by a new NGR-VEGI fusion protein (26). In addition to its ability to inhibit neovascularization, VEGI can play an important role in immune response. VEGI can induce dendritic cell maturation (27), and the interaction between VEGI and DR3 can modulate the adaptive immune response by suppressing the proliferation of human activated B cells (28).

In Crohn's disease, VEGI has been shown to down-regulate the activation of T helper-1 cells and T helper-17 cells (29).

The expression of *VEGI* can affect the vessel formation of breast tumor (30). The mRNA and protein levels of VEGI in breast cancer patients were lower compared to the controls and patients with breast cancer who expressed more VEGI protein had a more favorable prognosis than patients who expressed less VEGI protein (14). Thus, the gene variants of *VEGI* may play a very important role in the development of breast cancer. Our study indicates that some SNPs in the *VEGI* gene may affect the development of breast cancer in Chinese Han women.

Our data indicates that rs6478106 is related to the risk of breast cancer in Chinese Han women. The CT genotype and T allele of rs6478106 were related to an increased risk of breast cancer. In the Crohn's disease, rs6478106 was proven to be a really significant locus (31). It is noteworthy that rs6478106 is located in the 3'-flanking region of the *VEGI* gene, and the 3' flanking region of gene often contains sequences that can influence the formation of 3' end of the message. It may also contain the sites where proteins may bind or enhancers. Thus, the genetic variants in these regions may affect the transcription of the gene. The T allele of rs6478106 can be treated as a potential marker to inform the prognosis of breast cancer patients. In addition, breast cancer patients have a higher frequency of expression of $C_{rs6478106}G_{rs4263839}$ than healthy controls, indicating that the $C_{rs6478106}G_{rs4263839}$ haplotype might also have potential to predict breast cancer development. The intron 1 of gene contains many splicing control elements and regulatory elements that can affect the expression of genes. The SNP in this location may affect its alternative splicing function. The location of rs4979462 is in intron 1 of *VEGI* gene. In our research, no significant difference was found in the genotype distribution of rs4979462 between healthy controls and breast cancer patients. However, based on the analysis of the association between gene polymorphisms in *VEGI* and the clinical features of breast cancer patients, we found that rs4979462 and rs6478106 are related to the expression of Her-2. The TT genotype of rs4979462 and rs6478106 was more frequent in the Her-2 (+) patients. According to researches involved Her-2 indicated that Her-2 could regulate cell growth and proliferation through many pathways in different diseases (32). It acts as a key marker in diagnosis and predicts the prognosis of cancers (33). The TT genotype in rs6478106 and rs4979462 may be a potential indicator to predict the prognosis of breast cancer patients.

In summary, we have found an association between *VEGI* gene polymorphisms and the risk of breast cancer and the clinical pathologic features of breast cancer in Chinese Han women.

Conclusions

Our study indicates that *VEGI* gene polymorphisms may be associated with the risk of breast cancer in Chinese Han women in northeast China. Our results show an association between *VEGI* gene polymorphisms and the Her-2 status of breast cancer patients as well. This analysis was the first to involve *VEGI* gene polymorphisms and breast cancer risk in Chinese Han women. However, further functional studies need to be conducted in our subsequent research.

Acknowledgements

We thank all patients and healthy volunteers for providing blood samples. We are grateful for the collaboration of the participating hospitals and their staff.
Funding: This study was supported by a grant from the National Natural Science Foundation of China (31070780) and the Major Project of Technology Department, Heilongjiang Province (GB05C402).

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Ng CK, Pemberton HN, Reis-Filho JS. Breast cancer intratumor genetic heterogeneity: causes and implications. Expert Rev Anticancer Ther 2012;12:1021-32.
2. Khan MA, Hanif S, Iqbal S, et al. Presentation delay in breast cancer patients and its association with sociodemographic factors in North Pakistan. Chin J Cancer Res 2015;27:288-93.
3. Ban KA, Godellas CV. Epidemiology of breast cancer. Surg Oncol Clin N Am 2014;23:409-22.
4. Byler S, Goldgar S, Heerboth S, et al. Genetic and epigenetic aspects of breast cancer progression and therapy. Anticancer Res 2014;34:1071-7.
5. Wang H, Liu L, Lang Z, et al. Polymorphisms of ERBB2 and breast cancer risk: a meta-analysis of 26 studies involving 35,088 subjects. J Surg Oncol 2013;108:337-41.

6. Li D, Zhang Q, Xu F, et al. Association of CTLA-4 gene polymorphisms with sporadic breast cancer risk and clinical features in Han women of northeast China. Mol Cell Biochem 2012;364:283-90.
7. Yu J, Tian S, Metheny-Barlow L, et al. Modulation of endothelial cell growth arrest and apoptosis by vascular endothelial growth inhibitor. Circ Res 2001;89:1161-7.
8. Hou W, Medynski D, Wu S, et al. VEGI-192, a new isoform of TNFSF15, specifically eliminates tumor vascular endothelial cells and suppresses tumor growth. Clin Cancer Res 2005;11:5595-602.
9. Prehn JL, Thomas LS, Landers CJ, et al. The T cell costimulator TL1A is induced by FcgammaR signaling in human monocytes and dendritic cells. J Immunol 2007;178:4033-8.
10. Migone TS, Zhang J, Luo X, et al. TL1A is a TNF-like ligand for DR3 and TR6/DcR3 and functions as a T cell costimulator. Immunity 2002;16:479-92.
11. Bamias G, Martin C 3rd, Marini M, et al. Expression, localization, and functional activity of TL1A, a novel Th1-polarizing cytokine in inflammatory bowel disease. J Immunol 2003;171:4868-74.
12. Liang PH, Tian F, Lu Y, et al. Vascular endothelial growth inhibitor (VEGI; TNFSF15) inhibits bone marrow-derived endothelial progenitor cell incorporation into Lewis lung carcinoma tumors. Angiogenesis 2011;14:61-8.
13. Zhang N, Sanders AJ, Ye L, et al. Vascular endothelial growth inhibitor, expression in human prostate cancer tissue and the impact on adhesion and migration of prostate cancer cells in vitro. Int J Oncol 2009;35:1473-80.
14. Parr C, Gan CH, Watkins G, et al. Reduced vascular endothelial growth inhibitor (VEGI) expression is associated with poor prognosis in breast cancer patients. Angiogenesis 2006;9:73-81.
15. Haridas V, Shrivastava A, Su J, et al. VEGI, a new member of the TNF family activates nuclear factor-kappa B and c-Jun N-terminal kinase and modulates cell growth. Oncogene 1999;18:6496-504.
16. Lu Y, Gu X, Chen L, et al. Interferon-γ produced by tumor-infiltrating NK cells and CD4+ T cells downregulates TNFSF15 expression in vascular endothelial cells. Angiogenesis 2014;17:529-40.
17. Jia W, Sander AJ, Jia G, et al. Vascular endothelial growth inhibitor (VEGI) is an independent indicator for invasion in human pituitary adenomas. Anticancer Res 2013;33:3815-22.
18. Yang SK, Lim J, Chang HS, et al. Association of TNFSF15 with Crohn's disease in Koreans. Am J Gastroenterol 2008;103:1437-42.
19. Zucchelli M, Camilleri M, Andreasson AN, et al. Association of TNFSF15 polymorphism with irritable bowel syndrome. Gut 2011;60:1671-7.
20. Képíró L, Széll M, Kovács L, et al. Genetic risk and protective factors of TNFSF15 gene variants detected using single nucleotide polymorphisms in Hungarians with psoriasis and psoriatic arthritis. Hum Immunol 2014;75:159-62.
21. Zhang N, Sanders AJ, Ye L, et al. Vascular endothelial growth inhibitor in human cancer (Review). Int J Mol Med 2009;24:3-8.
22. Ge Z, Sanders AJ, Ye L, et al. Aberrant expression and function of death receptor-3 and death decoy receptor-3 in human cancer. Exp Ther Med 2011;2:167-172.
23. Kang YJ, Kim WJ, Bae HU, et al. Involvement of TL1A and DR3 in induction of pro-inflammatory cytokines and matrix metalloproteinase-9 in atherogenesis. Cytokine 2005;29:229-35.
24. Qi JW, Qin TT, Xu LX, et al. TNFSF15 inhibits vasculogenesis by regulating relative levels of membrane-bound and soluble isoforms of VEGF receptor 1. Proc Natl Acad Sci U S A 2013;110:13863-8.
25. Wu L, Li X, Ye L, et al. Vascular endothelial growth inhibitor 174 is a negative regulator of aggressiveness and microvascular density in human clear cell renal cell carcinoma. Anticancer Res 2014;34:715-22.
26. Ma W, Li G, Wang J, et al. In vivo NIRF imaging-guided delivery of a novel NGR-VEGI fusion protein for targeting tumor vasculature. Amino Acids 2014;46:2721-32.
27. Tian F, Grimaldo S, Fujita M, et al. The endothelial cell-produced antiangiogenic cytokine vascular endothelial growth inhibitor induces dendritic cell maturation. J Immunol 2007;179:3742-51.
28. Cavallini C, Lovato O, Bertolaso A, et al. The TNF-family cytokine TL1A inhibits proliferation of human activated B cells. PLoS One 2013;8:e60136.
29. Takedatsu H, Michelsen KS, Wei B, et al. TL1A (TNFSF15) regulates the development of chronic colitis by modulating both T-helper 1 and T-helper 17 activation. Gastroenterology 2008;135:552-67.
30. Gao Y, Ge Z, Zhang Z, et al. Vascular endothelial growth inhibitor affects the invasion, apoptosis and vascularisation in breast cancer cell line MDA-MB-231. Chin Med J (Engl) 2014;127:1947-53.
31. Yamazaki K, Umeno J, Takahashi A, et al. A genome-wide association study identifies 2 susceptibility Loci for

Crohn's disease in a Japanese population. Gastroenterology 2013;144:781-8.
32. Kumari N, Kapoor VK, Krishnani N, et al. Role of C-erbB2 expression in gallbladder cancer. Indian J Pathol Microbiol 2012;55:75-9.
33. Bayrak M, Olmez OF, Kurt E, et al. Prognostic significance of c-erbB2 overexpression in patients with metastatic gastric cancer. Clin Transl Oncol 2013;15:307-12.

Cite this article as: Han S, Liu L, Xu F, Chen S, Yuan W, Fu Z, Li D, Li D. A case-control study about the association between vascular endothelial growth inhibitor gene polymorphisms and breast cancer risk in female patients in Northeast China. Chin J Cancer Res 2016;28(4):435-443. doi:10.21147/j.issn.1000-9604.2016.04.07

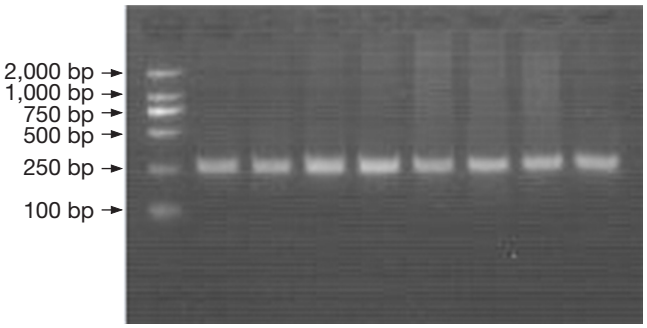

Figure S1 Agarose gel electrophoresis of rs6478106 after PCR reaction. PCR, polymerase chain reaction.

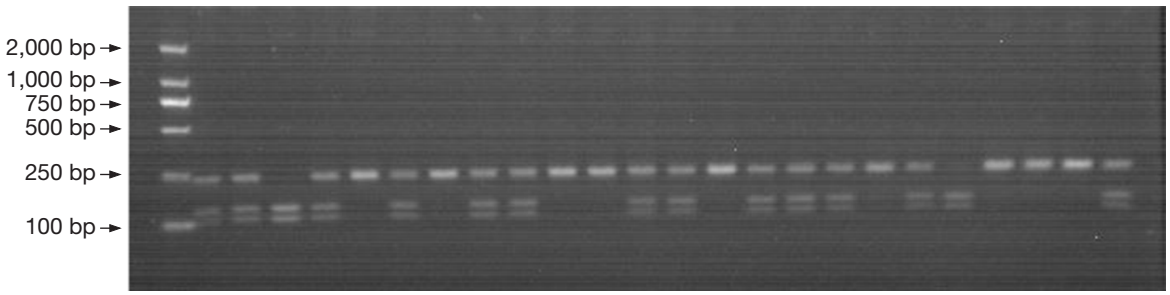

Figure S2 Agarose gel electrophoresis of rs6478106 digested by *Eco*53KI. Lane 1 is DNA marker; lanes 4, 21 are TT homozygote; lanes 6, 8, 11, 12, 15, 19, 22-24 are CC homozygote; lanes 2, 3, 5, 7, 9, 10, 13, 14, 16-18, 20, 25 are CT heterozygote.

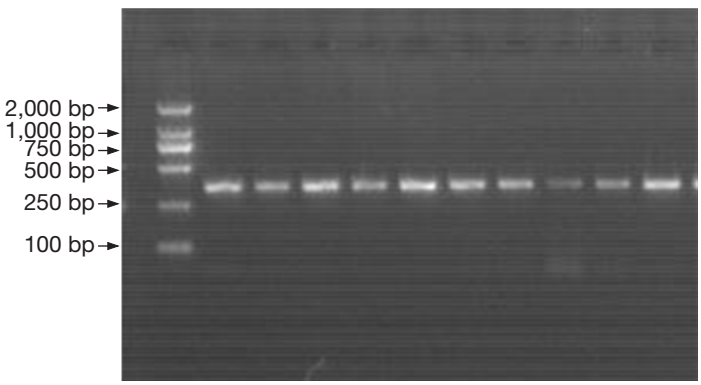

Figure S3 Agarose gel electrophoresis of rs4263839 after PCR reaction. PCR, polymerase chain reaction.

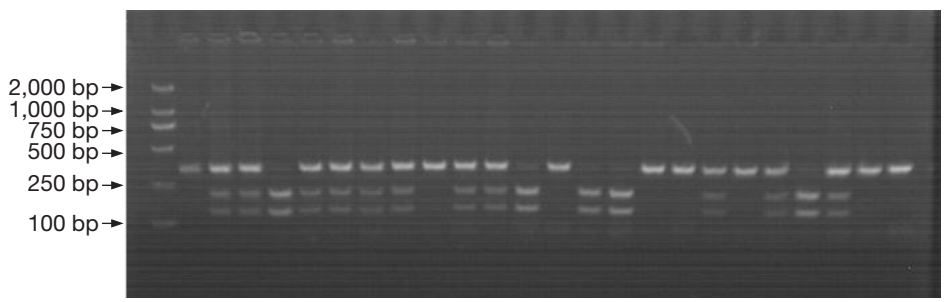

Figure S4 Agarose gel electrophoresis of rs4263839 digested by *Apo*I. Lane 1 is DNA marker; lanes 2, 10, 14, 17, 18, 20, 24, 25 are GG homozygote; lanes 5, 13, 15, 16, 22 are AA homozygote; lanes 3, 4, 6-9, 11, 12, 19, 21, 23 are GA heterozygote.

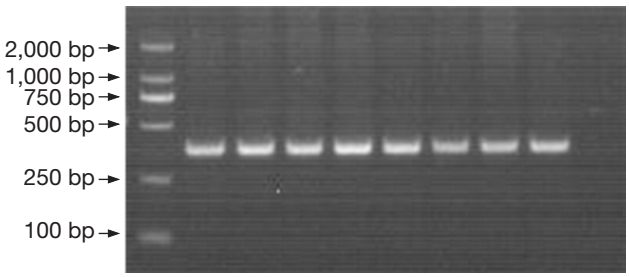

Figure S5 Agarose gel electrophoresis of rs4979462 after PCR reaction. PCR, polymerase chain reaction.

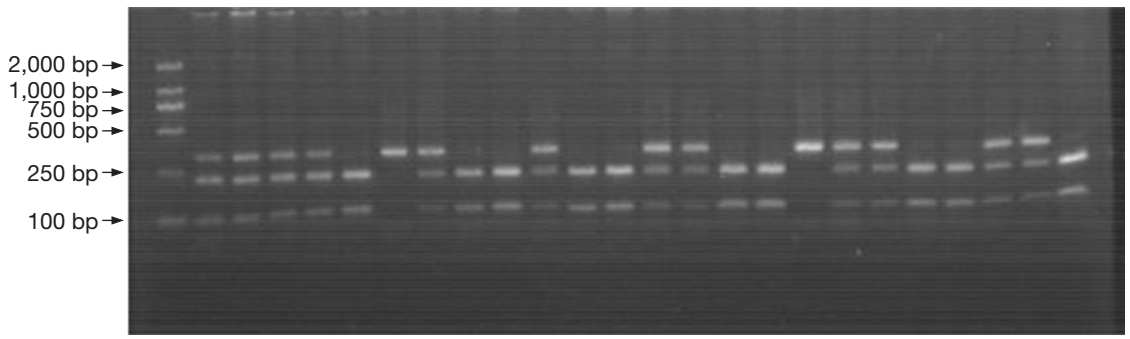

Figure S6 Agarose gel electrophoresis of rs4979462 digested by *Msc*I. Lane 1 is DNA marker; lanes 7, 18 are TT homozygote; lanes 6, 9, 10, 12, 13, 16, 17, 21, 22, 25 are CC homozygote; lanes 2-5, 8, 11, 14, 15, 19, 20, 23, 24 are CT heterozygote.

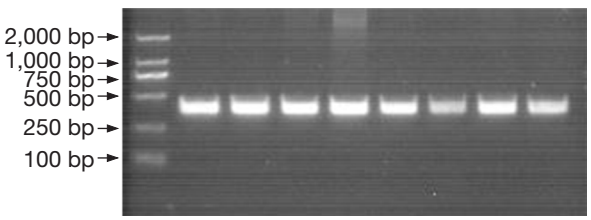

Figure S7 Agarose gel electrophoresis of rs7848647 after PCR reaction. PCR, polymerase chain reaction.

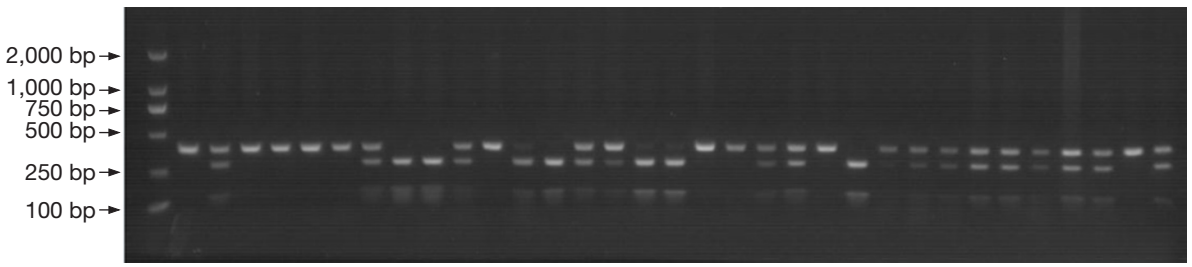

Figure S8 Agarose gel electrophoresis of rs7848647 digested by *Cvi*QI. Lane 1 is DNA marker; lanes 2, 4-7, 12, 19, 20, 23, 25, 33 are TT homozygote; lanes 9, 10, 13, 14, 17, 18, 24 are CC homozygote; lanes 3, 8, 11, 15, 16, 21, 22, 26-32, 34 are TC heterozygote.

Table S1 Genotype and allele frequencies of *VEGI* gene polymorphisms and LN metastasis status

SNP ID	Genotype	LN (+) No. (%)	LN (–) No. (%)	OR (95% CI)	P value
rs6478106	CC	118 (46.46)	164 (42.60)	Reference	
	CT	117 (46.06)	188 (48.83)	0.865 (0.622-1.204)	0.389
	TT	19 (7.48)	33 (8.57)	0.800 (0.434-1.476)	0.475
	Dominant			0.855 (0.622-1.176)	0.336
	Recessive			0.862 (0.479-1.553)	0.622
	Allelic			0.892 (0.701-1.135)	0.353
rs4263839	GG	59 (22.87)	116 (29.74)	Reference	
	GA	149 (57.75)	209 (53.59)	1.402 (0.961-2.044)	0.079
	AA	50 (19.38)	65 (16.67)	1.512 (0.932-2.454)	0.093
	Dominant			1.428 (0.994-2.052)	0.054
	Recessive			1.202 (0.799-1.807)	0.376
	Allelic			1.213 (0.970-1.517)	0.090
rs4979462	CC	148 (58.73)	200 (51.55)	Reference	
	CT	86 (34.13)	162 (41.75)	0.717 (0.512-1.005)	0.053
	TT	18 (7.14)	26 (6.70)	0.936 (0.495-1.770)	0.838
	Dominant			0.748 (0.543-1.030)	0.075
	Recessive			1.071 (0.574-1.997)	0.829
	Allelic			0.839 (0.648-1.085)	0.181
rs7848647	TT	67 (26.07)	86 (22.40)	Reference	
	TC	142 (55.25)	209 (54.43)	0.872 (0.594-1.280)	0.485
	CC	48 (18.68)	89 (23.18)	0.692 (0.431-1.113)	0.128
	Dominant			0.818 (0.567-1.182)	0.285
	Recessive			0.761 (0.514-1.128)	0.173
	Allelic			0.849 (0.679-1.062)	0.151

VEGI, vascular endothelial growth inhibitor; LN, lymph node.

Table S2 Genotype and allele frequencies of *VEGI* gene polymorphisms and ER status

SNP ID	Genotype	ER (+) No. (%)	ER (–) No. (%)	OR (95% CI)	P value
rs6478106	CC	175 (45.45)	95 (43.18)	Reference	
	CT	177 (45.97)	109 (49.55)	0.882 (0.624-1.245)	0.474
	TT	33 (8.57)	16 (7.27)	1.120 (0.586-2.139)	0.732
	Dominant			0.912 (0.653-1.273)	0.589
	Recessive			1.670 (0.894-3.119)	0.105
	Allelic			0.978 (0.760-1.257)	0.861
rs4263839	GG	113 (28.83)	57 (25.33)	Reference	
	GA	203 (51.79)	134 (59.56)	0.764 (0.519-1.124)	0.172
	AA	76 (19.39)	34 (15.11)	1.128 (0.674-1.887)	0.648
	Dominant			0.841 (0.580-1.219)	0.360
	Recessive			1.351 (0.868-2.103)	0.182
	Allelic			1.016 (0.805-1.282)	0.894
rs4979462	CC	213 (54.48)	113 (52.31)	Reference	
	CT	150 (38.36)	89 (41.20)	0.894 (0.632-1.266)	0.528
	TT	28 (7.16)	14 (6.48)	1.061 (0.537-2.096)	0.865
	Dominant			0.917 (0.657-1.279)	0.609
	Recessive			1.113 (0.573-2.162)	0.752
	Allelic			0.963 (0.739-1.255)	0.780
rs7848647	TT	95 (24.55)	50 (22.73)	Reference	
	TC	210 (54.26)	122 (55.45)	0.906 (0.602-1.363)	0.636
	CC	82 (21.19)	48 (21.82)	0.899 (0.549-1.474)	0.673
	Dominant			0.904 (0.611-1.337)	0.613
	Recessive			0.980 (0.656-1.465)	0.922
	Allelic			0.952 (0.753-1.203)	0.681

VEGI, vascular endothelial growth inhibitor; ER, estrogen receptor.

Table S3 Genotype and allele frequencies of *VEGI* gene polymorphisms and PR status

SNP ID	Genotype	PR (+) No. (%)	PR (–) No. (%)	OR (95% CI)	P value
rs6478106	CC	176 (44.22)	93 (45.37)	Reference	
	CT	187 (46.98)	99 (48.29)	0.998 (0.703-1.416)	0.992
	TT	35 (8.79)	13 (6.34)	1.423 (0.718-2.820)	0.311
	Dominant			1.047 (0.746-1.470)	0.789
	Recessive			1.424 (0.736-2.756)	0.292
	Allelic			1.087 (0.840-1.406)	0.525
rs4263839	GG	116 (28.86)	53 (24.88)	Reference	
	GA	214 (53.23)	122 (57.28)	0.801 (0.541-1.188)	0.270
	AA	72 (17.91)	38 (17.84)	0.866 (0.520-1.442)	0.579
	Dominant			0.817 (0.560-1.192)	0.294
	Recessive			1.005 (0.651-1.550)	0.983
	Allelic			0.924 (0.730-1.170)	0.513
rs4979462	CC	214 (53.77)	111 (53.62)	Reference	
	CT	157 (39.45)	82 (39.61)	0.993 (0.698-1.412)	0.969
	TT	27 (6.78)	14 (6.76)	1.000 (0.504-1.984)	0.999
	Dominant			0.994 (0.710-1.392)	0.973
	Recessive			1.003 (0.514-1.958)	0.992
	Allelic			0.997 (0.762-1.304)	0.981
rs7848647	TT	94 (23.62)	51 (24.64)	Reference	
	TC	218 (54.77)	113 (54.59)	1.047 (0.695-1.577)	0.827
	CC	86 (21.61)	43 (20.77)	1.085 (0.658-1.789)	0.749
	Dominant			1.057 (0.715-1.564)	0.780
	Recessive			1.051 (0.696-1.587)	0.812
	Allelic			1.038 (0.818-1.316)	0.759

VEGI, vascular endothelial growth inhibitor; PR, progestrogen receptor.

Table S4 Genotype and allele frequencies of *VEGI* gene polymorphisms and p53 status

SNP ID	Genotype	p53 (+) No. (%)	p53 (–) No. (%)	OR (95% CI)	P value
rs6478106	CC	36 (45.00)	229 (44.47)	Reference	
	CT	37 (46.25)	245 (47.57)	0.961 (0.587-1.573)	0.873
	TT	7 (8.75)	41 (7.96)	1.086 (0.453-2.606)	0.853
	Dominant			0.979 (0.609-1.571)	0.929
	Recessive			1.109 (0.479-2.564)	0.810
	Allelic			1.006 (0.707-1.438)	0.974
rs4263839	GG	23 (28.40)	144 (27.38)	Reference	
	GA	46 (56.79)	286 (54.37)	1.007 (0.587-1.726)	0.980
	AA	12 (14.81)	96 (18.25)	0.783 (0.372-1.647)	0.518
	Dominant			0.951 (0.565-1.598)	0.848
	Recessive			0.779 (0.406-1.495)	0.452
	Allelic			0.914 (0.654-1.276)	0.596
rs4979462	CC	38 (48.72)	282 (54.34)	Reference	
	CT	33 (42.31)	203 (39.11)	1.206 (0.732-1.989)	0.462
	TT	7 (8.97)	34 (6.55)	1.528 (0.633-3.688)	0.343
	Dominant			1.252 (0.778-2.017)	0.354
	Recessive			1.406 (0.601-3.293)	0.430
	Allelic			1.267 (0.874-1.836)	0.210
rs7848647	TT	14 (17.72)	128 (24.71)	Reference	
	TC	47 (59.49)	280 (54.05)	1.535 (0.815-2.888)	0.128
	CC	18 (22.78)	110 (21.24)	1.496 (0.711-3.147)	0.286
	Dominant			1.524 (0.827-2.807)	0.174
	Recessive			1.094 (0.621-1.928)	0.755
	Allelic			1.186 (0.848-1.659)	0.317

VEGI, vascular endothelial growth inhibitor.

Table S5 Genotype and allele frequencies of *VEGI* gene polymorphisms and TNBC status

SNP ID	Genotype	TNBC No. (%)	Non-TNBC No. (%)	OR (95% CI)	P value
rs6478106	CC	42 (43.30)	227 (44.86)	Reference	
	CT	51 (52.58)	235 (46.44)	1.173 (0.750-1.835)	0.484
	TT	4 (4.12)	44 (8.70)	0.491 (0.168-1.440)	0.265
	Dominant			1.065 (0.687-1.651)	0.777
	Recessive			0.452 (0.158-1.287)	0.153
	Allelic			0.932 (0.668-1.301)	0.680
rs4263839	GG	22 (21.57)	147 (28.65)	Reference	
	GA	60 (58.82)	276 (53.80)	1.453 (0.857-2.463)	0.164
	AA	20 (19.61)	90 (17.54)	1.485 (0.768-2.873)	0.238
	Dominant			1.461 (0.878-2.430)	0.143
	Recessive			1.146 (0.669-1.965)	0.619
	Allelic			1.202 (0.890-1.624)	0.230
rs4979462	CC	49 (50.52)	276 (54.33)	Reference	
	CT	43 (44.33)	196 (38.58)	1.236 (0.789-1.935)	0.355
	TT	5 (5.15)	36 (7.09)	0.782 (0.293-2.092)	0.624
	Dominant			1.165 (0.755-1.800)	0.490
	Recessive			0.713 (0.272-1.864)	0.488
	Allelic			1.049 (0.743-1.482)	0.785
rs7848647	TT	28 (28.28)	117 (23.12)	Reference	
	TC	52 (52.53)	279 (55.14)	0.779 (0.469-1.299)	0.334
	CC	19 (19.19)	110 (21.74)	0.722 (0.381-1.366)	0.315
	Dominant			0.763 (0.470-1.237)	0.271
	Recessive			0.855 (0.497-1.472)	0.571
	Allelic			0.857 (0.631-1.163)	0.321

VEGI, vascular endothelial growth inhibitor; TNBC, triple negative breast cancer.

Table S6 The haplotype of the *VEGI* gene polymorphisms and the ER status

Haplotype		Frequency	ER (+)	ER (–)	P value
rs6478106	rs4263839				
C	A	0.430	0.422	0.434	0.6727
T	G	0.295	0.294	0.296	0.9442
C	G	0.253	0.257	0.251	0.8022
T	A	0.022	0.027	0.019	0.3695
rs4979462	rs7848647				
C	T	0.490	0.486	0.492	0.8293
C	C	0.244	0.243	0.245	0.9511
T	C	0.244	0.252	0.239	0.5963
T	T	0.022	0.018	0.024	0.5239

VEGI, vascular endothelial growth inhibitor; ER, estrogen receptor.

Table S7 The haplotype of the *VEGI* gene polymorphisms and the PR status

Haplotype		Frequency	PR (+)	PR (–)	P value
rs6478106	rs4263839				
C	A	0.430	0.446	0.422	0.4264
T	G	0.294	0.285	0.299	0.6133
C	G	0.253	0.250	0.2555	0.846
T	A	0.022	0.019	0.024	0.5924
rs4979462	rs7848647				
C	T	0.491	0.503	0.484	0.5273
C	C	0.244	0.232	0.251	0.4716
T	C	0.243	0.248	0.240	0.7465
T	T	0.022	0.016	0.025	0.3227

VEGI, vascular endothelial growth inhibitor; PR, progestrogen receptor.

Table S8 The haplotype of the *VEGI* gene polymorphisms and the p53 status

Haplotype		Frequency	p53 (+)	p53 (–)	P value
rs6478106	rs4263839				
C	A	0.431	0.433	0.414	0.6481
T	G	0.296	0.296	0.300	0.9007
C	G	0.252	0.250	0.268	0.6244
T	A	0.021	0.022	0.018	0.7629
rs4979462	rs7848647				
C	T	0.490	0.496	0.452	0.2954
T	C	0.245	0.240	0.277	0.309
C	C	0.244	0.243	0.251	0.8237
T	T	0.021	0.021	0.020	0.9448

VEGI, vascular endothelial growth inhibitor.

Table S9 The haplotype of the *VEGI* gene polymorphisms and the Her-2 status

Haplotype		Frequency	Her-2 (+)	Her-2 (–)	P value
rs6478106	rs4263839				
C	A	0.430	0.442	0.386	0.1026
T	G	0.296	0.282	0.343	0.0532
C	G	0.253	0.256	0.243	0.6577
T	A	0.022	0.020	0.029	0.4201
rs4979462	rs7848647				
C	T	0.490	0.502	0.449	0.1212
T	C	0.244	0.230	0.295	0.0258*
C	C	0.244	0.246	0.237	0.7719
T	T	0.023	0.023	0.019	0.6911

*, P=0.0986 (P>0.05) after multiple testing by Haploview program using 10,000 permutations. VEGI, vascular endothelial growth inhibitor; Her-2, human epidermal growth factor receptor 2.

Table S10 The haplotype of the *VEGI* gene polymorphisms and the LN metastasis status

Haplotype		Frequency	LN (+)	LN (–)	P value
rs6478106	rs4263839				
C	A	0.431	0.413	0.459	0.0998
T	G	0.296	0.308	0.279	0.2654
C	G	0.249	0.257	0.237	0.4136
T	A	0.023	0.022	0.025	0.7551
rs4979462	rs7848647				
C	T	0.487	0.468	0.516	0.0865
C	C	0.250	0.257	0.241	0.5062
T	C	0.238	0.248	0.222	0.2755
T	T	0.024	0.027	0.021	0.4978

VEGI, vascular endothelial growth inhibitor; LN, lymph node.

Table S11 The haplotype of the *VEGI* gene polymorphisms and the TNBC status

Haplotype		Frequency	TNBC (+)	TNBC (–)	P value
rs6478106	rs4263839				
C	A	0.430	0.423	0.463	0.2805
T	G	0.295	0.298	0.280	0.5893
C	G	0.253	0.258	0.230	0.3961
T	A	0.022	0.021	0.027	0.5821
rs4979462	rs7848647				
C	T	0.490	0.483	0.526	0.2508
C	C	0.244	0.253	0.204	0.1353
T	C	0.244	0.243	0.248	0.866
T	T	0.022	0.022	0.022	0.9737

VEGI, vascular endothelial growth inhibitor; TNBC, triple negative breast cancer.