Vascular Endothelial Growth Factor Polymorphisms in Relation to Breast Cancer Development and Prognosis

Qianren Jin,[1] Kari Hemminki,[1,2] Kerstin Enquist,[4] Per Lenner,[5] Ewa Grzybowska,[6] Rüdiger Klaes,[3] Roger Henriksson,[5] Bowang Chen,[2] Jolanta Pamula,[6] Wioletta Pekala,[6] Helena Zientek,[6] Jadwiga Rogozinska-Szczepka,[7] Beata Utracka-Hutka,[8] Göran Hallmans,[4] and Asta Försti[1,2]

Abstract

Purpose: Angiogenesis is a necessary step in tumor growth and metastasis. Vascular endothelial growth factor (VEGF) is a major mediator of breast cancer angiogenesis. Therefore, we investigated the association of polymorphisms in the *VEGF* gene with breast cancer risk and prognostic characteristics of the tumors in a large case-control study.

Experimental Design: We examined three polymorphisms in the *VEGF* gene (−2578C/A, −1154G/A, and +936C/T) in 571 familial breast cancer cases from Poland and Germany and −2578C/A, −634G/C, and +936C/T polymorphisms in 974 unselected breast cancer cases from Sweden together with ethnically and geographically selected controls.

Results: None of the polymorphisms or any haplotype was significantly associated with either familial or unselected breast cancers. Our study suggests that the +936C/T polymorphism is unlikely to be associated with breast cancer. We also analyzed the unselected cases for genotypes or haplotypes that associated with tumor characteristics. The −634CC genotype and the −2578/−634 CC haplotype were significantly associated with high tumor aggressiveness (large tumor size and high histologic grade, $P < 0.01$) and the −2578AA genotype and the −2578/−634 AG haplotype with low histologic grade tumors ($P = 0.04$). The genotypes and haplotypes were not related with other tumor characteristics such as regional or distant metastasis, stage at diagnosis, or estrogen or progesterone receptor status.

Conclusions: Although none of the polymorphisms studied in the *VEGF* gene was found to influence susceptibility to breast cancer significantly, some of the *VEGF* genotypes and haplotypes may influence tumor growth through an altered expression of VEGF and tumor angiogenesis.

Angiogenesis is an important step in the development of cancer and is necessary for primary tumor growth, invasiveness, and metastasis (1). Vascular endothelial growth factor (VEGF) is believed to be important for the process of initiation of angiogenesis and is a major mediator of breast cancer angiogenesis (2). Overexpression of VEGF has been shown in various cancers (3). Several polymorphisms in the *VEGF* gene have been reported to affect the expression of the gene. The −2578CC, −2549 del/del, −1154GG, and −634CC have been shown associated with a higher VEGF production (4–7), whereas the *+936 T* allele has been shown to correlate with lower VEGF plasma levels (8, 9). In addition, the −634G/C polymorphism is located within a potential binding site of the MZF1 transcription factor (10) and the +936C/T polymorphism leads to a loss of a potential AP-4 binding site (8, 9). Recent studies have shown that some *VEGF* polymorphisms are associated with the development of cancer. The −1154AA genotype, for example, is associated with a decreased prostate cancer risk and less advanced melanomas (11, 12) and the +936 C/T polymorphism with a decreased breast cancer risk (9). In breast cancer, VEGF has been shown to be of prognostic importance (13). These data suggest that the polymorphisms involved in the angiogenic pathway may affect the progression or aggressiveness of the tumor, including breast tumors.

In the present study, we investigated the relationship between genetic polymorphisms in the *VEGF* gene and the development of breast cancer in patients from Poland, Germany, and Sweden. Five of the polymorphisms were located in the promoter region at positions −2578, −2549, −2489, −2447, and −1154, one in the 5′ untranslated region at position −634 relative to the translation starting site, and the seventh one in the 3′ untranslated region at position +936, according to the numbering used by Renner et al. (ref. 8; Fig. 1). From these seven polymorphisms, four were selected for further analysis to evaluate their possible influence on the risk to breast cancer and the prognostic characteristics of the tumors.

Authors' Affiliations: [1]Department of Biosciences at Novum, Karolinska Institute, Huddinge, Sweden, [2]Division of Molecular Genetic Epidemiology, German Cancer Research Center and [3]Institute of Human Genetics, University of Heidelberg, Heidelberg, Germany, [4]Department of Public Health and Clinical Medicine/Nutritional Research, Umeå University and [5]Department of Oncology, Norrlands University Hospital, Umeå, Sweden, [6]Department of Tumor Biology, [7]I Clinics of Radiotherapy, and [8]Clinics of Chemotherapy, Centre of Oncology, Maria Sklodowska-Curie Institute, Gliwice, Poland

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Requests for reprints: Qianren Jin, Department of Biosciences at Novum, Karolinska Institute, SE-141 57, Huddinge, Sweden. Phone: 46-8-608-9238; Fax: 46-8-608-1501; E-mail: qianren.jin@cnt.ki.se.

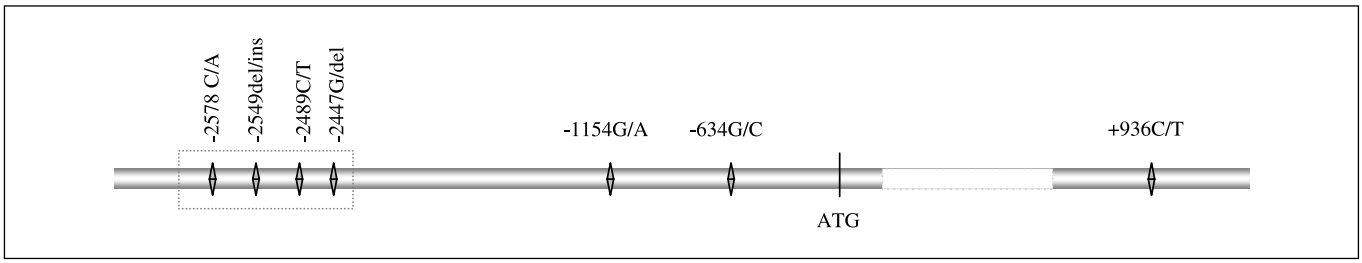

Fig. 1. Structure of the *VEGF* gene. Polymorphisms included in the present study. The translation starting site is marked by the ATG codon. The polymorphisms within the box are in complete linkage. The −2578, −1154, and +936 polymorphism were studied in the Polish and German familial breast cancer cases and controls and the −2578, −634, and +936 polymorphisms were analyzed in the Swedish unselected breast cancer series.

Materials and Methods

Subjects. Totally, 571 familial breast cancer cases (including 412 cases from Poland and 159 cases from Germany) as well as 974 unselected breast cancer cases together with ethnically and geographically selected controls were used in the study. To a large extent, the study populations consisted of Caucasians. The Polish and German cases were incident cases collected during the years 1997 to 2003 according to the criteria described earlier (14) through the Chemotherapy Clinics and the Genetic Counseling (Gliwice, Poland) and the Institute of Human Genetics, University Heidelberg (Germany). About 90% of patients approved participation in the study. The controls were recruited to earlier studies with comparable participation rate. Thirteen percent of the Polish breast cancer cases were *BRCA1/2* mutation carriers. All the German cases used in this study were tested *BRCA1/2* negative. All the familial cases were unrelated. These familial cases together with controls were analyzed at Karolinska Institute, Huddinge, Sweden. Unselected breast cancer cases together with controls were collected during the time period January 1990 to January 2001 by the Department of Public Health and Clinical Medicine/Nutritional Research in Umeå University, Sweden (15). The samples, including controls, within the Västerbotten intervention cohort, the mammary screening cohort, and the MONICA cohort were collected in a population-based manner. The cancer cases were retrieved by linkage to the regional cancer registry. Samples (n = 268) were taken before breast cancer diagnosis, and 449 were collected after diagnosis, being mainly prevalent cases. Samples (n = 257) were collected in a hospital-based manner from untreated patients referred to the Department of Oncology for newly diagnosed breast cancer; the controls were selected from the Västerbotten intervention cohort. Clinical data for the unselected breast cancer cases were retrieved from the registry managed by the Northern Sweden Breast Cancer Group (Table 1). Altogether, only 12 of 782 breast cancer cases had distant metastasis indicating that even the prevalent cases were recruited relatively shortly after diagnosis, excluding biases due to preferential survival. The unselected cases together with controls were analyzed at Umeå University. After DNA digestion, the coded samples were divided on the plates by randomly mixing cases and controls. The study was approved by the ethical committee of Karolinska Institute Syd.

PCR amplification. The primer sequences for the polymorphisms in the *VEGF* gene were designed based on the published Genbank sequences M63971 and AF024710 and are available by the corresponding author. PCR was carried out as described earlier (16).

RFLP analysis. The −2578C/A, as a marker for the completely linked polymorphisms −2578C/A, −2549del/ins 18 bp, −2489C/T, and −2447del G, and +936C/T polymorphisms were analyzed in the familial sample sets using RFLP analysis. The assays were set up after PCR as described (16). PCR products were digested with the *Mva*I and *Hsp*92II restriction endonuleases, respectively, using the buffers and temperatures recommended by the manufacturers. The digested PCR products were resolved on a 10% polyacrylamide gel (Bio-Rad, Hercules, CA) and stained with ethidium bromide for visualization under UV light.

ALF express fragment analysis. ALF express fragment analysis was employed to analyze the −1154G/A polymorphism in the *VEGF* gene. The forward primer was labeled with a Cy5 fluorescence dye. After digestion with *Mnl*I, PCR products were electrophoresed and detected using an automated ALF Express sequencer (Amersham Pharmacia). The results were analyzed using software package Fragment Manager as described earlier (17).

The genotyping results after the RFLP and ALF Express analyses were read by two independent individuals. About 10% of the PCR-RFLP and PCR-ALF assays were randomly repeated and the results were checked for concordance.

Table 1. Characteristics of the Swedish breast cancer samples at diagnosis

Characteristic	Breast cancer patients, *n*
Age at diagnosis (y), median (range)	54.3 (27.1-75.9)
Distant metastasis	
Negative	770
Positive	12
Regional lymph node metastasis	
Negative	574
Positive	237
Stage at diagnosis	
0	10
I	421
II	320
III	21
IV	13
Histologic grade	
Grade 1	142
Grade 2	322
Grade 3	250
Estrogen receptors	
Negative	146
Positive	358
Progesterone receptor	
Negative	165
Positive	275
Tumor size (mm)	
In situ	9
‹20	510
21-50	223
›50	19

Taqman assay. The −2578C/A, −634G/C, +936C/T polymorphisms were analyzed in the Swedish populations by the Umeå Center for Genome Research, Medical and Clinical Genetics, Umeå University, Sweden, using Taqman PCR. The primers and probes were designed using the Assay-by-Design service (Applied Biosystems, Foster City, CA) and the sequences are available by the corresponding author upon request. The assays were carried out using the standard method recommended by Applied Biosystems. The results were automatically read using the ABI PRISM 7900 HT Sequence Detection System.

DNA sequencing. DNA sequencing on three random samples from each genotype for all the polymorphisms was used to confirm the genotypes following PCR-RFLP, PCR-ALF, and Taqman assays as described (16).

Haplotype and linkage disequilibrium analysis. Haplotypes and linkage disequilibrium between the polymorphisms of the *VEGF* gene were determined based on the genotypes of all individuals participating in the study using a Haploview program (version 2.05, http://www.broad.mit.edu/personal/jcbarret/haploview/).

Statistical analysis. The differences in the genotype and haplotype frequencies of the studied polymorphisms in the breast cancer cases and controls were compared for statistical significance by the Yates corrected χ^2 test. The statistical significance for deviations from Hardy-Weinberg equilibrium was tested using the Pearson χ^2 test. Odds ratios and 95% confidence intervals were calculated for associations between genotypes and breast cancer and tumor characteristics. The joint analyses were carried out using a Mantel-Haenszel adjustment with each series as a separate stratum. For a polymorphism with a variant allele frequency between 10% and 50%, the study had >90% power to detect a 1.8- to 1.5-fold increase of relative risk in all familial cases and a 1.5- to 1.3-fold increased risk in all cases. All the statistical tests were carried out using Epi Info 2000 software (http://www.cdc.gov/epiinfo).

Results

We examined the effect of seven common polymorphisms in the *VEGF* gene on breast cancer development (Fig. 1). The polymorphisms −2578C/A, −2549del/ins 18 bp, and −2489C/T were amplified in the same fragment. The −2549del/ins 18 bp polymorphism resulted in an 18-bp larger PCR fragment, and the −2489C/T polymorphism abolished a restriction site for *Mva*I. The results of the RFLP analysis suggested that the −2549 and −2489 polymorphisms are in

Table 2. Allele and genotype distributions of the polymorphisms in the *VEGF* gene in different populations

	Polish			German			Swedish			Joint
Polymorphism/ genotype	Cases (%)	Controls (%)	OR* (95% CI)	Cases (%)	Controls (%)	OR* (95% CI)	Cases (%)	Controls (%)	OR* (95% CI)	OR* (95% CI)
−2578C/A										
CC	104 (25.3)	106 (25.1)	1.00	44 (28.8)	50 (30.9)	1.00	258 (27.5)	257 (27.3)	1.00	1.00
CA	195 (47.4)	207 (48.9)	0.96 (0.68-1.36)	75 (49.0)	72 (44.4)	1.18 (0.68-2.06)	449 (47.8)	451 (48.0)	0.99 (0.79-1.24)	1.00 (0.84-1.19)
AA	112 (27.3)	110 (26.0)	1.04 (0.70-1.54)	34 (22.2)	40 (24.7)	0.97 (0.50-1.86)	232 (24.7)	232 (24.7)	1.00 (0.77-1.29)	1.00 (0.82-1.23)
A%	51.0	50.5		46.7	46.9		48.6	48.7		
−1154G/A										
GG	173 (42.1)	199 (47.0)	1.00	84 (52.8)	76 (46.6)	1.00				1.00
GA	189 (46.0)	178 (42.1)	1.22 (0.91-1.65)	58 (36.5)	69 (42.3)	0.76 (0.46-1.25)				1.07 (0.83-1.38)
AA	49 (11.9)	46 (10.9)	1.23 (0.76-1.97)	17 (10.7)	18 (11.0)	0.85 (0.39-1.89)				1.11 (0.74-1.66)
A%	34.9	31.9		28.9	32.2					
−634G/C										
GG							488 (52.1)	492 (52.3)	1.00	
GC							363 (38.8)	367 (39.0)	1.00 (0.82-1.21)	
CC							85 (9.1)	82 (8.7)	1.05 (0.74-1.47)	
C%							28.5	28.2		
+936C/T										
CC	298 (72.3)	297 (70.4)	1.00	120 (78.4)	128 (78.5)	1.00	708 (76.6)	720 (77.1)	1.00	1.00
CT	100 (24.3)	114 (27.0)	0.87 (0.63-1.21)	31 (20.3)	31 (19.0)	1.07 (0.59-1.93)	204 (22.1)	203 (21.7)	1.02 (0.81-1.28)	0.98 (0.82-1.17)
TT	14 (3.4)	11 (2.6)	1.27 (0.53-3.05)	2 (1.3)	4 (2.5)	0.53 (0.07-3.46)	12 (1.3)	11 (1.2)	1.11 (0.45-2.71)	1.10 (0.62-1.94)
T%	15.5	16.1		11.4	12.0		12.3	12.0		

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval.
*Comparison with the genotype indicated by an odds ratio of 1.00.

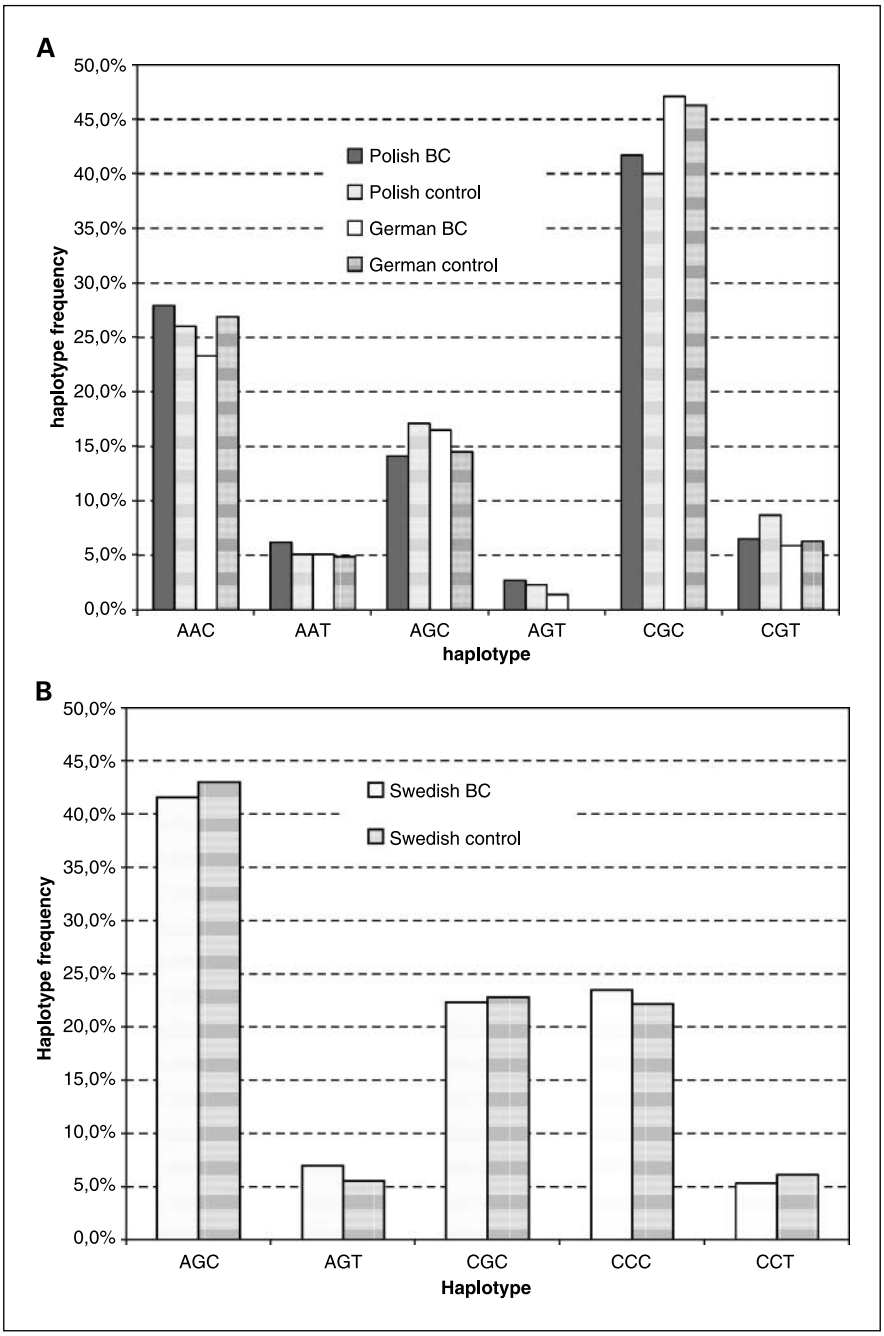

Fig. 2. The observed frequencies of the haplotypes in the *VEGF* gene in (*A*) familial and (*B*) unselected breast cancer series. Nucleotides are listed in the order −2578C/A, −1154G/A, and +936C/T in (*A*) and −2578C/A, −634G/C, and +936C/T in (*B*).

complete linkage disequilibrium. This was confirmed by sequencing 20 randomly chosen samples. Sequence analysis also confirmed the previously reported complete linkage between the −2578 and −2549 polymorphisms (18). A novel polymorphism, −2447 del G, which was completely linked to the *−2578A* allele, was also detected. Thus, the −2578C/A polymorphism was chosen as a marker for all the completely linked polymorphisms in this fragment. The polymorphisms −2578C/A, −1154G/A, and +936C/T were examined in familial breast cancer samples and controls using the RFLP assay. For the unselected breast cancer series, Taqman assay was used. Because the Taqman assay did not work for the −1154G/A polymorphism, we analyzed another functional polymorphism, −634G/C, instead (Fig. 1).

The genotype distribution of all studied polymorphisms followed the Hardy-Weinberg equilibrium in every sample set (P = 0.13-1.00). The genotype and allele distributions among the breast cancer cases and control subjects are shown in Table 2. The number of samples analyzed for each polymorphism was not exactly equal because of unsuccessful amplification of a few samples. No differences in the allele or genotype frequencies between the breast cancer cases and controls were detected in any population. Nor did the joint analysis show any differences between the breast cancer cases and controls (Table 2). The lack of association remained when the data were stratified by age (data not shown).

Haplotypes for the *VEGF* gene were determined for the familial and unselected breast cancer cases separately because

different polymorphisms were analyzed in these two series. The results revealed that the promoter polymorphisms −2578 and −1154 as well as the −2578 and −634 polymorphisms were in tight linkage disequilibrium ($|D'|$ = 0.95 and 1.00, respectively), consistent with a previous report (11). The +936 polymorphism was not linked to the other polymorphisms ($|D'|$ = 0.02-0.25). Haplotypes were created using genotyping data of the polymorphisms −2578C/A, −1154G/A, and +936C/T in the familial series and the polymorphisms −2578C/A, −634G/C, and +936C/T in the unselected series. Because of the tight linkage between the promoter polymorphisms, haplotypes CAC and CAT were only present among 0.8% of familial cases and controls. Similarly, haplotypes ACC and ACT were missing from the unselected breast cancer series, and haplotype CGT was present only in 0.3% of the samples. The frequencies of the haplotypes at positions −2578/−1154 and −2578/−634 in the cases and controls were similar as reported (11, 19). No significant differences in haplotype frequencies between the breast cancer cases and controls were detected (Fig. 2).

When we stratified the unselected breast cancer cases by all tumor characteristics listed in Table 1, two interesting associations emerged (Table 3). Because of missing data, the numbers of cases in Table 3 were less than in Table 2. The

Table 3. Associations of the *VEGF* genotypes with tumor characteristics in the Swedish population

	Polymorphism/genotype*					
	−634G/C			−2578C/A		
	GG (%)	GC (%)	CC (%)	CC (%)	CA (%)	AA (%)
Tumor size (mm)						
≤20	271 (53.8)	194 (38.5)	39 (7.7)	138 (27.3)	238 (47.0)	130 (25.7)
>20	101 (43.5)	99 (42.7)	32 (13.8)	73 (30.9)	106 (44.9)	57 (24.2)
OR (95% CI)	1.00	1.37 (0.97-1.94)	2.20 (1.27-3.82)	1.00	0.84 (0.58-1.23)	0.83 (0.53-1.29)
P		0.08	0.004		0.41	0.44
Histologic grade						
Grade 1	79 (57.2)	51 (37.0)	8 (5.8)	28 (20.4)	67 (48.9)	42 (30.7)
Grade 2	164 (52.1)	126 (40.0)	25 (7.9)	90 (28.7)	147 (46.8)	77 (24.5)
Grade 3	111 (46.2)	96 (40.0)	33 (13.8)	77 (31.4)	114 (46.5)	54 (22.0)
P_{trend}		0.44	0.009		0.19	0.04
Stage†						
0 + I	206 (48.8)	174 (41.2)	42 (10.0)	118 (27.8)	208 (49.1)	98 (23.1)
II-IV	183 (53.5)	130 (38.0)	29 (8.5)	96 (27.7)	159 (46.0)	91 (26.3)
OR (95% CI)	1.00	0.84 (0.61-1.15)	0.78 (0.45-1.34)	1.00	0.94 (0.66-1.34)	1.14 (0.76-1.72)
P		0.30	0.40		0.78	0.58
Regional lymph node metastasis						
Negative	281 (50.2)	224 (40.0)	55 (9.8)	158 (28.2)	266 (47.4)	137 (24.4)
Positive	114 (49.4)	92 (40.8)	25 (10.8)	65 (28.3)	116 (50.4)	49 (21.3)
OR (95% CI)	1.00	1.01 (0.72-1.42)	1.12 (0.64-1.94)	1.00	1.06 (0.73-1.55)	0.87 (0.55-1.38)
P		0.99	0.77		0.82	0.60
Distant metastasis‡						
Negative	377 (50.5)	297 (39.8)	72 (9.7)	211 (28.1)	353 (46.9)	188 (25.0)
Positive	8 (72.7)	1 (9.1)	2 (18.2)	5 (45.5)	4 (36.4)	2 (18.2)
OR (95% CI)	1.00	0.16 (0.00-1.20)	1.31 (0.13-6.74)	1.00	0.48 (0.09-2.25)	0.45 (0.04-2.79)
P		0.08	0.67		0.31	0.46
Estrogen receptors						
Positive	158 (45.0)	153 (43.6)	40 (11.4)	110 (31.3)	162 (46.0)	80 (22.7)
Negative	75 (56.0)	45 (33.6)	14 (10.4)	44 (31.9)	58 (42.0)	36 (26.1)
OR (95% CI)	1.00	0.62 (0.39-0.97)	0.74 (0.36-1.50)	1.00	0.90 (0.55-1.46)	1.13 (0.64-1.97)
P		0.04	0.46		0.72	0.76
Progesterone receptors						
Positive	123 (45.2)	119 (43.8)	30 (11.0)	85 (29.5)	121 (44.5)	66 (24.3)
Negative	78 (51.7)	59 (39.1)	14 (9.3)	46 (29.5)	73 (46.8)	37 (23.7)
OR (95% CI)	1.00	0.78 (0.50-1.22)	0.74 (0.35-1.55)	1.00	1.11 (0.68-1.82)	1.04 (0.58-1.84)
P		0.30	0.49		0.73	0.99

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval.
*Comparison with the genotype indicated by an odds ratio of 1.00.
† 54% of samples were stage I and 41% were stage II.
‡ Fisher exact test.

−634CC genotype showed statistically significant association to higher tumor aggressiveness (large tumor size and high grade, $P < 0.01$). The −2578AA genotype was correlated with low-grade tumors ($P = 0.04$). Because the −2578 and −634 polymorphisms were in tight linkage disequilibrium, whereas the +936 polymorphism was not linked to these polymorphisms, the haplotype analysis was restricted to the −2578C/A and −634G/C polymorphisms (Table 4). The haplotype −2578/−634 CC showed significant association with higher tumor aggressiveness (large tumor size and higher grade, $P \leq 0.005$). There was a significant trend to larger tumor size with the increasing copy number of the haplotype ($P_{trend} = 0.008$), and individuals with two copies of the haplotype had both significantly larger and higher-grade tumors ($P = 0.005$). A less significant association was observed between the haplotype −2578/−634 AG and a lower tumor grade ($P = 0.04$) and only in carriers of two copies of this haplotype. Carriers of the −634 C allele tended to have more often estrogen receptor–positive tumors than carriers of the GG genotype. Because this effect was linked only to heterozygous carriers, the results are difficult to interpret. There were no indications that the polymorphisms would have any effects on the other tumor characteristics (Table 3). Addition of the information of the +936C/T polymorphism data did not change the results (data not shown).

Discussion

Functional polymorphisms, which have an effect on the regulation of gene expression, can contribute to the differences between individuals in susceptibility to and severity of a disease. The effect may be seen by a 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 (4–10). We analyzed the familial series for the −2578, −1154, and +936 polymorphisms using RFLP assay. For the unselected breast cancer series, a Taqman assay was used. Because the Taqman assay did not work for the −1154 polymorphism, we analyzed the −634 polymorphism instead.

The allele and genotype distributions of the four polymorphisms were in close agreement with those previously published for healthy Caucasian individuals (8, 11, 12, 18–21). In a previous study among 500 Caucasian breast cancer cases and 500 controls, Krippl et al. have shown a decreased risk of breast cancer in individuals who were *+936 T* allele carriers (9). However, the genotypes in patients did not follow the Hardy-Weinberg equilibrium. In another study, no association between the +936 polymorphism and risk to breast cancer among 862 cases and 713 controls could be observed (21). Here, we did a large case-control study of 1,489 women with breast cancer, including 565 women with familial breast cancer from Poland and Germany and 924 unselected breast cancer cases from Sweden. The use of familial cases can substantially increase the power of association studies as shown earlier (22, 23). We observed no differences in the allele or genotype frequencies between either the familial or unselected breast cancer case and respective control groups, nor did the joint analyses show any differences between the cases and controls (odds ratio, 0.99; 95% confidence interval, 0.85-1.15; $P = 0.93$). To our knowledge, no other studies on the effect of the other polymorphisms on the risk of breast cancer have been published. In our study, no significant differences in the allele, genotype or haplotype distribution of the polymorphisms in the *VEGF* gene between the familial and unselected breast cancer cases and respective controls were detected. Being the largest study thus far, and with one third being familial cases, our study provided strong evidence that the *+936 T* allele or the other studied polymorphisms do not modify the risk of breast cancer. This result is not surprising, because VEGF, as a key mediator of angiogenesis, is more likely to alter the aggressiveness of the tumor than susceptibility to cancer.

Table 4. Associations of the *VEGF* haplotypes with tumor characteristics in the Swedish population

	Tumor size (mm)				Histologic grade			
	≤20	>20	OR for tumor size	*P*	Grade 1	Grade 2	Grade 3	P_{trend}
Haplotype −2578/−634*								
CC	263 (26.9)	153 (35.0)	1.47 (1.15-1.89)	0.002	60 (23.4)	166 (27.6)	159 (34.1)	0.005
AG	479 (49.0)	196 (45.0)	0.85 (0.67-1.07)	0.18	140 (54.6)	287 (47.6)	208 (44.6)	0.04
Haplotype in individuals†								
No. CC copies in an individual								
0	264 (54.0)	95 (43.6)	1.00		75 (58.6)	159 (52.8)	107 (45.9)	
1	187 (38.2)	93 (42.7)	1.38 (0.97-1.97)	0.08	46 (35.9)	118 (39.2)	93 (39.9)	0.33
2	38 (7.8)	30 (13.8)	2.19 (1.24-3.86)	0.005	7(5.5)	24 (8.0)	33(14.2)	0.005
No. AG copies in an individual								
0	134 (27.4)	71 (32.6)	1.00		26 (20.3)	87 (28.9)	75 (32.2)	
1	231 (47.2)	98 (45.0)	0.80 (0.54-1.18)	0.28	64 (50.0)	141 (46.8)	108 (46.4)	0.15
2	124 (25.4)	49 (22.5)	0.75 (0.47-1.18)	0.23	38 (29.7)	73 (24.3)	50 (21.5)	0.04

*Comparison with all the other haplotypes.
† Comparison with the noncarriers of the specific haplotype indicated by an odds ratio of 1.00.

Among the unselected breast cancer cases, we observed a significant correlation between the −634CC genotype and a larger tumor size and a higher histologic grade of the tumors. In addition, the haplotype −2578/−634 CC was associated with more aggressive tumors. In addition, our results showed that the −634GG genotype correlated with less aggressive tumors and the −2578AA genotype and the haplotype −2578/−634 AG with low-grade tumors. Our results are in contrast to the study of Howell et al. who have reported an association between the rare −2578/−1154/−634 CAC haplotype and a less advanced melanoma (11). However, our results are in agreement with the reports regarding the effect of the polymorphisms on VEGF production. The −634CC genotype has been reported to be associated with higher serum levels of VEGF than the GG genotype (7) and the two common haplotypes −2578/−1154/−634 AAG and AGG with a decreased transcription of the *VEGF* gene and lower circulating levels of VEGF (19). Moreover, the −2578CC genotype and the *−2578C* allele have been reported to correlate with a higher VEGF production than the A allele *in vitro* (5, 6).

In summary, the present study investigated polymorphisms in the *VEGF* gene in a large case-control study. None of the polymorphisms alone or in combination with each other was found to influence the risk of breast cancer, either in the familial or unselected cases. Our study provided evidence that the +936C/T polymorphism is not associated with breast cancer risk. However, some genotypes and haplotypes in the *VEGF* gene may have an effect on breast tumor growth. Functional studies of the haplotypes and an independent study are needed to confirm our results. The polymorphisms should also be studied in relation to metastasis and survival and whether they influence therapeutic effects.

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