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Genetic Variation in *VEGF* Family Genes and Breast Cancer Risk: A Report from the Shanghai Breast Cancer Genetics Study

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Abstract

Background: In addition to mediating aspects of physiologic and pathologic angiogenesis, the VEGF family also contributes to carcinogenesis.

Methods: We comprehensively characterized genetic variation across four *VEGF* family genes and evaluated associations with breast cancer risk with odds ratios (OR) and 95% CIs for participants of the two-stage case–control Shanghai Breast Cancer Genetics Study (SBCGS). Stage 1 evaluated 200 single nucleotide polymorphisms (SNP) across two *VEGF* ligands (*VEGFA* and *VEGFC*) and two *VEGF* receptors (*FLT1*/*VEGFR1* and *KDR*/*VEGFR2*) among 2,079 cases and 2,148 controls. Five SNPs with promising associations were assessed in stage 2 among 4,419 cases and 1,851 controls.

Results: Two SNPs were consistently associated with breast cancer risk across our two study stages and were significant in combined analyses. Compared with *FLT1 rs9551471* major allele homozygotes (*AA*), reduced risks were associated with *AG* (OR = 0.92, 95% CI: 0.84–1.00) and *GG* (OR = 0.78, 95% CI: 0.64–0.95) genotypes ($P_{trend} = 0.005$). Compared with *VEGFA rs833070* major allele carriers (*CC* or *CT*), increased risk was associated with *TT* genotypes (OR = 1.26, 95% CI: 1.05–1.52, $P = 0.016$).

Conclusion: Results from our study indicate that common genetic variation in *VEGFA* and *FLT1* (*VEGFR1*) may contribute to breast cancer susceptibility.

Impact: Our findings provide clues for future studies on *VEGF* family genes in relation to cancer susceptibility and survival. *Cancer Epidemiol Biomarkers Prev; 20(1); 33–41; ©2011 AACR.*

Introduction

The human VEGF family includes 5 growth factors and 3 tyrosine kinase receptors that mediate various aspects of both physiologic and pathologic angiogenesis, lymphangiogenesis, and vasculogenesis (1–3). In addition to normal processes, such as ovarian follicular development and embryogenesis, VEGF family members are also involved in several diseases, including macular degeneration, rheumatoid arthritis, endometriosis, and cardiovascular disease (2–6). In regards to cancer, the actions of the VEGF family are crucial for tumor growth and metastasis, as tumors cannot grow beyond a limited initial size without the establishment of blood flow for acquiring nutrients and carrying away debris (2, 3). In addition to this angiogenic switch, VEGF family members can also contribute to cancer development by promoting cell growth and enhancing cell survival (1, 2, 4, 6, 7).

The members of the VEGF family have both unique and overlapping functions, which are mediated by specific ligand–receptor interactions. VEGFA is a potent endothelial mitogen that also regulates vascular permeability, cellular motility, and survival (2–4, 7). VEGFC influences the migration and survival of lymphatic endothelial cells and supports the maintenance of differentiated lymphatic endothelium (2, 3). Other VEGF family growth factors include placental growth factor (PGF), which enhances VEGF signaling, FIGF/VEGFD (*c-fos*–induced growth factor), which contributes to lymphangiogenesis, and VEGFB, the function of which remains unclear (2, 3). Because of alternative splicing, multiple isoforms of VEGFA, VEGFB, and PlGF exist; these isoforms differ in their mRNA stability, amino acid sequences, protein localization, and functions (1–3). The

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VEGF receptor 1 (VEGFR1), also known as FMS-like tyrosine kinase-1 (FLT1), has highest affinity for VEGFA but can also bind VEGFB or PlGF (1, 2, 8). Because VEGFA binding results in only a small increase in kinase activity, FLT1 functions primarily as a negative regulator of angiogenesis by sequestering VEGFA; however, positive roles in the regulation of vascular permeability, cell motility, and apoptotic inhibition have also been identified (1–3, 8). FLT1 also undergoes alternative splicing to encode either a membrane-bound receptor or a soluble variant (sFLT1) that acts to bind VEGFA and block its signaling (1–3). VEGFR2, also known as KDR (kinase-insert domain containing receptor), has lower affinity for VEGFA than FLT1 but a much higher protein-tyrosine kinase activity (1–3). KDR is the primary mediator of normal angiogeneic signaling by VEGFA but can also bind VEGFC and FIGF (1–3). VEGFR3, also known as FLT4, binds to VEGFC and FIGF and contributes to angiogenesis, lymphangiogenesis, and cell survival (1, 2).

Thus, VEGFA is the key mediator of angiogenesis and therefore intertwined in cancer development via the angiogenic switch (1–4). Furthermore, *VEGFA* and other *VEGF* family genes also have crucial roles in cell survival, mitogenesis, migration, differentiation, vascular permeability, and mobilization and therefore may influence cancer development (1, 2, 4, 8). Common genetic variation in *VEGF* family genes may influence gene transcription, mRNA stability, protein splicing, and protein functions and therefore cancer risk. Several previous studies have examined genetic variation in 8 single nucleotide polymorphisms (SNP) in *VEGF-A* (*rs699947*, *rs1005230*, *rs833061*, *rs1570360*, *rs2010963*, *rs25648*, *rs10434*, and *rs3025039*) in relation to breast cancer risk; results have been inconsistent (9–18). Studies of genetic variation in other *VEGF* family genes and breast cancer risk are sparse; 1 *FLT1* SNP (*−962 C/T*) and 2 *KDR* SNPs (*rs2305948* and *rs1870377*) were evaluated in 2 studies with null results (18, 19). Furthermore, no studies employed a 2-stage genotyping approach to minimize false-positive findings. We therefore conducted a large, 2-stage study, in which we comprehensively evaluated genetic variation in 4 major genes in the *VEGF* family in relation to breast cancer risk among participants of the Shanghai Breast Cancer Genetics Study (SBCGS).

Materials and Methods

Study population

Subjects were participants of 3 population-based studies conducted among Chinese women in urban Shanghai: the Shanghai Breast Cancer Study (SBCS), the Shanghai Breast Cancer Survival Study (SBCSS), and the Shanghai Endometrial Cancer Study (SECS). Collectively, these studies comprise the SBCGS; detailed methods for these studies have been previously reported (20). Briefly, the SBCS is a large 2-stage (SBCS-I and SBCS-II), population-based, case–control study. Breast cancer cases were identified via a rapid case-ascertainment system and the Shanghai Cancer Registry; diagnoses were confirmed by 2 senior pathologists. Controls were randomly selected using the Shanghai Resident Registry. SBCS-I recruitment occurred between August 1996 and March 1998 and included women aged 25 to 65. SBCS-II recruitment occurred from April 2002 to February 2005 and was expanded to include women aged 20 to 70. Also included in the study were cases recruited between April 2002 and December 2006 as part of the SBCSS, a population-based study of newly diagnosed breast cancer cases identified by the Shanghai Cancer Registry, and controls recruited between January 1997 and December 2003 as part of the SECS, a population-based, case–control study of endometrial cancer that included women aged 25 to 70 that was conducted in a manner almost identical to the SBCS. Of those eligible, 1,459 cases (91.1%) and 1,556 controls (90.3%) from SBCS-I, 1,989 cases (83.7%) and 1,989 (70.4%) controls from SBCS-II, and 5,046 cases (80.1%) from the SBCSS and 1,212 controls (74.4%) from the SECS completed in-person interviews with structured questionnaires. Blood or buccal cell samples were donated and available for 1,193 cases (81.8%) and 1,310 controls (84.2%) from SBCS-I, 1,932 cases (97.1%) and 1,857 controls (93.4%) from SBCS-II, and 4,845 (96.0%) cases from the SBCSS and 1,039 (85.7%) controls from the SECS. Because of a time overlap in subject recruitment, 1,469 breast cancer patients participated in both the SBCS-II and the SBCSS and 109 controls participated in both the SBCS-I and SECS so that the actual number of participants included in the current analysis from SBCSS and SECS was 3,466 and 930, respectively. For breast cancer cases, clinical characteristics were ascertained by medical record abstraction using a standard protocol. Stage of disease was available for 5,992 cases (92.2%). Of these breast cancer cases, 36.1% were stage 0 or 1, 34.7% were stage 2, 18.6% were stage 3, and 10.7% were stage 4. Estrogen receptor (ER) status was available for 5,967 cases (91.8%); 64.2% of these cases were ER positive and 36.8% were ER negative. Progesterone receptor (PR) status was available for 5,941 cases (91.4%); 59.5% of these cases were PR positive and 40.6% were PR negative. Genomic DNA for all included participants was extracted using commercial DNA purification kits. Informed consent was granted by all included women, and approval was granted from relevant review boards in both China and the United States.

SNP selection and genotyping

Included in the current analysis were SNPs from 4 *VEGF* gene family members for which we had high genetic coverage of HapMap SNPs (>80% with a pairwise $r^2 = 0.8$) that had minimum minor allele frequencies (MAFs) of 0.05 among Han Chinese (HCB). Stage 1 genotyping included 4 methods; all available data were utilized to maximize our coverage of genetic variation. First, haplotype tagging SNPs (htSNP) were selected from HCB data from the HapMap Project, using the Tagger program, to capture SNPs with an MAF of

0.05 in the *VEGFA*, *VEGFC*, *FLT1*, and *KDR* genes (±5 kb), with an $r^2 \geq 0.90$. Seventy-four htSNPs were successfully designed and genotyped among 2,131 SBCS-I participants, using a Targeted Genotyping System (Affymetrix), in 2006. Second, to increase the density of genetic markers in this study, data from our Affymetrix Genome-Wide SNP Array 6.0 (Affymetrix) was included for 150 SNPs that were genotyped among 4,157 SBCS-I and SBCS-II participants. Third, 4 SNPs (*rs833061*, *rs1570360*, *rs2010963*, and *rs3025039*) were genotyped by TaqMan among 2,350 SBCS-I participants; *rs3025039* was additionally genotyped among 3,777 SBCS-II participants. Finally, 2 SNPs (*rs9554312* and *rs9582036*) were genotyped with the Sequenom iPLEX MassARRAY platform among 3,777 SBCS-II participants. Twenty-eight SNPs were genotyped by more than 1 method, generating a total of 200 SNPs. Stage 1 analysis was conducted for 132 SNPs, with a minimum MAF of 0.05 among genotyped controls. SNPs with promising associations were further evaluated for linkage disequilibrium (LD) and consistency of associations with breast cancer risk when stratified by SBCS population, when possible. Stage 2 genotyping among 3,453 SBCSS cases and 914 SECS controls and 966 cases and 937 controls from SBCS that were not genotyped in stage 1 was conducted with the Sequenom platform as previously described. For all genotyping methods, blinded duplicate samples and quality controls were included. All included SNPs had concordance rates of at least 95% among duplicates within each platform, as well as across genotyping platforms. Specifically, for Affymetrix targeted genotyping, the average call rate was 99.7%. Blinded ($n = 39$) and HapMap samples ($n = 12$) were included, consistency rates averaged 99.6%. For Affymetrix genome-wide association study (GWAS) SNPs analyzed in this study, the average call rate was 99.7%. Three quality control samples were included per plate (20); the average concordance rate was 99.9% for 150 SNPs. For TaqMan targeted genotyping, the average call rate was 96.7. Eight blinded and 8 unblinded quality control samples were included per plate; concordance rates were greater than 97% (13). For Sequenom targeted genotyping, the average call rate was 97.7%. Two blinded and 2 HapMap samples were included per plate; the average concordance rate was 99.5% for 7 SNPs. Laboratory personnel were blinded to the case–control status of all samples.

Statistical analysis

Characteristics between cases and controls were compared with the χ^2 test or t test for categorical or continuous variables, respectively. Stage 1 odds ratios (OR) and corresponding confidence intervals (95% CI) were determined by logistic regression that included adjustment for age and education. For SNPs genotyped in stage 2, pooled analyses of stage 1 and stage 2 data included additional adjustment for genotyping stage. Additive, dominant, and recessive models of effect were employed. Multiplicative interactions between genetic variants and family history of breast cancer or menopausal status were evaluated by comparing estimates of effect across strata for heterogeneity, as well as the statistical significance of interaction terms in logistic regression models. The majority of analyses were conducted with SAS v9.2. Permutation tests were conducted using the max(T) approach in PLINK v1.07 with 1,000 iterations to determine the family-wise corrected P value for SNP–breast cancer associations after correcting for the number of variants evaluated while considering their underlying LD structure (21). All statistical tests were 2-tailed, and $P \leq 0.05$ values were interpreted as statistically significant.

Results

In stage 1, inherited genetic variation in 4 *VEGF* family genes was comprehensively characterized (Table 1). A total of 200 SNPs were genotyped across 2 VEGF family ligands (*VEGFA* and *VEGFC*) and 2 receptors

Table 1. SNP information for included *VEGF* family gene members

VEGF family gene	Genomic location	Gene span, kb	SNPs in HapMap[a]	SNPs genotyped	Genetic variation coverage[b]	
					$r^2 = 0.8$	$r^2 = 0.9$
Ligands						
VEGFA	6p12	16.3	27	22	85.2%	77.8%
VEGFC	4q34	109.2	59	29	93.2%	78.0%
Receptors						
FLT1 / VEGFR1	13q12	193.4	127	119	94.5%	91.3%
(KDR / VEGFR2)	4q12	47.1	55	30	98.2%	96.4%

[a] HapMap data among HCB, SNPs with MAF greater than 0.05%.

[b] Coverage of HapMap CHB SNPs by our genotyped SNPs, using a pairwise tagging approach in Tagger.

(*FLT1/VEGFR1* and *KDR/VEGFR2*); of these, 132 had MAFs of at least 5% among controls. Using a pairwise tagging approach and an $r^2 = 0.9$ or 0.8, the coverage of SNPs with minimum MAF of 0.05% identified in HCB included in the HapMap project was greater than 77% or 85%, respectively. Twenty-eight SNPs were genotyped by more than 1 method, the majority of the overlap occurred between Affymetrix targeted genotyping and GWAS data ($N = 26$). Consistency rates ranged from 97.98% to 100% and averaged 99.62%; the average number of participants genotyped by both methods was 1,846.

A total of 10,497 women were included in the current study; 4,227 women in stage 1 and 6,270 women in stage 2. Participants genotyped in the 2 stages were generally comparable (Table 2). Established breast cancer risk factors, including early age at menarche, late age at menopause, late age at first live birth, a first-degree relative with breast cancer, history of fibroadenoma, high body mass index (BMI) or waist-to-hip ratio (WHR), and low physical activity, were found to be associated with breast cancer risk among SBCGS participants. Women who did not donate DNA samples ($N = 1,088$) were generally comparable with women who did, although these women were younger, less educated, had earlier ages at menopause, lower BMI and/or WHRs, and were also less likely to engage in regular physical activity than women who donated DNA samples.

Associations with breast cancer risk for 132 SNPs with MAF ≥ 0.05 genotyped in stage 1 yielded significant *P* values (≤ 0.05) in either additive, dominant, or recessive models for 12 SNPs (Supplementary Table 1). LD and consistency of associations between SBCS-I and SBCS-II, when possible, were evaluated for these 12 variants. Seven variants were not selected for additional genotyping because of 3 reasons: high LD with SNPs selected (*rs718273* and *rs10507385*), inconsistency of associations with breast cancer when stratified by SBCS population (*rs17063612*, *rs4771249*, *rs9508016*, and *rs12429309*), or high LD with variants that had inconsistent associations when stratified by SBCS population (*rs3794400*). Five SNPs were selected for additional genotyping in stage 2, including 1 in *VEGFA* (*rs8330070*), 3 in *FLT1* (*rs9551471*, *rs3812867*, and *rs9554312*), and 1 in *KDR* (*rs10006115*). Associations with breast cancer risk for these 5 SNPs are given in Table 3. Three SNPs showing significant associations in stage 1 were not replicated in stage 2 (*rs3812867*, *rs9554312*, and *rs10006115*), whereas 2 SNPs showed associations in both genotyping stages that were generally consistent (*rs833070* and *rs9551471*). Combined analyses of stage 1 and 2 data revealed an uncorrected significant association of breast cancer risk for *VEGFA rs833070* in recessive models and for *FLT1 rs9551471* regardless of genetic model. Permutation tests were employed to evaluate the corrected statistical significance of these associations. The permutation test corrected recessive *P* value for *VEGFA rs833070* was 0.13, and the permutation test corrected allelic *P* value for *FLT1 rs9551471* was 0.02.

The effects associated with *VEGFA rs833070* and *FLT1 rs9551471* were found to be independent ($P_{interaction} = 0.505$) and so a combined analysis was conducted by summing the total number of risk alleles/genotypes (Table 4). To accommodate differences in types and directions of associations with breast cancer risk, a risk allele/genotype was defined as a minor allele homozygote for *VEGFA rs833070* and increasing copies of the major allele for *FLT1 rs9551471*. Compared with women without any risk alleles/genotypes (4.3%), those with 1 (32.5%), 2 (60.0%), or 3 (3.2%) risk alleles/genotypes were greater than 30% (OR: 1.33, 95% CI: 1.13–1.57), 40% (OR: 1.45, 95% CI: 1.24–1.70), and 80% (OR: 1.88, 95% CI: 1.42–2.50) more likely to be breast cancer cases, respectively. This association followed a significant linear trend ($P = 0.0004$). Additional adjustment for menopausal status, hormone replacement therapy, and regular physical activity did not substantially alter these results (data not shown). When stratified, no effect measure heterogeneity was found by menopausal status or family history of breast cancer; multiplicative interaction terms were not statistically significant. In addition, cases were stratified by tumor stage and ER or PR status. The risk of breast cancer associated with *VEGFA rs833070* and *FLT1 rs9551471* was generally consistent by disease stage and ER or PR status, although effects seemed to be more pronounced among early-stage patients as well among patients with PR-negative tumors (Table 4).

Discussion

Inherited genetic variation in 2 *VEGF* family ligands (*VEGFA* and *VEGFC*) and 2 *VEGF* family receptors (*FLT1* and *KDR*) was comprehensively characterized among participants of the SBCGS. Two SNPs were found to have consistent relationships with breast cancer risk. Minor allele homozygotes of *VEGFA rs833070* had an increased risk of breast cancer, whereas the minor allele of *FLT1 rs9551471* was associated with decreasing breast cancer risk in a dose–response manner. To our knowledge, neither of these polymorphisms has been previously evaluated in relation to cancer susceptibility.

Previous studies of genetic variation in *VEGF* gene family members and breast cancer risk have included a limited number of polymorphisms with mixed findings. *VEGFA* SNPs discussed, including their names and relative locations, are shown in Figure 1. Two *VEGFA* SNPs (*rs1005230* and *rs10434*) have had null results in a single study (17), whereas 3 SNPs (*rs1570360*, *rs2010963*, and *rs25648*) have consistently not been associated with breast cancer risk in several studies (10–14, 17, 18). On the contrary, 3 SNPs (*rs699947*, *rs833061*, and *rs3025039*) have had inconsistent results. The *rs699947 CC* genotype was associated with a significantly increased risk of breast cancer (OR: 1.99, 95% CI: 1.06–3.74) in 1 study (18) but not associated in 4 other studies (11, 12, 14, 17). Similarly, the *rs833061 CC* genotype was associated with a significantly increased risk (OR: 2.01, 95% CI: 1.08–3.76) in 1 study (18)

Table 2. Demographic and other breast cancer risk factors, by genotyping stage: the SBCGS

Characteristic	Study Stage 1 (N = 4,227)			Study Stage 2 (N = 6,270)			By Genotyping Status (N = 11,585)		
	Cases (*N* = 2,079)	Controls (*N* = 2,148)	*P*	Cases (*N* = 4,419)	Controls (*N* = 1,851)	*P*	Genotyped (*N* = 10,497)	Not genotyped (*N* = 1,088)	*P*
Demographic factors									
Age, y	49.3 ± 8.3	49.1 ± 8.7	0.390	53.9 ± 10.2	53.3 ± 8.8	**0.016**	51.8 ± 9.5	50.6 ± 9.1	<**0.001**
Education (less than middle school)	206 (9.9%)	286 (13.3%)	<**0.001**	525 (11.9%)	304 (16.4%)	<**0.001**	1,255 (12.0%)	163 (15.0%)	**0.004**
Reproductive risk factors									
Age at menarche, y	14.5 ± 1.7	14.7 ± 1.8	<**0.001**	14.4 ± 1.7	14.7 ±1.8	<**0.001**	14.5 ± 1.7	14.5 ±1.7	0.902
Postmenopausal	804 (38.8%)	873 (40.7%)	0.195	2,257 (51.1%)	1,047 (56.6%)	<**0.001**	4,988 (47.5%)	507 (46.6%)	0.572
Age at menopause y[a]	48.4 ± 4.5	47.7 ± 4.8	**0.002**	49.0 ± 4.3	48.8 ± 4.1	0.162	48.6 ± 4.4	48.2 ± 4.5	**0.042**
Age at first live birth, y[b]	26.5 ± 3.9	26.0 ± 3.8	**0.001**	26.8 ± 3.9	25.5 ± 4.0	<**0.001**	26.8 ± 3.8	26.6 ± 4.2	0.158
Use of oral contraceptives[c]	419 (20.2%)	433 (20.2%)	0.997	166 (17.2%)	416 (22.5%)	**0.001**	1,434 (20.4%)	205 (20.9%)	0.696
Additional risk factors									
First-degree relative with breast cancer	92 (4.4%)	63 (2.9%)	**0.010**	246 (5.6%)	24 (2.6%)	<**0.001**	447 (4.3%)	37 (3.4%)	0.182
Use of hormone replacement therapy[d]	65 (3.1%)	51 (2.4%)	0.136	161 (5.7%)	70 (3.8%)	**0.003**	347 (3.9%)	26 (2.7%)	0.054
History of breast fibroadenomas[e]	201 (9.7%)	121 (5.6%)	<**0.001**	96 (10.0%)	48 (5.1%)	<**0.001**	514 (7.3%)	67 (6.9%)	0.614
BMI kg/m^2	23.8 ± 3.3	23.3 ± 3.4	<**0.001**	24.0 ± 3.5	23.6 ± 3.3	<**0.001**	23.7 ± 3.4	23.3 ± 3.4	<**0.001**
WHR	0.822 ± 0.06	0.808 ± 0.06	<**0.001**	0.834 ± 0.05	0.816 ± 0.05	<**0.001**	0.823 ± 0.06	0.812 ± 0.05	<**0.001**
Regular physical activity	521 (25.1%)	638 (29.7%)	<**0.001**	2,473 (56.0%)	622 (33.6%)	<**0.001**	4,245 (40.5%)	299 (27.5%)	<**0.001**

NOTE: Continuous variables: mean values ± SD, *P* value from *t* tests; categorical variables: numbers and percentages, *P* values from χ^2 test. Bold values considered to be significant $P \leq 0.05$

[a] Among postmenopausal women.

[b] Among parous women.

[c] Information unavailable for 3,456 participants genotyped in stage 2.

[d] Information unavailable for 1,601 participants genotyped in stage 2.

[e] Information unavailable for 4,375 participants genotyped in stage 2.

Table 3. Selected *VEGF* family SNPs and breast cancer risk: the SBCGS

Gene, SNP, Information[a]	N genotyped (cases/controls)	Genotype frequencies[b]	Breast cancer risk, Additive models[c]			Dominant models[d]		Recessive models[e]	
			AB OR (95% CI)	BB OR (95% CI)	*P*	AB/BB OR (95% CI)	*P*	BB OR (95% CI)	*P*
VEGFA rs833070 (C/T), 22.6%, intron 2									
Genotyping stage 1	2,066/2,141	58.3/37.0/4.7	0.96 (0.84–1.09)	**1.35 (1.03–1.77)**	0.378	1.00 (0.89–1.13)	0.994	**1.37 (1.05–1.79)**	**0.022**
Genotyping stage 2	4,329/1,796	60.9/34.4/4.7	1.05 (0.93–1.18)	1.18 (0.91–1.53)	0.189	1.06 (0.95–1.19)	0.284	1.16 (0.90–1.50)	0.251
Combined	6,395/3,937	59.5/35.8/4.7	1.01 (0.92–1.10)	**1.26 (1.05–1.53)**	0.107	1.04 (0.95–1.13)	0.398	**1.26 (1.05–1.52)**	**0.016**
					permutation corrected recessive p-value = 0.13				
FLT1 rs9551471 (A/G), 22.7%, intron 6									
Genotyping stage 1	2,073/2,081	59.1/36.0/4.9	**0.87 (0.76–0.99)**	0.81 (0.61–1.09)	**0.018**	**0.86 (0.76–0.97)**	**0.018**	0.86 (0.64–1.15)	0.296
Genotyping stage 2	4,301/1,786	60.5/34.2/5.3	0.96 (0.85–1.08)	**0.75 (0.58–0.98)**	0.081	0.93 (0.83–1.05)	0.231	**0.76 (0.59–0.99)**	**0.039**
Combined	6,374/3,867	61.3/34.2/4.5	0.92 (0.84–1.00)	**0.78 (0.64–0.95)**	**0.005**	**0.90 (0.83–0.98)**	**0.016**	**0.80 (0.66–0.97)**	**0.025**
					permutation corrected allelic p-value = **0.02**				
FLT1 rs3812867 (G/A), 9.4%, exon 30 (3' UTR)									
Genotyping stage 1	2,068/2,145	82.8/16.7/0.5	0.95 (0.81–1.12)	**2.26 (1.07–4.78)**	0.741	0.99 (0.84–1.16)	0.877	**2.28 (1.07–4.82)**	**0.032**
Genotyping stage 2	4,354/1,794	81.6/17.6/0.9	0.90 (0.78–1.05)	0.82 (0.45–1.51)	0.139	0.90 (0.78–1.04)	0.147	0.84 (0.46–1.53)	0.566
Combined	6,422/3,939	82.7/16.5/0.8	0.92 (0.83–1.03)	1.26 (0.78–2.03)	0.352	0.94 (0.84–1.04)	0.221	1.27 (0.79–2.06)	0.324
FLT1 rs9554312 (A/G), 30.2%, 3' FR[f]									
Genotyping stage 1	1,587/1,623	51.9/38.5/9.6	**1.21 (1.04–1.40)**	1.24 (0.98–1.58)	**0.010**	**1.21 (1.06–1.39)**	**0.006**	1.14 (0.91–1.44)	0.249
Genotyping stage 2	4,344/1,798	47.2/42.8/10.1	0.95 (0.85–1.07)	0.91 (0.75–1.11)	0.272	0.94 (0.85–1.06)	0.311	0.93 (0.78–1.12)	0.469
Combined	5,931/3,421	48.6/41.6/9.9	1.04 (0.95–1.14)	1.03 (0.88–1.19)	0.476	1.04 (0.95–1.13)	0.377	1.01 (0.87– 1.16)	0.924
KDR rs10006115 (G/T), 9.4%, intron 29									
Genotyping stage 1	1,917/1,924	83.3/16.0/0.8	**1.23 (1.04–1.46)**	0.98 (0.47–2.03)	**0.028**	**1.22 (1.04–1.44)**	**0.017**	0.94 (0.45–1.95)	0.870
Genotyping stage 2	4,340/1,797	80.4/19.0/0.7	0.91 (0.79–1.05)	1.18 (0.61–2.30)	0.345	0.92 (0.80–1.06)	0.260	1.20 (0.62–2.34)	0.586
Combined	6,257/3,721	81.6/17.7/0.8	1.03 (0.93–1.15)	1.08 (0.67–1.76)	0.521	1.03 (0.93–1.15)	0.538	1.08 (0.66–1.75)	0.765

NOTE: Estimates and *P* values in bold are significant at $P < 0.05$.

[a] SNP information includes alleles (major or reference allele / minor or test allele) as determined by allele frequency among all genotyped controls, minor allele frequency among all genotyped controls, and region of the gene where SNP is located.

[b] Genotype frequencies for AA (major allele homozygotes)/AB (heterozygotes)/BB (minor allele homozygotes) among controls.

[c] Breast cancer risk for heterozygotes (AB) and homozygotes (BB), each compared with major allele homozygotes (AA) in models adjusted for age, education, and genotyping stage when appropriate; P_{trend}.

[d] Breast cancer risk for minor alelle carriers (AB/BB) compared with major allele homozygotes (AA) in models adjusted for age, education, and genotyping stage when appropriate; *P* value for dominant association.

[e] Breast cancer risk for minor allele homozygotes (BB) compared with major allele carriers (AA/AB) in models adjusted for age, education, and genotyping stage when appropriate; *P* value for recessive association.

[f] 3' FR, 3' flanking region, downstream of the gene.

Table 4. *FLT1* (*VEGFR1*) *rs9551471* and *VEGFA rs833070* and breast cancer risk: the SBCGS

Analysis	Breast cancer risk[a] by risk alleles / genotypes of *VEGFR1* / *FLT1 rs9551471* and *VEGFA rs833070*[b]				P_{trend}
	0 (254/188)	1 (2,014/1,299)	2 (3,849/2,267)	3(225/102)	
All women	1.0 (reference)	**1.33 (1.13–1.57)**	**1.45 (1.24–1.70)**	**1.88 (1.42–2.50)**	**0.0004**
By Menopausal status					
Premenopausal	1.0 (reference)	1.21 (0.97–1.52)	**1.41 (1.14–1.75)**	**1.99 (1.32–2.99)**	**0.0090**
Postmenopausal	1.0 (reference)	**1.45 (1.13–1.86)**	**1.47 (1.16–1.87)**	**1.87 (1.25–2.78)**	**0.0132**
			P value for interaction = 0.87		
By Tumor stage					
Early (TNM < 3)	1.0 (reference)	**1.36 (1.14–1.63)**	**1.48 (1.25–1.76)**	**1.97 (1.46–2.66)**	**0.0006**
Late (TNM > 3)	1.0 (reference)	**1.45 (1.13–1.86)**	**1.47 (1.16–1.87)**	**1.87 (1.25–2.78)**	**0.0132**
			P value for case–case comparison = 0.81		
By ER/PR status					
ER positive, PR positive	1.0 (reference)	**1.31 (1.02–1.69)**	**1.39 (1.09–1.77)**	**1.80 (1.20–2.70)**	0.1411
ER positive, PR negative	1.0 (reference)	1.14 (0.76–1.72)	1.40 (0.95–2.06)	**2.06 (1.11–3.83)**	**0.0161**
ER negative, PR positive	1.0 (reference)	1.36 (0.97–1.91)	1.31 (0.95–1.82)	**1.98 (1.16–3.37)**	0.1867
ER negative, PR positive	1.0 (reference)	**1.41 (1.15–1.73)**	**1.54 (1.26–1.87)**	**2.07 (1.48–2.90)**	**0.0002**
			P value for case–case comparison = 0.73		

NOTE: Estimates and *P* values in bold are significant at $P < 0.05$.

[a] Odds ratio and 95% CI for the risk of breast cancer, adjusted for age, education, and genotyping stage.

[b] Number of risk alleles/genotyped defined as: *rs9551471 A* and *rs833070 TT*, (*N* cases / *N* controls).

but not associated with breast cancer risk in 4 other studies (12–14, 17). Three studies have reported protective effects for *rs3025039*: the *T* allele was associated with a decreased risk of breast cancer among 1,000 Caucasians (OR: 0.51, 95% CI: 0.38–0.70; ref. 9) and among 457 Caucasian *BRCA1* mutation carriers (OR: 0.63, 95% CI: 0.41–0.98; ref. 16), whereas premenopausal Chinese women with the *TT* genotype had a reduced risk of breast cancer (OR: 0.45, 95% CI: 0.25–0.79) in our previous report (13). However, additional genotyping among SBCS stage 2 participants did not confirm such an association (data not shown). This is in agreement with others who initially found a reduced risk associated with the *T* allele (9) but were unable to replicate findings in a second study population (17). In addition, 1 small study found that the *T* allele occurred more frequently among breast cancer cases (15), whereas 3 other studies found no association (11, 12, 18). Previous reports on other *VEGF* family polymorphisms and breast cancer risk are sparse. Schneider and colleagues evaluated 1 *FLT1* SNP (*−962 C/T*) and 2 *KDR* SNPs (*889 G/A*, *V2971I*, *rs2305948*, and *1416 A/T*, *Q472H*, *rs1870377*) and found no association among 520 breast cancer cases and 715 controls (18). Forsti and colleagues also evaluated these *KDR* SNPs and found no overall association with breast cancer risk (19).

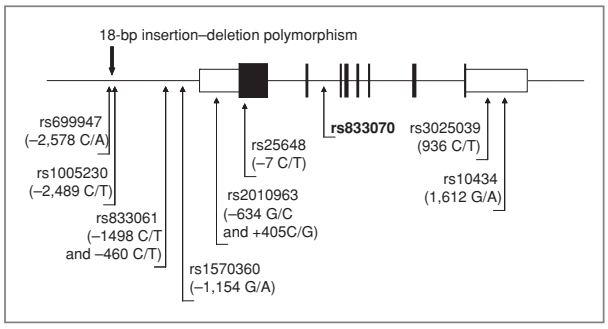

Figure 1. *VEGF* SNPs of interest: the SBCGS.

Of the *VEGF* family variants previously evaluated in the breast cancer risk literature, the majority (*VEGFA rs699947*, *rs833061*, *rs1570360*, *rs2010963*, and *rs3025039* and *KDR rs2305948* and *rs1870377*) were genotyped in the current study and were not found to be associated with breast cancer susceptibility. Three SNPs previously evaluated in only one study with null findings (17) were not genotyped in the current report (*rs1005230*, *rs25648*, and *rs10434*). Given our high coverage of genetic variation, it is likely that these SNPs were also not related to breast cancer risk in the current analysis. Several *VEGFA* variants have been reported to be functional. SNPs in the promoter, 5′-untranslated region (UTR), and 3′-UTR have been found to influence expression, although reports for both SNPs (22, 23) and haplotypes (24, 25) have been inconsistent. Associations between *VEGFA* polymorphisms and plasma or serum levels have also produced mixed findings (9, 14, 17, 26, 27). In the current study, 4 potentially functional *VEGFA* SNPs were genotyped (*rs699947*, *rs1570360*, *rs2010963*, and *rs3025039*); none were found be significantly associated with breast cancer risk. An 18-bp insertion–deletion in the *VEGFA* promoter has also had inconsistent

associations with *VEGFA* expression (23, 28, 29). This insertion–deletion polymorphism is reported to be in perfect LD with *rs699947* (30), which was genotyped in our study and not found to be associated with susceptibility to breast cancer. However, *rs699947* is in high LD with *rs833070* ($D' = 1.0$, $r^2 = 0.84$), so *rs833070* may also capture some of the variation due to the insertion–deletion polymorphism.

VEGFA is the primary mitogen of the VEGF family, and angiogenic signaling is normally mediated by interactions with KDR (VEGFR2; refs. 1–3). However, during pathogenic angiogenesis, signaling via FLT1 (VEGFR1) may be more important (1, 8); for example, FLT1 contributes to the development of the "premetastatic niche," whereas KDR does not (31). This is consistent with our finding of SNPs in *VEGFA* and *FLT1*, but not *KDR*, to be significantly associated with altered breast cancer susceptibility. Both of these polymorphisms found to be associated with breast cancer risk are located in introns: *rs833070* is in intron 2 of *VEGFA* and *rs9551471* is in intron 6 of *FLT1*. *In silico* analysis was conducted to assess the potential functional importance of these loci. No regulatory features were found surrounding or immediately adjacent to *rs9551471* in the Ensembl genome browser (32), whereas *rs833070* was found to be located within a large (1.8 kb) gene-associated putative regulatory element that is enriched with CTCF and DNaseI sites. In agreement with this, FASTSNP (33) scored *rs9551471* as 0 (intronic with no known function) and *rs833070* as 1–2 (possible intronic enhancer). Because it is possible that these SNPs are themselves not functional, but are in high LD with the true casual variants, SNPs in very high LD were also evaluated; no additional insights were revealed. Thus, we can conjecture that these intronic SNPs possibly influence gene expression or mRNA splicing, or are in LD with ungenotyped variations that do. Notably, *VEGFA rs833070* has recently been found to be associated with 2 other phenotypes. In a small study of healthy adults, *rs833070* minor allele carriers had significantly reduced hippocampus concentration, as assessed by high-resolution structural MRI scans (34), and among 175 Japanese patients with type 1 diabetes, *VEGFA rs833070* minor allele homozygotes had a significantly shorter time to nonproliferative diabetic retinopathy progression than major allele carriers (30). These findings highlight the diverse roles of the VEGF family, which is now known to extend far beyond angiogenesis.

Strengths of the current study include our comprehensive SNP coverage, a very large study population, and a multigenic approach to assessing breast cancer risk. To our knowledge, this is the largest and most comprehensive study on *VEGF* family polymorphisms and breast cancer to date. In summary, our data indicate that common genetic variation in *VEGF* family genes may contribute to altered breast cancer susceptibility among Chinese women. Specifically, we found that women with the *VEGFA rs833070 TT* genotype and/or increasing copies of the *FLT1* (*VEGFR1*) *rs9551471 A* allele had increasing risks of breast cancer in a dose–response manner. These findings are consistent with recent meta-analyses that reported no significant associations between *VEGFA −2578 C/A* (*rs699947*), *−460 C/T* (*rs833061*), and *+936 C/T* (*rs3025039*) variants and breast cancer risk (31–39). To date, no GWAS of breast cancer have detected significant associations between *VEGF* family variants and breast cancer risk. Given the *P* value threshold required by GWAS, and the possibly lower coverage of genetic variation, these results are not inconsistent with our findings. Additional studies are needed to confirm our findings and evaluate genetic variation in other *VEGF* family members, including *VEGFB*, *FIGF* (*VEGFD*), *PGF*, and *FLT4* (*VEGFR3*), in relation to breast cancer risk.

Disclosure of Potential Conflicts of Interest

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute or the NIH.

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