NIH Public Access
Author Manuscript
Cancer Epidemiol. Author manuscript; available in PMC 2014 October 01.

Published in final edited form as:
Cancer Epidemiol. 2013 October ; 37(5): 619–624. doi:10.1016/j.canep.2013.06.005.

No Association between Genetic Variants in Angiogenesis and Inflammation Pathway Genes and Breast Cancer Survival among Chinese Women

Tsogzolmaa Dorjgochoo[1], **Ying Zheng**[2], **Yu-Tang Gao**[3], **Xiangyu Ma**[1,4], **Jirong Long**[1], **Pingping Bao**[2], **Ben Zhang**[1], **Wanqing Wen**[1], **Wei Lu**[2], **Wei Zheng**[1], **Xiao Ou Shu**[1], and **Alicia Beeghly-Fadiel**[1]

[1]Division of Epidemiology, Department of Medicine, Vanderbilt Epidemiology Center, Vanderbilt University School of Medicine, Nashville, TN 37232, USA

[2]Shanghai Municipal Center for Disease Control and Prevention, Shanghai, 200336, People's Republic of China

[3]Department of Epidemiology, Shanghai Cancer Institute, Shanghai, 200032, People's Republic of China

[4]Visiting from Department of Epidemiology, College of Preventive Medicine, Third Military Medical University, Chongqing, China

Abstract

Background—Angiogenesis and inflammation are implicated in breast cancer prognosis; however, the role of individual germline variation in related genes is unknown.

Methods—A two-stage candidate pathway association study was conducted among 6,983 Chinese women. Stage 1 included 2,884 women followed for a median of 5.7 years; Stage 2 included 4,099 women followed for a median of 4.0 years. Cox proportional hazards regression was used to estimate the effects of genetic variants on disease-free survival (DFS) and overall survival (OS).

Results—Stage 1 included genotyping of 506 variants in 22 genes; analysis was conducted for 370 common variants. Nominally significant associations with DFS and/or OS were found for 20 loci in ten genes in Stage 1; variants in 19 loci were successfully genotyped and evaluated in Stage 2. In analyses of both study stages combined, nominally significant associations were found for nine variants in seven genes; none of these associations surpassed a significance threshold level corrected for the total number of variants evaluated in this study.

© 2013 Elsevier Inc. All rights reserved.

Corresponding Author: Alicia Beeghly-Fadiel, MPH, PhD, Assistant Professor, Division of Epidemiology, Department of Medicine, Vanderbilt University School of Medicine, 2525 West End Avenue, Suite 800, Nashville, TN 37203, Phone: 615 936 3512, alicia.beeghly@vanderbilt.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of Interest Disclosure: The authors declare that they have no potential conflicts of interest to disclose.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Conclusions—No association with survival was found for 370 common variants in 22 angiogenesis and inflammation pathway genes among Chinese women with breast cancer.

Impact—Our data do not support a large role for common genetic variation in 22 genes in breast cancer prognosis; research on angiogenesis and inflammation genes should focus on common variation in other genes, rare host variants, or tumor alterations.

Keywords

breast cancer survival; genetic variants; angiogenesis genes; inflammation pathway genes; Chinese women

Introduction

Breast cancer prognosis is largely determined by disease stage and tumor characteristics, such as estrogen receptor (ER) and human epidermal growth factor receptor 2 (HER2) status; however, considerable heterogeneity in disease outcome persists beyond categorization on such factors (1). As angiogenesis is critical for tumor growth (2) and inflammation can also promote cancer initiation and development (3), individual genetic variation in genes in these pathways may contribute to the variability of disease outcomes. Prior studies have reported associations between angiogenesis and inflammation related genes and breast cancer survival (4–9) but are generally limited by small sample size and/or lack of replication. Therefore, this study was undertaken in order to comprehensively evaluate genetic variants across 22 angiogenesis and inflammatory pathway genes for associations with breast cancer survival. To reduce the possibility of false positive findings, a two-stage study was undertaken in order to first identify, and then test for replication, associations with breast cancer survival. Genes evaluated included *CCL2, CCL5, CCR2, COL18A1, FGFR4, FLT1, HIF1A, HPGD, IL1B, IL6, KDR, MMP1, MMP3, MMP7, MMP9, PLAU, PTGES, PTGIS, PTGS2, SERPINE1, THBS1*, and *VEGFA*.

Subjects and Methods

Study Population

Breast cancer cases from the Shanghai Breast Cancer Study (SBCS), the Shanghai Breast Cancer Survival Study (SBCSS), and the Shanghai Women's Health Study (SWHS) were evaluated. Study design and data collection procedures have been previously described for the SBCS (10), the SBCSS (11), and the SWHS (12, 13). Cancer diagnoses were histologically confirmed; clinical characteristics and treatment information were obtained by medical records abstraction. Breast cancer outcomes were determined by active follow up surveys and linkage with the Vital Statistics Registry database from the Shanghai Center for Disease Control and Prevention. Survival time was defined as beginning at the time of cancer diagnosis and ending at either relapse or breast cancer death for disease-free survival (DFS), any death for overall survival (OS), or else censored at the date of last contact. Approval was granted by all relevant institutional review boards; all participants provided informed consent.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Genotyping and SNP Selection

Twenty-two genes related to angiogenesis and inflammatory pathways were selected for study based on a literature review conducted at the initiation of this study. Genes included *CCL2, CCL5, CCR2, COL18A1, FGFR4, FLT1, HIF1A, HPGD, IL1B, IL6, KDR, MMP1, MMP3, MMP7, MMP9, PLAU, PTGES, PTGIS, PTGS2, SERPINE1, THBS1,* and *VEGFA.* Details on methods and quality control procedures have been previously described (13, 14). Briefly, DNA was extracted from either blood or buccal cell samples and analyzed by either of four genotyping platforms. Stage 1 genotyping was conducted by Affymetrix Targeted Genotyping for 1,062 breast cancer cases or the Affymetrix Genome-Wide Human SNP Array 6.0 for 2,918 breast cancer cases. Stage 2 genotyping was conducted with a custom-designed Illumina iSelect Beadchip for 1,613 breast cancer cases or the Sequenom iPLEX MassArray platform for 2,601 breast cancer cases. To maximize our coverage of genetic variation across genes, all genetic variants in these genes (±5 kb) that were genotyped by either of our Stage 1 genotyping platforms with minor allele frequencies (MAF) > 5% were evaluated. Variants with nominally significant Stage 1 associations with DFS or OS were evaluated for inclusion in Stage 2; only those with consistent directions of associations between DFS and OS in independent genetic loci (r^2 <0.6) were selected for Stage 2.

Statistical Analysis

Analysis was limited to breast cancer cases with follow-up data available. Cox proportional hazards regression was used to evaluate associations between genetic variants and breast cancer outcomes using additive, dominant, and recessive models, with adjustment for age at diagnosis. Adjustment for study stage was included when appropriate using an indicator variable to adjust for unknown or unmeasured differences between the two study populations. Additional adjustment for disease stage and treatment (surgery, chemotherapy, radiotherapy, and tamoxifen) was also employed. Indicator variables were created for women with unknown information on these treatments. Sensitivity analyses were conducted by excluding either *in situ* breast cancer cases (N=192) or late stage (stages III and IV) breast cancer cases (N=698). Evaluation of the proportional hazards assumption was conducted using a test for interactions with survival times. Significance of statistical tests was based on two-tailed probability levels of 0.05; the Bonferroni correction was used to amend significance thresholds to address the issue of multiple comparisons. All analyses were performed using SAS 9.2 (SAS Institute, Cary, NC).

Results

A total of 6,983 Chinese women with breast cancer were included in the current analysis (Table 1). Stage 1 included a total of 2,884 women that were genotyped by either of our Stage 1 platforms; Stage 2 included a total of 4,099 women that were genotyped by either of our Stage 2 platforms. Stage 1 women were slightly younger than Stage 2 (means of 51.8 and 53.7 years, respectively), and were followed longer (means of 5.7 and 4.0 years, respectively). For all women, treatments included surgery (99.5%), chemotherapy (91.3%), tamoxifen (56.1%), and radiotherapy (32.0%). Of tumors with data available, 64.6% were ER positive, 59.4% were PR positive, and 29.2% were HER2 positive.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

As shown in Figure 1, a total of 506 SNPs in 22 genes related to angiogenesis and inflammation were genotyped, and 370 variants with MAF ≥5% were evaluated for associations with breast cancer outcomes. Nominally significant associations with either DFS and/or OS were found for 20 loci in 10 genes in Stage 1 analyses (Table 2). Stage 2 genotyping was successful for variants in 19 loci; no significant associations with breast cancer survival outcomes were found in Stage 2 analyses. Nine variants in seven genes (*CCL2 rs41416652, COL18A1 rs8126650, FLT1 rs3794396, rs9551471 and rs9319425, MMP7 rs643281, PTGIS rs522962, SERPINE1 rs2227672,* and *THBS rs2292305*) had nominally significant associations with DFS and/or OS in analyses of the two stages combined. Bonferroni corrected *P* value thresholds for the total number of variants evaluated in the entire study, or just in Stage 2 are 0.00014 and 0.0026, respectively. The strongest association found was for *rs8126650* and disease-free survival (*P*=0.008). Thus, no common genetic variants were significantly associated with breast cancer outcomes after considering the number of variants evaluated in this study.

These analyses included a small number of *in situ* breast cancer cases (N=192); when excluded from analyses, nominal significance was gained for two variants (*rs3794396* and *rs470215*). Analyses after excluding late stage (III and IV) breast cancer cases were also conducted (N=698). Nominal significance was attenuated for six variants (*rs41416652, rs3794396, rs9551471, rs9319425, rs522962*, and *rs2227672*) and gained for two variants (*rs470215* and *rs643281*) when late stage patients were excluded. One of these associations (*MMP7 rs643281* and disease-free survival) resulted in a *P* value of 0.0017; this surpassed our significance threshold for the number of variants evaluated in Stage 2, but not for the total number of variants evaluated in the entire study.

All regression models included adjustment for age at diagnosis, and study stage when appropriate; results were materially unaltered when additional adjustment for disease stage and treatment (surgery, chemotherapy, radiotherapy, and tamoxifen) were included. The proportional hazards assumption was evaluated for all genetic variants that were analyzed in Stage 2; all but one (*MMP7 rs643281*) were found to be compatible with the proportional hazards assumption.

Discussion

This large two-stage candidate pathway study comprehensively evaluated genetic variants in genes related to angiogenesis and inflammation pathways on breast cancer outcomes. Based on Stage 1 results, variants in 10 genes were selected for additional evaluation; however, no associations were replicated in Stage 2. In analyses of all women combined, nominally significant associations were found for nine genetic variants in seven genes; however, no associations retained statistical significance after considering the total number of variants evaluated.

Prior studies on germline variants in angiogenesis or inflammation related genes and breast cancer survival are limited. In one small study, an *IL6* variant was associated with markers of poor prognosis and a *VEGFA* variant was associated with markers of favorable prognosis (4). Another small study reported an association between a *VEGFA* variant and reduced

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

disease-free survival (5). A mid-sized study found no association between a variant in *PTGS2* and breast cancer survival, but a significant association for an *IL10* variant (6). Another mid-size study found no association between variants in *MMP1, MMP2, MMP3, MMP9,* and *MMP13* and breast cancer survival (7), but a significant association between a *SERPINE1* (*PAI1*) variant and worse survival (8). One larger study found no association between variants in the *KDR* and *POSTN* genes and breast cancer prognosis (9). Notably, a very large two-stage study failed to show replicated associations with breast cancer survival for the majority of nine variants previously reported to be associated with breast cancer survival, including variants in the *SERPINE1, TGFB1,* and *VEGFA* genes (15). Thus, without replication, it is likely that many of the previously reported associations with breast cancer survival may actually be false positive findings.

In addition to a two-stage study design, strengths of this study include a large sample size, genetically homogenous population (Han Chinese), and prospective investigation of disease outcomes. A limitation of this investigation is that variants in only 22 genes were evaluated; other genes related to angiogenesis and inflammation were not included in this study. However, inclusion of more genes or variants would also increase the significance threshold to account for multiple comparisons. Without consideration for adapting the significance threshold, this study, despite being very large, was somewhat underpowered to detect small effect sizes due to the low number of deaths that occurred (N=808). Given our total sample size, this study had greater than 80% power to detect an HRs of 1.20, 1.18, and 1.16 for variants with MAFs of 0.20, 0.25, and 0.30, respectively. Another limitation of this study is that only Chinese women were included; results may not be generalizable to other ethnic groups or populations.

In conclusion, this study is the first and largest two-stage candidate pathway study to examine associations between genetic variants in genes related to angiogenesis and inflammation in relation to breast cancer survival. Results indicate that common genetic variants within 22 angiogenesis and inflammation related genes (*CCL2, CCL5, CCR2, COL18A1, FGFR4, FLT1, HIF1A, HPGD, IL1B, IL6, KDR, MMP1, MMP3, MMP7, MMP9, PLAU, PTGES, PTGIS, PTGS2, SERPINE1, THBS1,* and *VEGFA*) are unlikely to play a major role in breast cancer survival among Chinese women.

Acknowledgments

The authors wish to thank the participants and research staff of the SBCS, SBCSS, and the SWHS for their contributions and commitment to this project, Regina Courtney for DNA preparation, and Bethanie Hull and Samantha Stansel for assistance with the submission of this manuscript.

Grant Support: This research was supported by grants from the NIH/NCI, including R01CA124558 and R01CA64277 (PI: W Zheng), R01CA90899 (PI: XO Shu) as well as a grant from the DAMD 170210607 (PI: XO Shu). Dr. Beeghly-Fadiel is supported in part by a grant from the NIH/NICHHD (5K12 HD043483-09; PI: K Hartmann). The content of this article is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. DNA extraction and genotyping was conducted at the Survey and Biospecimen and Functional Genomic Resource Laboratory, at the Vanderbilt Microarray Shared Resource, which is supported in part by the Vanderbilt Ingram Center (P30CA68485).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Abbreviations

CCL2	chemokine (C-C motif) ligand 2
CCL5	chemokine (C-C motif) ligand 5
CCR2	chemokine (C-C motif) receptor 2
COL18A1	collagen, type XVIII, alpha 1
DFS	disease-free survival
DNA	deoxyribonucleic acid
ER	estrogen receptor
FGFR4	fibroblast growth factor receptor 4
FLT1	fms-related tyrosine kinase 1
HER2	human epidermal growth factor receptor 2
HIF1A	hypoxia inducible factor 1, alpha subunit
HPGD	hydroxyprostaglandin dehydrogenase
IL1B	interleukin 1, beta
IL6	interleukin 6
IL10	interleukin 10
kb	kilobase
KDR	kinase insert domain receptor
MAF	minor allele frequency
MMP1	matrix metallopeptidase 1
MMP2	matrix metallopeptidase 2
MMP3	matrix metallopeptidase 3
MMP7	matrix metallopeptidase 7
MMP9	matrix metallopeptidase 9
MMP13	matrix metallopeptidase 13
OS	overall survival
PLAU	plasminogen activator, urokinase
POSTN	periostin, osteoblast specific factor
PR	progesterone receptor
PTGES	prostaglandin E synthase
PTGIS	prostaglandin I2 synthase
PTGS2	prostaglandin-endoperoxide synthase 2

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

SBCS	Shanghai Breast Cancer Study
SBCSS	Shanghai Breast Cancer Survival Study
SERPINE1	serpin peptidase inhibitor, clade E, member 1 (previously known as *PAI1*)
SWHS	Shanghai Women's Health Study
THBS1	thrombospondin 1
TGFB1	transforming growth factor, beta 1
VEGFA	vascular endothelial growth factor A

References

1. Bertos NR, Park M. Breast cancer - one term, many entities? J Clin Invest. 2011; 121(10):3789–3796. [PubMed: 21965335]
2. Miller KD, Dul CL. Breast cancer: the role of angiogenesis and antiangiogenic therapy. Hematol Oncol Clin North Am. 2004; 18(5):1071–1086. ix. [PubMed: 15474336]
3. DeNardo DG, Coussens LM. Inflammation and breast cancer. Balancing immune response: crosstalk between adaptive and innate immune cells during breast cancer progression. Breast Cancer Res. 2007; 9(4):212. [PubMed: 17705880]
4. Smith KC, Bateman AC, Fussell HM, Howell WM. Cytokine gene polymorphisms and breast cancer susceptibility and prognosis. Eur J Immunogenet. 2004; 31(4):167–173. [PubMed: 15265021]
5. Maae E, Andersen RF, Steffensen KD, et al. Prognostic impact of VEGFA germline polymorphisms in patients with HER2-positive primary breast cancer. Anticancer Res. 2012; 32(9):3619–3627. [PubMed: 22993299]
6. Gerger A, Renner W, Langsenlehner T, et al. Association of interleukin-10 gene variation with breast cancer prognosis. Breast Cancer Res Treat. 2010; 119(3):701–705. [PubMed: 19437115]
7. Lei H, Hemminki K, Altieri A, et al. Promoter polymorphisms in matrix metalloproteinases and their inhibitors: few associations with breast cancer susceptibility and progression. Breast Cancer Res Treat. 2007; 103(1):61–69. [PubMed: 17033924]
8. Lei H, Hemminki K, Johansson R, et al. PAI-1 -675 4G/5G polymorphism as a prognostic biomarker in breast cancer. Breast Cancer Res Treat. 2008; 109(1):165–175. [PubMed: 17616807]
9. Forsti A, Jin Q, Altieri A, et al. Polymorphisms in the KDR and POSTN genes: association with breast cancer susceptibility and prognosis. Breast Cancer Res Treat. 2007; 101(1):83–93. [PubMed: 16807673]
10. Zheng W, Long J, Gao YT, et al. Genome-wide association study identifies a new breast cancer susceptibility locus at 6q25.1. Nat Genet. 2009; 41(3):324–328. [PubMed: 19219042]
11. Shu XO, Zheng Y, Cai H, et al. Soy food intake and breast cancer survival. JAMA. 2009; 302(22): 2437–2443. [PubMed: 19996398]
12. Zheng W, Chow WH, Yang G, et al. The Shanghai Women's Health Study: rationale, study design, and baseline characteristics. Am J Epidemiol. 2005; 162(11):1123–1131. [PubMed: 16236996]
13. Long J, Cai Q, Sung H, et al. Genome-wide association study in east Asians identifies novel susceptibility loci for breast cancer. PLoS Genet. 2012; 8(2):e1002532. [PubMed: 22383897]
14. Zheng W, Long J, Gao YT, et al. Genome-wide association study identifies a new breast cancer susceptibility locus at 6q25.1. Nat Genet. 2009; 41(3):324–328. [PubMed: 19219042]
15. Beeghly-Fadiel A, Zheng W, Lu W, et al. Replication study for reported SNP associations with breast cancer survival. J Cancer Res Clin Oncol. 2012

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

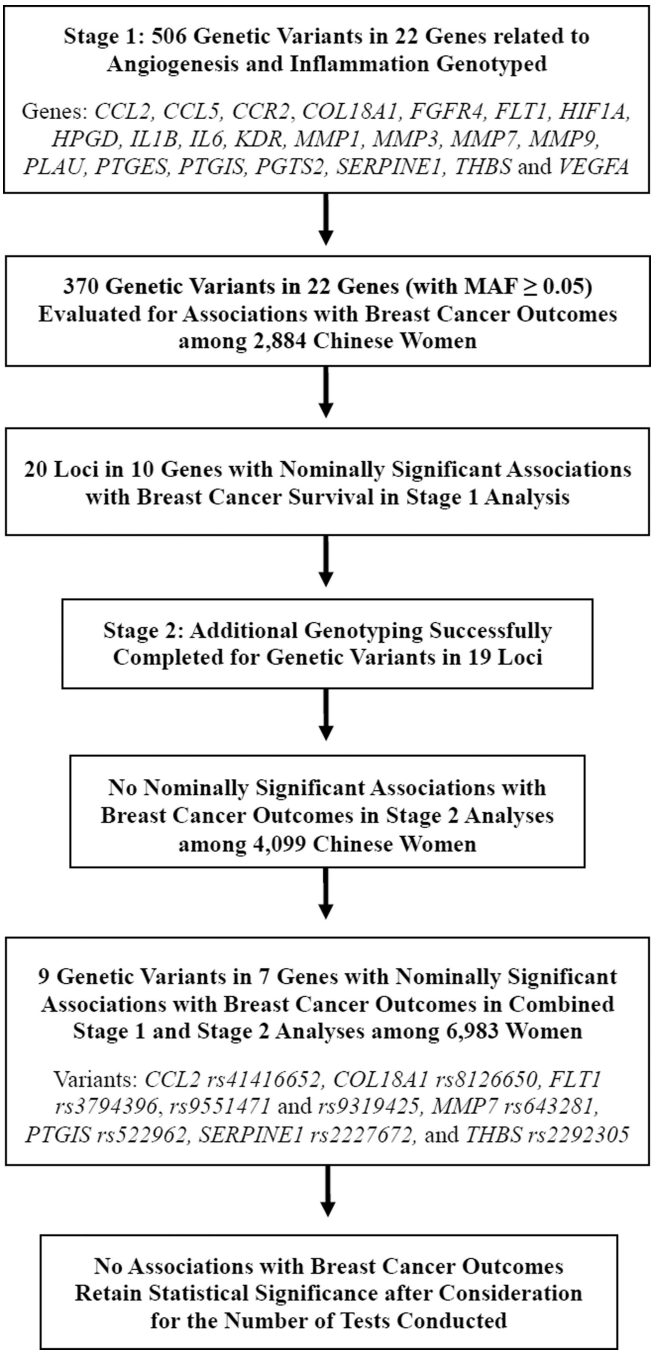

Figure 1.
Study Overview

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 1

Clinical Characteristics of Study Population (N=6,983 Chinese Women)

Characteristic*	Stage 1**	Stage 2**
Patients, N	2,884	4,099
Mean Follow-up Time, years	5.7 (1.9)	4.0 (1.4)
Age at Diagnosis, years	51.8 (9.6)	53.7 (10.1)
TNM Stage of Disease		
0–I	869 (32.9)	1,425 (37.7)
II	1,477 (56.0)	1,948 (51.6)
III–IV	292 (11.1)	406 (10.7)
Estrogen Receptor Status		
Positive	1,570 (65.1)	2,527 (64.3)
Negative	842 (34.9)	1,406 (35.8)
Progesterone Receptor Status		
Positive	1,482 (61.6)	2,271 (58.0)
Negative	923 (38.4)	1,647 (42.0)
Surgery		
Yes	2,853 (99.4)	4,053 (99.5)
No	16 (0.6)	22 (0.5)
Chemotherapy		
Yes	2,646 (92.4)	3,687 (90.5)
No	218 (7.6)	388 (9.5)
Radiotherapy		
Yes	883 (32.5)	1,288 (31.6)
No	1,837 (67.5)	2,785 (68.4)
Tamoxifen		
Yes	1,432 (65.4)	1,932 (50.8)
No	758 (34.6)	1,870 (49.2)

* Mean (standard error) or N (%) for each variable

** Column percents may not sum to 100 due to rounding error

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Cancer Epidemiol. Author manuscript; available in PMC 2014 October 01.

Table 2

Two Stage Candidate Pathway Analysis of Angiogenesis and Inflammation Variants and Breast Cancer Survival

			Disease Free Survival (DFS)						Overall Survival (OS)					
			HR (95% CI)***		P values****				HR (95% CI)***		P values****			
Gene, SNP, and Information	Genotyping Method**	Cases / Events	Heterozygotes	Homozygotes	Allelic	Dominant	Recessive	Cases / Events	Heterozygotes	Homozygotes	Allelic	Dominant	Recessive
CCL2 rs41416652 (T/C, 44.1)													
Study Stage 1	Affy 6.0	2,000 / 497	0.98 (0.80–1.20)	0.86 (0.66–1.11)	0.272	0.541	0.217	2,448 / 394	1.02 (0.82–1.27)	**0.71 (0.52–0.97)**	0.060	0.483	**0.012**
Study Stage 2	iSelect	1,292 / 225	0.91 (0.68–1.22)	0.78 (0.53–1.14)	0.202	0.324	0.260	1,324 / 197	1.08 (0.79–1.48)	0.98 (0.65–1.47)	0.989	0.748	0.718
Combined	NA	3,292 / 722	0.96 (0.82–1.13)	0.83 (0.67–1.03)	0.106	0.308	0.092	3,772 / 591	1.05 (0.87–1.25)	0.79 (0.62–1.01)	0.130	0.736	**0.022**
COL18A1 rs6518240 (A/G, 8.7)													
Study Stage 1	Affy 6.0	1,999 / 496	0.99 (0.78–1.27)	1.82 (0.75–4.39)	0.667	0.858	0.183	2,448 / 393	1.04 (0.79–1.36)	**2.97 (1.32–6.66)**	0.225	0.464	**0.009**
Study Stage 2	iSelect	1,301 / 226	1.04 (0.74–1.47)	2.11 (0.52–8.47)	0.587	0.701	0.299	1,333 / 198	0.95 (0.65–1.39)	0.00 (no events)	0.606	0.707	0.969
Combined	NA	3,300 / 722	1.01 (0.83–1.24)	1.94 (0.92–4.09)	0.472	0.679	0.081	3,781 / 591	1.02 (0.82–1.27)	2.17 (0.97–4.85)	0.442	0.655	0.060
COL18A1 rs8126650 (G/T, 26.0)													
Study Stage 1	Affy 6.0	1,895 / 453	0.84 (0.69–1.03)	**0.63 (0.41–0.97)**	**0.013**	**0.029**	0.072	2,326 / 364	0.80 (0.64–1.00)	**0.60 (0.37–0.99)**	**0.008**	**0.015**	0.091
Study Stage 2	Sequenom	2,152 / 265	0.86 (0.67–1.11)	0.90 (0.54–1.51)	0.319	0.251	0.868	2,344 / 207	0.85 (0.64–1.14)	1.19 (0.70–2.04)	0.779	0.448	0.376
Combined	NA	4,047 / 718	**0.85 (0.73–0.99)**	0.72 (0.52–1.00)	**0.009**	**0.013**	0.110	4,670 / 571	**0.81 (0.68–0.97)**	0.77 (0.54–1.11)	**0.015**	**0.011**	0.325
FLT1 rs3794396 (G/C, 6.1)													
Study Stage 1	Affy 6.0	1,993 / 492	1.26 (0.97–1.63)	**3.58 (1.34–9.58)**	**0.016**	**0.038**	**0.013**	2,440 / 391	1.30 (0.98–1.73)	**4.04 (1.51–10.83)**	**0.010**	**0.028**	**0.007**
Study Stage 2	iSelect	1,292 / 225	1.02 (0.69–1.52)	0.86 (0.12–6.11)	0.961	0.931	0.874	1,324 / 197	1.00 (0.65–1.54)	0.00 (no events)	0.688	0.844	0.976
Combined	NA	3,285 / 717	1.19 (0.96–1.48)	2.22 (0.92–5.36)	**0.037**	0.062	0.083	3,764 / 588	1.21 (0.95–1.53)	2.03 (0.76–5.44)	0.052	0.076	0.172
FLT1 rs7326277 (T/C, 31.3)													
Study Stage 1	Targeted	733 / 282	1.12 (0.88–1.44)	0.69 (0.45–1.08)	0.429	0.883	0.057	821 / 223	1.12 (0.86–1.48)	**0.52 (0.30–0.90)**	0.166	0.834	**0.010**
Study Stage 2	iSelect	1,298 / 225	0.94 (0.71–1.25)	1.06 (0.69–1.64)	0.998	0.797	0.676	1,330 / 197	0.94 (0.70–1.27)	1.10 (0.70–1.74)	0.906	0.854	0.579
Combined	NA	2,031 / 507	1.03 (0.86–1.24)	0.85 (0.62–1.15)	0.537	0.937	0.230	2,151 / 420	1.04 (0.85–1.27)	0.77 (0.54–1.09)	0.351	0.805	0.108
FLT1 rs9551471 (A/G, 20.9)													
Study Stage 1	Affy 6.0	1,991 / 497	0.86 (0.71–1.04)	0.69 (0.42–1.14)	**0.045**	0.065	0.204	2,440 / 394	1.00 (0.81–1.23)	0.92 (0.56–1.53)	0.829	0.902	0.752
Study Stage 2	Sequenom	3,355 / 472	0.86 (0.71–1.05)	0.87 (0.54–1.39)	0.152	0.126	0.695	3,448 / 383	0.95 (0.76–1.18)	1.02 (0.62–1.70)	0.758	0.669	0.871
Combined	NA	5,346 / 969	**0.86 (0.75–0.99)**	0.77 (0.55–1.09)	**0.016**	**0.019**	0.227	5,888 / 777	0.98 (0.84–1.14)	0.96 (0.67–1.37)	0.728	0.736	0.858
FLT1 rs9319425 (T/C, 49.8)													
Study Stage 1	Targeted	733 / 282	1.07 (0.81–1.41)	0.89 (0.64–1.24)	0.527	0.961	0.265	821 / 223	1.02 (0.75–1.38)	**0.66 (0.45–0.97)**	**0.047**	0.435	**0.012**
Study Stage 2	iSelect	1,304 / 226	1.07 (0.78–1.48)	1.04 (0.71–1.53)	0.834	0.695	0.953	1,336 / 198	1.06 (0.75–1.50)	1.01 (0.67–1.52)	0.951	0.789	0.861
Combined	NA	2,037 / 508	1.07 (0.86–1.31)	0.94 (0.73–1.21)	0.660	0.814	0.331	2,157 / 421	1.03 (0.82–1.29)	0.79 (0.60–1.05)	0.116	0.637	**0.035**
FLT1 rs9513116 (G/A, 37.4)													
Study Stage 1	Targeted	734 / 283	0.86 (0.67–1.11)	1.00 (0.71–1.43)	0.690	0.350	0.606	821 / 225	**0.72 (0.54–0.95)**	0.81 (0.54–1.21)	0.088	**0.025**	0.830

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Gene, SNP, and Information	Genotyping Method**	Disease Free Survival (DFS)						Overall Survival (OS)					
			HR (95% CI)***		P values****				HR (95% CI)***		P values****		
		Cases / Events	Heterozygotes	Homozygotes	Allelic	Dominant	Recessive	Cases / Events	Heterozygotes	Homozygotes	Allelic	Dominant	Recessive
Study Stage 2	iSelect	1,280 / 222	0.91 (0.68–1.21)	1.10 (0.75–1.63)	0.875	0.722	0.415	1,313 / 193	1.01 (0.74–1.37)	1.15 (0.75–1.75)	0.604	0.798	0.506
Combined	NA	2,014 / 505	0.89 (0.74–1.08)	1.07 (0.82–1.38)	0.956	0.424	0.309	2,134 / 418	0.85 (0.69–1.04)	0.96 (0.72–1.29)	0.436	0.177	0.717
MMP1 rs1939008 (A/G, 43.3)													
Study Stage 1	Affy 6.0	1,992 / 493	0.94 (0.77–1.15)	1.20 (0.94–1.52)	0.230	0.895	**0.045**	2,436 / 392	1.02 (0.81–1.28)	1.16 (0.88–1.53)	0.324	0.591	0.257
Study Stage 2	iSelect	1,300 / 226	1.10 (0.83–1.47)	0.77 (0.51–1.16)	0.369	0.949	0.092	1,332 / 198	1.06 (0.77–1.44)	0.77 (0.50–1.19)	0.349	0.858	0.142
Combined	NA	3,292 / 719	0.99 (0.84–1.17)	1.05 (0.86–1.30)	0.678	0.932	0.521	3,768 / 590	1.03 (0.85–1.23)	1.02 (0.81–1.29)	0.843	0.782	0.978
MMP1 rs470215 (A/G, 8.1)													
Study Stage 1	Affy 6.0	1,996 / 497	1.17 (0.93–1.49)	**2.14 (1.01–4.52)**	**0.046**	0.093	0.053	2,442 / 394	1.14 (0.88–1.49)	1.94 (0.80–4.69)	0.142	0.215	0.154
Study Stage 2	iSelect	1,291 / 225	1.17 (0.83–1.65)	0.57 (0.08–4.10)	0.556	0.439	0.562	1,323 / 197	1.18 (0.82–1.70)	0.68 (0.10–4.89)	0.512	0.424	0.683
Combined	NA	3,287 / 722	1.16 (0.96–1.42)	1.55 (0.77–3.11)	0.062	0.084	0.245	3,765 / 591	1.15 (0.93–1.43)	1.43 (0.64–3.20)	0.137	0.161	0.413
MMP7 rs11568818 (T/C, 8.9)													
Study Stage 1	Targeted	739 / 284	1.02 (0.75–1.40)	**4.31 (1.37–13.55)**	0.437	0.666	**0.013**	827 / 225	1.04 (0.73–1.47)	**6.04 (1.92–19.05)**	0.320	0.567	**0.002**
Study Stage 2	iSelect	1,289 / 223	0.96 (0.67–1.39)	0.60 (0.08–4.28)	0.699	0.768	0.614	1,321 / 195	0.91 (0.61–1.36)	0.75 (0.11–5.39)	0.590	0.607	0.791
Combined	NA	2,028 / 507	0.99 (0.78–1.26)	1.69 (0.63–4.52)	0.774	0.915	0.298	2,148 / 420	0.97 (0.75–1.27)	2.25 (0.84–6.04)	0.744	0.960	0.106
MMP7 rs643281 (G/A, 9.9)													
Study Stage 1	Affy 6.0	2,002 / 496	1.09 (0.87–1.37)	**2.35 (1.17–4.74)**	0.109	0.240	**0.019**	2,450 / 394	1.08 (0.84–1.38)	1.97 (0.88–4.42)	0.249	0.392	0.107
Study Stage 2	iSelect	1,300 / 226	1.33 (0.97–1.83)	0.92 (0.23–3.70)	0.132	0.092	0.840	1,332 / 198	1.09 (0.77–1.57)	1.11 (0.28–4.49)	0.616	0.610	0.900
Combined	NA	3,302 / 722	1.16 (0.97–1.40)	1.74 (0.93–3.25)	**0.032**	0.057	0.101	3,782 / 592	1.08 (0.88–1.32)	1.56 (0.78–3.15)	0.260	0.360	0.223
PTGES rs10448290 (C/T, 20.7)													
Study Stage 1	Affy 6.0	1,958 / 487	0.93 (0.77–1.13)	0.62 (0.37–1.02)	0.094	0.224	0.071	2,406 / 385	0.92 (0.74–1.14)	**0.51 (0.27–0.97)**	0.065	0.183	**0.047**
Study Stage 2	iSelect	1,299 / 225	0.99 (0.75–1.31)	0.81 (0.38–1.73)	0.704	0.819	0.589	1,331 / 197	1.14 (0.85–1.53)	0.96 (0.45–2.05)	0.557	0.434	0.813
Combined	NA	3,257 / 712	0.95 (0.81–1.11)	0.67 (0.44–1.01)	0.099	0.239	0.066	3,737 / 582	0.99 (0.83–1.17)	0.63 (0.39–1.03)	0.215	0.492	0.066
PTGIS rs522962 (T/C, 27.4)													
Study Stage 1	Affy 6.0	1,903 / 455	**0.81 (0.67–0.99)**	0.81 (0.56–1.18)	**0.042**	**0.027**	0.520	2,335 / 365	0.83 (0.67–1.04)	0.87 (0.57–1.32)	0.146	0.096	0.760
Study Stage 2	iSelect	1,298 / 225	0.82 (0.62–1.09)	1.15 (0.72–1.82)	0.683	0.305	0.339	1,330 / 197	0.95 (0.70–1.28)	1.32 (0.82–2.13)	0.557	0.950	0.204
Combined	NA	3,201 / 680	**0.82 (0.69–0.96)**	0.92 (0.69–1.24)	0.062	**0.018**	0.964	3,665 / 562	0.88 (0.74–1.05)	1.03 (0.75–1.41)	0.455	0.224	0.599
SERPINE1 rs2227672 (G/T, 8.9)													
Study Stage 1	Targeted	738 / 284	0.92 (0.67–1.25)	**3.27 (1.04–10.26)**	0.967	0.785	**0.040**	826 / 225	1.01 (0.72–1.42)	**4.75 (1.51–14.95)**	0.444	0.704	**0.008**
Study Stage 2	iSelect	1,304 / 226	1.20 (0.86–1.67)	0.66 (0.09–4.72)	0.417	0.336	0.656	1,336 / 198	1.24 (0.87–1.77)	1.47 (0.36–5.95)	0.200	0.210	0.625
Combined	NA	2,042 / 510	1.03 (0.82–1.29)	1.62 (0.61–4.35)	0.601	0.710	0.339	2,162 / 423	1.10 (0.86–1.40)	**2.53 (1.05–6.14)**	0.168	0.289	**0.043**
THBS1 rs2292305 (A/G, 32.1)													
Study Stage 1	Targeted	734 / 281	0.95 (0.75–1.21)	**0.58 (0.35–0.95)**	0.071	0.284	**0.033**	821 / 223	0.93 (0.71–1.21)	**0.32 (0.16–0.67)**	**0.009**	0.130	**0.003**
Study Stage 2	iSelect	1,297 / 224	0.97 (0.74–1.29)	0.97 (0.62–1.50)	0.838	0.834	0.917	1,328 / 197	0.99 (0.73–1.33)	1.05 (0.66–1.65)	0.917	0.998	0.820

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Gene, SNP, and Information	Genotyping Method**	Cases / Events	Disease Free Survival (DFS) HR (95% CI)*** Heterozygotes	Homozygotes	P values**** Allelic	Dominant	Recessive	Cases / Events	Overall Survival (OS) HR (95% CI)*** Heterozygotes	Homozygotes	P values**** Allelic	Dominant	Recessive
Combined	NA	2,031 / 505	0.97 (0.81–1.16)	0.75 (0.54–1.05)	0.159	0.397	0.096	2,149 / 420	0.96 (0.79–1.17)	**0.66 (0.45–0.96)**	0.075	0.292	**0.034**
VEGFA rs3024994 (C/T, 5.4)													
Study Stage 1	Affy 6.0	1,994 / 496	**1.31 (1.02–1.69)**	0.67 (0.09–4.76)	0.066	**0.046**	0.665	2,443 / 393	1.14 (0.84–1.54)	0.79 (0.11–5.60)	0.493	0.446	0.799
Study Stage 2	iSelect	1,300 / 225	1.00 (0.65–1.56)	0.00 (no events)	0.822	0.920	0.973	1,331 / 198	0.81 (0.49–1.35)	0.00 (no events)	0.338	0.375	0.976
Combined	NA	3,294 / 721	1.23 (0.98–1.53)	0.49 (0.07–3.48)	0.138	0.094	0.460	3,774 / 591	1.04 (0.80–1.35)	0.59 (0.08–4.21)	0.901	0.824	0.597
VEGFA rs3025035 (C/T, 14.6)													
Study Stage 1	Targeted	728 / 278	1.17 (0.90–1.53)	1.12 (0.53–2.39)	0.263	0.227	0.855	816 / 219	**1.36 (1.02–1.81)**	1.64 (0.81–3.35)	**0.019**	**0.023**	0.258
Study Stage 2	iSelect	1,305 / 225	0.78(0.56–1.07)	1.34 (0.60–3.04)	0.358	0.199	0.391	1,337 / 197	0.81 (0.58–1.14)	0.95 (0.35–2.58)	0.302	0.249	0.997
Combined	NA	2,033 / 503	0.98 (0.80–1.20)	1.19 (0.68–2.07)	0.877	0.973	0.525	2,153 / 416	1.07 (0.86–1.34)	1.29 (0.72–2.30)	0.336	0.412	0.423
VEGFA rs6905288 (A/G, 26.7)													
Study Stage 1	Affy 6.0	2,000 / 497	0.95 (0.79–1.14)	0.74 (0.50–1.10)	0.183	0.348	0.158	2,449 / 394	0.92 (0.75–1.13)	**0.57 (0.35–0.94)**	**0.048**	0.168	**0.034**
Study Stage 2	iSelect	1,300 / 226	0.89 (0.67–1.18)	1.24 (0.80–1.94)	0.842	0.692	0.232	1,332 / 198	0.93 (0.69–1.25)	1.25 (0.78–2.02)	0.705	0.915	0.276
Combined	NA	3,300 / 723	0.93 (0.80–1.09)	0.92 (0.68–1.23)	0.344	0.328	0.682	3,781 / 592	0.92 (0.78–1.09)	0.81 (0.58–1.14)	0.163	0.222	0.304

* SNP information includes (Major / Minor allele, and minor allele frequecy) as determined by all available genotyped breast cancer cases

** Genotyping Methods: Stage 1 genotyping by Affymetrix Targeted genotyping among 1,062 cases from the SBCS (Targeted) or the Affymetrix Genome Wide Array 6.0 among 2,918 cases from the SBCS (Affy 6.0); Stage 2 genotyping by Illumina iSelect Beadchip among 1,613 cases from the SBCS and SBCSS (iSelect) or by a Sequenom iPLEX platform among 2,601 cases from the SBCSS and SWHS (Sequenom)

*** Hazard Ratios (HR) and 95% Confidence Intervals (CI) from Cox Proportional Hazards Regression, including adjustment for age at diagnosis, and study stage when appropriate; Major allele homozygotes are referent, estimates are for heterozygotes and minor allele homozygotes

**** P values from tests for allelic associations (trend), dominant associations, and recessive associations (bold values denote significance at ≤ 0.05)