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Angiogenesis genes, dietary oxidative balance, and breast cancer risk and progression: The Breast Cancer Health Disparities Study

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Abstract

Angiogenesis is essential for tumor development and progression. Genetic variation in angiogenesis-related genes may influence breast carcinogenesis. We evaluated dietary factors associated with oxidative balance, *DDIT4* (1 SNP), *FLT1* (35 SNPs), *HIF1A* (4 SNPs), *KDR* (19 SNPs), *MPO* (1 SNP), *NOS2A* (15 SNPs), *TEK* (40 SNPs), and *VEGFA* (8 SNPs) and breast cancer risk among Hispanic (2111 cases, 2597 controls) and non-Hispanic white (NHW) (1481 cases, 1586 controls) women in the Breast Cancer Health Disparities Study. Adaptive Rank Truncated Product (ARTP) analysis was used to determine gene and pathway significance with breast cancer. *TEK* was associated with breast cancer overall (P_{ARTP} = 0.03) and with breast cancer survival (P_{ARTP} = 0.01). *KDR* was of borderline significance overall (P_{ARTP} = 0.07), although significantly associated with breast cancer in both low and intermediate Native American (NA) ancestry groups (P_{ARTP} = 0.02) and ER+/PR- tumor phenotype (P_{ARTP} = 0.008). Both *VEGFA* and *NOS2A* were associated with ER−/PR− tumor phenotype (P_{ARTP} = 0.01 and P_{ARTP} = 0.04 respectively). *FLT1* was associated with breast cancer survival among those with low NA ancestry (P_{ARTP} = 0.009). With respect to diet, having a higher dietary oxidative balance score

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(DOBS) was significantly associated with lower breast cancer risk (OR 0.74 95% CI 0.64–0.84), with the strongest associations observed for women with the highest NA ancestry (OR 0.44 95 %CI 0.30–0.65). We observed few interactions between DOBS and angiogenesis-related genes. Our data suggest that dietary factors and genetic variation in angiogenesis-related genes contribute to breast cancer carcinogenesis.

Keywords

Breast Cancer; *FLT1*; *KDR*; *NOS2A*; *TEK*; *VEGFA*; diet; antioxidants; survival; Hispanic

Introduction

Angiogenesis, or the development of new blood vessels, is essential for cancer progression by allowing tumor cells oxygen and nutrients needed for growth [1, 2]. Vascular endothelial growth factor A (VEGFA) and its receptors are major mediators of tumor angiogenesis [3]. As pro-angiogenic growth factors, VEGFA and its tyrosine kinase receptors, VEGFR-1 (alias FLT1) and VEGFR-2 (alias KDR), promote angiogenesis, vascular permeability, cell migration and gene expression and have been the target of anti-cancer therapy [1]. VEGF when released by various cells at the site of inflammation induces angiogenesis [4]. It is believed that VEGF signaling in angiogenesis is mainly mediated through KDR which stimulates endothelial cell survival, cell proliferation, migration and invasion, and capillary-like tube formation [5]. FLT1 is thought to modulate binding of KDR and VEGF. Endothelial tyrosine kinase (TEK) also known as TIE2, is involved in angiogenesis in conjunction with growth factors angiopoietin 1 and 2 [6]. Studies have linked TEK expression to breast cancer metastasis and bone metastasis in particular [7, 8].

Inflammation is closely linked to angiogenesis and a hallmark feature of tumorigenesis as inflammatory cells that infiltrate tissue can stimulate angiogenesis. One mechanism for this is the induction of nitric oxide synthase (NOS2) by inflammatory cytokines and hypoxia. NOS2 produces large amounts of nitric oxide which can increase apoptosis and inhibit carcinogenesis or promote carcinogenesis by increasing angiogenesis [9]. Hypoxia also can induce hypoxia-inducible factor-1A (HIFIA), which is a transcription factor involved in the regulation of the tumor microenvironment [10]. HIFIA has been linked to aggressive tumor phenotypes by promoting angiogenesis and tumor metastasis and invasion and is modulated by ROS in response to oxidative stress [11]. DNA Damage-Inducible transcript 4 (DDIT4 alias REDD1), is a HIF1A responsive protein that is induced by adverse environmental conditions and enhances oxidative stress-dependent cell death. It has been shown to be a negative feedback regulator of HIF1A that influences HIF1A expression and suppresses tumorigenesis [12]. Myeloperoxidase (*MPO)* generates reactive oxidant species as part of its function in innate host defense mechanisms that can lead to damage of normal tissue and contribute to inflammatory injury. Polymorphisms in *MPO* have been implicated in risk of lung and prostate cancers [13].

In this study we examined the role of genetic variation in a network of genes that play key roles in angiogenesis and related inflammatory processes in breast cancer risk. Specifically,

we investigated associations between genetic variation in *VEGFA*, *FLT1*, *KDR*, *TEK*, *DDIT4*, *HIF1A*, *MPO*, and *NOS2A* genes with risk of developing breast cancer in an admixed population of non-Hispanic white (NHW) and U.S. Hispanic and Mexican women. We evaluated associations with ER and PR tumor phenotype and survival as well as the interactive effects with dietary factors that have pro and anti-oxidative properties that could modify the effects of these genes. These included alcohol, polyunsaturated fat, beta carotene, alpha tocopherol (vitamin E), vitamin C, dietary fiber, and folic acid. We created a dietary oxidative balance score (DOBS) as previously described to estimate the dietary oxidative load derived from these nutrients [14]. We focused on main effects of genetic and dietary factors as well as their interactive effects to determine how these factors work together to alter risk of breast cancer risk.

Methods

The Breast Cancer Health Disparities Study includes participants from three population-based case-control studies, the 4-Corner's Breast Cancer Study, the Mexico Breast Cancer Study, and the San Francisco Bay Area Breast Cancer Study [15] who completed an in-person interview and who had a blood or mouthwash sample available for DNA extraction. In the 4- Corner's Breast Cancer Study, participants were between 25 and 79 years of age with a histological confirmed diagnosis of *in situ* (n=341) or invasive (n=1492) cancer between October 1999 and May 2004; controls were selected from the target populations of cases living in Arizona, Colorado, New Mexico, and Utah and were frequency matched to cases on ethnicity and 5-year age distribution[16]. Participants from the Mexico Breast Cancer Study were between 28 and 74 years of age. Eligible cases in Mexico were women diagnosed with either a new histologically confirmed *in situ* or invasive breast cancer between January 2004 and December 2007 at 12 participating hospitals from three main health care systems; controls were randomly selected from the catchment area of the 12 participating hospitals using a probabilistic multi-stage design and frequency matched to cases based on 5-year age distribution, membership in health care institution, and place of residence. The San Francisco Bay Area Breast Cancer Study included women aged 35 to 79 years from the San Francisco Bay Area diagnosed with a first primary histologically confirmed invasive breast cancer between April 1995 and April 2002; controls were identified by random-digit dialing (RDD) and frequency-matched to cases based on the expected race/ethnicity and 5-year age distribution [17, 18]. All participants signed informed written consent prior to participation and each study was approved by the Institutional Review Board for Human Subjects at each institution.

Data Harmonization

Data were harmonized across all study centers and questionnaires as previously described [15]. Women were classified as either pre-menopausal or post-menopausal based on responses to questions on menstrual history. Women who reported still having periods during the referent year (defined as the year before diagnosis for cases or before selection into the study for controls) were classified as pre-menopausal. Center-specific definitions were used to define post-menopausal women. Women were classified as post-menopausal if they reported either a natural menopause or If they reported taking hormone therapy (HT)

and were still having periods or were at or above the 95th percentile of age for those who reported having a natural menopause (i.e., ≥ 12 months since their last period. This age at menopause was site specific by ethnicity: 58 for NHW and 56 for Hispanic women from the 4-Corner's Breast Cancer Study; 54 for the Mexico Breast Cancer Study; and 55 for NHW and 56 for Hispanic women from the San Francisco Bay Area Breast Cancer Study. Dietary data were collected using detailed food frequency questionnaires or diet histories in all centers; the referent period was the year prior to diagnosis for both 4-Corner's Breast Cancer Study and the San Francisco Bay Area Breast Cancer Study, while in Mexico it was for a typical week in the year prior to diagnosis or initial symptoms.

Genetic Data

DNA was extracted from either whole blood or mouthwash samples; 7287 blood-derived and 634 mouthwash-derived samples were available. Whole Genome Amplification (WGA) was applied to the mouthwash-derived DNA samples prior to genotyping. A tagSNP approach was used to characterize variation across candidate genes. TagSNPs were selected using the following parameters: linkage disequilibrium (LD) blocks were defined using a Caucasian LD map and an r^2=0.8; minor allele frequency (MAF) >0.1; range= −1500 bps from the initiation codon to +1500 bps from the termination codon; and 1 SNP/LD bin. Additionally, 104 Ancestral Informative Markers (AIMs) were used to distinguish European and Native American ancestry in the study population [15]. All markers were genotyped using a multiplexed bead array assay format based on GoldenGate chemistry (Illumina, San Diego, California). A genotyping call rate of 99.93% was attained (99.65% for WGA samples). We included 132 blinded internal replicates representing 1.6% of the sample set. The duplicate concordance rate was 99.996% as determined by 193,297 matching genotypes among sample pairs. In the current analysis we evaluated *DDIT4* (1 SNP), *FLT1* (35 SNPs), *HIF1A* (4 SNPs), *KDR* (19 SNPs), *MPO* (1 SNP), *NOS2A* (15 SNPs), *TEK* (40 SNPs), and *VEGFA* (8 SNPs). A description of these genes and SNPs is shown in online Supplement 1.

Tumor Characteristics and Survival—Survival information and ER/PR tumor information were not available for cases from Mexico and therefore assessment of these variables is limited to data obtained from the 4-Corner's Breast Cancer Study and the San Francisco Bay Area Breast Cancer Study. Cancer registries in Utah, Colorado, Arizona, New Mexico, and California provided information on stage at diagnosis, months of survival after diagnosis, cause of death, and estrogen receptor (ER) and progesterone receptor (PR) status. Information on ER and PR status of tumors was available for 1019 (69%) NHW and 977 (75%) Hispanic cases. Surveillance Epidemiology and End Results (SEER) summary disease stage was available for breast cancer cases from the U.S. Staging is based on three codes of local, regional, and distant, where distant corresponds to AJCC stage 4, local is predominately AJCC stage 1 with some stage 2, and regional contains AJCC stage 2 and 3.

Statistical Methods

Genetic ancestry estimation—The program STRUCTURE was used to compute individual ancestry for each study participant assuming two founding populations [19, 20]. A three-founding population model was assessed but did not fit the population structure with the same level of repeatability and correlation among runs as the two-founding population

model. Participants were classified by level of percent Native American (NA) ancestry. Assessment across categories of ancestry was done using cut-points based on the distribution of genetic ancestry in the control population with the goal of creating distinct ancestry groups that had sufficient power to assess associations. Three strata, 0–28%, 29 to 70%, and 71 to 100%, were used to evaluate associations by level of NA ancestry. Genetic ancestry was used as a continuous variable when included in the models to adjust for possible confounding.

SNP Associations—Genes and SNPs were assessed for their association with breast cancer risk by strata of genetic ancestry and menopausal status in the whole population and by ER/PR status for the San Francisco Bay Area and 4-Corners studies. All statistical analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC). Logistic regression models were used to estimate odds ratios (OR) and 95% confidence intervals (CI) for breast cancer risk associated with SNPs, adjusting for age, study center, genetic ancestry, reference year BMI, and parity. Associations with SNPs were assessed assuming a co-dominant model. Based on the initial assessment, SNPs which appeared to have a dominant or recessive mode of inheritance were evaluated with those inheritance models in subsequent analyses. For stratified analysis test for interactions were calculated using a Wald 1 df test; adjustments for multiple comparisons within the gene used the step-down Bonferroni correction (i.e., Holm method) taking into account the correlated nature of the data using the SNP spectral decomposition method proposed by Nyholt [21] and modified by Li and Ji [22].

Dietary Analysis—Given the hypothesized pathway we evaluated nutrients with anti- or pro-oxidative balance properties. A dietary oxidative balance score (DOBS) was created based on each individual's ranking of each nutrient included in the score. Anti-oxidants included were vitamin C, vitamin E, beta carotene (data for beta carotene was not available for Mexico), folic acid, and dietary fiber; alcohol was treated as a pro-oxidant. To account for the different number of foods queried on the diet questionnaires used for each study, nutrients were evaluated as nutrient per 1000 calories and quartiles of intake and the DOBS were based on study-specific distributions; additional adjustment for calories did not alter findings. Long-term alcohol consumption was classified into three levels: the top 25th percentile of consumption, all other drinkers, and non-drinkers. In creating the DOBS, participants were assigned values of zero for low levels (first quartile) of exposure to anti-oxidants or high exposure to pro-oxidants (fourth quartile), one for intermediate levels (second and third quartiles) of exposure, and two for high levels (fourth quartile) of exposure to anti-oxidants and low exposure (first quartile) to pro-oxidants. We report ORs and 95% CI for each component part of the DOBS as well as associations for the overall summary score. DOBS trend p values and p values for interaction between the DOBS and SNPs were based on one degree of freedom (1-df) Wald chi-square test statistics as noted above.

Survival Analysis—Survival months were calculated based on month and year of diagnosis and month and year of death or date of last contact by SEER registry; all registry updates were through the spring of 2012. Associations between SNPs and risk of dying of

breast cancer among primary invasive cases were evaluated using Cox proportional hazards models to obtain multivariate hazard ratios (HR) and 95% CI by admixture strata. Since survival data were not available for the Mexico study site, the upper two admixture strata were combined to evaluate survival by ancestry groups. Individuals were censored when they died of causes other than breast cancer or were lost to follow-up. In addition to the minimal adjustments for age, study center, genetic ancestry, referent year BMI, and parity, models were also adjusted for SEER summary stage to estimate the HR. Interactions between genetic variants and genetic ancestry with survival were assessed using p values from 1-df Wald chi-square tests.

ARTP analysis—We used the adaptive rank truncated product (ARTP) method that utilizes a highly efficient permutation algorithm to determine the significance of association of each gene and of the angiogenesis pathway with breast cancer overall, by admixture, and by ER/PR strata. The gene p values were generated using the ARTP package in R, permuting outcome status 10,000 times while adjusting for age, reference year BMI, and genetic admixture [23, 24]. We also controlled for SEER summary stage when estimating the ARTP for breast cancer survival. We report both pathway and gene p values (P_{ARTP}). The original R program was modified to incorporate Cox Proportional Hazard modeling that permuted both vital status and survival months to estimate gene and pathway associations.

Results

The majority of breast cancer cases were Hispanic, under 60 years of age, and post-menopausal (Table 1). Among U.S. cases, most tumors were ER+/PR+. ER−/PR− tumors accounted for 18.4% of NHW and 23.4% of Hispanic cases. The majority of women who self-reported being NHW were estimated as having low NA Ancestry (99.5% of controls), while U.S. women who self-reported being Hispanic where divided between those with intermediate NA ancestry (64.9% of controls) and high NA ancestry (24.4% of controls). Intake of alcohol was very low in the study population and significantly lower among NHW and Hispanic controls than cases. Among women who self-reported being Hispanic or were from Mexico, median levels of all nutrients, except for vitamin C, were significantly different between cases and controls; no significant associations were observed for individual nutrients for NHW women.

Associations between genes and breast cancer risk overall and by admixture group showed that several genes in the pathway were statistically significantly associated as determined by ARTP (SNPs that showed statistical significant for *KDR, NOS2A, TEK* are shown in Table 2), whereas other genes, such as *FLT1* had several significant SNPs that did not maintain statistical significant using ARTP (Online Supplement Table 2). When considering all women together, *TEK* was associated with breast cancer risk (P_{ARTP}= 0.03) while *KDR* was of borderline significance (P_{ARTP} = 0.07). When stratified by NA ancestry, *KDR* was significantly associated with breast cancer risk among women in the low and middle NA ancestry groups (P_{ARTP} = 0.02 and 0.02 respectively), this reflects the strong association observed for rs12498529 and modest associations with both rs2219471 and rs1531290. Both *NOS2A* and *KDR* were associated with breast cancer risk in the middle NA ancestry group (P_{ARTP} = 0.04 and 0.02 respectively); *KDR* rs12498529 remained statistically different

between admixture groups after adjustment for multiple comparisons (P_{adj} = 0.03). The significant gene associations as determined by ARTP reflect both the numbers of SNPs associated within a gene as well as the strength of the SNP associations. For *KDR*, these include rs12498529, rs203465, and rs1531290; for *NOS2A*, these include rs7406657 and rs2297516. No genes were significantly associated in the highest NA ancestry group as determined by ARTP. The overall pathway P_{ARTP} was 0.25. Associations did not differ by menopausal status.

Four genes were associated with various ER/PR tumor sub-groups as determined by ARTP (Table 3). *KDR* was associated with ER+/PR- tumors with seven SNPs having significant associations with this tumor type (P_{ARTP} = 0.0008). *NOS2A* was associated with ER−/PR− tumors (2 SNPs) as was *VEGFA* (3 SNPs) (P_{ARTP} = 0.04 and 0.01 respectively). *TEK* was associated with ER+/PR+ tumors (P_{ARTP} = 0.048) having 4 SNPs significantly associated. *TEK* was of borderline significance (P_{ARTP} = 0.06 with ER−/PR+ tumors). Several SNPs in *FLT1* were associated with specific tumor phenotype, however the gene p value from ARTP was >0.05 for all tumor phenotypes (associations shown in online Supplemental Table 3).

Given the biological plausibility that dietary factors with pro- and anti-oxidant properties could modify breast cancer risk associated with angiogenesis-related genes, we evaluated dietary factors that have recognized pro- or anti-oxidant properties. Several of these factors were statistically significantly associated with breast cancer overall and by admixture groups (Table 4). These include alcohol, vitamin E, beta carotene, folic acid, dietary fiber, and the summary DOBS. For the most part, associations were strongest among women with the highest level of NA ancestry. For instance, highest level of alcohol intake was only associated with an increased risk among women with the highest NA ancestry, while vitamin E, folic acid, and the dietary oxidative balance score were more associated with decreased risk (DOBS interaction p value = 0.001). No association was observed for vitamin C for all groups.

Significant interaction was observed between the DOBS and the following SNPs: *FLT1* rs7987649; *KDR* rs1531289; *TEK* rs669102, rs12350649, rs17834811, rs7047856, and rs581724; and *VEGFA* rs3025033, although after adjustment for multiple comparisons only the *VEGFA* rs3025033 remained statistically significant (P_{adj}=0.03) (Table 5). The protective association observed for having a high DOBS was observed for all genotypes, however, the magnitude of that association differed by genotypes, and in some instances such as *TEK* rs17834811, rs7047856, and rs581724 there was no additional reduction in risk beyond that observed for the homozygote variant genotype group.

Angiogenesis genes also were associated with survival (Figure 1), however only *KDR* (P_{ARTP} = 0.04), and *TEK* (P_{ARTP} = 0.02) showed statistically significant p values for the association as estimated by ARTP and *FLT1* was of borderline significance (P_{ARTP} = 0.052). *FLT1* also was significantly associated with breast cancer survival among those women with the lowest level of NA ancestry (P_{ARTP} = 0.009) with the pathway p value among this group being 0.09. As shown in Figure 1, both of these genes had several SNPs that were associated with survival overall and within specific ancestry groups. The overall

pathway P_{ARTP} for survival was 0.06. Only *DDIT4* was of borderline significance among those with NA ancestry over 28% (P_{ARTP} = 0.07).

Discussion

Angiogenesis-related genes were associated with both breast cancer development and progression in this population of NHW and Hispanic/Mexican women. Some associations appeared stronger for specific tumor phenotype and others appeared to interact with dietary factors associated with oxidative balance. Of the genes assessed, *TEK* appeared to influence breast cancer the most, as seen by its association with breast cancer risk and survival. *KDR*, *NOS2A*, and *VEGFA* were associated with breast cancer for specific tumor phenotypes, while *FLT1* was associated with survival among women who were primarily NHW. We did not observe differences in association by menopausal status and most associations were strongest in groups that did not include high NA ancestry.

Angiogenesis is an essential component of the carcinogenic process. Increased vascularization allows tumors to obtain the necessary nutrients and oxygen needed for growth and invasion. As such, angiogenesis-related genes are potentially important in regulating breast cancer development and progression. Studies have evaluated angiogenesis genes with mixed results. *VEGFA* has been the focus of much research because of its well-documented role in angiogenesis and its potential as a treatment modality for cancer patients. Several polymorphisms have been associated with breast cancer. The Cancer Prevention Study II cohort examined three polymorphisms and found an association with invasive breast cancer for −2578 (rs699947) and −1154 (rs1570360) [25]. The −2578 polymorphism also was associated with increased breast cancer risk in a study of African American women by Schneider [26] but not in one by Langsenlehner [27] or Jin [28]. The +936 (rs3025039) was not associated with breast cancer risk in a study conducted by Oliveira [29], Balasubramanian [30], Langsenlehner [27], although Krippl [31], Rodrigues [32] , and Kataoka [33] saw an inverse association with the TT genotype. We did not observe a significant association with this polymorphism. Likewise, we did not observe a significant association for *VEGFA* rs25648 similar to what has reported by Langsenlehner [27]; Balasubramanian [30] only observed a significant association with survival. We only observed an association between *VEGFA* rs25648 and ER−/PR− tumors but not with breast cancer survival. Beeghly-Fadiel and colleagues in their study of Chinese women saw an increased risk with *VEGFA* rs833070 and *FLT1* rs9551471; we did not see an increased risk with either of these polymorphisms. We also did not observe an association between breast cancer survival and *VEGFA*.

Our findings suggest that *VEGFA* receptors may play a more important role in breast cancer carcinogenesis than *VEGFA* itself. KDR, a type 2 receptor, is primarily responsible for VEGF signaling in the angiogenesis process; VEGFA has been shown to induce tumor cell proliferation via activation of KDR [3]. Studies also have shown that drugs that inhibit VEGF signaling reduce phosphorylated VEGFR2 expression in patients with inflammatory breast cancer [34]. *KDR* was significantly associated with breast cancer for all groups except the highest NA ancestry group. It also was significantly associated with ER+/PR- tumors and was of borderline significant (P_{ARTP}= 0.07) for overall survival. The VEGFA type 1

receptor, FLT1, was significantly associated with survival among those with low NA ancestry. The role of FLT1 in angiogenesis is less well defined [3] although several studies have shown that FLT1 stimulates tumor growth [2]. Our data suggest that *FLT1* may be a tumor promoter, enhancing metastasis, given our observed association with survival.

TEK appeared to have the greatest overall impact on breast cancer in this population. It was associated with breast cancer risk overall as well as risk of dying from breast cancer after diagnosis. The strongest associations were for ER+/PR+ tumors, which represent the majority of breast cancer tumors. TEK regulates angiogenic growth factors, is a receptor for angiopoietin-1 and 2 (Ang1 and Ang2), and has been have linked to breast cancer metastasis including bone metastasis [7, 8]. The angiopoietin/TEK pathway is critical to the developing vasculature and vessel stabilization [35]. Recent studies also have shown the importance of TEK expression in distinct tie expressing monocytes, or TEMs, that play a key role in tumor promotion and angiogenesis [36, 37]. These TEMs cluster in hypoxic areas of solid tumors and migrate in response to angiopoietin-2, which modulate TEK-dependent signaling and regulates apoptosis [38].

Of the genes that could influence angiogenesis through their role in hypoxia and oxidative stress, only *NOS2A* was associated with breast cancer risk and only among women with intermediate NA ancestry and those with ER−/PR− tumors. Nitric oxide can affect cancer through many ways. It can increase apoptosis and inhibit carcinogenesis or promote carcinogenesis through increasing angiogenesis [9]. While we had hypothesized that *NOS2A* and *HIF1A* would interact with dietary antioxidants to alter breast cancer risk, as has been shown in other cancers [14], we did not observe the same level of association in this study.

However, dietary pro- and anti-oxidants were associated with breast cancer risk, especially among those with greater NA ancestry. While many nutrients had a strong association with breast cancer and the overall DOBS showed that those who consumed a diet that was high in anti-oxidants and low in pro-oxidants had a reduced risk of breast cancer, the influence of genetic factors on these associations was minimal. This suggests that oxidative processes are important and that dietary intake may play an important and robust role in this process despite genetic variation in related pathways.

The Breast Cancer Health Disparities Study has strengths, in that it is the largest collection of breast cancer cases of Hispanic and Mexican women reported to date. Additionally, we utilized information on genetic admixture to more accurately define NA ancestry and capture heterogeneity among Hispanics. All three contributing data sites collected extensive diet and lifestyle data, allowing us to utilize harmonized data to assess main effects as well as interaction with genes. While we were able to evaluate tumor phenotype and survival in the U.S. studies, we did not have those data available from Mexico. Thus, data on ER and PR tumor status and survival do not have the range of NA ancestry that is included in the main effect risk estimates and dietary associations. A smaller sample to evaluate these associations can also contribute to less power, which could limit our ability to detect some associations, especially for rarer variants. Additionally we lack complete treatment data which prohibits us from evaluating associations with these genes stratified by type of

treatment, which could be informative. Studies that focus on determining functionality of SNPs within these genes could importantly add to this work.

In summary, our data suggest that angiogenesis-related genes are important in both breast cancer risk and survival. Genetic variation in the tyrosine kinase receptors, *TEK*, *KDR*, and *FLT1* appear to have the most effect on disease risk and survival and thus these genes may be candidates for drug therapy targets. While dietary antioxidants were associated with breast cancer risk, the genes evaluated had little modifying effect on observed associations with diet. The findings from this study support the importance of these genes in breast cancer carcinogenesis and should be replicated in other population-based studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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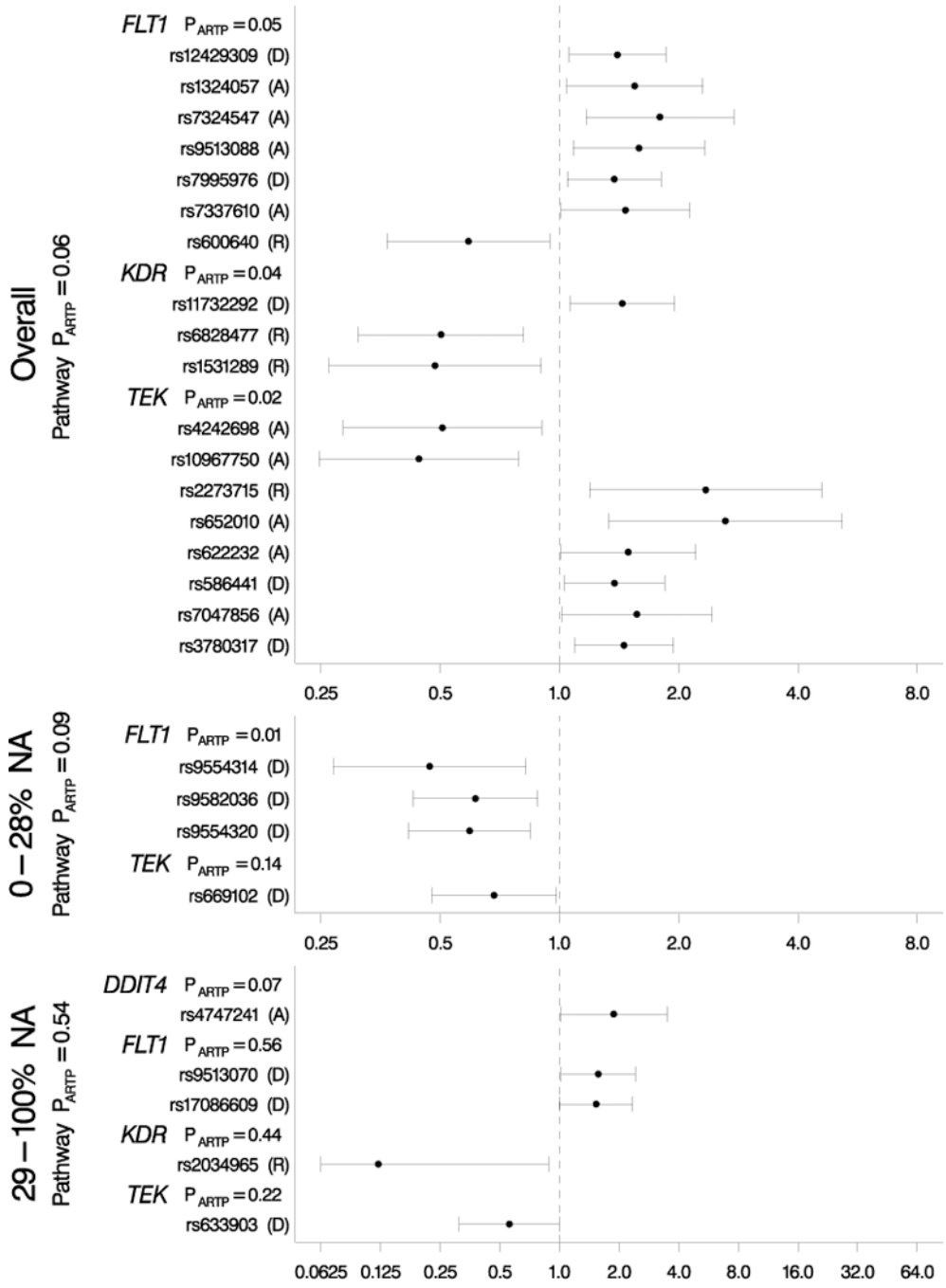

Figure 1.

Hazard ratios and 95% confidence bounds of angiogenesis-related genes associated with breast cancer survival

A=Additive model, D=Dominant Model, R= Recessive Model; p values are for ARTP

Table 1

Description of Study Population by Ethnicity

	Non-Hispanic White					U. S. Hispanic or Mexican				
	Controls		Cases		p value	Controls		Cases		p value
	N	%	N	%		N	%	N	%	
Total	1586	37.9	1481	41.2		2597	62.1	2111	58.8	
Study Site										
4-Corner's	1322	83.4	1227	82.8	NA[1]	723	27.8	597	28.3	NA
Mexico	0	0	0	0		994	38.3	816	38.7	
San Francisco Bay Area	264	16.6	254	17.2		880	33.9	698	33.1	
Age (years)					NA					NA
<40	116	7.3	89	6		311	12	200	9.5	
40–49	408	25.7	409	27.6		831	32	713	33.8	
50–59	409	25.8	413	27.9		756	29.1	617	29.2	
60–69	350	22.1	361	24.4		526	20.3	430	20.4	
≥70	303	19.1	209	14.1		173	6.7	151	7.2	
Mean	56.6		56			52.3		52.7		
Menopausal Status					NA					NA
Pre-menopausal	494	31.5	489	33.5		1027	40.7	836	40.9	
Post-menopausal	1076	68.5	970	66.5		1499	59.3	1210	59.1	
Estimated Native American Ancestry					NA					NA
Low (0 – 28%)	1578	99.5	1472	99.4		278	10.7	275	13	
Intermediate (29 – 70%)	7	0.4	7	0.5		1686	64.9	1393	66	
High (71 – 100%)	1	0.1	2	0.1		633	24.4	443	21	
ER/PR Status[2]					NA					NA
ER+/PR+	NA		695	68.2		NA		605	61.9	
ER+/PR−	NA		121	11.9		NA		115	11.8	
ER−/PR+	NA		15	1.5		NA		28	2.9	
ER−/PR−	NA		188	18.4		NA		229	23.4	
SEER Summary Stage[2,3]					NA					NA

	Non-Hispanic White					U. S. Hispanic or Mexican				
	Controls		Cases		p value	Controls		Cases		p value
	N	%	N	%		N	%	N	%	
Local	NA		829	71.1		NA		648	59.6	
Regional	NA		322	27.6		NA		430	39.6	
Distant	NA		15	1.3		NA		9	0.8	
Vital Status[2,3]					NA					NA
Deceased	NA		202	17.1		NA		202	17.5	
Alive	NA		982	82.9		NA		950	82.5	
Cause of Death[2,3]					NA					NA
Breast Cancer	NA		102	50.5		NA		115	56.9	
Other	NA		100	49.5		NA		87	43.1	
Alcohol Intake										
None	792	50.74	691	46.9	0.03	2011	78.07	1545	73.43	<.01
Any	769	49.26	784	53.2		565	21.93	559	26.57	
Daily Anti-oxidant Intake/1000 kcal)	Median					Median				
Vitamin C (mg)	75.96		78.05		0.54	80.91		81.8		0.85
Vitamin E (mg)	4.63		4.6		0.56	4.85		4.74		<.01
Beta Carotene (mcg)	2290.08		2266.01		0.73	1997.54		1838.54		<.01
Dietary Folate (mcg)	187.32		187.93		0.37	204.26		193.49		<.01
Dietary Fiber (g)	10.72		10.82		0.99	12.86		12.47		<.01

[1] p values not applicable

[2] Information unavailable for the Mexico study site.

[3] Among primary invasive breast cancer cases.

Table 2

Associations between angiogenesis-related genes and breast cancer risk, by Native American ancestry

	All				0 – 28% Native American Ancestry				29 – 70% Native American Ancestry				71 – 100% Native American Ancestry			
	Controls	**Cases**	**OR[1]**	**(95% CI)**	**Controls**	**Cases**	**OR**	**(95% CI)**	**Controls**	**Cases**	**OR**	**(95% CI)**	**Controls**	**Cases**	**OR**	**(95% CI)**
***KDR* P_{ARTP}**				**0.07**				**0.02**				**0.02**				**0.88**
(rs2219471)																
AA/AG	3998	3462	1.00		1731	1663	1.00		1644	1367	1.00		623	432	1.00	
GG	150	107	0.77	(0.60, 1.00)	110	79	0.75	(0.56, 1.01)	34	23	0.81	(0.47, 1.39)	6	5	1.05	(0.31, 3.56)
(rs12498529)[2]																
AA	2752	2300	1.00		1217	1068	1.00		1096	910	1.00		439	322	1.00	
AT	1242	1140	1.09	(0.99, 1.20)	562	592	1.21	(1.05, 1.40)	505	444	1.04	(0.88, 1.21)	175	104	0.80	(0.60, 1.07)
TT	154	126	0.97	(0.76, 1.24)	63	81	1.50	(1.07, 2.11)	76	34	0.51	(0.33, 0.77)	15	11	1.03	(0.46, 2.32)
(rs7692791)																
CC	1225	1051	1.00		392	387	1.00		543	474	1.00		290	190	1.00	
CT	2012	1745	0.97	(0.87, 1.08)	902	855	0.96	(0.81, 1.14)	830	696	0.92	(0.79, 1.09)	280	194	1.05	(0.81, 1.37)
TT	911	772	0.90	(0.79, 1.03)	547	500	0.92	(0.76, 1.11)	305	219	0.80	(0.64, 0.99)	59	53	1.30	(0.85, 1.98)
(rs2034965)																
GG/G																
A	3867	3360	1.00		1723	1635	1.00		1557	1315	1.00		587	410	1.00	
AA	283	208	0.84	(0.70, 1.01)	119	106	0.94	(0.71, 1.23)	122	75	0.73	(0.54, 0.99)	42	27	0.92	(0.55, 1.54)
(rs1531290)																
AA	1667	1487	1.00		470	491	1.00		778	700	1.00		419	296	1.00	
AG	1847	1545	0.87	(0.79, 0.96)	940	864	0.88	(0.75, 1.03)	720	560	0.86	(0.74, 1.00)	187	121	0.85	(0.64, 1.12)
GG	634	533	0.83	(0.72, 0.95)	431	385	0.85	(0.70, 1.03)	180	128	0.75	(0.58, 0.97)	23	20	1.01	(0.53, 1.92)
(rs12502008)																
GG	1176	1063	1.00		717	711	1.00		393	298	1.00		66	54	1.00	
GT	1978	1640	0.96	(0.87, 1.07)	881	802	0.92	(0.80, 1.07)	821	659	1.05	(0.87, 1.27)	276	179	0.87	(0.57, 1.32)
TT	996	863	1.07	(0.94, 1.22)	244	227	0.94	(0.76, 1.16)	465	432	1.25	(1.02, 1.53)	287	204	0.98	(0.65, 1.49)
***NOS2A* P_{ARTP}**				**0.25**				**0.86**				**0.04**				**0.66**
(rs7406657)																
GG	2080	1790	1.00		1033	988	1.00		802	618	1.00		245	184	1.00	

	All				0 – 28% Native American Ancestry				29 – 70% Native American Ancestry				71 – 100% Native American Ancestry			
	Controls	**Cases**	**OR[1]**	**(95% CI)**	**Controls**	**Cases**	**OR**	**(95% CI)**	**Controls**	**Cases**	**OR**	**(95% CI)**	**Controls**	**Cases**	**OR**	**(95% CI)**
GC	1717	1468	1.02	(0.93, 1.13)	685	652	1.00	(0.87, 1.14)	728	611	1.09	(0.94, 1.27)	304	205	0.93	(0.71, 1.22)
CC	351	308	1.06	(0.90, 1.25)	124	100	0.83	(0.63, 1.10)	148	160	1.42	(1.11, 1.83)	79	48	0.77	(0.51, 1.17)
(rs9906835)																
AA	1291	1110	1.00		628	591	1.00		515	397	1.00		148	122	1.00	
AG	2097	1742	0.98	(0.89, 1.09)	921	852	0.98	(0.85, 1.14)	829	672	1.06	(0.89, 1.25)	347	218	0.79	(0.59, 1.07)
GG	760	714	1.12	(0.98, 1.28)	293	296	1.06	(0.87, 1.29)	334	321	1.25	(1.02, 1.54)	133	97	0.88	(0.62, 1.27)
(rs2297516)																
AA	1462	1209	1.00		648	607	1.00		609	452	1.00		205	150	1.00	
AC	2046	1734	1.03	(0.93, 1.14)	914	842	0.98	(0.85, 1.13)	807	677	1.13	(0.96, 1.33)	325	215	0.90	(0.69, 1.19)
CC	642	624	1.18	(1.03, 1.35)	280	291	1.10	(0.90, 1.34)	263	261	1.34	(1.08, 1.66)	99	72	0.96	(0.66, 1.40)
(rs944725)																
CC	1497	1233	1.00		648	626	1.00		600	448	1.00		249	159	1.00	
CT	1958	1703	1.06	(0.96, 1.17)	892	820	0.95	(0.82, 1.10)	787	676	1.14	(0.97, 1.34)	279	207	1.15	(0.88, 1.52)
TT	694	633	1.12	(0.98, 1.27)	301	296	1.02	(0.84, 1.24)	292	266	1.24	(1.01, 1.53)	101	71	1.06	(0.73, 1.53)
TEK P_{ARTP}				**0.03**				**0.14**				**0.18**				**0.12**
(rs17834811)																
TT	2239	2020	1.00		892	898	1.00		950	831	1.00		397	291	1.00	
TG	1654	1325	0.86	(0.78, 0.95)	808	697	0.85	(0.74, 0.98)	641	491	0.84	(0.72, 0.98)	205	137	0.89	(0.68, 1.16)
GG	257	223	0.92	(0.76, 1.11)	142	146	1.01	(0.79, 1.30)	88	68	0.90	(0.64, 1.25)	27	9	0.45	(0.21, 0.99)
(rs7042119)																
CC	2747	2212	1.00		1054	933	1.00		1155	900	1.00		538	379	1.00	
CT/TT	1403	1357	1.15	(1.04, 1.27)	788	809	1.16	(1.01, 1.32)	524	490	1.17	(1.00, 1.36)	91	58	0.78	(0.54, 1.13)
(rs10967753)																
TT	1337	1119	1.00		465	433	1.00		610	473	1.00		262	213	1.00	
TC/CC	2811	2449	1.00	(0.91, 1.10)	1377	1308	1.01	(0.87, 1.18)	1068	917	1.09	(0.93, 1.27)	366	224	0.73	(0.57, 0.94)
(rs7047856)																
AA	1872	1728	1.00		797	841	1.00		785	671	1.00		290	216	1.00	
AG/GG	2278	1841	0.87	(0.80, 0.95)	1045	901	0.82	(0.72, 0.94)	894	719	0.93	(0.80, 1.07)	339	221	0.88	(0.68, 1.12)
(rs581724)																
AA	1089	974	1.00		350	347	1.00		488	453	1.00		251	174	1.00	

	All				0 – 28% Native American Ancestry				29 – 70% Native American Ancestry				71 – 100% Native American Ancestry			
	Controls	**Cases**	**OR[1]**	**(95% CI)**	**Controls**	**Cases**	**OR**	**(95% CI)**	**Controls**	**Cases**	**OR**	**(95% CI)**	**Controls**	**Cases**	**OR**	**(95% CI)**
AC/CC	3060	2595	0.90	(0.81, 1.00)	1492	1395	0.94	(0.80, 1.11)	1190	937	0.83	(0.71, 0.97)	378	263	0.95	(0.74, 1.23)
(rs3780317)																
GG	3072	2719	1.00		1299	1274	1.00		1272	1095	1.00		501	350	1.00	
GA/AA	1077	850	0.87	(0.78, 0.96)	543	468	0.88	(0.76, 1.02)	406	295	0.82	(0.69, 0.97)	128	87	0.97	(0.71, 1.33)
(rs3737188)																
AA	2763	2449	1.00		1111	1112	1.00		1190	1002	1.00		462	335	1.00	
AG	1238	999	0.89	(0.80, 0.98)	640	547	0.86	(0.75, 0.99)	448	353	0.92	(0.78, 1.08)	150	99	0.93	(0.69, 1.25)
GG	149	120	0.88	(0.68, 1.12)	91	82	0.92	(0.68, 1.26)	41	35	1.02	(0.64, 1.63)	17	3	0.24	(0.07, 0.85)

[1] Adjusted for age, study center, BMI in reference year, parity, and genetic admixture

[2] SNP association significantly different at the 0.05 level or less across admixture groups.

Table 3

Associations between angiogenesis genes and breast cancer defined by ER and PR tumor status

		Controls	ER + / PR +			ER + / PR −			ER − / PR +			ER − / PR −[4]		
		N	N	OR[1]	(95% CI)	N	OR	(95% CI)	N	OR	(95% CI)	N	OR	(95% CI)
***KDR* P_{ARTP}**					**0.19**			**0.0008**			**0.31**			**0.20**
(rs2219471)[2]	AA	2064	837	1.00		129	1.00		27	1.00		271	1.00	
	AG/GG	1100	461	1.00	(0.87, 1.15)	106	1.55	(1.18, 2.03)	16	1.20	(0.64, 2.28)	144	1.03	(0.82, 1.28)
(rs7692791)[2]	CC	836	351	1.00		51	1.00		8	1.00		130	1.00	
	CT	1536	632	0.96	(0.82, 1.12)	119	1.28	(0.91, 1.80)	25	1.82	(0.81, 4.09)	198	0.84	(0.66, 1.07)
	TT	792	315	0.89	(0.74, 1.07)	65	1.32	(0.89, 1.94)	10	1.41	(0.54, 3.65)	86	0.69	(0.52, 0.93)
(rs12498529)	AA	2077	819	1.00		134	1.00		27	1.00		265	1.00	
	AT/TT	1087	478	1.12	(0.98, 1.28)	100	1.43	(1.09, 1.87)	16	1.14	(0.61, 2.13)	149	1.08	(0.87, 1.34)
(rs17709898)	AA	1619	673	1.00		104	1.00		23	1.00		216	1.00	
	AG/GG	1547	625	0.93	(0.82, 1.07)	131	1.32	(1.01, 1.74)	20	0.98	(0.52, 1.82)	199	0.99	(0.80, 1.22)
(rs10020464)	CC	1596	638	1.00		101	1.00		16	1.00		215	1.00	
	CT/TT	1568	660	1.05	(0.92, 1.19)	133	1.34	(1.02, 1.75)	27	1.74	(0.93, 3.25)	198	0.94	(0.77, 1.16)
(rs6837735)	CC	2069	829	1.00		136	1.00		27	1.00		264	1.00	
	CT/TT	1097	469	1.08	(0.95, 1.24)	99	1.40	(1.07, 1.84)	16	1.08	(0.58, 2.02)	151	1.07	(0.86, 1.32)
(rs2034965)[2]	GG	1762	708	1.00		107	1.00		25	1.00		243	1.00	
	GA/AA	1404	590	1.04	(0.91, 1.18)	127	1.48	(1.13, 1.94)	18	0.87	(0.47, 1.60)	172	0.87	(0.71, 1.08)
(rs1531290)[2]	AA	1083	470	1.00		103	1.00		19	1.00		152	1.00	
	AG/GG	2081	826	0.86	(0.75, 0.99)	131	0.63	(0.47, 0.83)	24	0.68	(0.36, 1.27)	263	0.91	(0.73, 1.14)
***NOS2A* P_{ARTP}**					**0.72**			**0.48**			**0.93**			**0.04**
(rs8072199)[2]	CC	1332	534	1.00		101	1.00		18	1.00		206	1.00	
	CT	1392	585	1.00	(0.86, 1.15)	107	0.98	(0.73, 1.31)	22	1.19	(0.63, 2.27)	165	0.75	(0.60, 0.94)
	TT	442	179	0.94	(0.76, 1.16)	27	0.78	(0.49, 1.22)	3	0.54	(0.16, 1.91)	44	0.64	(0.45, 0.91)
(rs3729508)	GG/GA	2708	1105	1.00		195	1.00		35	1.00		372	1.00	
	AA	457	193	1.00	(0.83, 1.21)	40	1.19	(0.83, 1.70)	8	1.38	(0.63, 3.02)	43	0.69	(0.49, 0.96)
(rs3729508)	CC	1248	507	1.00		96	1.00		17	1.00		138	1.00	
	CT/TT	1911	787	1.06	(0.93, 1.21)	137	0.97	(0.73, 1.27)	25	0.92	(0.49, 1.72)	276	1.29	(1.04, 1.61)

		Controls	ER + / PR +			ER + / PR −			ER − / PR +			ER − / PR −[4]		
		N	N	OR[1]	(95% CI)	N	OR	(95% CI)	N	OR	(95% CI)	N	OR	(95% CI)
TEK P_{ARTP}					**0.05**			**0.58**			**0.06**			**0.52**
(rs4242698)[2]	AA/AC	2781	1169	1.00		221	1.00		35	1.00		360	1.00	
	CC	385	128	0.82	(0.66, 1.01)	14	0.47	(0.27, 0.81)	8	1.66	(0.76, 3.62)	54	1.10	(0.81, 1.49)
(rs586441)[2]	AA/AG	3123	1267	1.00		230	1.00		41	1.00		410	1.00	
	GG	43	31	1.83	(1.14, 2.93)	5	1.64	(0.64, 4.20)	2	3.90	(0.91, 16.78)	5	0.93	(0.37, 2.38)
(rs7042119)[2]	CC	1966	741	1.00		137	1.00		23	1.00		243	1.00	
	CT/TT	1200	557	1.20	(1.05, 1.37)	98	1.15	(0.87, 1.51)	20	1.53	(0.83, 2.83)	172	1.18	(0.96, 1.46)
(rs7047856)[2]	AA	1402	634	1.00		124	1.00		23	1.00		180	1.00	
	AG/GG	1764	664	0.83	(0.73, 0.95)	111	0.71	(0.54, 0.93)	20	0.70	(0.38, 1.28)	235	1.05	(0.85, 1.29)
(rs3780317)[2]	GG/GA	3105	1265	1.00		230	1.00		38	1.00		409	1.00	
	AA	60	33	1.40	(0.91, 2.15)	5	1.21	(0.48, 3.05)	5	7.72	(2.89, 20.64)	6	0.79	(0.34, 1.85)
VEGFA P_{ARTP}					**0.11**			**0.64**			**0.70**			**0.01**
(rs25648)[2]	CC	2178	854	1.00		169	1.00		27	1.00		307	1.00	
	CT	874	381	1.11	(0.96, 1.28)	55	0.80	(0.59, 1.10)	14	1.32	(0.69, 2.53)	97	0.79	(0.62, 1.01)
	TT	90	48	1.44	(1.00, 2.07)	6	0.93	(0.40, 2.16)	2	1.93	(0.45, 8.27)	4	0.33	(0.12, 0.90)
(rs833070)[2]	GG	920	339	1.00		65	1.00		9	1.00		151	1.00	
	GA	1579	650	1.10	(0.94, 1.29)	120	1.06	(0.77, 1.45)	24	1.67	(0.77, 3.62)	198	0.79	(0.63, 0.99)
	AA	666	308	1.22	(1.01, 1.47)	50	1.04	(0.71, 1.53)	10	1.70	(0.68, 4.23)	66	0.62	(0.46, 0.85)
(rs3025010)[2,3]	TT	1279	503	1.00		94	1.00		17	1.00		195	1.00	
	TC	1440	619	1.09	(0.95, 1.26)	106	0.99	(0.74, 1.32)	19	1.03	(0.53, 1.99)	180	0.83	(0.67, 1.04)
	CC	446	176	1.02	(0.83, 1.25)	35	1.09	(0.73, 1.64)	7	1.20	(0.49, 2.93)	40	0.59	(0.41, 0.84)

[1] Odds ratios (OR) and 95% confidence intervals (CI) adjusted for age, study center, BMI in reference year, parity, and genetic admixture (continuous).

[2] SNP association significantly different at the 0.05 level or less across ER/PR groups.

[3] Similar associations for rs2146323 (r^2 values range from 0.88 to 0.94 across admixture groups).

[4] pathway P_{ARTP}= ER+/PR+ = 0.44; ER+/PR- = 0.01; ER−/PR+ = 0.52; ER−/PR− = 0.06.

Table 4

Dietary factors associated with oxidative balance and breast cancer risk, by Native American ancestry

	All				0% – 28% Native American Ancestry				29% – 70% Native American Ancestry				71% – 100% Native American Ancestry			
	Controls	Cases	OR[1]	(95% CI)	Controls	Cases	OR	(95% CI)	Controls	Cases	OR	(95% CI)	Controls C	cases	OR	(95% CI)
Alcohol[2]																
None	2780	2216	1.00		940	838	1.00		1276	1007	1.00		564	371	1.00	
Low/Moderate	998	963	1.06	(0.95, 1.19)	644	631	1.06	(0.91, 1.23)	305	292	1.07	(0.89, 1.29)	49	40	1.24	(0.79, 1.96)
High	334	377	1.21	(1.03, 1.43)	236	267	1.21	(0.98, 1.48)	82	85	1.22	(0.88, 1.68)	16	25	2.32	(1.21, 4.47)
Vitamin C per 1000 Cal																
Low	1015	881	1.00		457	430	1.00		427	367	1.00		131	84	1.00	
Moderate	2028	1696	0.95	(0.85, 1.06)	915	883	1.04	(0.88, 1.22)	814	624	0.87	(0.73, 1.04)	299	189	0.98	(0.70, 1.37)
High	1025	931	1.02	(0.89, 1.16)	467	425	0.96	(0.79, 1.16)	407	368	1.03	(0.84, 1.26)	151	138	1.35	(0.93, 1.95)
Vitamin E per 1000 Cal[2]																
Low	1015	991	1.00		441	424	1.00		412	406	1.00		162	161	1.00	
Moderate	2040	1700	0.84	(0.75, 0.94)	907	867	0.99	(0.84, 1.17)	847	641	0.76	(0.64, 0.91)	286	192	0.68	(0.51, 0.91)
High	1013	822	0.79	(0.70, 0.90)	491	447	0.92	(0.77, 1.11)	389	315	0.79	(0.64, 0.97)	133	60	0.42	(0.29, 0.62)
Beta-Carotene per 1000 Cal																
Low	797	725	1.00		430	392	1.00		343	309	1.00		24	24	1.00	
Moderate	1578	1381	0.95	(0.84, 1.08)	904	874	1.08	(0.91, 1.27)	604	467	0.84	(0.69, 1.03)	70	40	0.51	(0.24, 1.07)
High	790	658	0.89	(0.77, 1.03)	496	450	1.01	(0.84, 1.23)	267	191	0.77	(0.60, 0.98)	27	17	0.63	(0.26, 1.56)
Folic Acid per 1000 Cal[2]																
Low	1011	997	1.00		551	529	1.00		348	360	1.00		112	108	1.00	
Moderate	2037	1764	0.90	(0.80, 1.00)	894	859	1.01	(0.87, 1.18)	830	682	0.82	(0.68, 0.98)	313	223	0.76	(0.55, 1.05)
High	1019	750	0.77	(0.67, 0.88)	394	350	0.93	(0.77, 1.13)	470	319	0.69	(0.56, 0.85)	155	81	0.53	(0.36, 0.79)
Dietary Fiber per 1000 Cal																
Low	1013	997	1.00		563	577	1.00		341	330	1.00		109	90	1.00	
Moderate	2031	1718	0.89	(0.79, 0.99)	891	826	0.93	(0.80, 1.08)	839	686	0.88	(0.73, 1.06)	301	206	0.84	(0.60, 1.19)
High	1024	797	0.82	(0.72, 0.94)	385	335	0.87	(0.72, 1.06)	468	346	0.81	(0.65, 1.00)	171	116	0.81	(0.55, 1.19)

	All				0% – 28% Native American Ancestry				29% – 70% Native American Ancestry				71% – 100% Native American Ancestry			
	Controls	**Cases**	**OR[1]**	**(95% CI)**	**Controls**	**Cases**	**OR**	**(95% CI)**	**Controls**	**Cases**	**OR**	**(95% CI)**	**Controls C**	**cases**	**OR**	**(95% CI)**
Dietary Oxidative Balance Score[2]																
Quartile 1	960	984	1.00		477	490	1.00		355	371	1.00		128	123	1.00	
Quartile 2	946	863	0.91	(0.80, 1.04)	466	456	0.98	(0.82, 1.17)	368	328	0.91	(0.73, 1.12)	112	79	0.73	(0.49, 1.07)
Quartile 3	1142	925	0.82	(0.72, 0.93)	456	432	0.94	(0.78, 1.13)	494	353	0.72	(0.58, 0.88)	192	140	0.73	(0.52, 1.03)
Quartile 4	970	714	0.74	(0.64, 0.84)	412	350	0.85	(0.70, 1.03)	411	299	0.73	(0.59, 0.90)	147	65	0.44	(0.30, 0.65)
Trend P			<.0001				0.10				<0.01				<0.01	

[1] Odds ratios (OR) and 95% confidence intervals (CI) adjusted for age, study center, BMI in reference year, parity, and genetic admixture (continuous). Low = bottom quartile; Moderate = middle two quartiles, High = upper quartile

[2] Associations were significantly different at the <0.05 level by ancestry group

Table 5

Interaction between angiogenesis-related genes and dietary oxidative balance score

	Dietary Oxidative Balance Score (DOBS)																
	Quartile 1				Quartile 2				Quartile 3				Quartile 4				Interaction
	Controls	Cases	OR[1]	(95% CI)	Controls	Cases	OR	(95% CI)	Controls	Cases	OR	(95% CI)	Controls	Cases	OR	(95% CI)	P-value
FLT1 (rs7987649)																	
AA	381	381	1.00		375	341	0.95	(0.77, 1.17)	432	385	0.93	(0.76, 1.14)	447	276	0.64	(0.52, 0.78)	0.03
AG	416	415	1.01	(0.82, 1.23)	409	350	0.89	(0.72, 1.09)	512	371	0.75	(0.62, 0.91)	361	285	0.81	(0.65, 1.00)	
GG	110	104	0.95	(0.70, 1.29)	105	82	0.81	(0.58, 1.12)	119	100	0.88	(0.65, 1.19)	92	92	1.03	(0.74, 1.42)	
KDR (rs1531289)																	
GG	530	480	1.00		511	441	0.99	(0.83, 1.19)	607	483	0.92	(0.77, 1.09)	522	373	0.80	(0.67, 0.97)	0.04
GA	364	423	1.27	(1.06, 1.54)	370	363	1.08	(0.89, 1.31)	437	366	0.94	(0.78, 1.14)	361	294	0.92	(0.75, 1.12)	
AA	65	81	1.37	(0.96, 1.95)	65	59	1.01	(0.69, 1.47)	97	75	0.87	(0.63, 1.21)	86	47	0.61	(0.41, 0.89)	
TEK (rs669102)																	
GG	293	261	1.00		272	255	1.07	(0.84, 1.36)	327	232	0.84	(0.66, 1.06)	270	224	0.96	(0.75, 1.23)	0.02
GA	469	502	1.24	(1.01, 1.53)	464	409	1.06	(0.85, 1.31)	556	465	1.00	(0.81, 1.23)	469	350	0.89	(0.71, 1.10)	
AA	198	221	1.36	(1.05, 1.75)	210	198	1.17	(0.90, 1.52)	259	228	1.10	(0.86, 1.41)	231	140	0.75	(0.57, 0.98)	
TEK (rs12350649)																	
AA	598	575	1.00		603	529	0.93	(0.79, 1.10)	676	532	0.85	(0.72, 1.00)	566	446	0.84	(0.71, 1.00)	0.01
AT	305	338	1.23	(1.01, 1.49)	276	272	1.13	(0.92, 1.39)	380	323	0.97	(0.80, 1.18)	336	229	0.77	(0.62, 0.95)	
TT	53	66	1.45	(0.98, 2.13)	64	58	1.11	(0.76, 1.62)	82	66	0.99	(0.70, 1.41)	65	36	0.68	(0.44, 1.04)	
TEK (rs17834811)																	
TT	506	578	1.00		506	478	0.85	(0.71, 1.01)	599	527	0.8	(0.67, 0.94)	543	384	0.63	(0.53, 0.76)	0.01
TG	388	345	0.75	(0.62, 0.91)	374	333	0.78	(0.64, 0.94)	477	353	0.65	(0.54, 0.78)	373	268	0.62	(0.51, 0.76)	
GG	66	61	0.76	(0.52, 1.10)	66	52	0.68	(0.46, 1.00)	66	44	0.58	(0.39, 0.87)	54	62	0.98	(0.66, 1.44)	
TEK (rs7047856)																	
AA	416	493	1.00		412	423	0.89	(0.73, 1.07)	520	452	0.75	(0.63, 0.91)	459	324	0.61	(0.50, 0.74)	0.005
AG	437	394	0.75	(0.62, 0.91)	437	363	0.71	(0.59, 0.86)	507	389	0.67	(0.55, 0.81)	424	308	0.62	(0.51, 0.76)	
GG	107	97	0.75	(0.55, 1.02)	97	77	0.69	(0.49, 0.95)	115	84	0.63	(0.46, 0.87)	87	82	0.81	(0.58, 1.12)	
TEK (rs581724)																	
AA	245	281	1.00		248	237	0.86	(0.67, 1.11)	295	250	0.77	(0.61, 0.99)	249	178	0.65	(0.50, 0.84)	0.04

	Dietary Oxidative Balance Score (DOBS)																
	Quartile 1				Quartile 2				Quartile 3				Quartile 4				Interaction
	Controls	Cases	OR[1]	(95% CI)	Controls	Cases	OR	(95% CI)	Controls	Cases	OR	(95% CI)	Controls	Cases	OR	(95% CI)	P value
AC	471	477	0.86	(0.69, 1.06)	434	415	0.82	(0.66, 1.03)	577	451	0.68	(0.55, 0.85)	512	358	0.60	(0.48, 0.75)	
CC	243	226	0.76	(0.59, 0.98)	264	211	0.67	(0.52, 0.87)	270	224	0.70	(0.55, 0.90)	209	178	0.72	(0.55, 0.94)	
VEGFA (rs3025033)																	
AA	584	588	1.00		569	537	0.96	(0.81, 1.14)	625	556	0.91	(0.77, 1.07)	553	453	0.83	(0.70, 0.98)	0.005
AG	325	327	1.03	(0.85, 1.25)	318	284	0.94	(0.77, 1.15)	427	312	0.78	(0.65, 0.95)	349	226	0.68	(0.56, 0.84)	
GG	50	68	1.46	(0.99, 2.16)	59	42	0.78	(0.52, 1.19)	88	54	0.70	(0.48, 1.00)	68	34	0.58	(0.38, 0.90)	

[1] Odds ratios (OR) and 95% confidence intervals (CI) adjusted for age, study center, BMI in reference year, parity and genetic admixture (continuous).