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Diet and lifestyle factors modify immune/inflammation response genes to alter breast cancer risk and prognosis: The Breast Cancer Health Disparities Study

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Abstract

Tumor necrosis factor-α (TNF) and toll-like receptors (TLR) are important mediators of inflammation. We examined 10 of these genes with respect to breast cancer risk and mortality in a genetically admixed population of Hispanic/Native American (NA) (2111 cases, 2597 controls) and non-Hispanic white (NHW) (1481 cases, 1585 controls) women. Additionally, we explored if diet and lifestyle factors modified associations with these genes. Overall, these genes (collectively) were associated with breast cancer risk among women with >70% NA ancestry (P_{ARTP} = 0.0008), with *TLR1* rs7696175 being the primary risk contributor (OR 1.77, 95% CI 1.25, 2.51). Overall, *TLR1* rs7696175 (HR 1.40, 95% CI 1.03, 1.91; P_{adj}=0.032), *TLR4* rs5030728 (HR 1.96, 95% CI 1.30, 2.95; P_{adj}=0.014), and *TNFRSF1A* rs4149578 (HR 2.71, 95% CI 1.28, 5.76; P_{adj}=0.029) were associated with increased breast cancer mortality. We observed several statistically significant interactions after adjustment for multiple comparisons, including interactions between our dietary oxidative balance score and *CD40LG* and *TNFSF1A*; between

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The authors have no conflict of interest to report.

cigarette smoking and *TLR1*, *TLR4*, and *TNF*; between body mass index (BMI) among pre-menopausal women and *TRAF2*; and between regular use of aspirin/non-steroidal anti-inflammatory drugs and *TLR3* and *TRA2*. In conclusion, our findings support a contributing role of certain *TNF*-α and *TLR* genes in both breast cancer risk and survival, particularly among women with higher NA ancestry. Diet and lifestyle factors appear to be important mediators of the breast cancer risk associated with these genes.

Keywords

Breast cancer; TLR; TNF; TRAIL; TRAF2; survival; cigarettes; oxidative stress

Introduction

Tumor necrosis factor-α (TNF), a pro-inflammatory cytokine, stimulates cell proliferation and induces cell differentiation and is thought to be one of the most important promoters of inflammation. Additionally, TNF is a modulator of insulin resistance, especially among individuals who are obese or have chronic inflammation conditions; TNF has been reported to inhibit insulin-induced glucose uptake by targeting components of the insulin-signaling cascade [1–5]. TNF mediates cell survival and apoptosis through TNF receptors by activating at least two major signaling pathways, NFκB and the p38 mitogen-activated protein (MAP) kinase pathway. Tumor necrosis factor receptor superfamily member 1A *(TNFRSF1A or TNFR1)* is a major receptor for TNF-alpha that activates NFκB, mediates apoptosis, and functions as a regulator of inflammation. TNF receptor-associated factor 2 (TRAF2) is a member of the TRAF protein family that interacts with TNF receptors. TRAF2 is required for TNF activation of mitogen activated protein kinase 8 (MAPK8 alias JNK1) as well as NFκB and therefore is thought to influence the apoptotic effects of TNF. TNFSF10 (TRAIL) protein expression has been elevated in adriamycin-treated breast cells [6]. This protein preferentially induces apoptosis in transformed and tumor cells. CD40LG, also known as TNFSF5 and TRAP, is involved in TNF-signaling pathway and related cytokine activity. Toll-like receptors (TLR) also are mediators of inflammation and potentially important modulators of cancer risk through their involvement in the NFκB-signaling pathway [7,8]. TLR4 specifically has been linked to breast cancer [9] and to colon tumor progression and metastatic potential [10,11]. TRAIL has been designated CD253 (cluster of differentiation 253); TLR2 has been designated as CD282; and TLR3 has been designated as CD283.

In this study we examine genetic variation in *TLR* and *TNF*-related genes as they relate to breast cancer risk and survival. *TNF* rs1800629 has been associated with breast cancer risk in a small case-control study of Mexican women [12], suggesting that this gene and possibly its related pathway are important for breast cancer risk in Latina women. We evaluate associations by genetic ancestry since breast cancer incidence rates differ between non-Hispanic white (NHW), Hispanic, and Native American (NA) women living in the Southwestern United States [13]. We also evaluate associations by lifestyle factors that are associated with inflammation and insulin and could therefore modify risk associated with these genes and pathway. Factors we evaluate include dietary oxidative balance score

(DOBS) [14], body mass index (BMI), regular cigarette smoking, use of aspirin or other non-steroidal anti-inflammatory drugs (NSAIDs), and having been diagnosed with diabetes. Given the association of these genes with apoptosis and metastatic potential, we evaluate their association with breast cancer mortality.

Methods

The Breast Cancer Health Disparities Study includes participants from three population-based case-control studies [13], the 4-Corners Breast Cancer Study (4-CBCS) [15], the Mexico Breast Cancer Study (MBCS)[16], and the San Francisco Bay Area Breast Cancer Study (SFBCS) [17,18], who completed an in-person interview and who had a blood or mouthwash sample available for DNA extraction. Information on exposures was collected up to the referent year, defined as the calendar year before diagnosis for cases or before selection into the study for controls. 4-CBCS participants were between 25 and 79 years; MBCS participants were between 28 and 74 years; and SFBCS participants were between 35 to 79 years. All participants signed informed written consent prior to participation and each study was approved by their Institutional Review Board for Human Subjects.

Data Harmonization

Data were harmonized across all study centers and questionnaires as previously described [13]. Women were classified as either pre-menopausal or post-menopausal based on responses to questions on menstrual history. Pre-menopausal women were those 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). Post-menopausal women were those who 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). Women in 4-CBCS and SFBCS were asked to self-identify their race/ethnicity and were classified as non-Hispanic white (NHW), Hispanic, Native American (NA) or a combination of these groups. Women in MBCS were not asked their race or ethnicity.

Lifestyle variables included BMI calculated as self-reported weight (kg) during the referent year divided by measured height squared (m^2) and categorized as normal (<25 kg/m^2), overweight (25–29.9 kg/m^2), and obese (≥30 kg/m^2). Cigarette smoking was evaluated as current, former, or never a regular smoker, where regular was defined as having smoked one or more cigarettes for six months or longer in 4-CBCS and SFBCS (data available for a subset of subjects only) or having smoked 100 or more cigarettes in MCBCS. A dietary oxidative balance score (DOBS) that included nutrients with anti- or pro-oxidative balance properties was developed as previously reported [14]. Dietary information was collected via a computerized validated diet history questionnaire in 4-CBCS [19,20], a 104-item semi-quantitative Food Frequency Questionnaire (FFQ) in MBCS [21], and a modified version of the Block Food Frequency Questionnaire in SFBCS [22]. Alcohol consumption was based on long-term use; consumption during the referent year was used for a subset of SFBCS women without information on long-term use. Regular use of aspirin or NSAIDS defined as three or more times a week for at least one month was available for the 4-CBCS only. A

history of diabetes was defined as ever being told by a health care provider that you had diabetes or high blood sugar (available only for a subset of SFBCS participants).

Genetic Data

DNA was extracted from either whole blood (n=7287) or mouthwash (n=634) samples. Whole Genome Amplification (WGA) was applied to the mouthwash-derived DNA samples prior to genotyping. A tagSNP approach was used to capture variation across the entire candidate genes. Genes were selected based on the literature available at the time the platform was developed that indicated a potential effect on inflammation. TagSNPs were selected using the following parameters: linkage disequilibrium (LD) blocks were defined using a Caucasian LD map in concordance with the custom-made GoldenGate chemistry array 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 Ancestry Informative Markers (AIMs) were used to distinguish European and NA ancestry [13]. 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 tagSNPs for *CD40LG* alias *TNFSF5* and *TRAP* (3 SNPs), *TLR1* (1 SNP), *TLR2* (4 SNPs), *TLR3* (4 SNPs), *TLR4* (8 SNPs), *TNF* (2 SNPs on Illumina and 1 taqman), *TNFRSF1A* (4 SNPs), *TNFRSF11A* (25 SNPs), *TNFSF10* (12 SNPs), and *TRAF2* (4 SNPs). Online Supplement 1 provides a description of these genes and SNPs.

Tumor Characteristics and Survival

Data on estrogen receptor (ER) and progesterone receptor (PR) tumor status and survival were available for cases from 4-CBCS and SFBCS only. 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 ER and PR status. Surveillance Epidemiology and End Results (SEER) disease stage was categorized as local, regional, or distant.

Statistical Methods

Genetic ancestry estimation

The program STRUCTURE was used to estimate individual ancestry for each study participant assuming two founding populations [23,24]. A three-founding population model was assessed but did not fit the population structure. Participants were classified by level of percent NA ancestry (≤28%, >28–70%, and >70%), based on the distribution of genetic ancestry in the control population [13].

SNP Associations

Genes and SNPs were assessed for their association with breast cancer risk overall, by strata of genetic ancestry, and by menopausal status in the whole population and by ER/PR status

for the 4-CBCS and SFBCS. 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. Confounding variables adjusted in these analyses were study, BMI in the referent year, and parity as a categorical variables and age (five-year categories) and genetic ancestry as continuous variables. A p value of <0.05 was considered statistically significant, although results are presented for those where the unadjusted p values was <0.05 and the multiple comparison adjusted p value was <0.15. Associations at this level are presented since group sample sizes vary and these associations could be relevant for replication in other populations. Associations with SNPs were assessed assuming a co-dominant model. Based on the initial assessment, SNPs that appeared to have a dominant or recessive mode of inheritance were evaluated with those inheritance models in subsequent analyses. For stratified analyses, the p value was based on the Wald chi-square test comparing the homozygote rare to the homozygote common when presenting the co-dominant model. Tests for interactions were evaluated using Wald one degree of freedom (1-df) tests. The multinomial p value reported for ER/PR status using the glogit link in the logistic procedure excludes controls. Adjustments for multiple comparisons within the gene used the step-down Bonferroni correction, taking into account the degree of correlation of the SNPs within genes using the SNP spectral decomposition method proposed by Nyholt [25] and modified by Li and Ji [26].

Interactions

We assessed gene by environment interactions among environmental and lifestyle factors that could influence candidate genes given their potential involvement in inflammation, including BMI (separately for pre- and post-menopausal women), smoking (current, former, or never smokers), dietary oxidative balance score, and regular use of aspirin/NSAID (for 4-CBCS participants only). The dietary oxidative balance score (DOBS) was based on each individual's ranking of anti-oxidants (vitamin C, vitamin E, beta carotene (data for beta carotene were not available for MBCS), folic acid, and dietary fiber) and pro-oxidants (alcohol). Nutrients were evaluated as nutrient per 1000 calories and quartiles of intake and the DOBS were based on study-specific distributions. Alcohol consumption was classified into three levels: the top 25^{th} 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.

Survival Analysis

Survival months were calculated based on month and year of diagnosis and month and year of death or last contact. Survival updates were received in the winter of 2013 which included complete survival surveillance through December of 2012. Associations between SNPs and breast cancer-specific mortality among cases with a first primary invasive breast cancer were evaluated using Cox proportional hazards models to obtain multivariate hazard ratios (HR) and 95% confidence intervals (CI) among all women and by genetic ancestry strata. Since survival data were not available for MBCS, the upper two ancestry strata were

combined to evaluate survival by genetic ancestry. Individuals were censored when they died of causes other than breast cancer or were lost to follow-up. We present Wald p values for all women and by ancestry strata based on the comparison between the homozygote rare and common genotypes using models adjusted for age, study, genetic ancestry, BMI, and SEER stage. 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 all genes combined with breast cancer risk by genetic ancestry and by ER/PR status. Case/control status was permuted 10,000 times within R version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria) and p values based on 1-df Wald chi-square tests were generated from logistic regression models. We also assessed associations with mortality using the ARTP method, permuting vital status and survival months together. Likelihood-ratio test p values were calculated from Cox proportional hazard models. We controlled the logistic and Cox models using the adjustment variables previously stated. We report both pathway and gene p values based on the ARTP method (P_{ARTP}) [27,28]. Since ARTP has not been developed to incorporate lifestyle factors when evaluating interactions, results for interactions were adjusted for multiple comparisons as described above.

Results

The majority of women were Hispanic/NA, post-menopausal, had ER+/PR+ tumors, and were diagnosed with local stage disease (Table 1). Among NHW women 21.4% had died, compared to 19.8% of Hispanic/NA women; 47.6% of deaths among NHW women were from breast cancer, compared to 55.9% of deaths among Hispanic/NA women. Among NHW women, 44.4 to 45.9% had a BMI of <25 kg/m^2, compared to 17.6 to 23.5% among Hispanic/NA women.

Few genes and SNPs were significantly associated with breast cancer risk (Table 2, shows those with an adjusted p value of <0.15). *TLR1*, *TLR2*, and *TNFRSF11A* had the strongest association among women with the highest level of NA ancestry. Of the 25 SNPs evaluated for *TNFRSF11A*, five were associated with breast cancer risk among those with high NA ancestry. Of these, rs7237982 (OR_{GG} 2.34, 95% CI 1.05, 5.21), rs17069845 ($OR_{TC/CC}$ 0.74, 95% CI 0.57, 0.97), and rs8083511 (OR_{CC} 1.74, 95% CI 1.12, 2.70) were significantly associated with breast cancer risk and the ORs were significantly different from those in other ancestry groups prior to adjustment for multiple comparisons (data not shown in table). *CD40LG* rs1126535 was significantly associated with breast cancer risk among those with low NA ancestry and *CD40LG* rs5939073 was associated with breast cancer risk among those with intermediate ancestry.

No differences in risk were identified by menopausal status (data not shown), and only two significant associations were identified by ER/PR phenotype (Table 3). *TLR3* rs5743305 was associated with ER−/PR+ tumors and *TNFRSF1A* rs4149578 was significantly

associated with ER−/PR− tumors. Both of these genes had statistically significant ARTP p values of 0.011 and 0.023, respectively.

Two of the three SNPs analyzed in *CD40LG* and one of four SNPs in *TNFRSF1A* significantly interacted with DOBS (Table 4). *CD40LG* homozygote variant was associated with increased risk of breast cancer only among those with low DOBS. For the homozygote common genotype of *TNFRSF1A* rs4149570, breast cancer risk decreased with increasing DOBS. *TLR1*, *TLR4* (1 of 4 SNPs) and *TNF* (1 of 4 SNPs) interacted with cigarette smoking. *TLR1* homozygote rare genotype significantly increased risk only among never-smokers; *TLR4* rs111536898 rare allele decreased risk among never-smokers; *TNF* rs1800630 rare allele increased risk among current smokers only. Three of four SNPs in *TRAF2* were associated with BMI among pre-menopausal women only. For these SNPs, the rare allele was associated with a reduced risk of breast cancer among obese women. Two SNPs of *TLR3* interacted with aspirin/NSAIDs with the greatest effect among regular users. *TRAF2* rs4880073 also interacted with aspirin/NSAID use with the AA genotype reducing breast cancer risk among non-regular users. After adjustment for multiple comparisons, no significant interactions between having diabetes and any SNPs were observed.

TLR1 rs7696175, *TRL4* rs5030728, *TNRFSF1A* rs4149578, *TNFSF10* rs231985, rs3136597, and rs231983 were associated with breast cancer-specific mortality (Table 5). In all instances the rare genotype was associated with poorer survival. Associations with *TLR1*, *TLR4*, *TNFRSF1A*, and *TNFRSF10* rs231985 were slightly stronger among those with greater NA ancestry; however, there were no statistically significant differences in mortality by NA ancestry. Associations with all-cause mortality were similar as those presented for breast cancer-specific mortality (data not shown) with a few exceptions; *TLR1* rs7696175, *TLR4* rs5030728, and *TNFSF10* rs231985 were uniquely associated with breast cancer-specific mortality. On the other hand, *TLR4* rs10759932 was strongly associated with overall mortality (OR_{CC} = 1.94, 95% CI 1.14, 3.32) but not with breast cancer-specific mortality (OR=0.93, 95% CI 0.70, 1.24).

Discussion

Major contributions of this paper are the identification of important diet and lifestyle factors that modify associations between breast cancer risk and TNF and TLR-related genes and SNPs and of the finding that variants in these genes are associated with both breast cancer risk and mortality in a genetically admixed population. However, few genes and SNPs were associated with either breast cancer risk or mortality. We confirmed that *TLR1*, which was previously identified in a breast cancer GWAS, was associated with breast cancer risk and mortality, especially among women with greater NA ancestry. Additionally, *TLR4*, *TNFRSF1A*, and *TNFS10 w*ere associated with breast cancer-specific mortality. *TLR3* rs5743305 was associated with ER−/PR+ tumors and *TNFRSF1A* rs4149578 was significantly associated with ER−/PR− tumors. Diet and lifestyle factors associated with oxidative stress, inflammation, and insulin significantly interacted with several SNPs in these genes.

TLRs are a set of innate immunity genes involved in the activation of NFKB and MAPK, thereby mediating immune/inflammatory response [29]. TLRs can promote inflammation, cell survival and tumor progression [30]. Studies have shown associations between *TLR4* Asp299Gly (rs4986790) with increased breast cancer risk and lower metastasis-free survival, although *TLR4* rs1927911 and rs10759932 were not associated with survival [31]. *TLR4* rs4986970 affects the extracellular domain of TLR4 and is associated with reduced endotoxin responses [31]; *TLR4* rs4986971 (in perfect LD with rs4986970) in the promoter region also has been shown to affect gene function [8]. Reduced expression of TLR4 has been shown to inhibit breast cancer cell proliferation; knock out of *TLR4* gene can actively inhibit breast cancer cell survival [30]. TLR3 has been shown to directly trigger apoptosis in human breast cancer cells [30,32]. *TLR1* was the only gene in this study associated with breast cancer risk based on the ARTP results while both *TLR1* rs7696175 and *TLR4* rs5030728 were associated with breast cancer-specific mortality. We did not detect any significant associations with previously identified functional SNPs in either *TLR4* or *TNF*. *TLR1* rs7696175, which we and others have previously reported being associated with breast cancer risk [33,34], was identified as being a major contributor to risk within the pathway and also associated with mortality in this study.

The TNF family is a group of cytokines associated with apoptosis and antitumor activity; however, they also are involved in inflammation, immunity, and tumor progression [35]. A previous study in Mexico women found that *TNF* -308 G>A polymorphism (rs1800629) was associated with breast cancer risk [12]. We did not confirm this association. In our study the MAF was 0.079 among Hispanic controls, the majority of whom were from Mexico, while the MAF in NHWs was 0.17. The Mexico study was based on 294 controls (1% AA genotype) and 465 cases (14% AA genotype). Our data are in HWE and show 1 case and no controls with this genotype in the highest NA ancestry group (423 cases, 608 controls) that is predominately from Mexico; our numbers are consistent with allele frequencies reported for Hispanic populations in National Center for Biotechnology Information. A meta-analysis of this SNP and breast cancer risk showed a null association as we observed in this study [36].

TNF apoptosis inducing ligand (TNFSF10 or TRAIL) has been shown to activate apoptosis upon binding to its receptor and has been shown to influence survival among those with metastatic colon cancer [37–39]; it was associated with breast cancer-specific mortality in this study. Triple negative breast cancer cell lines have been shown to be sensitive to TNFSF10, whereas other tumor phenotypes are not [38,40]. While we did not see an association between any SNPs in this gene and ER−/PR− tumors, HER2 data were not available and we did observe an association between ER−/PR− tumors and TNFRSF1A (TNFR superfamily receptor 1A or p60). *TNFRSF1A* rs4149570 and rs12426675 have been associated with hepatocellular carcinoma cancer and these SNPs in the promoter have high transcriptional activity [41]. Functionality has been assigned to *TNFRSF1A* rs4149570 in the promoter that results in repression of TNFR1 [41]. We observed a significant interaction with DOBS and this SNP. *TNFRSF1A* rs4149578 was associated with breast cancer-specific mortality in our study.

We examined several diet and lifestyle factors that could influence oxidative stress, the functional role of these genes. Two of the three SNPs analyzed in *CD40LG* and one of four SNPs in *TNFRSF1A* interacted significantly with DOBS, while *TLR1* (1), *TLR4* (1 of 4 SNPs) and *TNF* (1 of 4 SNPs) interacted with cigarette smoking. *CD40LG* is an immune response gene and involved in thrombo-inflammatory reactions by up-regulating cell adhesion molecules and increased production of pro-inflammatory cytokines and reactive oxygen species [42,43]. Higher intake of dietary antioxidants modified the risk associated with the variant allele in two of the *CD40LG* SNPs. Vitamin C, which is a component of our DOBS, has been shown to suppress NFκB activation by inhibiting TNF activation of IKK [44]. TNF also has been shown to be able to induce reactive oxygen species [12]. Cigarette smoking has been shown to reduce innate immune response by suppressing inflammatory mediators [45], and a high oxidant/free radical burden in cigarette smoke has been correlated with increased expression of inflammatory mediator TNF [46]. The interaction we observed between TLRs and TNF and cigarette smoking has biological plausibility, given the influence of cigarette smoking on immune response and free radical burden and the key role of TLR and TNF in mediators of immune response.

Two SNPs of *TLR3* interacted with aspirin/NSAIDs with the greatest effect found among regular users; *TRAF2* rs4880073 also interacted with aspirin/NSAIDs. Aspirin has been shown to interfere with the NFκB complex [47]. TRAF2 is required for an NFκB independent signal that protects against TNF-induced apoptosis and TLR3 signaling activates the transcription of NFκB and interferon regulatory factor 3 [48]. *TLR3* rs3775291 has been associated with aspirin-exacerbated respiratory disease where eosinophils are activated via TLR3 and then recruit leukocytes to sites of inflammation as part of an inflammatory response.

Three of four SNPs in *TRAF2* were associated with BMI among pre-menopausal women, but not among post-menopausal women. The immune system has been shown to play a role in obesity and insulin resistance. The CD40 signaling intermediary is TRAF2 and it has been shown that CD40-mice have worsened insulin resistance. Thus the CD40/TRAF2 signaling pathway is thought to protect against adipose tissue inflammation and metabolic complications associated with obesity [49].

This study represents one of the largest studies of breast cancer in Hispanics, a genetically admixed population of European and NA ancestry. The pooling of data from three studies allowed us to evaluate associations with risk, mortality, and lifestyle factors that could mediate genetic risk. We have pooled our populations to test the hypothesis that differences in risk are associated with ancestry, thus using the population only as a replication from one to the other could yield misleading results. While we have tried to target key genes and SNPs in the candidate pathway, there may be other important genes and SNPs that are not included here. We utilized the Illuminia platform that was based on a Caucasian population LD structure, which could result in not capturing the entire variation in populations with more NA ancestry. Likewise, since we used a tagSNP approach to capture variation across the gene, we have detected associations with SNPs that we do not know their functional significance. Other variables such as persistent infections or country of nativity might be important confounders which we were not able to adjust in our data. Although we used

several statistical methods to adjust for the associations observed among our candidate genes, associations could still be chance findings that need replication in other similar populations.

Several genes and SNPs were associated with breast cancer risk and mortality, although the pathway was only significant for women with the highest NA ancestry Additionally, DOBS, cigarette smoking, pre-menopausal BMI, and use of aspirin/NSAID significantly interacted with several SNPs within the pathway. This study suggests the importance of incorporating diet and lifestyle factors to obtain a better understanding of the total underlying genetic risk associated with breast cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Gao Z, Zuberi A, Quon MJ, Dong Z, Ye J. Aspirin Inhibits Serine Phosphorylation of Insulin Receptor Substrate 1 in Tumor Necrosis Factor-treated Cells through Targeting Multiple Serine Kinases. J Biol Chem. 2003; 278:24944–24950. [PubMed: 12714600]
2. Xu H, Hotamisligil GS. Signaling pathways utilized by tumor necrosis factor receptor 1 in adipocytes to suppress differentiation. FEBS Lett. 2001; 506:97–102. [PubMed: 11591379]
3. Hotamisligil GS. The role of TNFalpha and TNF receptors in obesity and insulin resistance. J Intern Med. 1999; 245:621–625. [PubMed: 10395191]
4. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, et al. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. Science. 1996; 271:665–668. [PubMed: 8571133]

5. Hofmann C, Lorenz K, Braithwaite SS, Colca JR, Palazuk BJ, et al. Altered gene expression for tumor necrosis factor-alpha and its receptors during drug and dietary modulation of insulin resistance. Endocrinology. 1994; 134:264–270. [PubMed: 8275942]
6. Kuribayashi K, Krigsfeld G, Wang W, Xu J, Mayes PA, et al. TNFSF10 (TRAIL), a p53 target gene that mediates p53-dependent cell death. Cancer Biol Ther. 2008; 7:2034–2038. [PubMed: 19106633]
7. Fukata M, Abreu MT. TLR4 signalling in the intestine in health and disease. Biochem Soc Trans. 2007; 35:1473–1478. [PubMed: 18031248]
8. Resler AJ, Malone KE, Johnson LG, Malkki M, Petersdorf EW, et al. Genetic variation in TLR or NFkappaB pathways and the risk of breast cancer: a case-control study. BMC Cancer. 2013; 13:219. [PubMed: 23634849]
9. Mai CW, Kang YB, Pichika MR. Should a Toll-like receptor 4 (TLR-4) agonist or antagonist be designed to treat cancer? TLR-4: its expression and effects in the ten most common cancers. Onco Targets Ther. 2013; 6:1573–1587. [PubMed: 24235843]
10. Simiantonaki N, Kurzik-Dumke U, Karyofylli G, Jayasinghe C, Michel-Schmidt R, et al. Reduced expression of TLR4 is associated with the metastatic status of human colorectal cancer. Int J Mol Med. 2007; 20:21–29. [PubMed: 17549384]
11. Niedzielska I, Niedzielski Z, Tkacz M, Orawczyk T, Ziaja K, et al. Toll-like receptors and the tendency of normal mucous membrane to transform to polyp or colorectal cancer. J Physiol Pharmacol. 2009; 60(Suppl 1):65–71. [PubMed: 19609015]
12. Gomez Flores-Ramos L, Escoto-De Dios A, Puebla-Perez AM, Figuera-Villanueva LE, Ramos-Silva A, et al. Association of the tumor necrosis factor-alpha -308G>A polymorphism with breast cancer in Mexican women. Genet Mol Res. 2013; 12:5680–5693. [PubMed: 24301937]
13. Slattery ML, John EM, Torres-Mejia G, Lundgreen A, Herrick JS, et al. Genetic variation in genes involved in hormones, inflammation and energetic factors and breast cancer risk in an admixed population. Carcinogenesis. 2012; 33:1512–1521. [PubMed: 22562547]
14. Slattery ML, John EM, Torres-Mejia G, Lundgreen A, Lewinger JP, et al. Angiogenesis genes, dietary oxidative balance, and breast cancer risk and progression: The breast cancer health disparities study. Int J Cancer. 2013
15. Slattery ML, Sweeney C, Edwards S, Herrick J, Baumgartner K, et al. Body size, weight change, fat distribution and breast cancer risk in Hispanic and non-Hispanic white women. Breast Cancer Res Treat. 2007; 102:85–101. [PubMed: 17080310]
16. Angeles-Llerenas A, Ortega-Olvera C, Perez-Rodriguez E, Esparza-Cano JP, Lazcano-Ponce E, et al. Moderate physical activity and breast cancer risk: the effect of menopausal status. Cancer Causes Control. 2010; 21:577–586. [PubMed: 20084545]
17. John EM, Horn-Ross PL, Koo J. Lifetime physical activity and breast cancer risk in a multiethnic population: the San Francisco Bay area breast cancer study. Cancer Epidemiol Biomarkers Prev. 2003; 12:1143–1152. [PubMed: 14652273]
18. John EM, Phipps AI, Davis A, Koo J. Migration history, acculturation, and breast cancer risk in Hispanic women. Cancer Epidemiol Biomarkers Prev. 2005; 14:2905–2913. [PubMed: 16365008]
19. Slattery ML, Caan BJ, Duncan D, Berry TD, Coates A, et al. A computerized diet history questionnaire for epidemiologic studies. J Am Diet Assoc. 1994; 94:761–766. [PubMed: 8021418]
20. Murtaugh MA, Sweeney C, Giuliano AR, Herrick JS, Hines L, et al. Diet patterns and breast cancer risk in Hispanic and non-Hispanic white women: the Four-Corners Breast Cancer Study. Am J Clin Nutr. 2008; 87:978–984. [PubMed: 18400722]
21. Hernandez-Avila M, Romieu I, Parra S, Hernandez-Avila J, Madrigal H, et al. Validity and reproducibility of a food frequency questionnaire to assess dietary intake of women living in Mexico City. Salud publica de Mexico. 1998; 40:133–140. [PubMed: 9617194]
22. Horn-Ross PL, John EM, Lee M, Stewart SL, Koo J, et al. Phytoestrogen consumption and breast cancer risk in a multiethnic population: the Bay Area Breast Cancer Study. Am J Epidemiol. 2001; 154:434–441. [PubMed: 11532785]
23. Falush D, Stephens M, Pritchard JK. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics. 2003; 164:1567–1587. [PubMed: 12930761]

24. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000; 155:945–959. [PubMed: 10835412]
25. Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. American journal of human genetics. 2004; 74:765–769. [PubMed: 14997420]
26. Li J, Ji L. Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. Heredity. 2005; 95:221–227. [PubMed: 16077740]
27. Yu K, Li Q, Bergen AW, Pfeiffer RM, Rosenberg PS, et al. Pathway analysis by adaptive combination of P-values. Genetic epidemiology. 2009; 33:700–709. [PubMed: 19333968]
28. Kai Yu, OL.; Wheeler, William. R package. 2.0.0. 2011. ARTP Gene and Pathway p-values computed using the Adaptive Rank Truncated Product.
29. Shatz M, Menendez D, Resnick MA. The human TLR innate immune gene family is differentially influenced by DNA stress and p53 status in cancer cells. Cancer Res. 2012; 72:3948–3957. [PubMed: 22673234]
30. Yang H, Zhou H, Feng P, Zhou X, Wen H, et al. Reduced expression of Toll-like receptor 4 inhibits human breast cancer cells proliferation and inflammatory cytokines secretion. J Exp Clin Cancer Res. 2010; 29:92. [PubMed: 20618976]
31. Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat Med. 2007; 13:1050–1059. [PubMed: 17704786]
32. Salaun B, Coste I, Rissoan MC, Lebecque SJ, Renno T. TLR3 can directly trigger apoptosis in human cancer cells. J Immunol. 2006; 176:4894–4901. [PubMed: 16585585]
33. Fejerman L, Stern MC, Ziv E, John EM, Torres-Mejia G, et al. Genetic ancestry modifies the association between genetic risk variants and breast cancer risk among Hispanic and non-Hispanic white women. Carcinogenesis. 2013
34. Barnholtz-Sloan JS, Shetty PB, Guan X, Nyante SJ, Luo J, et al. FGFR2 and other loci identified in genome-wide association studies are associated with breast cancer in African-American and younger women. Carcinogenesis. 2010; 31:1417–1423. [PubMed: 20554749]
35. Wu Y, Zhou BP. TNF-alpha/NF-kappaB/Snail pathway in cancer cell migration and invasion. Br J Cancer. 2010; 102:639–644. [PubMed: 20087353]
36. Guo J, Meng H, Pei J, Zhu M. Association between the TNF-alpha-238G>A and TGF-beta1 L10P Polymorphisms and Breast Cancer Risk: A Meta-Analysis. Breast Care (Basel). 2011; 6:126–129. [PubMed: 21673823]
37. Bisgin A, Kargi A, Yalcin AD, Aydin C, Ekinci D, et al. Increased serum sTRAIL levels were correlated with survival in bevacizumab-treated metastatic colon cancer. BMC Cancer. 2012; 12:58. [PubMed: 22313795]
38. Rahman M, Pumphrey JG, Lipkowitz S. The TRAIL to targeted therapy of breast cancer. Adv Cancer Res. 2009; 103:43–73. [PubMed: 19854352]
39. Thorburn A, Behbakht K, Ford H. TRAIL receptor-targeted therapeutics: resistance mechanisms and strategies to avoid them. Drug Resist Updat. 2008; 11:17–24. [PubMed: 18374623]
40. Rahman M, Davis SR, Pumphrey JG, Bao J, Nau MM, et al. TRAIL induces apoptosis in triple-negative breast cancer cells with a mesenchymal phenotype. Breast Cancer Res Treat. 2009; 113:217–230. [PubMed: 18266105]
41. Kim S, Moon SM, Kim YS, Kim JJ, Ryu HJ, et al. TNFR1 promoter -329G/T polymorphism results in allele-specific repression of TNFR1 expression. Biochem Biophys Res Commun. 2008; 368:395–401. [PubMed: 18243134]
42. Bou Khzam L, Hachem A, Zaid Y, Boulahya R, Mourad W, et al. Soluble CD40 ligand impairs the anti-platelet function of peripheral blood angiogenic outgrowth cells via increased production of reactive oxygen species. Thromb Haemost. 2013; 109:940–947. [PubMed: 23426185]
43. Bou Khzam L, Boulahya R, Abou-Saleh H, Hachem A, Zaid Y, et al. Soluble CD40 ligand stimulates the pro-angiogenic function of peripheral blood angiogenic outgrowth cells via increased release of matrix metalloproteinase-9. PLoS One. 2013; 8:e84289. [PubMed: 24358353]

44. Carcamo JM, Pedraza A, Borquez-Ojeda O, Golde DW. Vitamin C suppresses TNF alpha-induced NF kappa B activation by inhibiting I kappa B alpha phosphorylation. Biochemistry. 2002; 41:12995–13002. [PubMed: 12390026]

45. Metcalfe HJ, Lea S, Hughes D, Khalaf R, Abbott-Banner K, et al. Effects of cigarette smoke on TLR activation of COPD macrophages. Clin Exp Immunol. 2014

46. Bozinovski S, Vlahos R, Zhang Y, Lah LC, Seow HJ, et al. Carbonylation caused by cigarette smoke extract is associated with defective macrophage immunity. Am J Respir Cell Mol Biol. 2011; 45:229–236. [PubMed: 20935190]

47. Brummelkamp TR, Nijman SM, Dirac AM, Bernards R. Loss of the cylindromatosis tumour suppressor inhibits apoptosis by activating NF-kappaB. Nature. 2003; 424:797–801. [PubMed: 12917690]

48. Palikhe NS, Kim SH, Kim JH, Losol P, Ye YM, et al. Role of Toll-like Receptor 3 Variants in Aspirin-Exacerbated Respiratory Disease. Allergy Asthma Immunol Res. 2011; 3:123–127. [PubMed: 21461252]

49. Chatzigeorgiou A, Seijkens T, Zarzycka B, Engel D, Poggi M, et al. Blocking CD40-TRAF6 signaling is a therapeutic target in obesity-associated insulin resistance. Proc Natl Acad Sci U S A. 2014; 111:2686–2691. [PubMed: 24492375]

Highlights

- Associations were stronger among women with greater Native American ancestry.
- *TLR1* rs7696175 had the strongest influence on risk.
- *TLR1*, *TLR4*, *TNFRSF1A* were associated with increased breast cancer mortality.
- Diet and lifestyle factors mediated breast cancer risk associated with these genes.

Table 1

Description of study population by self-reported race/ethnicity

	U.S. non-Hispanic White				U. S. Hispanic/Native American or Mexican			
	Controls		Cases		Controls		Cases	
	N	%	N	%	N	%	N	%
Total	1585	37.9	1481	41.2	2597	62.1	2111	58.8
Study								
4-CBCS	1321	83.3	1227	82.8	723	27.8	597	28.3
MBCS	0	0	0	0	994	38.3	816	38.7
SFBCS	264	16.7	254	17.2	880	33.9	698	33.1
Age (years)								
<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	349	22	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								
Pre-menopausal	494	31.5	489	33.5	1027	40.7	836	40.9
Post-menopausal	1075	68.5	970	66.5	1499	59.3	1210	59.1
Estimated Percent Native American Ancestry								
0–28	1577	99.5	1472	99.4	278	10.7	275	13
29–70	7	0.4	7	0.5	1686	64.9	1393	66
71–100	1	0.1	2	0.1	633	24.4	443	21
ER/PR Status[2]								
ER+/PR+	NA[1]		695	68.2	NA[1]		605	61.9
ER+/PR-	NA[1]		121	11.9	NA[1]		115	11.8
ER−/PR+	NA[1]		15	1.5	NA[1]		28	2.9
ER−/PR−	NA[1]		188	18.4	NA[1]		229	23.4
Vital Status[2,3]								

	U.S. non-Hispanic White				U. S. Hispanic/Native American or Mexican			
	Controls		Cases		Controls		Cases	
	N	%	N	%	N	%	N	%
Deceased	NA[1]		255	21.4	NA[1]		229	19.8
Alive	NA[1]		935	78.6	NA[1]		929	80.2
Cause of Death[2,3]								
Breast Cancer	NA[1]		121	47.5	NA[1]		128	55.9
Other	NA[1]		134	52.5	NA[1]		101	44.1
SEER Summary Stage[2,3]								
Local	NA[1]		831	71	NA[1]		650	59.6
Regional	NA[1]		325	27.8	NA[1]		432	39.6
Distant	NA[1]		15	1.3	NA[1]		9	0.8
Smoking Status[4]								
Never	794	60.3	688	56.1	1616	72.1	1298	70.1
Former	360	27.3	386	31.5	347	15.5	322	17.4
Current	163	12.4	152	12.4	278	12.4	231	12.5
BMI (kg/m^2)								
<25	699	44.4	678	45.9	453	17.6	492	23.5
25–29.9	465	29.5	433	29.3	951	36.9	768	36.7
≥30	412	26.1	367	24.8	1172	45.5	832	39.8
NSAID use[5]								
No	708	53.7	670	54.7	446	61.7	395	66.2
Yes	610	46.3	554	45.3	277	38.3	202	33.8
Dietary Oxidative Balance Score[6] [mean (SD)]								
4-CBCS	6.3 (2.7)		6.3 (2.6)		6.7 (2.5)		6.5 (2.6)	
MBCS	NA[1]		NA[1]		5.9 (2.0)		5.7 (2.0)	
SFBCS	5.6 (2.6)		5.7 (2.6)		6.9 (2.5)		6.1 (2.5)	

[1] Data not applicable (NA)

[2] Data unavailable from Mexico Breast Cancer Study (MBCS).

[3] Includes first primary invasive breast cancer cases from the 4-Corners Breast Cancer Study (4-CBCS) and San Francisco Bay Area Breast Cancer Study (SFBCS) only

[4] Data available for a subset of SFBCS participants only

[5] Data only available for the 4-CBCS

[6] Dietary Oxidative Balance Score (DOBS) includes alcohol (pro-oxidant), vitamin C, vitamin E, beta carotene (data not available for MBCS), folic acid, and dietary fiber (anti-oxidants).

Table 2

Summary of significant associations between pathway genes and breast cancer risk by percent Native American ancestry

	<=28% Native American Ancestry			>28 – 70% Native American Ancestry			>70% Native American Ancestry			
	Controls	**Cases**	**OR[1] (95% CI)**	**Controls**	**Cases**	**OR (95% CI)**	**Controls**	**Cases**	**OR (95% CI)**	**Interaction P-value (raw; adjusted)[2]**
CD40LG (rs1126535)										0.014, 0.036
TT	1232	1136	1.00	516	457	1.00	81	68	1.00	
TC	552	526	1.05 (0.90, 1.21)	824	662	0.91 (0.77, 1.07)	291	182	0.77 (0.52, 1.13)	
CC	57	78	1.47 (1.03, 2.10)	337	270	0.94 (0.76, 1.16)	256	187	0.96 (0.65, 1.41)	
P-value (raw; adjusted) [2]			0.032, 0.083			0.576, 0.940			0.821, 1.000	
CD40LG (rs5930973)										0.022, 0.036
GG	1637	1560	1.00	1630	1327	1.00	623	433	1.00	
GA/AA	205	182	0.93 (0.75, 1.15)	49	63	1.63 (1.11, 2.40)	6	4	0.79 (0.21, 2.94)	
P-value (raw; adjusted)			0.519, 0.855			0.014, 0.036			0.730, 1.000	
TLR1 (rs7696175)										<.001, <.001
CC/CT	1472	1444	1.00	1462	1174	1.00	550	355	1.00	
TT	370	298	0.82 (0.69, 0.97)	215	216	1.25 (1.01, 1.53)	78	82	1.77 (1.25, 2.51)	
P-value (raw; adjusted)			0.020, 0.020			0.038, 0.038			0.001, 0.001	
TLR2 (rs4696483)										0.012, 0.036
CC/CT	1796	1714	1.00	1637	1352	1.00	617	422	1.00	
TT	46	27	0.61 (0.38, 0.99)	42	38	1.13 (0.72, 1.78)	12	15	1.83 (0.84, 4.00)	
P-value (raw; adjusted)			0.047, 0.142			0.583, 1.000			0.131, 0.392	
TNFRSF11A (rs8099222)										0.227, 1.000
GG	1055	994	1.00	1028	877	1.00	494	320	1.00	
GA	681	640	1.00 (0.87, 1.15)	546	453	0.97 (0.83, 1.13)	131	102	1.11 (0.82, 1.50)	
AA	97	92	1.01 (0.75, 1.36)	103	60	0.69 (0.49, 0.96)	4	15	4.66 (1.51, 14.40)	
P-value (raw; adjusted)			0.970, 1.000			0.028, 0.493			0.008, 0.133	
TNFRSF11A (rs8089829)										0.004, 0.070
AA	525	532	1.00	644	499	1.00	324	203	1.00	
AG	916	826	0.88 (0.75, 1.02)	759	653	1.11 (0.95, 1.31)	272	189	1.06 (0.82, 1.38)	
GG	398	380	0.94 (0.78, 1.13)	274	236	1.10 (0.89, 1.36)	33	44	1.92 (1.17, 3.16)	
P-value (raw; adjusted)			0.495, 1.000			0.381, 1.000			0.010, 0.161	

[1] Odds Ratios (OR) and 95% Confidence Intervals (CI) adjusted for age, study, BMI during referent year, parity, and genetic ancestry.

[2] P values in table for SNPs are unadjusted and adjusted for multiple comparisons using the step down Bonferroni correction. $ARTP_p$ for <=28% NA ancestry = 0.267 for *CD40LG*, 0.019 for TLR1, 0.205 for *TLR2*, and 0.93 for *TNFRSF11A*; for >28–70% NA ancestry = 0.0498 for *CD40LG*, 0.037 for *TLR1*, 0.822 for *TLR2* and 0.348 for *TNFRSF11A*; for >70% NA ancestry *CD40LG* = 0.762, *TLR1* = 0.0008, *TLR2*= 0.446, and *TNFRSF11A* = 0.176. Pathway p value only significant for >70% NA = 0.015.

Table 3

Associations between *TLR3* and *TNFRSF1* and breast cancer risk, by ER/PR tumor phenotype.

	Controls	ER+/PR+		ER+/PR−		ER−/PR+		ER−/PR−		Multinomial P-values (raw; adjusted)[2,3]
	N	N	OR[1] (95% CI)	N	OR (95% CI)	N	OR (95% CI)	N	OR (95% CI)	
TLR3 (rs5743305)[4]										0.011, 0.040
TT	1321	528	1.00	101	1.00	10	1.00	157	1.00	
TA	1415	597	1.04 (0.91, 1.20)	106	0.97 (0.73, 1.29)	21	1.98 (0.93, 4.23)	201	1.20 (0.96, 1.50)	
AA	430	172	0.97 (0.79, 1.19)	28	0.82 (0.53, 1.27)	12	3.70 (1.58, 8.67)	57	1.11 (0.80, 1.53)	
P-value (raw; adjusted) [2]			0.774, 1.000		0.387, 1.000		0.003,0.010		0.534, 1.000	
TNFRSF1A (rs4149578)[4]										0.018, 0.053
GG	2487	1000	1.00	184	1.00	33	1.00	350	1.00	
GA/AA	678	295	1.09 (0.93, 1.27)	50	0.99 (0.72, 1.38)	10	1.13 (0.55, 2.30)	64	0.67 (0.51, 0.89)	
P-value (raw; adjusted)			0.284, 0.851		0.971, 1.000		0.747, 1.000		0.006, 0.017	

[1] Odds Ratios (OR) and 95% Confidence Intervals (CI) adjusted for age, study, BMI during referent year, parity, and genetic ancestry.

[2] P values for SNPs in table are unadjusted and adjusted for multiple comparisons using the step down Bonferroni correction.

[3] Multinomial p values exclude controls.

[4] ARTP p value for *TLR3* for ER−/PR+ tumors was 0.011; ARTP p value for ER−/PR− tumors for *TNFRSF1A* was 0.023.

Table 4

Gene and environment interactions associated with breast cancer risk.

	Dietary Oxidative Balance Score (DOBS)[1]												
	Quartile 1			Quartile 2			Quartile 3			Quartile 4			
	Controls	Cases	OR[2] (95% CI)	Controls	Cases	OR (95% CI)	Controls	Cases	OR (95% CI)	Controls	Cases	OR (95% CI)	Interaction P-value (raw; adjusted)[3]
CD40LG (rs1126535)													0.014, 0.029
TT	477	462	1.00	461	416	0.96 (0.80, 1.15)	475	429	0.96 (0.80, 1.15)	402	339	0.88 (0.72, 1.07)	
TC	359	373	1.15 (0.95, 1.40)	373	335	1.02 (0.83, 1.24)	523	346	0.75 (0.62, 0.91)	376	289	0.88 (0.72, 1.08)	
CC	129	147	1.35 (1.02, 1.78)	119	112	1.12 (0.83, 1.51)	213	154	0.89 (0.69, 1.15)	156	103	0.81 (0.60, 1.08)	
CD40LG (rs3092936)													0.011, 0.029
TT	620	599	1.00	604	566	1 (0.85, 1.18)	693	571	0.88 (0.75, 1.03)	545	458	0.89 (0.75, 1.05)	
TC	276	302	1.21 (0.99, 1.49)	289	243	0.95 (0.77, 1.17)	403	282	0.79 (0.65, 0.97)	313	221	0.81 (0.65, 1.00)	
CC	68	81	1.42 (1.00, 2.01)	60	56	1.09 (0.74, 1.62)	117	76	0.8 (0.58, 1.10)	78	52	0.81 (0.55, 1.18)	
TNFRSF1A (rs4149570)													0.011, 0.032
GG	399	428	1.00	413	376	0.87 (0.71, 1.06)	564	439	0.75 (0.62, 0.90)	450	309	0.65 (0.53, 0.80)	
GT	441	436	0.90 (0.74, 1.09)	421	386	0.85 (0.69, 1.03)	530	385	0.68 (0.56, 0.82)	392	335	0.8 (0.65, 0.98)	
TT	125	116	0.81 (0.61, 1.09)	119	102	0.78 (0.58, 1.06)	119	105	0.81 (0.60, 1.09)	92	88	0.86 (0.62, 1.19)	

	Smoking Status[4]									
	Never-Smoker			Former Smoker			Current Smoker			Interaction P-value
TLR1 (rs7696175)										0.032, 0.032
CC	895	662	1.00	271	271	1.26 (1.03, 1.54)	136	124	1.16 (0.89, 1.52)	
CT	1130	954	1.13 (0.99, 1.29)	323	321	1.25 (1.04, 1.51)	225	192	1.09 (0.87, 1.35)	
TT	368	355	1.29 (1.08, 1.54)	111	110	1.18 (0.88, 1.57)	75	66	1.05 (0.74, 1.49)	
TLR4 (rs11536898)										0.012, 0.069
CC	1962	1654	1.00	573	550	1.06 (0.92, 1.21)	372	313	0.93 (0.79, 1.10)	
CA/AA	433	317	0.83 (0.70, 0.97)	132	152	1.22 (0.95, 1.56)	64	69	1.15 (0.81, 1.63)	
TNF (rs1800630)										0.005, 0.013
CC	1803	1505	1.00	532	508	1.07 (0.93, 1.24)	339	269	0.88 (0.74, 1.05)	
CA/AA	592	466	0.93 (0.81, 1.07)	173	194	1.21 (0.97, 1.51)	97	112	1.31 (0.99, 1.74)	

	BMI Among Pre-Menopausal Women									
	Normal (< 25 kg/m^2)			Overweight (25 to <30 kg/m^2)			Obese (>= 30 kg/m^2)			
TRAF2 (rs2784075)[5]										0.004, 0.008
GG	244	244	1.00	229	170	0.78 (0.60, 1.03)	195	183	1.02 (0.77, 1.34)	
GA/AA	222	268	1.25 (0.97, 1.62)	295	245	0.94 (0.72, 1.22)	328	208	0.73 (0.56, 0.95)	
TRAF2 (rs7027246)										<.001, 0.002
GG	230	220	1.00	222	159	0.8 (0.60, 1.06)	183	179	1.1 (0.83, 1.47)	
GA/AA	235	288	1.32 (1.02, 1.70)	300	255	0.99 (0.76, 1.30)	337	209	0.74 (0.57, 0.98)	
TRAF2 (rs908831)										0.018, 0.021
AA	152	168	1.00	143	105	0.7 (0.50, 0.99)	124	128	1.00 (0.71, 1.41)	
AG	227	236	0.96 (0.72, 1.28)	261	209	0.8 (0.59, 1.07)	263	184	0.70 (0.52, 0.95)	
GG	87	108	1.17 (0.81, 1.68)	120	101	0.85 (0.59, 1.21)	136	79	0.61 (0.42, 0.88)	

	Aspirin/NSAID Use[6]						
	Non-Regular Users			Regular Users			
	Controls	Cases	OR (95% CI)	Controls	Cases	OR (95% CI)	Interaction P value
TLR3 (rs11721827)							<.001, 0.003
AA	879	779	1.00	631	579	1.07 (0.92, 1.24)	
AC	239	251	1.17 (0.96, 1.43)	226	164	0.83 (0.66, 1.04)	
CC	24	29	1.33 (0.76, 2.30)	26	11	0.48 (0.23, 0.97)	
TLR3 (rs3775291)							0.014, 0.038
GG	552	521	1.00	479	358	0.82 (0.68, 0.98)	
GA	482	438	0.98 (0.82, 1.17)	346	324	1.04 (0.85, 1.26)	
AA	108	101	1.00 (0.75, 1.35)	58	71	1.36 (0.94, 1.97)	
TRAF2 (rs4880073)							0.016, 0.049
GG	330	357	1.00	315	243	0.72 (0.58, 0.91)	
GA	589	518	0.81 (0.67, 0.98)	409	375	0.87 (0.71, 1.07)	
AA	223	185	0.76 (0.59, 0.97)	159	136	0.81 (0.62, 1.07)	

[1] Dietary Oxidative Balance Score (DOBS) includes alcohol (pro-oxidant), vitamin C, vitamin E, beta carotene (data not available for MBCS), folic acid, and dietary fiber (anti-oxidants).

[2] Odds Ratios (OR) and 95% Confidence Intervals (CI) adjusted for age, study, BMI during referent year (where appropriate), parity, and genetic ancestry.

[3] P values are unadjusted and adjusted for multiple comparisons using the step down Bonferroni correction.

[4] Data unavailable for subset of SFBCS participants.

[5] In high LD with rs7027246: r^2 of 0.79, 0.93, and 0.96 among women with ≤28% NA ancestry, 29–70% NA ancestry, and ≥71% NA ancestry, respectively.

[6] Data available for 4-CBCS participants only.

Table 5

Associations between genes and breast cancer-specific mortality by percent Native American Ancestry.

			≤28% Native American Ancestry		>28% Native American Ancestry		
	Deaths/Person Years	**HR[1] (95% CI)**	**Deaths/Person Years**	**HR (95% CI)**	**Deaths/Person Years**	**HR (95% CI)**	**Interaction P-value (raw, adjusted)[2]**
TLR1 (rs7696175)[3]							0.880, 0.880
CC/CT	198 / 20050	1.00	114 / 11827	1.00	84 / 8223	1.00	
TT	51 / 3708	1.40 (1.03, 1.91)	31 / 2374	1.40 (0.94, 2.09)	20 / 1334	1.48 (0.91, 2.42)	
P-value (raw; adjusted)[2]		0.032, 0.032		0.100, 0.100		0.116, 0.116	
TLR4 (rs5030728)							0.252, 1.000
GG	114 / 12578	1.00	65 / 6971	1.00	49 / 5608	1.00	
GA	106 / 9413	1.24 (0.95, 1.61)	64 / 6015	1.18 (0.83, 1.66)	42 / 3398	1.32 (0.87, 2.01)	
AA	29 / 1767	1.96 (1.30, 2.95)	16 / 1216	1.59 (0.92, 2.76)	13 / 552	2.70 (1.46, 5.01)	
P-value (raw; adjusted)		0.001, 0.008		0.096, 0.506		0.002, 0.010	
TNFRSF1A (rs4149578)							0.356, 1.000
GG/GA	240 / 23463	1.00	140 / 14012	1.00	100 / 9451	1.00	
AA	7 / 253	2.71 (1.27, 5.76)	4 / 174	1.97 (0.72, 5.37)	3 / 80	4.34 (1.34, 14.06)	
P-value (raw; adjusted)		0.010, 0.029		0.184, 0.553		0.014, 0.043	
TNFSF10 (rs231985)							0.348, 1.000
AA/AT	243 / 23464	1.00	143 / 13979	1.00	100 / 9486	1.00	
TT	5 / 219	2.99 (1.23, 7.28)	2 / 169	2.15 (0.52, 8.78)	3 / 50	5.26 (1.63, 16.93)	
P-value (raw; adjusted)		0.016, 0.096		0.288, 0.622		0.005, 0.038	
TNFSF10 (rs3136597)							0.354, 1.000
CC	153 / 15330	1.00	81 / 8852	1.00	72 / 6478	1.00	
CA	78 / 7407	1.14 (0.86, 1.50)	53 / 4694	1.34 (0.94, 1.89)	25 / 2713	0.88 (0.55, 1.40)	
AA	18 / 1021	2.01 (1.23, 3.29)	11 / 655	2.14 (1.13, 4.06)	7 / 366	1.99 (0.91, 4.36)	
P-value (raw; adjusted)		0.006, 0.039		0.020, 0.140		0.084, 0.508	
TNFSF10 (rs231983)							0.326, 1.000
CC	48 / 5967	1.00	17 / 2607	1.00	31 / 3360	1.00	
CA/AA	200 / 17713	1.44 (1.04, 1.98)	128 / 11541	1.76 (1.06, 2.92)	72 / 6172	1.28 (0.83, 1.97)	
P-value (raw; adjusted)		0.027, 0.138		0.030, 0.179		0.269, 1.000	

[1] Breast cancer survival among primary invasive cases; Hazard Ratios (HR) and 95% Confidence Intervals (CI) adjusted for age, study, BMI during referent year, genetic ancestry, and SEER summary stage. Deaths are for breast cancer deaths only; other causes of death are censored.

[2] P values for SNPs are unadjusted and adjusted for multiple comparisons using the step down Bonferroni correction.

[3] ARTP p values for genes among all cases: *TLR1* = 0.037; *TLR4* = 0.032; *TNFRSF1A*= 0.151; *TNFSF10*= 0.122. Pathway p value is 0.152.