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Associations between *ALOX*, *COX*, and *CRP* polymorphisms and breast cancer among Hispanic and non-Hispanic white women: The Breast Cancer Health Disparities Study

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Abstract

Chronic inflammation is suggested to be associated with specific cancer sites, including breast cancer. Recent research has focused on the roles of genes involved in the leukotriene/lipoxygenase and prostaglandin/cyclooxygenase pathways in breast cancer etiology. We hypothesized that genes in *ALOX*/*COX* pathways and *CRP* polymorphisms would be associated with breast cancer risk and mortality in our sample of Hispanic/Native American (NA) (1,430 cases, 1,599 controls) and non-Hispanic white (NHW) (2,093 cases, 2,610 controls) women. A total of 104 Ancestral Informative Markers was used to distinguish European and NA ancestry. The adaptive rank truncated product (ARTP) method was used to determine the significance of associations for each gene and the inflammation pathway with breast cancer risk and by NA ancestry. Overall, the pathway was associated with breast cancer risk (P_{ARTP} =0.01). Two-way interactions with NA ancestry (p_{adj}< 0.05) were observed for *ALOX12* (rs2292350, rs2271316) and *PTGS1* (rs10306194). We observed increases in breast cancer risk in stratified analyses by tertiles of polyunsaturated fat intake for *ALOX12* polymorphisms; the largest increase in risk was among women in the highest tertile with *ALOX12* $rs9904779_{CC}$ (Odds Ratio (OR), 1.49; 95% Confidence Interval (CI) 1.14–1.94, p_{adj}=0.01). In a sub-analysis stratified by NSAIDs use, two-way interactions with NSAIDs use

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were found for *ALOX12* rs9904779 (p_{adj}= 0.02), rs434473 (p_{adj}= 0.02), and rs1126667 (p_{adj}= 0.01); ORs for *ALOX12* polymorphisms ranged from 1.55–1.64 among regular users. Associations were not observed with breast cancer mortality. These findings could support advances in the discovery of new pathways related to inflammation for breast cancer treatment.

Keywords

chronic inflammation; ethnicity; genetic variants

Introduction

It has been suggested that chronic inflammation is associated with specific sites of cancer, including cancer of the liver and colon, and in more recent research, cancer of the breast [1]. The direct relationship between inflammation and cancer is broadly accepted; yet, many of the molecular and cellular mechanisms facilitating this relationship remain uncertain [1]. A number of inflammatory cells, oxidants, growth factors, cytokines, and proinflammatory lipid mediators have been identified as factors associated with chronic inflammation [2]. For example, arachidonic acid (AA), a polyunsaturated omega-6 fatty acid, when oxygenated is transformed into numerous products which mediate or modify inflammatory reactions [3]. Two critical pathways involved in modifying the inflammatory response are the leukotriene and prostaglandin pathways; both of these pathways use AA as their primary precursor [4]. In the leukotriene pathway, arachidonate lypoxygenases (*ALOX*s) convert AA into leukotrienes, a class of paracrine hormones included in the inflammatory response, as well as other inflammation-mediating eicosanoids which are suspected to be involved in several inflammatory diseases [4]. In the prostaglandin pathway, cellular cyclooxygenases (*COX*) convert AA into an intermediate prostaglandin, PG-G2. The metabolites of the prostaglandin pathway are produced in human tissues and regulate physiological processes including angiogenesis, coagulation, proliferation, immune response, and inflammation [5].

Specific genes involved in the leukotriene/lipoxygenase and prostaglandin/cyclooxygenase pathways have been implicated in carcinogenesis, and recent research has focused on the roles of these genes in breast cancer etiology. Arachidonate 12-lipoxygenase (*ALOX12*) has been described as pro-carcinogenic, as it converts AA to 12-hydroperoxyeicosatetraenoic acid (12-HPETE) and increases expression of proinflammatory cytokine genes, such as tumor necrosis factor-alpha [4,6], while *ALOX15* has an anti-carcinogenic role, as it decreases cancer cell proliferation and increases apoptosis [4,7]. Very few studies to date have investigated the relationships of *ALOX* genes with breast cancer risk [8–10]. In a case-control study of Indian women conducted by Prasad et al., the functional *ALOX12* polymorphism rs1126667 (Gln261Arg) was found to be significantly associated with an increase in breast cancer risk [odds ratio (OR) $rs1126667_{AG/GG}$, 3.78; 95% confidence interval (CI) 2.37–6.04)] [10]. The study also revealed differences in genotype frequencies for rs1126667 among various racial/ethnic populations, suggesting that racial/ethnic differences in genotypes also may contribute to racial/ethnic differences in risk of breast cancer.

Cyclooxygenase occurs in several isoforms, including *COX*-1 and *COX*-2. *COX*-1 is the key enzyme in prostaglandin synthesis, while *COX*-2 is unexpressed under normal conditions and up-regulated by cytokines, growth factors, and tumor promoters. *COX*-2 expression is increased in the earlier stages of carcinogenesis and tumor development or growth [11]. *PTGIS*, also known as prostacyclin synthase, is produced by cyclooxygenase and converts prostaglandin H2 to prostaglandin. Some studies have examined the associations between *COX* polymorphisms and breast cancer risk but findings have been inconsistent [11–15]. Abraham et al. [15] examined the associations between common polymorphisms in the prostaglandin pathway (including *COX*-1/2 and *PTGIS*) and breast cancer risk and survival; their results were not indicative of significant associations with *COX*-1 and *COX*-2 polymorphisms and breast cancer risk or survival; but the homozygous variant genotype of *PTGIS* rs5602 did show a modest increase in breast cancer risk [15]. Although findings have been inconsistent, numerous epidemiologic studies have established that use of aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs) decreases the risk of breast cancer [16]. NSAIDs function by inhibiting *COX* genes, suggesting that the positive effect of NSAIDs in the reduction of breast cancer risk may be linked to suppression of *COX* overexpression [17].

Another marker of inflammation is C-reactive protein (*CRP*). *CRP* is an acute phase protein characterized by increases in its plasma concentration in response to acute inflammation, tissue damage, or infection. *CRP* has also been shown to be associated with chronic low-grade inflammation in diseases such as diabetes, obesity, heart disease, and specific types of cancers [18]. The physiological role of circulating *CRP* has been investigated in several studies of breast cancer outcomes and survivorship [19–21]; however, few studies have examined the associations between genetic variation in the *CRP* gene and breast cancer risk [22] and to date, no published studies have investigated *CRP* genetic associations with breast cancer survival.

As previously mentioned, AA is a polyunsaturated omega-6 fatty acid involved in the leukotriene/lipoxygenase and prostaglandin/cyclooxygenase pathways. Both animal and human studies have indicated that high intakes of omega-polyunsaturated fatty acids (PUFAs), regulate various stages in the development of breast and colon cancer [23]. Case-control studies have shown modest positive associations with high-fat diets and postmenopausal breast cancer risk, and strong correlations have been found between fat intake and breast cancer rates [23]. Previous studies suggest a role for dietary fats, such as polyunsaturated fats, as risk modifiers in breast cancer associations [23].

We hypothesized that polymorphisms in the *ALOX12*, *ALOX15*, *CRP*, *PTGS1* (*COX-1*), *PTGS2* (*COX-2*), and *PTGIS* genes would be associated with breast cancer risk and breast cancer-specific mortality in our sample of Hispanic and non-Hispanic white (NHW) women from the Breast Cancer Health Disparities Study (BCHDS). As a secondary aim, we evaluated the hypothesized associations between these genes and breast cancer risk by subgroups of Native American (NA) ancestry, menopausal status, body mass index (BMI), history of NSAIDs and aspirin use, and dietary fat intake.

Materials and methods

The BCHDS consists of participants from three population-based case-control studies: the 4-Corners Breast Cancer Study (4-CBCS), the San Francisco Bay Area Breast Cancer Study (SFBCS), and the Mexico Breast Cancer Study (MBCS) [24]. All participants signed informed written consent prior to participation, completed an interview, and had a blood or mouth sample available for DNA extraction. The study was approved by the Institutional Review Board for Human Subjects at each institution.

The 4-CBCS participants were Hispanic, NA (non-reservation living), and NHW women between 25 and 79 years of age with a histological confirmed diagnosis of *in situ* or invasive 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 [25]. Only 2.5% of the total study population for the 4-CBCS was NA, therefore, these women were analyzed with Hispanic women. The SFBCS included Hispanic and NHW 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 and frequency-matched to cases based on the expected race/ethnicity and 5-year age distribution [26,27]. Participants from the MBCS were between 28 and 74 years of age, living in one of three states, Monterrey, Veracruz and Mexico City, for the past five years. Participants from MBCS were not asked race or ethnicity. 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 [28].

Data Harmonization

Interview data were harmonized across the three studies [24]. The present analyses considered adjusting for BMI (kg/m^2) calculated as self-reported weight during the referent year (or more distantly recalled weight if referent year weight was not available or measured weight if neither were available) divided by measured height squared, parity (number of live births and stillborn pregnancies), self-reported ethnicity in the U.S. studies (all women in Mexico were considered Hispanic in analysis focusing on ethnicity), and highest level of education. The referent year was defined as the calendar year prior to diagnosis for cases or selection into the study for controls.

Genetic Data

DNA extraction occurred from either whole blood (n=7,286) or mouthwash (n=637) samples. Whole Genome Amplification (WGA) was applied to the mouthwash-derived samples prior to genotyping. A tagSNP approach was used to characterize variation across candidate genes. TagSNPs were selected based on the following: 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. A total of 104 Ancestral Informative Markers (AIMs) was used

to distinguish European and NA ancestry in the study population [24]. 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 reached (99.65% for WGA samples). We included 132 internal replicates that were blinded representing 1.6% of the sample set. The duplicate concordance rate was 99.996% as determined by 193,297 matching genotypes among sample pairs [24].

In the current analysis, we examined polymorphisms in the *ALOX12* (n=6 single nucleotide polymorphisms (SNPs)), *ALOX15* (n=5), *CRP* (n=3), *PTGIS* (n=2), *PTGS1* (n=5), and *PTGS2* (n=5) genes. Table 1 describes the 26 SNPs in detail, including the MAFs and adjusted Hardy-Weinberg equilibrium (HWE) p values.

Survival Data

Survival status was available for the Utah, New Mexico, Colorado, Arizona, and California study centers. Each center's respective cancer registry provided information on date of death or last follow-up (month and year). Survival (in months) was calculated as the difference between diagnosis date and date of death or last follow-up. The cause of death was classified as breast cancer if either the primary or contributing cause of death noted on the death certificate was breast cancer. Survival data were not available for the MBCS.

Statistical Methods

STRUCTURE was used to compute individual ancestry assuming two founding populations [29,30] and each study participant was classified by level of percent NA ancestry. The following strata for percent NA ancestry were created using cut-points based on the distribution of NA ancestry in the control population: 0–28%, 29–70%, and 71–100%. The groups were categorized in this manner to ensure sufficient power to assess associations. For stratified analyses in the present analyses, two groups were used for comparisons: low NA ancestry: < 29% vs. high NA ancestry: ≥ 29%. When used as an adjusting variable to assess confounding, NA ancestry was modeled as a continuous variable.

Power calculations were performed utilizing online software, The Genetic Power Calculator, which is located at the following: http://pngu.mgh.harvard.edu/~purcell/gpc/cc2.html. This software is used for the analysis of discrete traits in case-control studies [31]. The following parameters were considered to estimate the power using the study-specific median MAF=0.23 of the polymorphisms combined (see Table 1): number of cases, ratio of controls to cases, prevalence of breast cancer in U.S. population based on SEER age-adjusted prevalence, genotype point estimate (2-degree of freedom test/co-dominant model), D prime/r^2 (LD) =0.8, and defined type 1 error rate=0.05. Using the median MAF to detect an odds ratio (OR) of 1.20 and 1.50, under the above conditions, the overall power would be equal to 43% and 98%, respectively.

Descriptive statistics were calculated for all covariates and t-tests and chi-square tests were used to assess differences between groups. The homozygous common genotypes for each polymorphism were used as the referent categories. Using co-dominant models, genotype associations for all SNPs were estimated as ORs with 95% confidence intervals (CIs) by

unconditional logistic regression with adjustments for age, study center, and percent NA ancestry. Based on initial assessment of the co-dominant associations, dominant and recessive models were also examined. Potential confounders included BMI, menopausal status, parity, ethnicity, education, menopausal hormone therapy use, physical activity, caloric intake per day, and smoking status (ever or never). These covariates were included in multivariable models if their univariate P values were ≤ 0.20 and if they changed the point estimate for the main effects of the genotypes by ≥ 10% for SNPs that were found to be statistically significant prior to multiple comparisons [32]. However, there was no evidence of confounding and the models were adjusted for age, study center, and percentage of NA ancestry. Interactions between the pathway genes, NA ancestry, BMI (normal: < 25 kg/m^2 vs. overweight or obese: ≥ 25 kg/m^2), menopausal status, and dietary fat intake were assessed using the likelihood-ratio test comparing the model including an interaction term with a reduced model without the term. A subset analysis (n= 3,771) was conducted to evaluate interactions between SNPs and history of regular NSAIDs and aspirin use using the 4-CBCS sample, due to data not collected for these variables in the MBCS and the SFBCS. To account for the different number of foods queried on the diet questionnaires used for each study, dietary fat (total fat and polyunsaturated fat) intakes were evaluated as grams of fat per 1,000 calories and tertiles of intake based on the study-specific distribution of among controls.

For survival analyses, hazard ratios (HR) and 95% CIs were estimated using multivariable Cox proportional hazard models and were adjusted for SEER disease stage at diagnosis, age, NA ancestry, and study center. Stratified analyses were also conducted for survival analyses to determine if there was evidence of effect modification by NA ancestry.

Women were classified as either premenopausal or postmenopausal based on self-reported responses to questions on menstrual history. Women were classified postmenopausal using study-specific criteria. Those who were taking (HT) and still having periods and were at or above the 95^{th} percentile of age for ethnicity of those who reported having a natural menopause among their study center, were classified as postmenopausal. This age was 58 years for NHWs and 56 for Hispanics in the 4-CBCS, age 54 in the MBCS, and 55 for NHWs and 56 for Hispanics in the SFBCS.

Results were adjusted for multiple comparisons taking into account tagSNPs within each gene using the step-down Bonferroni correction (i.e., Holm's method) based on the effective number of independent SNPs as determined using the SNP spectral decomposition method proposed by Nyholt [33] and modified by Li and Ji [34]. The interaction p values, based on 1-df likelihood-ratio tests, were adjusted using the step-down Bonferroni correction or the Holm's test [35]. We considered an adjusted p value < 0.05 as potentially important for main effects and for interactions. The adaptive rank truncated product (ARTP) method that uses a highly efficient permutation algorithm to determine the significance of association of each gene and of the inflammation pathway with breast cancer risk overall and by NA ancestry was also utilized. The gene p values were generated using the ARTP package in R, permuting outcome status 1,000 times while adjusting for age, study center, and NA ancestry [36]. We report both pathway and gene p values (P_{ARTP}). All other data analyses were performed using SAS version 9.3 (SAS Institute, Cary NC).

Results

The distributions of the demographic and major risk factors for breast cancer in the BCHDS have been previously reported [24,37]. A total of 7,732 breast cancer cases and controls were included in analyses that evaluated breast cancer risk. Table 2 describes the distribution of selected variables of importance to the present analysis. More Hispanic women were overweight or obese compared to NHW women, regardless of case-control status ($p < 0.001$); however, NHW women consumed more dietary fats compared to Hispanic women in our study ($p<0.001$).

Associations of several of the genes with breast cancer risk were statistically significant both overall and by NA ancestry group as established by ARTP. For breast cancer risk among all women combined (Table 3), P_{ARTP} values were significant for the following genes: *ALOX12* ($P_{ARTP}= 0.01$), *PTGS1* ($P_{ARTP}= 0.01$), and *PTGS2* ($P_{ARTP}= 0.01$). When stratified by NA ancestry, *ALOX12* was significantly associated with breast cancer risk among both the low ($P_{ARTP}= 0.01$) and high ($P_{ARTP}= 0.01$) ancestry groups. While *ALOX15* ($P_{ARTP}= 0.01$) and *PTGS1* ($P_{ARTP}= 0.01$) were significantly associated with breast cancer risk among women with low ancestry, *PTGS2* was significantly associated with breast cancer risk among the high ancestry group ($P_{ARTP}=0.03$). Significant two-way interactions with NA ancestry ($p_{adj}< 0.05$) were observed for *ALOX12* polymorphisms (rs2292350, rs2271316) and for *PTGS1* (rs10306194). We did not find significant associations with *CRP* or *PTGIS* for breast cancer risk overall or by NA ancestry (data not shown). The overall pathway P_{ARTP} was 0.01.

Significant two-way interactions were observed between *PTGS2* (rs20417) and menopausal status ($p_{adj}= 0.02$) and also between *CRP* (rs1130864) and BMI ($p_{adj}= 0.02$) (data not shown) for breast cancer risk. In analyses stratified by menopausal status, decreased breast cancer risk was associated with the CC vs. GG genotype of *PTGS2* rs20417 among premenopausal women (OR, 0.60; 95% CI 0.37–0.99, $p_{adj}=0.05$). Decreased breast cancer risk also was associated with the CT/TT vs. CC genotype of *CRP* rs1130861 among women with normal BMI (OR, 0.79; 95% CI 0.67–0.93, $p_{adj}=0.04$).

Since dietary fat intake could modify breast cancer risk associated with leukotriene and prostaglandin pathway-related genes, we examined interaction effects between total dietary fat and polyunsaturated fat intakes. We did not observe significant two-way interactions between total fat and the *ALOX*, *COX*, or *CRP* genes; however, interactions were observed for *ALOX12* polymorphisms rs434473 ($p_{adj}=0.05$) and rs1126667 ($p_{adj}=0.05$) (Table 4). Among women in the highest tertile of polyunsaturated fat intake, the largest increase in risk was observed for *ALOX12* $rs9904779_{CC}$ (OR, 1.49; 95% CI 1.14–1.94, $p_{adj}=0.01$). No significant interactions were identified between polyunsaturated fat intake and *ALOX15*, *COX*, and *CRP* SNPs.

Table 5 shows genes with significant two-way interactions with regular use of NSAIDs for the 4-CBCS sample. Among regular users, statistically significant interactions were observed for *ALOX12* polymorphisms rs9904779 ($p_{adj}= 0.02$), rs434473 ($p_{adj}= 0.02$), and rs1126667 ($p_{adj}= 0.01$). Significant ORs ranged from 1.55–1.64 among regular NSAIDs

users. We did not identify significant interactions for *ALOX15*, *CRP*, or the *COX* genes. No significant interactions were found with evaluation of regular aspirin use (data not shown).

Lastly, we examined the associations between the *ALOX*, *CRP*, and *COX* genes with risk of breast cancer-specific mortality for all invasive breast cancer cases and by NA ancestry. After adjustment for multiple comparisons, we did not find any of the polymorphisms to be associated with breast cancer mortality (data not shown).

Discussion

In this study, we observed that specific genes involved in the inflammation-related leukotriene/lipoxygenase and prostaglandin/cyclooxygenase pathways were significantly associated with breast cancer risk in our admixed population of Hispanic and NHW women. When stratified by level of NA ancestry, we found significant interactions among *ALOX12* SNPs (rs2292350, rs2271316) and *PTGS1* rs10306194. Although we did not find significant interactions between total dietary fat intake and the inflammation genes, our results stratified by intake of polyunsaturated fat showed two-way interactions for *ALOX12* polymorphisms rs434473 and rs1126667, and significant increases in breast cancer risk were observed among women with the highest tertile of polyunsaturated fat intake for three SNPs of *ALOX12*. In our subset analysis of the 4-CBCS, regular use of NSAIDs significantly interacted with *ALOX12* polymorphisms (rs9904779, rs434473, rs1126667), with increases in breast cancer risk observed among regular users only. No significant interactions or associations were observed with regular aspirin use. We also considered the outcome of breast cancer-specific mortality, and no significant associations were identified for any of the genes.

The majority of associations were observed with *ALOX12* polymorphisms. As previously mentioned, *ALOX12* has been described as pro-carcinogenic, as it converts AA to 12-HPETE and increases expression of proinflammatory cytokine genes, such as tumor necrosis factor-alpha [4,6]. Another *ALOX12* product, 12-HETE, has also been found to be an eicosanoid that can stimulate cancer cells by up-regulating the expression and secretion of cathepsin B and by increasing the invasiveness and migration of cancer cells [8,38]; more specifically with breast cancer, 12-HETE has been found to increase proliferation and invasion of breast cells by inducing collagenase secretion from cells [39].

We also observed significant associations with overall breast cancer risk using ARTP for *COX-1* and *COX-2* genes; most of the associations were centered around *PTGS1* rs10306194 and *PTGS2* rs5277. *PTGS1* rs10306194 is located in the 3-prime UTR region, and *PTGS2* rs5277, one of the most studied *COX*-2 variants, is located in a coding region. Findings of previous studies investigating *PTGS1* and *PTGS2* have been lacking; however, a recent meta-analysis examining the relationship between *COX*-2 SNPs and breast cancer risk only identified a borderline significant increased risk of breast cancer with rs5277 in a recessive model (OR, 1.22; 95% CI 0.96–1.55) [40]. Our results indicate that $rs5277_{cc}$ is associated with a reduced risk (OR, 0.64; 95% CI 0.44–0.94). Furthermore, in a report by Cox and colleagues, $rs5275_{cc}$ was found to be inversely associated with breast cancer risk among a sample of predominantly NHW women from the Nurses' Health Study and the

Harvard Women's Health Study (pooled OR, 0.80; 95% CI 0.66–0.97, p trend=0.02) [41]. Reportedly, rs5275 is in high LD with rs5277 and with the other highly known polymorphisms on PTGS2 [41,42]. Our findings for PTGS2 and breast cancer risk require replication in future studies and other potentially functional polymorphisms in the COX-2 gene should be examined.

Use of NSAIDs has been associated with modest decreases in breast cancer risk in some epidemiological studies [43]. NSAIDs have an anti-inflammatory effect mainly because they bind with *COX-2*, and block the catalysis of AA to pro-inflammatory prostaglandins [44]. Several studies have examined the interaction between NSAIDs use and *COX* genes with breast cancer risk [12,44]; however, interaction effects between *ALOX* polymorphisms and NSAIDs use do not appear to have been investigated. Our results not only suggest that significant interactions exist between *ALOX12* SNPs rs9904779, rs434473, and rs1126667, but that these specific SNPs significantly increase risk of breast cancer among regular NSAIDs users. Since the leukotriene and prostaglandin pathways are in competition, it has been speculated that *ALOX* polymorphisms might indirectly interact with NSAID use to modify the protective effect which could have pharmacogenetic repercussions for prescribing NSAIDs for long-term use in some individuals [4].

The study conducted by Prasad et al. revealed differences in genotype frequencies for *ALOX12* rs1126667 among various racial/ethnic populations, including Indians, Caucasians, Chinese, Blacks, Koreans, and Spanish [10], suggesting that this polymorphism may contribute to racial/ethnic differences in breast cancer risk. Markers of inflammation, such as *CRP* levels, have also been found to be higher among minority populations, including Hispanics, when compared to individuals of European descent [45]. Although we did not observe associations between *CRP* variants and breast cancer risk overall or by NA ancestry, we found significant interactions with percent of NA ancestry and *ALOX12* SNPs rs2292350 and rs2271316. Most notably with *PTGS1* rs10306194, we observed among women with high NA ancestry, a significant increase in breast cancer risk (OR, 1.45; 95% CI 1.23–1.70 p_{adj}=0.0003) and a significant two-way interaction.

Certain breast cancer-related genes may modify the effects of hormonal risk factors, such as menopausal status, on breast cancer risk [46]. *PTGS2* rs20417 significantly interacted with menopausal status and risk of breast cancer, and decreased risk of breast cancer was associated with the CC genotype among premenopausal women. In previous reports, *ALOX* genes have been found to be associated with the occurrence of natural menopause [47,48]. Xiao et al. identified several *ALOX12* SNPs (rs2292350, rs312470, and rs312462) to be associated with age at natural menopause in postmenopausal women [47]. We did not observe any significant associations between *ALOX12* or *ALOX15* polymorphisms and breast cancer risk stratified by menopausal status.

The present analysis has several strengths and some limitations. Our study was able to compare breast cancer associations with 26 SNPs across six genes involved in several inflammation pathways and is the first to investigate associations of these specific inflammation genes and breast cancer risk by levels of NA ancestry. We were able to characterize the overall association of the combined pathway with breast cancer risk using

the ARTP method. Given the comprehensive data on lifestyle data, we were able to examine interaction effects between inflammation-related variables, including BMI and dietary polyunsaturated fat intake, and the genes. However, data for aspirin and NSAIDs use were not collected for Mexico and the SFBCS studies; but overall breast cancer risk estimates for the 4-CBCS were comparable to those for BCHDS. As with many epidemiologic studies, specific subject responses for variables included in subgroup analyses could be affected by recall bias. Our subgroup analyses and interactions should be examined further in future epidemiologic studies with larger sample sizes.

In summary, we observed significant associations between the *ALOX12*, *PTGS1*, and *PTGS2* genes and breast cancer risk; and overall, the inflammation-related pathway is significantly associated with breast cancer risk. Interestingly, many of the associations were with SNPs in *ALOX12*, and these were primarily observed among women who reported a history of regular NSAID use. We identified interactions with NA ancestry and dietary intake of polyunsaturated fats, and identified suggestive interactions between *CRP* and BMI and between *PTGS2* and menopausal status. To the best of our knowledge, this is the first report to characterize the genetic variation of the *ALOX* genes and other specific genes involved in inflammation-related pathways using a tagSNP approach with breast cancer risk among an admixed population of women from the U.S. and Mexico. These findings could support advances in the discovery of new pathways related to inflammation for breast cancer treatment.

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Abbreviations

ARTP	Adaptive rank truncated product

AA	Arachidonic acid
ALOX	Arachidonate lypoxygenases
BMI	Body mass index
BCHD	Breast Cancer Health Disparities Study
CRP	C-reactive protein
COX	Cyclooxygenase
MAF	Minor allele frequency
MBCS	Mexico Breast Cancer Study
NA	Native American
NSAIDs	Nonsteroidal anti-inflammatory drugs
NHW	Non-Hispanic white
SFBCS	San Francisco Bay Area Breast Cancer Study
SNPs	Single nucleotide polymorphisms
4-CBCS	4-Corners Breast Cancer Study

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Table 1

Description of *ALOX12*, *ALOX15*, *CRP*, *PTGIS*, *PTGS1*, and *PTGS2* polymorphisms, by ethnicity

						non-Hispanic Whites			Hispanics			
Symbol	**SNP ID**	**Chromosome Location**	**Coordinate**	**Region**	**Major/ Minor Allele[1]**	**Major allele freq.**	**Minor allele freq.**	**FDR adjusted HWE p value**	**Major allele freq.**	**Minor allele freq.**	**FDR adjusted HWE p value**	**Proportion Missing**
ALOX12	rs9904779	17p13.1	6898615	INTERGENIC	G/C	0.63	0.37	0.98	0.68	0.32	0.73	0.0005
ALOX12	rs434473	17p13.1	6904934	CODING	A/G	0.60	0.40	0.96	0.76	0.24	0.16	0.0014
ALOX12	rs1126667	17p13.1	6902760	CODING	G/A	0.60	0.40	0.96	0.68	0.32	0.32	0.0095
ALOX12	rs2292350	17p13.1	6901672	INTRON	G/A	0.56	0.44	0.96	0.74	0.26	0.13	0.0012
ALOX12	rs312462	17p13.1	6913652	CODING	C/T	0.91	0.09	0.96	0.90	0.10	0.15	0
ALOX12	rs2271316	17p13.1	6915401	INTERGENIC	G/C	0.63	0.37	0.98	0.52	0.48	0.19	0.004
ALOX15	rs2664592	17p13.3	4545163	INTERGENIC	G/C	0.79	0.21	0.96	0.74	0.26	0.92	0.0002
ALOX15	rs11568131	17p13.3	4534608	UTR	C/T	0.83	0.17	0.99	0.86	0.14	0.39	0.0594
ALOX15	rs916055	17p13.3	4534834	UTR	T/C	0.66	0.34	1.00	0.61	0.39	0.95	0.0007
ALOX15	rs11078527	17p13.3	4540647	INTERGENIC	C/T	0.84	0.16	1.00	0.86	0.14	0.56	0.0014
ALOX15	rs8182325	17p13.3	4544551	INTERGENIC	C/T	0.87	0.13	0.89	0.87	0.13	0.59	0.0689
CRP	rs1130864	1q21-q23	159683091	UTR	C/T	0.69	0.31	0.86	0.63	0.37	0.53	0.0007
CRP	rs2808630	1q21-q23	159680868	INTERGENIC	T/C	0.71	0.29	0.96	0.81	0.19	0.81	0.0002
CRP	rs1205	1q21-q23	159682233	UTR	C/T	0.79	0.21	0.67	0.89	0.11	0.73	0.0002
PTGIS	rs5602	20q13.13	48121978	UTR	C/T	0.52	0.48	0.62	0.55	0.45	0.12	0
PTGIS	rs6125671	20q13.13	48175598	INTRON	C/T	0.70	0.30	0.96	0.55	0.45	0.61	0
PTGS1	rs4240474	9q32-q33.3	125145619	INTRON	G/A	0.88	0.12	0.72	0.78	0.22	0.30	0.0005
PTGS1	rs3842798	9q32-q33.3	125145743	INTRON	T/C	0.82	0.18	1.00	0.72	0.28	0.52	0.0002
PTGS1	rs4273915	9q32-q33.3	125145329	INTRON	G/C	0.84	0.16	0.96	0.75	0.25	0.68	0.0002
PTGS1	rs10306135	9q32-q33.3	125137695	INTRON	A/T	0.87	0.13	0.96	0.88	0.12	0.68	0.0007
PTGS1	rs10306194	9q32-q33.3	125157198	UTR	C/A	0.84	0.16	0.96	0.91	0.09	0.70	0
PTGS2	rs20417	1q25.2-q25.3	186650321	INTERGENIC	G/C	0.84	0.16	0.96	0.82	0.18	0.92	0.0005
PTGS2	rs5275	1q25.2-q25.3	186643058	UTR	T/C	0.65	0.35	0.93	0.70	0.30	0.94	0.0002
PTGS2	rs5277	1q25.2-q25.3	186648197	CODING	G/C	0.84	0.16	0.86	0.91	0.09	0.36	0.0005
PTGS2	rs2745557	1q25.2-q25.3	186649221	INTRON	G/A	0.83	0.17	0.96	0.90	0.10	0.01	0
PTGS2	rs689466	1q25.2-q25.3	186650751	INTERGENIC	A/G	0.82	0.18	0.96	0.68	0.32	0.36	0.0005

[1] Major/minor allele reported for NHW population; minor allele frequency and Hardy-Weinberg Equilibrium (HWE) based on control population.

Mol Carcinog. Author manuscript; available in PMC 2016 December 01.

Table 2

Characteristics of study population, stratified by ethnicity and case-control status, The Breast Cancer Health Disparities Study *(n=7,732)*

	Non-Hispanic Whites (n=3,029)					Hispanics (n= 4,703)					
	Cases		Controls			Cases		Controls			
	No.	%	No.	%	p value[1]	No.	%	No.	%	p value[1]	p value[2]
Total Subjects	***1,430***		***1,599***			***2,093***		***2,610***			
Study Site											
4-CBCS	1,176	82.2	1,335	83.5	0.36	579	27.7	736	28.2	0.94	<0.001
Mexico	-		-			816	39.0	994	38.1		
SFBCS	254	17.8	264	16.5		698	33.4	880	33.7		
Age (years)											
<40	87	6.1	117	7.3	0.04	198	9.5	313	12.0	0.22	<0.001
40–49	400	28.0	409	25.6		708	33.8	834	32.0		
50–59	403	28.2	410	25.6		614	29.3	758	29.0		
60–69	340	23.8	356	22.3		425	20.3	530	20.3		
≥70	200	14.0	307	19.2		148	7.1	175	6.7		
Percentage of Native American ancestry											
< 0.29	1,419	99.2	1,591	99.5	0.34	276	13.2	280	10.7	0.01	<0.001
≥ 0.29	11	0.8	8	0.5		1,817	86.8	2,330	89.3		
Menopausal status											
Premenopausal	474	34.0	494	31.5	0.14	831	41.2	1,027	40.7	0.71	<0.001
Postmenopausal	919	66.0	1,075	68.5		1,186	58.8	1,499	59.3		
Body mass index (kg/m^2)											
Normal (< 25)	650	46.1	699	44.4	0.35	482	23.4	453	17.6	<0.001	<0.001
Overweight or obese (≥25)	761	53.9	877	55.7		1,580	76.6	2,123	82.4		
Fat intake/1000 kcal per day	Median		Median		p value[3]	Median		Median		p value[3]	p value[4]
Total fat (g)	38.45		38.83		0.19	36.63		36.74		0.44	<0.001
Polyunsaturated fat (g)	7.85		7.88		0.59	7.29		7.69		0.001	0.12
History of aspirin use[5]											
Yes	262	22.6	324	24.5	0.26	84	14.8	140	19.4	0.03	<0.001

	Non-Hispanic Whites (n=3,029)					Hispanics (n= 4,703)					
	Cases		Controls			Cases		Controls			
	No.	%	No.	%	p value[1]	No.	%	No.	%	p value[1]	p value[2]
No	898	77.4	997	75.5		483	85.2	583	80.6		
History of NSAIDs use[5]											
Yes	363	31.3	409	31.0	0.86	137	24.2	201	27.8	0.14	0.002
No	797	68.7	912	69.0		430	78.8	522	72.2		

Missing information: menopausal status n=227; body mass index n=107; total fat n=202; polyunsaturated fat n=202

[1] Case-control comparison within ethnicity. *p* values from chi-square tests.

[2] Ethnic group comparison, regardless of case-control status. *p* values from chi-square tests.

[3] Case-control comparison within ethnicity. *p* values from t-tests.

[4] Ethnic group comparison, regardless of case-control status. *p* values from t- tests.

[5] Data on regular use of aspirin and NSAIDs were collected in the 4-CBCS only.

Mol Carcinog. Author manuscript; available in PMC 2016 December 01.

Table 3

Associations between *ALOX12/15*, *PTGS1/2 (COX1/2)* polymorphisms, and breast cancer risk stratified by percent of Native American ancestry

		Cases		Controls		All women (n=7,732)			< 29% Native American ancestry (n=3,566)			≥29% Native American ancestry (n=4,166)				
Gene/SNP	**Genotype**	**N**	**%**	**N**	**%**	**OR[1]**	**(95% CI)**	**P_{ARTP}**	**OR[2]**	**95% CI**	**P_{ARTP}**	**OR[2]**	**95% CI**	**P_{ARTP}**	**$p_{int.}$**	**p_{adj}**
ALOX12 (rs9904779)								0.01			0.01			0.01		
	GG	1428	(43.71)	1839	(56.29)	1.00			1.00			1.00			0.657	
	GC	1614	(46.38)	1866	(53.62)	1.10	(1.00 - 1.21)		1.10	(0.95 - 1.28)		1.11	(0.97 - 1.26)			
	CC	480	(48.88)	502	(51.12)	1.20	(1.04 - 1.38)		1.28	(1.04 - 1.56)		1.13	(0.91 - 1.39)			
P-trend; p_{adj}						0.01;	0.03		0.02;	0.10		0.11				
ALOX12 (rs434473)																
	AA	1599	(43.18)	2104	(56.82)	1.00			1.00			1.00			0.778	
	AG/GG	1919	(47.76)	2099	(52.24)	1.17	(1.07 - 1.28)		1.20	(1.05 - 1.38)		1.16	(1.02 - 1.31)			
Wald p; p_{adj}						0.001;	0.008		0.01;	0.05		0.02;	0.10			
ALOX12 (rs1126667)																
	GG	1359	(43.05)	1798	(56.95)	1.00			1.00			1.00			0.408	
	GA/AA	2120	(47.21)	2371	(52.79)	1.16	(1.05 - 1.27)		1.22	(1.06 - 1.40)		1.12	(0.99 - 1.27)			
Wald p; p_{adj}						0.002;	0.005		0.01;	0.05		0.07				
ALOX12 (rs2292350)																
	GG	1679	(46.11)	1962	(53.89)	1.00			1.00			1.00			0.002	0.01
	GA/AA	1837	(45.04)	2242	(54.96)	0.92	(0.84 - 1.01)		0.80	(0.69 - 0.91)		1.05	(0.93 - 1.19)			
Wald p; p_{adj}						0.08			0.001;	0.005		0.44				
ALOX12 (rs2271316)																
	GG/GC	2713	(45.59)	3238	(54.41)	1.00			1.00			1.00			0.006	0.02
	CC	802	(45.67)	954	(54.33)	1.04	(0.93 - 1.16)		1.25	(1.04 - 1.51)		0.93	(0.81 - 1.06)			
Wald p; p_{adj}						0.50			0.02;	0.10		0.27				
ALOX15 (rs8182325)								0.10			0.01			0.96		
	CC/CT	3150	(45.06)	3840	(54.94)	1.00			1.00			1.00			0.033	0.12
	TT	48	(37.80)	79	(62.20)	0.73	(0.51 - 1.05)		0.47	(0.27 - 0.83)		1.07	(0.66 - 1.74)			
Wald p; p_{adj}						0.09			0.01;	0.04		0.79				

		Cases		Controls		All women (n=7,732)			< 29% Native American ancestry (n=3,566)			≥29% Native American ancestry (n=4,166)				
Gene/SNP	**Genotype**	**N**	**%**	**N**	**%**	**OR[1]**	**(95% CI)**	**P_{ARTP}**	**OR[2]**	**95% CI**	**P_{ARTP}**	**OR[2]**	**95% CI**	**P_{ARTP}**	**$p_{int.}$**	**p_{adj}**
PTGS1 (rs10306194)								0.01			0.01			0.33		
	CC	2668	(44.55)	3321	(55.45)	1.00			1.00			1.00			0.001	0.002
	CA/AA	854	(49.02)	888	(50.98)	1.16	(1.04 - 1.29)		0.98	(0.85 - 1.14)		1.45	(1.23 - 1.70)			
Wald p; p_{adj}						0.01;	0.03		0.79			<.0001;	0.0003			
PTGS2 (rs5277)								0.01			0.14			0.03		
	GG	2713	(45.16)	3295	(54.84)	1.00			1.00			1.00			0.316	
	GC	767	(47.91)	834	(52.09)	1.08	(0.97 - 1.21)		1.06	(0.91 - 1.23)		1.12	(0.95 - 1.33)			
	CC	43	(35.54)	78	(64.46)	0.64	(0.44 - 0.94)		0.54	(0.33 - 0.86)		0.91	(0.49 - 1.71)			
P-trend; p_{adj}						0.98			0.48			0.31				

[1] Models are adjusted for age, study center, and Native American ancestry.

[2] Models are adjusted for age and study center.

* Adaptive rank truncated product (ARTP)

Table 4

Associations between *ALOX12* polymorphisms and breast cancer risk stratified by dietary polyunsaturated fat intake (tertiles)

Gene/SNP	Genotype	Tertile 1						Tertile 2						Tertile 3						p-int; p_{adj}
		Cases		Controls				Cases		Controls				Cases		Controls				
		N	%	N	%	OR	95% CI	N	%	N	%	OR	95% CI	N	%	N	%	OR	95% CI	
ALOX12 (rs9904779)																				
	GG	508	(41.33)	573	(41.98)	1.00		479	(39.95)	585	(42.98)	1.00		395	(39.46)	625	(45.72)	1.00		0.12
	GC	560	(45.57)	609	(44.62)	1.04	(0.88 - 1.24)	558	(46.54)	608	(44.67)	1.12	(0.95 - 1.33)	458	(45.75)	595	(43.53)	1.15	(0.96 - 1.37)	
	CC	161	(13.10)	183	(13.41)	1.01	(0.79 - 1.29)	162	(13.51)	168	(12.34)	1.19	(0.92 - 1.52)	148	(14.79)	147	(10.75)	1.49	(1.14 - 1.94)	
P-trend; p_{adj}						0.81						0.11						0.004	0.01	
ALOX12 (rs434473)																				0.01;
	AA	579	(47.19)	667	(48.90)	1.00		541	(45.16)	663	(48.75)	1.00		419	(41.90)	702	(51.43)	1.00		0.05
	AG/GG	648	(52.81)	697	(51.10)	1.08	(0.92 - 1.27)	657	(54.84)	697	(51.25)	1.16	(0.98 - 1.36)	581	(58.10)	663	(48.57)	1.34	(1.13 - 1.59)	
Wald p; p_{adj}						0.33						0.08						0.001	0.01	
ALOX12 (rs1126667)																				0.01;
	GG	494	(40.69)	553	(41.05)	1.00		450	(38.04)	565	(41.76)	1.00		368	(37.13)	624	(45.98)	1.00		0.05
	GA/AA	720	(59.31)	794	(58.95)	1.02	(0.87 - 1.20)	733	(61.96)	788	(58.24)	1.16	(0.99 - 1.37)	623	(62.87)	733	(54.02)	1.34	(1.13 - 1.59)	
Wald p; p_{adj}						0.82						0.06						0.001	0.01	

Models are adjusted for age, study center, and Native American ancestry.

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Table 5

Associations between *ALOX12* polymorphisms and breast cancer risk stratified by history of regular use of NSAIDs, 4-CBCS subset analysis *(n=3,826)*

Gene/SNP	Genotype	Cases		Controls		All 4-CBCS women (n=3,826)		Regular use of NSAIDs (n=1,110)		Non-regular use of NSAIDs (n=2,661)		p-int	p_{adj}
		N	%	N	%	OR	95 % CI	OR	95% CI	OR	95% CI		
ALOX12 (rs9904779)													
	GG	651	(43.52)	845	(56.48)	1.00		1.00		1.00		0.005	0.02
	GC/CC	1075	(47.27)	1199	(52.73)	1.17	(1.03 - 1.34)	1.57	(1.23 - 2.01)	1.04	(0.89 - 1.22)		
Wald p; p_{adj}						0.02;	0.06	0.0003;	0.002	0.62			
ALOX12 (rs434473)													
	AA	668	(43.32)	874	(56.68)	1.00		1.00		1.00		0.007	0.02
	AG/GG	1057	(47.46)	1170	(52.54)	1.19	(1.04 - 1.36)	1.55	(1.21 - 1.99)	1.06	(0.90 - 1.24)		
Wald p; p_{adj}						0.01;	0.05	0.001;	0.006	0.48			
ALOX12 (rs1126667)													
	GG	606	(42.98)	804	(57.02)	1.00		1.00		1.00		0.002	0.01
	GA/AA	1108	(47.31)	1234	(52.69)	1.19	(1.04 - 1.37)	1.64	(1.28 - 2.11)	1.05	(0.89 - 1.23)		
Wald p; p_{adj}						0.01;	0.05	<.0001;	0.001	0.59			
ALOX12 (rs2292350)													
	GG	688	(48.18)	740	(51.82)	1.00		1.00		1.00		0.292	
	GA/AA	1039	(44.34)	1304	(55.66)	0.85	(0.74 - 0.97)	0.74	(0.58 - 0.95)	0.90	(0.77 - 1.05)		
Wald p; p_{adj}						0.02;	0.06	0.02;	0.10	0.18			
ALOX12 (rs312462)													
	CC	1408	(45.40)	1693	(54.60)	1.00		1.00		1.00		0.038	0.09
	CT/TT	319	(47.61)	351	(52.39)	1.10	(0.93 - 1.30)	1.47	(1.06 - 2.02)	0.99	(0.81 - 1.20)		
Wald p; p_{adj}						0.26		0.02;	0.10	0.89			
ALOX12 (rs2271316)													
	GG/GC	1414	(44.96)	1731	(55.04)	1.00		1.00		1.00		0.298	
	CC	313	(50.08)	312	(49.92)	1.24	(1.04 - 1.47)	1.48	(1.07 - 2.06)	1.16	(0.95 - 1.42)		
Wald p; p_{adj}						0.02;	0.06	0.02;	0.10	0.16			

Models are adjusted for age and Native American ancestry.

*4-Corners Breast Cancer Study (4-CBCS); Nonsteroidal anti-inflammatory drugs (NSAIDs)