IL6, Aspirin, Nonsteroidal Anti-inflammatory Drugs, and Breast Cancer Risk in Women Living in the Southwestern United States

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Abstract

Interleukin-6 is a cytokine thought to be involved in inflammation, insulin, and estrogen-related pathways. We evaluate genetic variation in the *IL6* gene with risk of breast cancer. We also evaluate breast cancer associations with aspirin and nonsteroidal anti-inflammatory drugs. A breast cancer case-control study (*n* = 1,527 non-Hispanic white cases, 1,601 non-Hispanic white controls, 798 Hispanic/Native American cases, and 924 Hispanic/Native American controls) was conducted among women living in the southwestern United States (4-Corner's Breast Cancer Study). Five *IL6* single nucleotide polymorphisms (SNP) and *IL6* haplotypes based on these SNPs were evaluated. Allele frequencies were significantly different between non-Hispanic white and Hispanic/Native American women. Among postmenopausal women not recently exposed to hormones, the AG/GG genotypes of rs1800797 (−596A>G) and the GC/CC genotypes of rs1800795 (−174G>C) significantly reduced risk of breast cancer among non-Hispanic white women [odds ratio (OR), 0.69; 95% confidence interval (95% CI), 0.48-1.00 and OR, 0.68; 95% CI, 0.47-0.99, respectively] and Hispanic/Native American women (OR, 0.48; 95% CI, 0.28-0.83 and OR, 0.44; 95% CI, 0.26-0.99, respectively). Haplotypes of the five *IL6* SNPs further defined these associations. Recent aspirin use significantly decreased risk of breast cancer among postmenopausal Hispanic/Native American women not recently exposed to hormones (OR, 0.56; 95% CI, 0.33-0.96). Among non-Hispanic white, the inverse association with aspirin was not statistically significant. *IL6* genotype and haplotype significantly modified the association between aspirin and breast cancer, with the greatest effect modification being among women not recently exposed to hormones [*P* interaction = 0.06 (for non-Hispanic white) and 0.04 (for Hispanic/Native American) and SNP rs1800796 or −572G>C]. These data suggest that *IL6* is associated with breast cancer risk and modifies the association between estrogen and aspirin and breast cancer risk. (Cancer Epidemiol Biomarkers Prev 2007;16(4):747–55)

Introduction

Inflammation signaling pathways, as indicated by serum levels of various cytokines as well as other biomarkers of inflammation, seem to influence insulin signaling pathways resulting in insulin resistance, as reflected in serum levels of insulin and glucose. In women, estrogen may modulate the interaction of these pathways. Evidence for the interrelatedness of inflammation and insulin pathways includes epidemiologic studies showing strong associations between biomarkers of inflammation [e.g., serum interleukin-6 (IL-6), tumor necrosis factor-α, and a heightened risk for obesity-related insulin resistance (1-4)]. IL-6 affects lipid and glucose metabolism (5), factors thought to be important in the etiology of cancer. IL-6 stimulates liver secretion of C-reactive protein, an important biomarker for proinflammatory status in several diseases (6). Elevated serum IL-6 is associated with incidence or clinical outcome in several cancers, including prostate, bladder, colon, and breast cancer (7-9). IL-6 is a pleiotropic cytokine that can stimulate as well as repress tumor cell proliferation and growth, inhibit apoptosis, and enhance invasiveness and metastasis (10-12).

Estrogen plays a role in inflammation signaling pathways by repressing production of IL-6 through an estrogen receptor–dependent mechanism. Serum levels of IL-6 increase following menopause in healthy women and with age in both men and women (13, 14). IL-6 is also known to increase the expression of aromatase in breast cancer cells, thereby enhancing the conversion of androgens to estrogens (15). It has been suggested that the normal feedback regulation between estrogens and IL-6 is disrupted in cancer. In breast cancer, it has been shown that serum IL-6 is increased specifically in postmenopausal women with estrogen receptor–negative tumors (8, 9). *In vitro* studies suggest that various tumor cells have increased production of IL-6; therefore, it is generally assumed that elevated serum IL-6 levels reflect disease and the trophic effects of IL-6 on tumor proliferation and growth are primarily autocrine in nature (8).

Polymorphisms in the *IL6* gene promoter have been reported to be related to levels of circulating C-reactive protein (16), to be associated with different profiles of plasma IL-6 response to immunization (17), and to modify the association between high body mass index (BMI) and incident type 2 diabetes (18).

In this article, the *IL6* promoter G>C −174 single nucleotide polymorphism (SNP), implicated in breast cancer by these published studies, as well as four other polymorphisms within

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the *IL6* gene not studied previously were examined to evaluate associations with genotypes and haplotypes with breast cancer. We evaluated associations in a large case-control study of non-Hispanic white and Hispanic/Native American women living in the southwestern United States. We evaluate associations by menopausal status and by exposure to hormones because estrogen has been shown to influence IL-6 production. We also investigated associations with aspirin and nonsteroidal anti-inflammatory drugs (NSAID), which may be associated with breast cancer through an inflammation pathway.

Materials and Methods

Study participants were women living in Cochise, Coconino, Maricopa, Pima, Pinal, Santa Cruz, and Yuma Counties in Arizona or the states of Colorado, New Mexico, or Utah at the time of diagnosis or selection; American Indian women living on reservations were not eligible. Study hypotheses focused specifically on breast cancer in Hispanic women; therefore, sampling was stratified on ethnicity to select Hispanic women in larger proportion than their representation in the population. All Hispanic women diagnosed with breast cancer during the study period were selected for the study. An age-matched sample of non-Hispanic white women was randomly selected on a 1:1 ratio to the distribution of Hispanic cases in Arizona and Colorado; at a 4:1 ratio to the distribution of Hispanic cases in Utah; all Hispanic and non-Hispanic cases ages 50 years and under in New Mexico; and a at 1:1 ratio for women >50 years in New Mexico. The Generally Useful Ethnic Search System program was used to identify women who were Hispanic. A detailed description of study methods and response rates has been published and is briefly described below (19).

Cases were histologically confirmed *in situ* and invasive breast cancer (International Classification of Diseases for Oncology sites C50.0-C50.6 and C50.8-C50.9) diagnosed between October 1999 and May 2004. State tumor registries were used to initially identify and subsequently confirm case eligibility. The Utah and New Mexico state cancer registries are National Cancer Institute–funded Surveillance Epidemiology and End Results registries; Arizona and Colorado registries are part of the Centers for Disease Control and Prevention National Program of Cancer Registries. Of cases identified, 873 Hispanic and Native American and 1,683 non-Hispanic white women participated (68% of women contacted). Of these cases, 798 Hispanic/Native American and 1,527 non-Hispanic white women were diagnosed with first primary breast cancer and 576 Hispanic and Native American and 1,169 non-Hispanic white cases had DNA available for analyses.

Controls were selected from the target populations to match ethnicity and 5-year age distribution of cases. In Arizona and Colorado, participants <65 years of age were randomly selected from a commercial mailing list; in New Mexico and Utah controls, <65 years of age were randomly selected from driver's license lists. In all states, women ≥65 years were randomly selected from Center for Medicare Services lists. Of controls identified, 935 Hispanic/Native American and 1,671 non-Hispanic white women participated (42% of participants contacted), of which 725 Hispanic/Native American and 1,330 non-Hispanic white controls had DNA available for analyses.

All women identified were screened for eligibility before study enrollment. As part of the screening, women were asked to self-identify their race and ethnicity. Women initially identified as being Hispanic by the Generally Useful Ethnic Search System program who were subsequently found not to be Hispanic or Native American were ineligible for the study. All participants signed informed written consent before participation; the study was approved by the Institutional Review Board for Human Subjects at each institution.

Diet and lifestyle data were collected by trained and certified interviewers using an interviewer-administered computerized questionnaire (19). The referent period was the year before diagnosis for cases or selection for controls. Respondents were given the option of having the interview administered in either English or Spanish. Respondents were asked to self-identify their ethnicity and race as part of the study questionnaire. If a respondent described herself as belonging to more than one race or ethnic group, all were recorded. The questionnaire included information about medical history, reproductive history, family history, diet, and physical activity, use of tobacco, medication use, diabetes history, and weight history. BMI was calculated using the formula of weight in kilograms (kg)/height in meters $(m)^2$. Women were asked to ''best describe your menstrual status on (referent date)'' by selecting response from a card; this information was used to define individual menopausal status. Regular use (at least thrice weekly for at least 1 month) of aspirin and NSAIDs was obtained along with date of last use of these medications.

Dietary intake data were collected using an extensive diet history questionnaire that was modified to incorporate foods commonly eaten in the southwestern United States (20-22). An extensive physical activity questionnaire was modified from the Cross-Cultural Activity Participation Survey (23) and used to collect information on activities done at home, at work, and during leisure and included intensity of the activity and frequency at which activities were done during the referent year, at ages 15, 30, and 50 years. Total MET-hours of activity during the referent year was calculated based on the compendium of MET values for physical activities (24).

Genotyping. DNA was extracted from blood drawn from 76.6% of participants in Arizona, 74.8% of participants in Colorado, 75% of participants in New Mexico, and 93.6% of participants in Utah. *IL6* genotypes were obtained for 1,174 first primary cases and 1,329 controls (non-Hispanic whites), 555 cases and 683 controls (Hispanics), and 21 cases and 43 controls (Native Americans). All five *IL6* SNPs were genotyped using Taqman-based assays. For SNPs rs1800795 (−174G>C) and rs1800797 (−596A>G), assays were done according to Watanabe et al. (25). For marker rs1800795 (−174G>C), primers 5′-TAGCCTCAATGACGACCTAAGCT-3′ (IL6-174F) and 5′ GGGCTGATTGGAAACCTTATTAAG-3′ (IL6-174R) and probes 5′-VIC-TGTCTTGC(G)ATGCTA-MGB-3′ (IL6-174G) and 5′-6FAM-TGTCTTGC(C)ATGCTA-MGB-3′ (IL6-174C) were used. For marker rs1800797 (−596A>G), primers 5′-GCCTTGAAGTAACTGCACGAAATT-3′ (IL6-596F) and 5′-TGTTCTGGCTCTCCCTGTGA-3′ (IL6-596R) and probes 5′-VIC-CCTGGCCA(C)CCTCA-MGB-3′ (IL6-596G) and 5′-6FAM-CTGGCCA(T)CCTCA-MGB-3′ (IL6-596A) were used. For SNPs rs2069849 (C/T coding exon 5), rs2069832 (G>A intron 2), and rs1800796 (−572G>C, also known as −634C>G), assays were purchased as complete assays from Applied Biosystems (Foster City, CA).

Briefly, each 5 μL PCR contained 20 ng genomic DNA, 900 nmol/L of each primer, 125 nmol/L of each Taqman probe, and 2.5 μL Taqman Universal PCR Master Mix (contains AmpErase UNG and AmpliTaq Gold enzymes, deoxynucleotide triphosphates, and reaction buffer). PCR was carried out under the following conditions: 50°C for 2 min to activate UNG, 95°C for 10 min followed by 40 cycles of 92°C for 15 s, and 60°C for 1 min using a 384-well dual block ABI 9700. Fluorescence end point of the Taqman reaction was measured using an ABI 7900HT real-time PCR instrument. Control samples representing all three possible genotypes were included at four positions each in every 384-well tray. In addition, internal replicates representing >1% of the sample set were blinded and included. There were no discordant pairs from these quality control samples.

Statistical Methods. Statistical Analysis System statistical package version 9.1 was used to conduct the analyses. Both *IL6* genotypes and haplotypes were evaluated. As the number of subjects reporting Native American ancestry was small, these women were combined with women reporting Hispanic ethnicity for analyses by race/ethnicity.

We used genotype data from control subjects, considering non-Hispanic whites and Hispanic/Native Americans separately to infer chromosomal phase of linked loci and to estimate haplotype frequencies. Haplotypes were assessed to estimate the *IL6* association with breast cancer to account for multiple SNPs within a gene. We used the expectation-maximization algorithm (26, 27), implemented in Statistical Analysis System/Genetics software (SAS, Cary, NC), to develop maximum likelihood estimates of population haplotype frequencies. The algorithm converges on haplotype frequencies that have the highest probability of generating the observed genotypes. An omnibus test implementing a model-free statistical comparison of haplotype distribution in two groups (28) was used to test for differences by race/ethnicity and by case-control status within race/ethnicity. In addition, χ^2 association tests between individual haplotypes and case-control status were done. Lewontin's D′ standardized statistic for linkage disequilibrium was calculated for each pair of polymorphisms. The values of Lewontin's D′ can range from −1 to 1; for absolute value of D′, 0 indicates complete independence between two SNPs and 1 represents maximum linkage disequilibrium. The expectation-maximization algorithm output also estimates each subject's probabilities of carrying particular pairs of haplotypes based on his or her genotypes. These probabilities can be used in regression models to evaluate disease association (29). Haplotype probabilities for cases were assigned according to the estimated haplotype probabilities of a control from the same race/ethnic group with the same genotypes. Fifteen haplotypes with an estimated population frequency of >0.0001 in non-Hispanic whites were possible based on the five polymorphisms considered. The haplotype probabilities were used to create continuous haplotype dose variables for each haplotype for each subject (30, 31). For haplotypes that can be unambiguously resolved, values of haplotype dose for an individual are zero copy (indicating that the haplotype is not possible based on the subject's genotypes), one copy (indicating heterozygosity at one marker), and two copies (indicating homozygosity for the haplotype). In individuals heterozygous for more than one polymorphism, noninteger haplotype dose variables (range between zero and two copies) reflect estimated probabilities of each possible haplotype, given observed individual genotypes and predicted population haplotype frequencies. For each subject, the sum of probabilities across all haplotypes equals two. We selected the four most common haplotypes, with combined population frequencies representing ≥97%, for analysis in case-control comparisons. The remaining haplotypes were estimated to occur very infrequently, and we were unable to analyze them with reasonable power.

We evaluated the distribution of the genotypes and haplotypes in the population, the independent associations of genetic polymorphisms and haplotypes with breast cancer risk, and the joint effect of genotypes and hormone replacement therapy and aspirin/NSAIDs on breast cancer risk. For independent associations, multivariate logistic regression models were used; haplotype associations were modeled as described previously (32). For joint effects, multivariate logistic regression models were used to calculate odds ratios (OR) for each category of exposure and each genotype or number of copies of each haplotype (0, 1, or 2). In subjects with ambiguous assignment (<5% of all subjects due to high linkage disequilibrium between markers), haplotype dose was rounded to the nearest integer, either zero, one, or two copies. As risk estimates were similar for heterozygous and homozygous genotypes for the minor allele, those groups were combined and compared with homozygous wild-type for dominant inheritance models. We evaluated Hispanic women separately from non-Hispanic whites because describing possible differences in pathways leading to breast cancer for these two groups was a primary aim of this case-control study. We considered premenopausal and perimenopausal women separately from postmenopausal women because of the differences in hormonal environment for tumor development and the differences in the role of obesity and breast cancer in these two groups. Additionally, among postmenopausal women, we evaluated subgroups of women who have become postmenopausal within 2 years of the referent year or were on hormone replacement therapy and those who were not exposed to hormones within 2 years. This 2-year cut point for hormone exposure yielded the most robust results in our data. Adjustment variables in these models include age, center, genetic admixture, parity, BMI, and long-term physical activity. We also evaluated associations by genetic admixture, an indication of population ancestry. Genetic admixture was determined using 15 loci selected to discriminate between European and Native American ancestry. The statistical program STRUCTURE was used to estimate the proportion of ancestry from each of two ancestor populations (33, 34). Effect modification between genotypes or haplotypes and exposure variables was evaluated by likelihood ratio test for a multiplicative interaction term in the logistic regression model.

Results

The majority of women were >50 years of age at the time of diagnosis (Table 1). A larger proportion of Hispanic/Native American women were premenopausal than non-Hispanic white women. The majority of tumors were invasive among both non-Hispanic white and Hispanic/Native American women. Non-Hispanic white women reported higher levels of education and were more likely to use hormone replacement therapy than Hispanic/Native American women.

The distribution of the five *IL6* polymorphisms evaluated is shown in Table 2 along with the minor allele frequency (MAF) for each of the SNPs for non-Hispanic white and Hispanic/Native American women. Among Hispanic/Native American women, we report distribution of genotype and MAF by genetic admixture. There was a significant difference in MAF for all SNPs between non-Hispanic white and Hispanic/Native American women. Allele frequencies were substantially lower in Hispanic/Native American than non-Hispanic white women for the rs1800797, rs18000795, and rs2069832 markers but higher for the rs1800796 and rs2069849 polymorphisms, and the frequencies were correlated with American Indian ancestry.

The absolute value of D′ between all pairs of markers ranged from 0.93 to 1.00 in non-Hispanic white and from 0.91 to 1.00 in Hispanic/Native American, with the exception of D′ between rs1800796 and rs2069849 in non-Hispanic white, which was 0.01. We show calculated ORs for the five *IL6* SNPs in Table 3. Among postmenopausal women not recently exposed to hormones, we observed a significant reduced risk of breast cancer associated with the rs1800797, rs1800795, and rs2069849 SNPs; these associations were slightly stronger among Hispanic/Native American women. Among Hispanic/Native American women, there was a significant interaction between recent hormone exposure and *IL6* genotype among postmenopausal women (P for interaction = 0.05, 0.03, and 0.02, respectively). Neither the rs1800796 nor the rs2069849 SNPs were significantly associated with breast cancer risk nor was there a significant interaction between recent hormone exposure and *IL6* and risk of breast cancer. None of the SNPs

Table 1. Description of population

	Non-Hispanic white		Hispanic/Native American		*P**
	Case, *n* (%)†	Control, *n* (%)	Case, *n* (%)	Control, *n* (%)	
Total	1,527 (48.8)	1,601 (51.2)	798 (46.3)	924 (53.7)	
Center					
Arizona	232 (15.2)	305 (19.1)	169 (21.2)	208 (22.5)	
Colorado	318 (20.8)	301 (18.8)	165 (20.7)	201 (21.8)	
New Mexico	645 (42.2)	618 (38.6)	362 (45.4)	324 (35.1)	
Utah	332 (21.7)	377 (23.5)	102 (12.8)	191 (20.7)	
Age (y)					
25-39	99 (6.5)	116 (7.2)	93 (11.7)	97 (10.5)	
40-49	433 (28.4)	420 (26.2)	266 (33.3)	252 (27.3)	
50-59	453 (29.7)	411 (25.7)	228 (28.6)	242 (26.2)	
60-69	356 (23.3)	371 (23.2)	148 (18.5)	214 (23.2)	
70-79	186 (12.2)	283 (17.7)	63 (7.9)	119 (12.9)	
Menopause status					
Pre/peri	538 (35.3)	492 (30.8)	333 (41.8)	333 (36.2)	<0.01
Post	858 (56.3)	1,008 (63.0)	399 (50.1)	522 (56.7)	
Recent post	128 (8.4)	100 (6.3)	64 (8.0)	65 (7.1)	
Tumor status					
In situ	264 (17.5)		131 (16.6)		0.58
Invasive	1,246 (82.5)		660 (83.4)		
Education level					
<High school graduate/GED	26 (1.7)	32 (2.1)	192 (25.9)	187 (22.3)	<0.01
High school graduate/GED	308 (20.7)	344 (22.1)	218 (29.4)	242 (28.8)	
College/trade school	561 (37.7)	599 (38.4)	218 (29.4)	256 (30.5)	
BA/BS degree or more	593 (39.9)	584 (37.5)	114 (15.4)	154 (18.4)	

*Compares non-Hispanic white and Hispanic control participants.
†Percentages may not add up to 100 due to rounding.

significantly altered risk among premenopausal women. Among postmenopausal women not recently exposed to hormones, the GGCAC haplotype (based on major or minor allele as shown in Table 2) reduced risk of breast cancer among non-Hispanic white women. Among postmenopausal Hispanic/Native American women not recently exposed to hormones, the AGGGC haplotype increased risk of breast cancer. Of these SNPs, there was a significant trend in risk across level of genetic admixture for the rs2069849 marker (*P* for linear trend = 0.02). Among premenopausal women, having a T allele increased risk for those with low levels (<33%) of Native American ancestry [OR, 2.0; 95% confidence interval (95% CI), 0.91-4.41] and was very protective among those women with >67% Native American ancestry (OR, 0.31; 95% CI, 0.14-0.70).

There were no significant associations between any regular use of either aspirin or NSAIDs and breast cancer among non-Hispanic white women (Table 4). Among Hispanic/Native American postmenopausal women not recently exposed to hormones, we observed a reduced risk of breast cancer for women who reported any regular use of aspirin. We also assess associations for recent aspirin and NSAID use; results were very similar to those in Table 4.

Aspirin use interacted significantly with the rs1800796 *IL6* marker to alter breast cancer risk among postmenopausal Hispanic/Native American women (Table 5). Among these women, the GG/GC genotypes were associated with reduced breast cancer risk only if aspirin was used, whereas among women who did not use aspirin on a regular basis having a C allele was inversely associated with breast cancer risk. The expected joint effect of aspirin and GC/CC under a multiplicative model would have been $0.55 \times 0.68 = 0.37$; however, the observed effect of 0.97 was 2.6-fold higher than expected, resulting in a significant departure from a multiplicative model ($P = 0.04$). Among non-Hispanic white women, there was a borderline significant association between aspirin and the rs1800796 *IL6* marker; however, associations were opposite of those observed for Hispanic/Native American women. None of the other *IL6* markers interacted significantly with aspirin in either premenopausal or postmenopausal women.

We evaluated the interaction between haplotypes and aspirin use and breast cancer risk to obtain a better understanding of how SNPs may operate together to alter risk. Significant associations were observed only in postmenopausal women, with the strongest associations being observed for Hispanic/Native American women who recently were exposed to hormones (Table 6). Of the most common haplotypes, there were significant interactions with the AGGGC and the ACGGC haplotypes and aspirin use among Hispanic/Native American women, indicating that, for postmenopausal women with recent hormone exposure who did not use aspirin, the haplotype was associated with increased risk of breast cancer in a similar manner to the association observed for women with no recent hormone use, but the association was absent (or possibly inverse) among women with recent hormone exposure who used aspirin. There were no significant interactions between haplotypes and aspirin use among non-Hispanic white women.

We evaluated associations between *IL6* genotypes and haplotypes and estrogen receptor and progesterone receptor status in tumors. We did not observe any unique associations with estrogen receptor or progesterone receptor tumor status and any individual *IL6* genotypes or haplotypes (data not shown in table).

Discussion

We observed significant associations between *IL6* and breast cancer risk, although the association varied with the marker assessed. Of the five SNPs evaluated, three SNPs, the rs1800797 (−596A/C), rs1800795 (−174G>C), and rs2069832 (G>A intron 2), showed similar results. The minor allele of each of these three SNPs was associated with reduced breast cancer risk, with stronger associations being observed for Hispanic/Native American women. Additionally, we observed that certain haplotypes of the five *IL6* SNPs were associated with breast cancer risk, although the haplotype most strongly associated varied somewhat between non-Hispanic white and Hispanic/Native American women. These

results suggest the importance of *IL6* in breast cancer and suggest several potential biological mechanisms that may be relevant to these observations.

IL-6 may be associated with breast cancer through several mechanisms, including regulation of insulin, inflammation, and estrogen, all factors that may importantly influence breast cancer risk. IL-6 has been shown to influence lipid and glucose metabolism (5) as well as to stimulate secretion of C-reactive protein, an indicator of inflammation, from the liver. Polymorphisms in the *IL6* gene promoter, described as −572G>C and −174G>C, which are rs18000796 and rs1800795, have been reported to be related to levels of circulating C-reactive protein, with the rs1800796 C and the rs1800795G alleles being considered the higher risk allele (16). The rs1800795 polymorphism also has been associated with different profiles of plasma IL-6 response to immunization, where the G allele was associated with increased *IL6* levels (17). On the other hand, the C allele of the rs1800795 *IL6* polymorphism was reported to increase risk of type 2 diabetes in obese subjects, which is inconsistent with the expectation of lower IL-6 levels with this allele (18). However, that study found a stronger positive association between BMI and serum IL-6 in carriers of the C compared with the G allele, whereas lean carriers of the G allele had somewhat higher IL-6 levels, which is consistent with the reported increased risk for type 2 diabetes in those with the G allele regardless of BMI.

We observed that the C allele of the rs1800795 *IL6* polymorphism reduced risk of breast cancer, especially among Hispanic women, which could be a reflection of the previously shown inverse association between BMI and breast cancer risk in Hispanic women (19). Others that have examined *IL6* genotypes and breast cancer incidence and survival have findings different than those reported here. Hefler et al. (12) reported an OR of 2.0 (95% CI, 1.1-3.6) for the *IL6* C allele with breast cancer in a small case-control study of Caucasian women conducted in Germany and Austria. Gonzalez-Zuloeta et al. (35) reported a nonsignificant 24% increased risk of breast cancer with *IL6* in a nested case-control analysis in the Rotterdam Study. Two studies that have reported associations with survival after diagnosis with breast cancer have contradictory results with regard to the direction of effect of the C allele (36, 37). Unfortunately, little is known about functionality of haplotypes, and we can only hypothesize that the function of the haplotype that includes multiple SNPs is similar in action to the functional SNPs within it. Not all *IL6* polymorphisms and haplotypes assessed were associated with breast cancer risk, suggesting different functionality with specific polymorphisms.

In this study, we focused on a possible inflammation role, given use of aspirin and NSAIDs may indicate levels of inflammation. We also evaluated associations influenced by estrogen through examination of the influence of *IL6* polymorphisms and haplotypes on breast cancer risk by menopausal status. Our results suggest that *IL6* may influence both pathways examined (i.e., both inflammation and estrogen), given the interaction with aspirin and the differences in association by hormone exposure among postmenopausal women.

Estrogen represses production of IL-6 through an estrogen receptor–dependent mechanism, and serum levels of IL-6 increase following menopause in healthy women and with age in both men and women (13, 14). IL-6 is known to increase the expression of aromatase in breast cancer cells, thereby enhancing the conversion of androgens to estrogens (15). In our study, we observed differences in association by recent hormone exposure in postmenopausal women. Associations

Table 2. Frequencies of five *IL6* SNPs and haplotypes in 4-Corner's Study controls

Marker*	Genotype†	Self-reported race/ethnicity				Hispanic/Native American, by tertile of percentage of Native American ancestry					
		Non-Hispanic white (n = 1,329)		Hispanic/Native American (n = 726)		Low (n = 247)		Intermediate (n = 234)		High (n = 245)	
		Frequency (%)	P HWE	Frequency (%)	P HWE	Frequency (%)	P HWE	Frequency (%)	P HWE	Frequency (%)	P HWE
rs1800797	AA	32.3		64.6		62.0		61.1		70.6	
(−596A>G)	AG	49.0		32.2		33.1		35.5		28.2	
	GG	18.8		3.2		4.9		3.4		1.2	
	MAF	43.2	0.94	19.3	0.35	21.4	0.78	21.2	0.33	15.3	0.18
rs1800796	GG	89.4		55.9		64.0		52.6		51.0	
(−572G>C)	GC	10.3		36.9		30.0		41.5		39.6	
	CC	0.3		7.2		6.1		6.0		9.4	
	MAF	5.5	0.98	25.6	0.40	21.1	0.12	26.7	0.37	29.2	0.51
rs1800795	GG	30.8		63.4		60.3		60.3		69.4	
(−174G>C)	GC	49.7		32.9		34.8		35.5		28.6	
	CC	19.5		3.7		4.9		4.3		2.0	
	MAF	44.4	0.78	20.2	0.55	22.3	0.93	22.0	0.61	16.3	0.47
rs2069832, G>A	GG	30.7		63.0		59.9		60.3		68.6	
intron 2	GA	49.9		33.2		34.8		35.5		29.4	
	AA	19.5		3.9		5.3		4.3		2.0	
	MAF	44.4	0.73	20.5	0.59	22.7	0.91	22.0	0.61	16.7	0.39
rs2069849,	CC	95.6		87.2		90.7		85.8		84.8	
exon 5 C/T	CT	4.4		12.6		8.9		13.7		15.2	
	TT	0.1		0.3		0.4		0.4		0.0	
	MAF	2.3	0.69	6.6	0.50	4.8	0.56	7.3	0.82	7.6	0.20
Haplotypes‡	AGGGC	47.7		47.5		52.0		44.0		46.9	
	GGCAC	42.7		19.1		21.5		20.8		15.2	
	ACGGC	5.3		25.4		20.4		26.4		28.9	
	AGGGT	2.1		6.6		4.9		7.3		7.6	

Abbreviation: HWE, Hardy-Weinberg equilibrium.

**IL6* gene region: rs1800797, rs1800796, and rs1800795, 5′ promoter; rs2069832, intron 2; rs2069849, coding exon 5.

† Mantel-Haenszel χ^2 test, genotype, and self-reported race ($P < 0.0001$ for all SNPs genotype) and percentage Native American ancestry categories ($P < 0.0001$ for all SNPs).

‡ Log likelihood omnibus test (28), haplotype distribution, and self-reported race ($P < 0.0001$) and percentage Native American ancestry (lowest versus highest tertile; $P < 0.01$).

Table 3. *IL6* association with breast cancer in the 4-Corner's Study

IL6 marker*	Race/ethnicity	Genotype†	Premenopausal		
			Cases, *n* (%)	Controls, *n* (%)	OR (95% CI)
rs1800797 (−596A>G)	NHW	AA	150 (36.5)	127 (30.8)	1.00
		AG/GG	261 (63.5)	285 (69.2)	0.77 (0.57-1.04)
	H/NA	AA	160 (67.5)	175 (66.0)	1.00
		AG/GG	77 (32.5)	90 (34.0)	0.98 (0.66-1.44)
rs1800796 (−572G>C)	NHW	GG	356 (85.8)	372 (90.1)	1.00
		GC/CC	59 (14.2)	41 (9.9)	1.53 (0.99-2.37)
	H/NA	GG	135 (56.7)	154 (58.3)	1.00
		GC/CC	103 (43.3)	110 (41.7)	1.09 (0.75-1.58)
rs1800795 (−174G>C)	NHW	GG	149 (36.2)	123 (29.8)	1.00
		GC/CC	263 (63.8)	290 (70.2)	0.76 (0.56-1.02)
	H/NA	GG	159 (66.8)	173 (65.3)	1.00
		GC/CC	79 (33.2)	92 (34.7)	0.97 (0.66-1.42)
rs2069832, G>A intron	NHW	GG	147 (35.8)	121 (29.5)	1.00
		GA/AA	264 (64.2)	289 (70.5)	0.76 (0.56-1.03)
	H/NA	GG	157 (66.0)	172 (64.9)	1.00
		GA/AA	81 (34.0)	93 (35.1)	0.98 (0.67-1.44)
rs2069849, Exon 5 C>T	NHW	CC	390 (94.4)	396 (96.1)	1.00
		CT/TT	23 (5.6)	16 (3.9)	1.35 (0.70-2.63)
	H/NA	CC	214 (89.9)	229 (86.4)	1.00
		CT/TT	24 (10.1)	36 (13.6)	0.68 (0.39-1.20)
		Haplotype‡,§			
	NHW	AGGGC	(48.9)	(47.3)	1.06 (0.87-1.30)
		GGCAC	(39.0)	(44.1)	0.83 (0.68-1.01)
		ACGGC	(6.8)	(5.0)	1.48 (0.97-2.27)
		AGGGT	(2.9)	(1.7)	1.45 (0.72-2.91)
	H/NA	AGGGC	(50.8)	(49.6)	1.01 (0.79-1.29)
		GGCAC	(18.5)	(19.1)	1.02 (0.74-1.41)
		ACGGC	(25.0)	(23.6)	1.12 (0.83-1.49)
		AGGGT	(5.0)	(6.8)	0.66 (0.37-1.15)

NOTE: ORs and 95% CIs adjusted for age, study center, referent year BMI, lifetime physical activity score, parity, and percentage Native American ancestry.
Abbreviations: NHW, Non-Hispanic white; H/NA, Hispanic/Native American.
**IL6* gene region: rs1800797, rs1800796, and rs1800795, 5′ promoter; rs2069832, intron + 180 of exon 2; rs2069849, coding exon 5.
†Referent genotype is homozygous wild-type for the major allele in our study population (dominant inheritance model).
‡Allele designation for markers in order: rs1800797, rs1800796, rs1800795, rs2069832, and rs2069849.
§Haplotype dose based on probability of carrying the haplotype (from 0 to 2 copies), estimated from controls, as a continuous variable; risk of carrying one copy versus not carrying the haplotype for the four most common haplotypes.

were strongest among postmenopausal women without recent hormone exposure. Our data suggest that, in the absence of estrogen, alleles associated with lower IL-6 production and reduced inflammation are protective. Estrogen itself may have a modifying effect on IL-6 levels so that the effect is only observed in the absence of estrogen.

Associations between use of aspirin and NSAIDs and breast cancer are inconsistent in the literature (38-45). Some studies suggest stronger associations with greater duration of use and for aspirin rather than NSAIDs (45). We observed similar associations for ever use of aspirin as for current use. In our data, the majority of women who reported ever using aspirin also were current aspirin users. We only observed significant inverse associations with aspirin among postmenopausal Hispanic women not recently exposed to hormones. Our study population, recruited in 2000 to 2004, had a very high prevalence of use of hormone replacement therapy, higher than most studies have reported. If the association between aspirin use and breast cancer is modified by use of exogenous hormones, as our data suggest, inconsistencies between results in our overall study population and other studies could be explained in part by the high prevalence of hormone replacement therapy use. The number of women in our study who were postmenopausal without recent hormone use was relatively small, and confidence intervals on the OR were relatively wide. We observed no significant associations between either aspirin or NSAIDs among non-Hispanic white women, which is similar to the findings reported by Marshall et al. (39). It has been suggested that aspirin and other cyclooxygenase-2 inhibitors may reduce risk of breast cancer by inhibiting aromatase activity (46).

As with the overall associations between *IL6* genotypes and breast cancer and aspirin and breast cancer, the interaction between aspirin and *IL6* genotypes was only significant among postmenopausal Hispanic not recently exposed to hormones. However, the interaction of *IL6* with aspirin use was only significant for the rs1800796 (−572G>C) polymorphism, suggesting that some polymorphisms, although not independently associated with breast cancer, may work jointly with other factors to alter breast cancer risk. The G allele was only associated with reduced risk of breast cancer in the presence of aspirin, whereas having a C allele was associated with reduced risk among both users and nonusers of aspirin. Differences in risk among aspirin users and nonusers for the −596A>G and the −174G>C, which was in linkage disequilibrium with the −596A>G, showed similar associations as were observed for the −72G>C, although the *P* for interaction was not statistically significant. Aspirin had the greatest reduction in risk in the presence of the high-risk allele and a more modest effect in the presence of the lower-risk allele. In this study, the joint effects of *IL6* genotype and aspirin use were to attenuate the expected risk on a multiplicative scale.

Of interest is the differences in association observed between Hispanic and non-Hispanic white women. There were significantly different minor allele frequencies between these two groups of women as well as among Hispanic women when stratified by their genetic ancestry. A study of Pima Indians that examined the −174G>C (rs1800795) *IL6* polymorphism

Table 3. *IL6* association with breast cancer in the 4-Corner's Study (Cont'd)

Postmenopausal					
Recent hormone exposure			No recent hormone exposure		
Cases, *n* (%)	Controls, *n* (%)	OR (95% CI)	Cases, *n* (%)	Controls, *n* (%)	OR (95% CI)
195 (37.0)	190 (31.9)	1.00	91 (41.7)	106 (34.3)	1.00
332 (63.0)	405 (68.1)	0.80 (0.63-1.03)	127 (58.3)	203 (65.7)	0.69 (0.48-1.00)
139 (66.2)	176 (64.9)	1.00	93 (76.2)	112 (61.5)	1.00
71 (33.8)	95 (35.1)	0.91 (0.62-1.35)	29 (23.8)	70 (38.5)	0.48 (0.28-0.83)
469 (88.3)	536 (89.6)	1.00	189 (86.3)	273 (87.8)	1.00
62 (11.7)	62 (10.4)	1.12 (0.77-1.64)	30 (13.7)	38 (12.2)	1.33 (0.78-2.26)
120 (57.4)	145 (53.1)	1.00	76 (62.3)	105 (57.4)	1.00
89 (42.6)	128 (46.9)	0.84 (0.58-1.22)	46 (37.7)	78 (42.6)	0.77 (0.47-1.28)
190 (35.8)	180 (30.1)	1.00	88 (40.2)	102 (32.8)	1.00
340 (64.2)	418 (69.9)	0.78 (0.61-1.00)	13 (159.8)	20 (967.2)	0.68 (0.47-0.99)
135 (64.6)	173 (63.6)	1.00	92 (75.4)	109 (59.6)	1.00
74 (35.4)	99 (36.4)	0.93 (0.63-1.37)	30 (24.6)	74 (40.4)	0.44 (0.26-0.77)
190 (35.9)	179 (29.9)	1.00	86 (39.4)	102 (33.0)	1.00
339 (64.1)	419 (70.1)	0.77 (0.60-1.00)	132 (60.6)	207 (67.0)	0.70 (0.48-1.02)
132 (63.5)	172 (63.2)	1.00	92 (76.0)	108 (59.0)	1.00
76 (36.5)	100 (36.8)	0.96 (0.65-1.41)	29 (24.0)	75 (41.0)	0.43 (0.25-0.74)
507 (95.5)	566 (94.6)	1.00	213 (97.3)	300 (96.8)	1.00
24 (4.5)	32 (5.4)	0.83 (0.48-1.44)	6 (2.7)	10 (3.2)	0.94 (0.33-2.70)
185 (88.1)	237 (87.5)	1.00	112 (91.8)	161 (88.0)	1.00
25 (11.9)	34 (12.5)	0.94 (0.53-1.67)	10 (8.2)	22 (12.0)	0.62 (0.27-1.43)
(50.7)	(47.4)	1.15 (0.97-1.35)	(53.1)	(49.1)	1.19 (0.93-1.54)
(39.0)	(42.5)	0.87 (0.74-1.04)	(36.2)	(41.9)	0.77 (0.59-0.99)
(5.9)	(4.8)	1.20 (0.84-1.74)	(6.8)	(6.3)	1.21 (0.72-2.02)
(1.3)	(2.5)	0.80 (0.46-1.42)	(1.3)	(1.5)	1.03 (0.35-3.05)
(48.5)	(45.0)	1.15 (0.88-1.49)	(59.1)	(48.4)	1.55 (1.09-2.19)
(20.0)	(18.9)	1.12 (0.81-1.56)	(13.6)	(19.9)	0.70 (0.44-1.11)
(24.5)	(28.3)	0.85 (0.63-1.13)	(22.3)	(23.2)	0.92 (0.61-1.39)
(5.8)	(6.7)	0.83 (0.48-1.44)	(4.1)	(6.0)	0.57 (0.25-1.30)

also showed significant differences in allele frequency between American Indians and Spanish Caucasian subjects (47). The G allele was much more common among individuals with mixed American Indian ancestry, and among individuals who reported full Pima Indian heritage, only the GG genotype existed. People with the G allele were more likely to develop type 2 diabetes. Our associations with Hispanic women may imply that other genes with different allele frequencies by ethnicity may be confounding the association or working in conjunction with *IL-6* to alter risk.

It is important to recognize that IL-6 is a pleiotropic cytokine with proinflammatory versus anti-inflammatory action. The direction of the effect may depend on other factors, including mode of production (autocrine versus paracrine/endocrine),

Table 4. Association with aspirin and NSAID use and breast cancer in the 4-Corner's Study

	Non-Hispanic white			Hispanic/Native American		
	Controls, *n* (%)	Cases, *n* (%)	OR (95% CI)[1]	Controls, *n* (%)	Cases, *n* (%)	OR (95% CI)
Premenopausal/perimenopausal						
Aspirin use						
No	426 (86.9)	445 (83.2)	1.00	299 (90.6)	303 (92.1)	1.00
Yes	64 (13.1)	90 (16.8)	1.32 (0.92-1.89)	31 (9.4)	26 (7.9)	0.87 (0.50-1.53)
NSAID use						
No	357 (72.7)	393 (73.5)	1.00	246 (74.5)	258 (78.4)	1.00
Yes	134 (27.3)	142 (26.5)	0.96 (0.73-1.28)	84 (25.5)	71 (21.6)	0.87 (0.60-1.27)
Postmenopausal with no recent hormone exposure						
Aspirin use						
No	273 (71.5)	187 (68.5)	1.00	181 (75.1)	153 (84.5)	1.00
Yes	109 (28.5)	86 (31.5)	1.14 (0.80-1.62)	60 (24.9)	28 (15.5)	0.56 (0.33-0.96)
NSAID use						
No	286 (74.7)	204 (74.7)	1.00	184 (76.3)	146 (80.7)	1.00
Yes	97 (25.3)	69 (25.3)	0.95 (0.65-1.38)	57 (23.7)	35 (19.3)	0.80 (0.48-1.34)
Postmenopausal with recent hormone exposure						
Aspirin use						
No	492 (69.8)	516 (74.2)	1.00	250 (75.8)	217 (80.1)	1.00
Yes	213 (30.2)	179 (25.8)	0.82 (0.64-1.04)	80 (24.2)	54 (19.9)	0.86 (0.57-1.30)
NSAID use						
No	456 (64.8)	455 (65.5)	1.00	221 (67.0)	197 (72.7)	1.00
Yes	248 (35.2)	240 (34.5)	1.03 (0.82-1.29)	109 (33.0)	74 (27.3)	0.80 (0.56-1.15)

NOTE: ''Yes'' represents any regular use (at least thrice weekly for at least 1 mo). ORs and 95% CIs adjusted for age, study center, referent year BMI, lifetime physical activity score, parity, and percentage Native American ancestry.

Table 5. Interaction of regular aspirin use and *IL6* rs1800796 postmenopausal women in the 4-Corner's Study

		Non-Hispanic white						Hispanic/Native American					
		rs1800796 GG			rs1800796 GC/CC			rs1800796 GG			rs1800796 GC/CC		
		Cases, *n*	Controls, *n*	OR (95% CI)	Cases, *n*	Controls, *n*	OR (95% CI)	Cases, *n*	Controls, *n*	OR (95% CI)	Cases, *n*	Controls, *n*	OR (95% CI)
Postmenopausal women, recent hormone exposure													
Aspirin use	No	362	374	1.00	42	43	0.98 (0.62-1.55)	102	106	1.00	66	103	0.68 (0.44-1.03)
	Yes	109	163	0.71 (0.53-0.95)	20	18	1.14 (0.59-2.21)	19	39	0.55 (0.30-1.04)	24	27	0.97 (0.52-1.81)
	P interaction						0.24						0.04
Postmenopausal women, no recent hormone exposure													
Aspirin use	No	131	196	1.00	24	24	1.83 (0.97-3.45)	66	73	1.00	36	64	0.58 (0.33-1.01)
	Yes	60	77	1.22 (0.80-1.86)	6	14	0.68 (0.24-1.86)	11	32	0.37 (0.17-0.82)	11	16	0.77 (0.32-1.84)
	P interaction						0.06						0.04

NOTE: ORs and 95% CIs adjusted for age, study center, referent year BMI, lifetime physical activity score, parity, and percentage Native American ancestry.

presence of binding proteins (gp130 and IL-6 receptor), and circulating levels of other cytokines and hormones. Because most of these factors act at the cellular level, it is difficult to assess, and although cytokines and hormones could be assessed, the timing of measurements would be difficult in a case-control study. Additionally, cytokines rarely work alone. There could be ethnic differences in genes for other cytokines, such as *IL10*, which is anti-inflammatory that interacts with *IL6*, which explain these differences.

This study is one of the largest conducted among Hispanic breast cancer cases, although there are study limitations. Perhaps the most significant limitation was difficulty in enrolling Hispanic cases and controls. Our assessment of participation suggests that participation was influenced by age, education, and ethnicity. We have adjusted for age and education to help remove their effect on biased associations. Studies also have shown that participation is generally not linked to genotype (48). Another limitation is that our assessment of aspirin and NSAIDs was based on recall, and our information on duration and dose has potential for errors. However, we focused on analysis of reported ever regular use and were able to detect associations in Hispanic women, suggesting that our measure of exposure, although limited, was suitable for risk factor detection. We assessed *IL6* haplotypes as well as genotypes, adding support to the role of *IL6* in breast cancer etiology; however, we are limited in our knowledge of the functionality of SNPs and haplotypes. Although we have made multiple comparisons in this study, our goal was to describe breast cancer risk for Hispanic/Native American and non-Hispanic white women. The comparisons that were made were a priori comparisons given our knowledge of differences in breast cancer risk by menopausal status and by estrogen exposure in postmenopausal women (19).

In conclusion, our data suggest that *IL6* is associated with breast cancer risk. Given the associations with both estrogen and aspirin, it is likely that both estrogen and inflammation-related pathways are involved. Our data also suggest that aspirin reduces risk of breast cancer among postmenopausal women not recently exposed to hormones. These associations

Table 6. *IL6* haplotype interaction with aspirin use and breast cancer risk in the 4-Corner's Study

	Haplotype	Postmenopausal, recent hormone exposure			Postmenopausal, no recent hormone exposure		
	AGGGC	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
		0 Copy	1 Copy	2 Copies	0 Copy	1 Copy	2 Copies
Non-Hispanic white							
Aspirin use	No	1.00	1.17 (0.96-1.43)	1.38 (0.92-2.05)	1.00	1.15 (0.85-1.56)	1.33 (0.73-2.42)
	Yes	0.79 (0.50-1.25)	0.87 (0.63-1.21)	0.95 (0.61-1.49)	1.06 (0.54-2.05)	1.23 (0.75-2.03)	1.45 (0.74-2.83)
P interaction				0.72			0.95
Hispanic/Native American							
Aspirin use	No	1.00	1.32 (0.98-1.78)	1.74 (0.96-3.15)	1.00	1.56 (1.06-2.31)	2.45 (1.13-5.32)
	Yes	1.64 (0.78-3.43)	1.12 (0.66-1.92)	0.77 (0.33-1.78)	0.68 (0.23-2.00)	0.95 (0.46-1.96)	1.34 (0.48-3.73)
P interaction				0.05			0.81
	GGCAC						
Non-Hispanic white							
Aspirin use	No	1.00	0.87 (0.71-1.06)	0.75 (0.50-1.13)	1.00	0.76 (0.56-1.04)	0.58 (0.32-1.08)
	Yes	0.76 (0.51-1.14)	0.64 (0.46-0.88)	0.53 (0.32-0.88)	0.96 (0.54-1.71)	0.84 (0.53-1.34)	0.74 (0.37-1.47)
P interaction				0.83			0.62
Hispanic/Native American							
Aspirin use	No	1.00	1.05 (0.73-1.51)	1.10 (0.54-2.27)	1.00	0.69 (0.42-1.13)	0.48 (0.18-1.28)
	Yes	0.82 (0.48-1.40)	1.16 (0.58-2.31)	1.64 (0.43-6.19)	0.63 (0.31-1.26)	0.36 (0.13-1.01)	0.21 (0.03-1.66)
P interaction				0.48			0.78
	ACGGC						
Non-Hispanic white							
Aspirin use	No	1.00	1.06 (0.69-1.63)	1.12 (0.47-2.67)	1.00	1.61 (0.87-2.97)	2.58 (0.75-8.84)
	Yes	0.70 (0.53-0.94)	1.21 (0.62-2.33)	2.07 (0.56-7.68)	1.23 (0.81-1.86)	0.64 (0.24-1.68)	0.33 (0.05-2.26)
P interaction				0.24			0.06
Hispanic/Native American							
Aspirin use	No	1.00	0.69 (0.50-0.97)	0.48 (0.25-0.94)	1.00	0.84 (0.53-1.32)	0.71 (0.29-1.75)
	Yes	0.60 (0.33-1.10)	0.88 (0.51-1.51)	1.27 (0.47-3.42)	0.44 (0.21-0.95)	0.72 (0.33-0.58)	1.17 (0.26-5.27)
P interaction				0.04			0.20

NOTE: ORs and 95% CIs adjusted for age, study center, referent year BMI, lifetime physical activity score, parity, and percentage Native American ancestry. Labeling indicates base at each of five SNPs in order of chromosomal location: rs1800797, rs1800796, rs1800795, rs2069832, and rs2069849.

seem to be restricted to Hispanic women, suggesting involvement in other genes that regulate cytokine levels that also differ by Hispanic and non-Hispanic white women.

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