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Genetic variation in genes involved in hormones, inflammation and energetic factors and breast cancer risk in an admixed population

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Breast cancer incidence rates are characterized by unique racial and ethnic differences. Native American ancestry has been associated with reduced breast cancer risk. We explore the biological basis of disparities in breast cancer risk in Hispanic and non-Hispanic white women by evaluating genetic variation in genes involved in inflammation, insulin and energy homeostasis in conjunction with genetic ancestry. Hispanic (2111 cases, 2597 controls) and non-Hispanic white (1481 cases, 1586 controls) women enrolled in the 4-Corner's Breast Cancer Study, the Mexico Breast Cancer Study and the San Francisco Bay Area Breast Cancer Study were included. Genetic admixture was determined from 104 ancestral informative markers that discriminate between European and Native American ancestry. Twenty-one genes in the CHIEF candidate pathway were evaluated. Higher Native American ancestry was associated with reduced risk of breast cancer (odds ratio = 0.79, 95% confidence interval 0.65, 0.95) but was limited to postmenopausal women (odds ratio = 0.66, 95% confidence interval 0.52, 0.85). After adjusting for genetic ancestry and multiple comparisons, four genes were significantly associated with breast cancer risk, *NFκB1, NFκB1A, PTEN* and *STK11*. Within admixture strata, breast cancer risk among women with low Native American ancestry was associated with *IkBKB, NFκB1, PTEN* and *RPS6KA2,* whereas among women with high Native American ancestry, breast cancer risk was associated with *IkBKB, mTOR, PDK2, PRKAA1, RPS6KA2* and *TSC1*. Higher Native American ancestry was associated with reduced breast cancer risk. Breast cancer risk differed by genetic ancestry along with genetic variation in genes involved in inflammation, insulin, and energy homeostasis.

Introduction

Breast cancer incidence rates are characterized by unique racial and ethnic differences, not only between countries but also between populations within countries. In the southwestern United States and Mexico, breast cancer incidence rates are highest among non-Hispanic white (NHW), intermediate among US Hispanic and Mexican women and lowest among Native American women (1). Differences in incidence rates are most probably influenced by differences in genetic and lifestyle factors. Hispanic women are the resultant of generations of admixture of European, Native American and African individuals, with varying degrees of admixture across Latin America and US Hispanics. Among Hispanic women, higher Native American genetic ancestry has been shown to reduce breast cancer risk (2–4).

The association between genetic admixture, as shown by degree of European or Native American ancestry, and risk of breast cancer can represent many factors, including both genetic and lifestyle. In the study by Fejerman and colleagues, socioeconomic status was the major confounder of the association of genetic admixture with breast cancer (2). This implies that differences in breast cancer risk can be attributed, at least in part, to unmeasured factors associated with education and other sociodemographic factors. However, the observed associations with genetic admixture and breast cancer also suggest a biological basis. Therefore, it is probable that genetic ancestry is associated with genetic and lifestyle factors that have evolved over time.

Variation in genes regulating key pathways linked to breast cancer may influence breast cancer risk differently in various groups of admixed populations. One plausible candidate is the CHIEF pathway, which has been introduced as a pathway where key factors such as hormones, inflammation and energy-related factors converge (5). Central to the CHIEF pathway are tumor suppressor genes, such as STK11 or LKB1 (serine/threonine protein kinase 11), which governs whole-body insulin sensitivity (6,7); mTOR (mammalian Target of Rapamycin), which is involved in normal energy homeostasis and inhibition of tumor growth (8); and PTEN (*p*hosphatase *ten*sin homolog deleted on chromosome *10*), which is a regulator of metabolic signaling and cell growth in the insulin/insulin-like growth factor (IGF) signaling pathway. PTEN also acts as a metabolic regulator by modulating signaling via the PI3K (phosphatidylinositol 3-kinase) and the Akt1 (v-akt murine thymoma viral oncogene homolog 1) pathway, downstream of the insulin receptor. Akt1-dependent phosphorylation negatively regulates the functioning of TSC1 and TSC2 (tuberous sclerosis complex) and links to inflammation via NFκB (9). TSC1 and TSC2 are also involved in the insulin signaling. Also, the CHIEF pathway plays a critical role in energy homeostasis through genes such as the *STK11*, *AMPKα*, *TSC1 & TSC2*, *mTOR*, *S6K* (*RPS6KB1* and *RPS6KB2*) component of the pathway, which senses and responds to changes in cellular ATP levels. Cells with low ATP and excess AMP activate STK11 at the apex of this pathway (10–13) to repress anabolic processes (ATP utilization) and enhance catabolic processes (ATP generation). In cells with excess adenosine monophosphate (AMP) from altered energy homeostasis, STK11 phosphorylates AMP-dependent kinase (*PRKAA1 and PRKAA2*) (10–12,14). Many of the CHIEF pathway components are gaining momentum in their associations with cancer, in particular with breast cancer. Specifically, PIK3CA, PTEN, STK11, mTOR, TSC1, TSC2, AKT, AMPK, S6K1, RSK (RPS6KA1) and NFkB have been studied jointly for their potential role in breast cancer development and treatment (15–29).

In this article, we evaluate the role of genetic ancestry and the influence of genetic variation in genes central to the CHIEF pathway to explore the biological basis of disparities in breast cancer risk in a large sample of Hispanic (2111 cases and 2597 controls) and NHW (1481 cases and 1586 controls) women. We evaluate key genes and determine whether their association with breast cancer risk differs by Native American ancestry and menopausal status.

Materials and methods

The population included in this study consists of participants in three population-based case-control studies, including the 4-Corner's Breast Cancer Study, the Mexico Breast Cancer Study and the San Francisco Bay Area

Abbreviations: AMP, adenosine monophosphate; ATP, adenosine triphosphate; BMI, body mass index; LD, linkage disequilibrium; NHW, non-Hispanic white; OR, Odds ratios; CI, confidence intervals.

Breast Cancer Study. All participants signed informed written consent prior to participation; the study was approved by the Institutional Review Board for Human Subjects at each institution. These studies have been previously described and are briefly described below.

4-Corner's Breast Cancer Study

Participants were NHW, Hispanic or Native American women living in non-reservation areas in the states of Arizona, Colorado, New Mexico or Utah at the time of diagnosis or selection (1). Eligible female breast cancer cases were between 25 and 79 years of age with a histological confirmed diagnosis of *in situ* ($n = 337$) or invasive cancer ($n = 1466$) (International Classification of Diseases of Oncology sites C50.0–C50.6 and C50.8–C50.9) between October 1999 and May 2004 who had provided a blood sample. Controls were selected from the target populations and were frequency matched to cases on the expected ethnicity and 5-year age distribution. In Arizona and Colorado, controls under age 65 years were randomly selected from a commercial mailing list; in New Mexico and Utah, they were randomly selected from driver's license lists. In all states, women 65 years and older were randomly selected from Center for Medicare Services lists. Women were screened by telephone for eligibility and self-identified their race/ethnicity prior to study enrollment. Of cases contacted, 852 Hispanic, 22 American Indian and 1683 NHW women participated. Of controls contacted, 913 Hispanic, 23 American Indian and 1669 NHW women participated. Blood was collected and DNA extracted for 76% of participants in Arizona, 71% of participants in Colorado, 75% of participants in New Mexico and 94% of participants in Utah. Lifestyle data were collected by trained and certified interviewers using the same interviewer-administered computerized questionnaire (30–32). The referent year for the study was the calendar year 1 year prior to diagnosis for cases or selection for controls. The physical activity questionnaire collected detailed information on activity performed at home, work, volunteer and leisure and included information on intensity, duration and frequency of activity performed (33). Height, weight and waist and hip circumference measurements were taken at the time of interview. Quality control was done centrally at the coordinating center at the University of Utah.

Mexico Breast Cancer Study

Participants were between 28 and 74 years of age, living in one of the three states, Monterrey, Veracruz and Mexico City, for the past 5 years as described previously (34). Eligible cases 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 in Mexico, IMSS, ISSTE and SS. *In situ* and invasive cancers were not distinguished in the study database. Controls were randomly selected from the catchment area of the 12 participating hospitals using a probabilistic multistage design. A total of 1000 cases and 1074 controls were recruited, and blood was collected and DNA extracted from 85 and 96% of women, respectively. Physical activity data were collected using a semistructured interview based on the 7-day recall questionnaire developed by Sallis *et al.* (35,36). Body-size measurements, recalled weight history, medical history, family history and reproductive history components of the study questionnaire were patterned after the 4-Corner's Breast Cancer Study questionnaire. Standing height, weight and hip and waist circumferences were measured by nurses at the hospitals. The study referent year was the year prior to diagnosis for cases or prior to selection for controls.

San Francisco Bay Area Breast Cancer Study

Participants were Hispanic, African American and NHW women aged 35–79 from the San Francisco Bay Area diagnosed with a first primary histologically confirmed invasive breast cancer between 1995 and 2002; controls were identified by random-digit dialing (37,38). This analysis was limited to women who participated in the biospecimen component of the parent study that was initiated in 1999 (39). Eligible cases were Hispanic women diagnosed between April 1997 and April 2002 and a 10% random sample of NHW women diagnosed between April 1997 and April 1999. Random-digit dialing controls were frequency matched to cases based on race/ethnicity and the expected 5-year age distribution of cases. Women participated in a telephone screening interview that assessed study eligibility and self-identified race/ethnicity. Those selected into the study completed a structured questionnaire in English or Spanish administered by professionally trained interviewers and participated in measurements of standing height, weight waist and hip circumferences. Physical activity information was collected on lifetime histories of sports and exercise, transportation, indoor and outdoor chores and occupational activity (37). The study reference year was the calendar year prior to diagnosis for cases or selection for controls. DNA was available for 93% of cases and 92% of controls of the 1105 cases (793 Hispanics, 312 NHW) and 1318 controls (998 Hispanics, 320 NHW) interviewed.

Data harmonization

Data were harmonized across all study centers and questionnaires. Key variables for harmonization were identified based on study hypotheses and the genetic pathway of interest. Data harmonization involved assessment of study-specific questions, creating derived variables that used the same or the closest information possible for each variable and assessing the distribution of variables across studies for comparability. The distributions of the study variables were very similar across the three studies providing validity to the harmonization process. Variables used in the analyses included body mass index (BMI) 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 (ht^2). Parity was defined as the number of live and stillborn births, age at first birth was defined as age at first live birth or still birth, self-reported race/ethnicity in US studies (all women in Mexico were classified as Hispanic), and highest level of education. Grams of alcohol intake consumed over the lifetime were available for all except for 600 cases and controls from California. For those women, we used alcohol consumption during the referent year as an adjustment variable. Physical activity was harmonized as hours of vigorous intensity activity performed during the referent year and analyzed using center-specific cutpoints to accommodate the level of inquiry of each study questionnaire.

Genetic data

DNA was derived from either whole blood or mouthwash samples obtained from study participants. A total of 7286 blood-derived and 637 mouthwash-derived samples were studied. Whole genome amplification was applied to the mouthwash-derived DNA samples prior to genotyping. Genotyping was conducted as part of a larger study of 1466 single nucleotide polymorphism (SNP) in 205 candidate genes in several inflammation-related pathway arms hypothesized to be involved in breast carcinogenesis. A tagSNP approach was used to characterize variation across candidate genes. TagSNPs were selected using the following parameters: linkage disequilibrium (LD) blocks were defined using a Caucasian LD map and an $r^2 = 0.8$; minor allele frequency >0.1; range $= -1500$ bps from the initiation codon to +1500 bps from the termination codon; and 1 SNP/LD bin. For genes where a functional SNP was identified, that SNP was included in the platform. Additionally, 104 ancestral informative markers were used to distinguish European and Native American ancestry in the study population (see Supplementary Table 1, available at Carcinogenesis Online, for list of ancestral informative markers used). All markers were genotyped using a multiplexed bead array assay format based on GoldenGate chemistry (Illumina, San Diego, CA). A genotyping call rate of 99.93% was attained (99.65% for whole genome amplification 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.

In the current analysis, we evaluated 21 genes central to the CHIEF pathway. These include *mTOR* (3 SNPs), *PDK1* (3 SNPs), *PIK3CA* (7 SNPs), *PIK3CB* (2 SNPs), *NFκB1* (11 SNPs), *NFκB1A* (3 SNPS), *PRKAA1* (7 SNPs), *PRKAG2* (13 SNPs), *PIK3CG* (8 SNPs), *IκBKB* (4 SNPs), *TSC1* (9 SNPs), *TSC2* (5 SNPs), *AKT1* (1 SNP), *AKT2* (1 SNPs), *PTEN* (5 SNPs), *PDK2* (2 SNPs), *STK11* (2 SNPs), *RPS6KB1* (5 SNPs), *RPS6KB2* (1 SNP), *RPS6KA1* (1) and *RPS6KA2* (10). Several tagSNPs had similar LD structure and allele frequency for the NHW and Hispanic women and by genetic ancestry, and others were different based on reported ethnicity and by degree of Native American ancestry. The LD structure for the SNPs assessed in this analysis is available in the Supplementary Table 2, available at *Carcinogenesis* Online, 'LD structure of candidate genes by self-reported ethnicity'; Supplementary Table 3, available at *Carcinogenesis* Online, has information on all SNPs assessed.

Statistical methods

The program STRUCTURE was used to compute individual ancestry for each study participant assuming two founding populations (40,41). A three-founding population model was assessed but did not fit the population structure with the same level of repeatability and correlation among runs as the two-founding population model. Participants were classified by level of percent Native American ancestry. Assessment across categories of ancestry was done using cutpoints based on the distribution of genetic ancestry in the total population with the goal of creating distinct ancestry groups that had sufficient power to assess associations. Use of traditional cutpoints such as tertiles would not generate distinct ancestry groups, given the non-linear association with ancestry from the underlying population. Ancestry was used as a continuous variable to adjust for associations with candidate genes. Associations were assessed within ancestry groups to further distinguish differences in risk for subjects with more Native American versus more European ancestry.

Table I. Description of study population

		Self-reported race/ethnicity							
		NHW				Hispanic			
		Controls		Cases		Controls		Cases	
		N	%	*N*	%	*N*	%	*N*	%
Total sample		1586	37.9	1481	41.2	2597	62.1	2111	58.8
Study site	4-Corner's	1322	83.4	1227	82.9	723	27.8	597	28.3
	Mexico	NA				994	38.3	816	38.7
	San Francisco Bay Area	264	16.7	254	17.2	880	33.9	698	33.1
Age (years)	<40	116	7.3	89	6.0	311	12.0	200	9.5
	40–49	408	25.7	409	27.6	831	32.0	713	33.8
	50–59	409	25.8	413	27.9	756	29.1	617	29.2
	60–69	350	22.1	361	24.4	526	20.3	430	20.4
	70+	303	19.1	209	14.1	173	6.7	151	7.2
	Mean	56.7		56.0		52.3		52.7	
Menopausal status[a]	Pre/Peri	494	31.5	489	33.5	1027	40.7	836	40.9
	Post	1076	68.5	970	66.5	1499	59.3	1210	59.1
Family history of breast cancer[b] (1°)	No	1290	84.5	1122	77.5	2326	91.8	1818	87.8
	Yes	237	15.5	326	22.5	208	8.2	252	12.2
% Native American ancestry	0–28	1578	99.5	1472	99.4	278	10.7	275	13.0
	29–70	7	0.4	7	0.5	1686	64.9	1393	66.0
	71–100	1	0.1	2	0.1	633	24.4	443	21.0
Education[c]	<High school	79	5.0	73	4.9	1538	60.2	1098	52.6
	H.S. grad/GED	339	21.4	300	20.3	419	16.4	385	18.4
	>High school	1168	73.6	1107	74.8	597	23.4	606	29.0
BMI[d] (kg/m^2)	<25	700	44.4	678	45.9	453	17.6	492	23.5
	25–29	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
Age at first birth[e] (years)	Nulliparous	249	15.7	261	17.7	181	7.0	229	11.0
	<20	199	12.6	178	12.0	825	31.9	508	24.3
	20–24	609	38.5	522	35.3	922	35.7	732	35.0
	25–29	342	21.6	333	22.5	451	17.5	359	17.2
	30+	184	11.6	184	12.5	204	7.9	264	12.6
Number of full-term Pregnancies[f]	Nulliparous	249	15.7	261	17.6	181	7.0	229	10.9
	1–2	638	40.3	646	43.7	790	30.5	786	37.2
	3–4	529	33.4	462	31.2	997	38.4	738	35.0
	5+	167	10.6	111	7.5	626	24.1	358	17.0
Alcohol consumption[g] (gm/day)	None	793	50.8	691	46.9	2151	83.5	1707	81.1
	<5	381	24.4	382	25.9	244	9.5	210	10.0
	5 up to 10	156	10.0	166	11.3	100	3.9	109	5.2
	10+	232	14.9	236	16.0	81	3.1	78	3.7
Vigorous-intensity physical activity[h] (hours/week)	4 Corner's: None	461	34.9	440	35.9	319	44.1	287	48.1
	Mean[i]	2.2		2.1		1.8		2.3	
	Mexico: None					827	83.2	555	68.1
	Mean[i]					8.8		4.5	
	California: None	142	53.8	131	51.6	682	77.5	523	74.9
	Mean[i]	4.8		4.0		4.3		4.3	

[a]174 observations without menopausal status.
[b]196 observations without family history of breast cancer data.
[c]66 observations without education data.
[d]52 observations without BMI measurements.
[e]39 observations without age at first birth data.
[f]7 observations without number of full-term pregnancies data.
[g]Based on long-term alcohol consumption except for a subgroup of California subjects for whom data on alcohol consumption were available only for reference year; 58 observations without alcohol data.
[h]1 observation without information on physical activity.
[i]Excludes subjects with no vigorous physical activity a week.

Genes were assessed for their association with breast cancer risk by menopausal status and by genetic ancestry. Women were classified as either pre-/perimenopausal or postmenopausal based on responses to questions on menstrual history. Women who reported still having periods during the referent year were classified as premenopausal. Center-specific definitions were used to define postmenopausal women. Women were classified as postmenopausal if they were taking hormone replacement therapy and still having periods if they were at or above the 95th percentile of age for race/ethnicity of those who reported having a natural menopause (i.e. ≥12 months since their last period) within their study center. This age was 58 for NHW and 56 for Hispanics from the 4-Corner's Breast Cancer Study, 54 for the Mexico Breast Cancer Study and 55 for NHW and 56 for Hispanics from the San Francisco Bay Area Breast Cancer Study.

Table II. Description of candidate genes

Gene	Alias	Chromosome	SNP	Major/Minor allele[a]	MAF NHW	MAF HISP/NA	FDR HWE P NHW	FDR HWE P HISP/NA
mTOR	*FLJ44809, FRAP, FRAP1, FRAP2, RAFT1, RAPT1*	1p36.2	rs2295080	T/G	0.30	0.30	0.97	0.91
PDK1		2q31.1	rs11904366	G/T	0.16	0.10	1.00	0.65
PIK3CA	*MGC142161, MGC142163*	3q26.3	rs2699905	G/A	0.24	0.16	0.96	0.85
	PI3K, p110-alpha DKFZp779K1237, MGC133043, PI3Kbeta, PI3K		rs6443624	C/A	0.23	0.26	1.00	0.71
PIK3CB	*PIK3C1, p110-BETA*	33q22.3	rs10513055	A/C	0.22	0.13	0.96	0.41
NFκB1	*DKFZp686C01211, EBP-1*	4q24	rs3774964	A/G*	0.34	0.47	0.92	0.52
	KBF1, MGC54151, NF-kappa-B		rs4648090	G/A	0.14	0.08	0.66	0.34
	NFKB-p105, NFKB-p50		rs4648110	T/A	0.21	0.12	0.96	0.23
PRKAA1	*AMPK*	5p12	rs11749437	T/G	0.18	0.06	0.96	0.38
	AMPKa1		rs10074991	G/A	0.26	0.25	0.96	0.61
	MGC33776		rs3805486	T/C	0.13	0.15	0.98	0.13
	MGC57364		rs10035235	C/T	0.25	0.44	0.89	0.48
RPS6KA2	*HU-2*	6q27	rs12200581	A/T	0.21	0.10	0.99	0.32
	MAPKAPK1C		rs12199759	A/G	0.16	0.24	0.96	0.62
	RSK, RSK3		rs3778405	A/G	0.10	0.35	0.62	0.07
	S6K-alpha		rs3778401	G/A	0.49	0.24	0.08	0.02
	S6K-alpha2		rs7766723	G/A	0.49	0.3	0.96	0.38
	p90-RSK3, pp90RSK3		rs7745781	A/G	0.14	0.21	0.96	0.62
PRKAG2	*AAKG, AAKG2, CMH6*	7q36.1	rs1001117	C/T	0.31	0.24	0.73	0.56
	CMH6, H91620p, WPWS		rs10236110	G/A	0.26	0.17	0.98	0.75
IκBKB	*FLJ40509*	8.p11.2	rs3747811	T/A*	0.48	0.39	0.86	0.20
	IKK-beta, IKK2, IKKB		rs5029748	C/A*	0.26	0.49	0.96	0.02
	MGC131801, NFKBIKB		rs13278372	C/A	0.11	0.10	0.68	0.32
TSC1	*KIAA0243, LAM*	9q34	rs2250057	T/G	0.36	0.48	0.96	0.01
	MGC86987, TSC		rs7870151	C/A	0.14	0.09	1.00	0.68
PTEN	*BZS, MGC11227, MHAM, MMAC1, PTEN1, TEP1*	10q23.3	rs2735343	G/C	0.34	0.44	0.94	0.73
NFκBIA	*IKBA, MAD-3, NFKBI*	14q13	rs696	G/A	0.39	0.31	0.96	0.89
TSC2	*FLJ43106, LAM, TSC4*	16p13.3	rs1051771	G/C	0.09	0.03	0.96	0.61
PDK2		17q21.3	rs4794096	T/G	0.4	0.43	0.96	0.71
			rs1063647	T/C	0.46	0.48	0.96	0.75
STK11	*LKB1*	19p13.3	rs8111699	C/G	0.45	0.46	0.96	0.85
	PJS		rs741765	G/A	0.23	0.25	0.97	0.2

[a]Major/Minor allele reported for NHW population; different major/minor allele for Hispanic population denoted by an asterisk; MAF, minor allele frequency; HWE, Hardy-Weinberg Equilibrium based on control population.

Logistic regression models were used to estimate the age- and study-center-adjusted odds ratios for breast cancer risk associated with SNPs. Additionally, we adjusted for potential confounding variables of BMI, parity, age at first birth, hours of vigorous-intensity physical activity and alcohol consumption. SNPs were assessed assuming a codominant model. Based on the initial assessment, those which appeared to have a dominant or recessive mode of inheritance were evaluated with those inheritance models in subsequent analyses.

All statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC). Interactions between genetic variants and genetic ancestry and menopausal status were assessed using *P* values from a likelihood ratio test comparing a full model that included an ordinal interaction term with a reduced model without an interaction term.

The *P* values based on one degree of freedom Wald test statistics for the main effect models were adjusted for multiple comparisons taking into account tagSNPs within the gene, using the methods of Conneely and Boehnke (42) via R version 2.12.0 (R Foundation for Statistical Computing, Vienna, Austria). The interaction *P* values, based on one degree of freedom likelihood ratio tests, were adjusted using the step-down Bonferroni correction or the Holm's test (43). This method of correction for multiple comparisons is very conservative, especially for correlated variables such as SNPs within a gene. Given that we are assessing hypothesized associations within a candidate pathway and candidate genes, we considered a pACT of 0.10 or less as potentially important for main effects and a Holm's *P* value of 0.15 or less as potentially important for interaction tests.

Results

The majority of women were postmenopausal (NHW 68.7% of controls and 66.7% of cases; Hispanics 60.0% of controls and 59.8 % of cases) (Table I). More NHW cases reported a family history of breast cancer in first-degree relatives than did Hispanic cases (22.5 versus 12.2%). Genes and SNPs associated with breast cancer in this population are described in Table II.

Higher Native American ancestry was associated with reduced risk of breast cancer (Table III) but was limited to postmenopausal women. Associations were identical whether the entire population was evaluated or the assessment was limited to women who self-reported being Hispanic (data not shown for Hispanic women only). Additionally, assessment of *in situ* versus invasive cancers within the SHINE data did not show appreciable differences in breast cancer associations.

Table III. Associations between genetic admixture and breast cancer risk

Percent Native American ancestry	Controls *N*	Cases *N*	OR[a]	(95% CI)[a]	OR[b]	(95% CI)[b]	OR[c]	(95% CI)[c]
All women								
0–28	1856	1747	1.00		1.00		1.00	
29–70	1693	1400	0.84	(0.75, 0.94)	0.94	(0.83, 1.05)	0.98	(0.87, 1.10)
71–100	634	445	0.66	(0.55, 0.79)	0.77	(0.63, 0.93)	0.79	(0.65, 0.95)
Pre-/Perimenopausal women								
0–28	589	584	1.00		1.00		1.00	
29–70	658	541	0.89	(0.74, 1.08)	0.98	(0.80, 1.19)	1.08	(0.88, 1.33)
71–100	274	200	0.80	(0.60, 1.07)	0.92	(0.68, 1.24)	0.99	(0.73, 1.33)
Postmenopausal women								
0–28	1237	1122	1.00		1.00		1.00	
29–70	982	821	0.83	(0.72, 0.95)	0.91	(0.78, 1.06)	0.94	(0.81, 1.09)
71–100	356	237	0.56	(0.44, 0.72)	0.64	(0.50, 0.83)	0.66	(0.52, 0.85)

[a]Adjusted for age and study center.
[b]Adjusted for age, study center and education.
[c]Adjusted for age, study center, BMI, parity, age at first birth, alcohol consumption and physical activity.

Table IV. Overall associations between candidate pathway genes and breast cancer

		Overall				Wald *P* value	P_{ACT}
		Controls	Cases	OR[a]	(95% CI)		
NFκB1							
rs3774964[b]	AA	1288	1061	1.00		0.007	0.054
	AG/GG	2893	2530	1.15	(1.04, 1.27)		
rs3755867	AA	1435	1209	1.00		0.025	0.120
	AG/GG	2747	2382	1.12	(1.01, 1.24)		
rs4648090	GG/GA	4118	3559	1.00		0.009	0.062
	AA	64	32	0.56	(0.36, 0.86)		
rs4648110	TT/TA	4060	3513	1.00		0.018	0.104
	AA	123	79	0.70	(0.52, 0.94)		
NFκBIA							
rs696	GG	1851	1676	1.00		0.012	0.033
	GA/AA	2331	1916	0.89	(0.81, 0.97)		
PTEN							
rs1903858[b]	TT	1591	1493	1.00		0.008	0.032
	TC/CC	2591	2097	0.88	(0.80, 0.97)		
rs2735343	GG	1491	1398	1.00		0.009	0.035
	GC/CC	2692	2192	0.88	(0.80, 0.97)		
TSC1							
rs7870151	CC	3300	2752	1.00		0.017	0.103
	CA	816	757	1.08	(0.96, 1.21)		
	AA	57	76	1.62	(1.13, 2.31)		
TSC2							
rs1051771	GG	3509	2871	1.00		0.038	0.164
	GC/CC	428	423	1.17	(1.01, 1.36)		
STK11							
rs8111699	CC	1239	971	1.00		0.009	0.103
	CG/GG	2942	2619	1.14	(1.03, 1.26)		
RPS6KA2							
rs7766723	GG/GA	3538	3062	1.00		0.044	0.346
	AA	642	530	0.87	(0.77, 1.00)		
rs7745781	AA	2813	2382	1.00		0.046	0.327
	AG	1235	1066	1.05	(0.95, 1.16)		
	GG	135	143	1.32	(1.03, 1.70)		

[a]OR (odds ratios) and 95% confidence interval (CI) adjusted for age, genetic ancestry, study center, BMI, parity, age at first birth, alcohol consumption and physical activity.
[b]*NFκB1* rs3774964 and rs3755867 $r^2 = 0.83$ for NHW and 0.91 for Hispanic; rs4648090 and rs4648110 $r^2 = 0.61$ for NHW and 0.67 for Hispanic; *PTEN* rs1903858 and rs2735343 $r^2 = 0.93$ for NHW and 0.95 for Hispanic.

Table V. Associations between candidate pathway genes and genetic admixture

% Native American ancestry		0–28		29–70		71–100		Wald *P* Value (P_{ACT})[b]			Interaction[c] *P* value
		OR[a]	(95% CI)	OR	(95% CI)	OR	(95% CI)	0–28	29–70	70–100	
IκBKB											
rs3747811	AA	1.00		0.93	(0.76, 1.15)	0.72	(0.55, 0.93)	0.960	0.574	0.020	0.030
	AT	1.10	(0.92, 1.30)	1.01	(0.84, 1.23)	0.84	(0.64, 1.12)			(0.056)	(0.032)
	TT	1.00	(0.83, 1.21)	0.99	(0.78, 1.26)	1.30	(0.81, 2.08)				
rs5029748[d]	CC	1.00		1.05	(0.88, 1.25)	1.21	(0.82, 1.77)	0.180	0.755	0.018	0.004
	CA/AA	1.11	(0.97, 1.26)	1.02	(0.88, 1.17)	0.78	(0.64, 0.96)			(0.055)	(0.011)
rs10958713	CC	1.00		1.07	(0.88, 1.30)	1.11	(0.74, 1.66)	0.191	0.693	0.114	0.016
	CT/TT	1.10	(0.96, 1.26)	1.03	(0.88, 1.19)	0.81	(0.65, 1.00)				(0.032)
rs13278372	CC/CA	1.00		0.96	(0.85, 1.08)	0.77	(0.64, 0.93)	0.003	0.076	0.031	<0.001
	AA	0.35	(0.18, 0.69)	1.82	(0.87, 3.81)	5.96	(0.69, 51.83)	(0.009)		(0.059)	(<0.001)
mTOR											
rs1057079[e]	AA	1.00		0.94	(0.81, 1.09)	0.71	(0.56, 0.89)	0.518	0.323	0.027	0.035
	AG	0.93	(0.81, 1.08)	0.95	(0.81, 1.11)	0.79	(0.61, 1.02)			(0.057)	(0.104)
	GG	1.00	(0.78, 1.30)	1.09	(0.82, 1.43)	1.32	(0.80, 2.17)				
NFκB1											
rs4648090	GG/GA	1.00		0.96	(0.86, 1.08)	0.77	(0.64, 0.94)	0.007	0.541	0.980	0.080
	AA	0.48	(0.29, 0.81)	0.70	(0.29, 1.66)	Too few to analyze		(0.050)			(0.879)
PDK2											
rs4794096	TT	1.00		1.00	(0.83, 1.19)	0.93	(0.71, 1.22)	0.914	0.525	0.042	0.159
	TG	1.01	(0.87, 1.17)	0.94	(0.80, 1.11)	0.80	(0.62, 1.02)			(0.042)	
	GG	1.01	(0.83, 1.23)	1.10	(0.89, 1.36)	0.62	(0.44, 0.87)				
rs1063647	TT	1.00		1.00	(0.82, 1.21)	0.59	(0.43, 0.81)	0.537	0.248	0.024	0.144
	TC	0.93	(0.80, 1.09)	0.93	(0.78, 1.10)	0.77	(0.60, 0.99)			(0.035)	
	CC	0.95	(0.79, 1.15)	0.88	(0.72, 1.08)	0.89	(0.66, 1.19)				
PIK3CA											
rs6443624	CC	1.00		1.07	(0.93, 1.24)	0.90	(0.72, 1.12)	0.277	0.080	0.027	0.013
	CA/AA	1.09	(0.95, 1.24)	0.95	(0.82, 1.11)	0.70	(0.54, 0.91)		(0.333)	(0.121)	(0.091)
PIK3CB											
rs10513055	AA	1.00		0.94	(0.82, 1.08)	0.77	(0.63, 0.94)	0.667	0.099	0.397	0.046
	AC	1.02	(0.89, 1.18)	1.04	(0.87, 1.25)	0.90	(0.61, 1.33)		(0.176)		(0.092)
	CC	0.83	(0.61, 1.13)	1.34	(0.83, 2.14)	0.68	(0.06, 7.60)				
PRKAA1											
rs10074991	GG	1.00		1.11	(0.96, 1.29)	0.97	(0.77, 1.21)	0.021	0.107	0.015	<0.001
	GA/AA	1.17	(1.03, 1.34)	0.99	(0.84, 1.15)	0.71	(0.55, 0.91)	(0.109)		(0.081)	(0.002)
	TT	1.00		1.05	(0.92, 1.20)	0.86	(0.70, 1.06)	0.286	0.028	0.072	0.011
rs3805486	TC/CC	1.09	(0.94, 1.27)	0.88	(0.74, 1.04)	0.67	(0.49, 0.90)		(0.143)	(0.295)	(0.067)
PRKAG2											
rs10236110	GG	1.00		0.92	(0.80, 1.06)	0.70	(0.57, 0.87)	0.571	0.280	0.010	0.007
	GA	0.93	(0.80, 1.06)	1.03	(0.86, 1.22)	0.91	(0.68, 1.22)			(0.118)	(0.086)
	AA	1.01	(0.77, 1.32)	0.98	(0.67, 1.45)	1.96	(0.71, 5.41)				
PTEN											
rs1903858[f]	TT	1.00		0.91	(0.77, 1.07)	0.79	(0.59, 1.04)	0.006	0.558	0.332	0.468
	TC/CC	0.82	(0.72, 0.94)	0.86	(0.75, 1.00)	0.68	(0.55, 0.84)	(0.021)			
RPS6KA2											
rs12199759	AA	1.00		1.08	(0.94, 1.24)	0.89	(0.71, 1.12)	<0.001	0.443	0.533	0.001
	AG	1.22	(1.05, 1.41)	1.01	(0.86, 1.19)	0.81	(0.63, 1.03)	(0.001)			(0.005)
rs3778405	GG	1.95	(1.32, 288)	1.01	(0.73, 1.41)	0.81	(0.48, 1.36)				
	AA	1.00		0.95	(0.83, 1.10)	1.09	(0.83, 1.43)	0.981	0.358	0.001	0.009
	AG/GG	1.04	(0.88, 1.22)	1.02	(0.88, 1.17)	0.71	(0.57, 0.87)			(0.012)	(0.084)
rs3778401	GG	1.00		1.06	(0.90, 1.26)	0.72	(0.58, 0.90)	0.855	0.319	<0.001	0.032
	GA	1.04	(0.89, 1.21)	0.87	(0.73, 1.05)	1.08	(0.79, 1.48)			(0.002)	(0.193)
	AA	1.00	(0.84, 1.20)	1.16	(0.87, 1.54)	2.56	(0.95, 6.86)				
TSC1											
rs2250057	TT/TG	1.00		1.00	(0.88, 1.13)	0.71	(0.58, 0.87)	0.204	0.074	0.015	0.090
	GG	0.90	(0.74, 1.09)	0.85	(0.71, 1.02)	0.95	(0.72, 1.26)		(0.310)	(0.099)	(0.810)
rs7870151	CC	1.00		0.96	(0.84, 1.09)	0.82	(0.67, 1.01)	0.109	0.008	0.448	
	CA	1.06	(0.91, 1.24)	1.15	(0.94, 1.41)	0.71	(0.50, 1.03)		(0.058)		0.909
	AA	1.49	(0.98, 2.25)	2.52	(1.12, 5.68)	0.90	(0.20, 4.10)				

[a]Odds ratios (OR) and 95% confidence intervals (CI) adjusted for age, study, BMI, parity, age at first birth, alcohol consumption and physical activity level.
[b]Main effect *P* value: multiple-comparison adjusted *P* value, P_{ACT}, shown in parenthesis.
[c]Interaction *P* value (gene × admixture); Holm's adjusted *P* value for interaction, a step-down Bonferroni adjustment, shown in parenthesis.
[d]rs55029748 has similar associations with rs10958713; their r^2 is 0.61 for NHW and 0.82 for Hispanic women.
[e]rs2295080 has similar associations as rs1057079; their r^2 is 0.78 for NHW and 0.90 for Hispanic women.
[f]rs2735343 has similar associations as rs1903858; their r^2 is 0.93 for NHW and 0.95 for Hispanic women.

Adjustment for possible confounding factors, including age at diagnosis or selection, study center, education, BMI, parity, age at first birth, alcohol consumption, and physical activity, slightly attenuated the associations although they remained statistically significant.

Of the 21 genes evaluated, we observed statistically significant associations for tagSNPs in only seven of them (Table IV). *NFκB1, NFκB1A, PTEN, TSC1, TSC2, STK11* and *RPS6KA2* were associated with breast cancer risk for all women combined after adjusting for genetic ancestry and other potentially confounding factors. Associations were generally modest. After adjusting for multiple comparisons, the following associations had pACT values of <0.10: *NFκB1* rs3774964 (pACT = 0.054) and rs4648090 (pACT = 0.062); *NFκB1A* rs696 (pACT = 0.033); and *PTEN* rs1903858 (pACT = 0.032), rs2735343 (pACT = 0.035).

Evaluation within admixture strata, with those in the bottom stratum having higher European ancestry and those in the top stratum having higher Native American ancestry (Table V), showed numerous associations within specific ancestry groups or for differences in association across ancestry groups (*P* value two-way interaction). We considered potentially meaningful strata-specific associations of 0.10 level or less after adjustment for multiple comparisons (Table V); those with significant Wald *P* values but with pACT values greater than 0.10 are shown in Supplementary Table 4, available at *Carcinogenesis* Online. The most common pattern of association observed was statistically significant associations between several SNPS and breast cancer risk among women in the highest category of Native American ancestry (71–100%). Genes showing this pattern of risk with an adjusted pACT of <0.10 were *IkBKB* (3 SNPs), *mTOR*, *PDK2* (2 SNPs), *PRKAA1*, *RPS6KA2* (2 SNPs) and *TSC1*. Only three genes, *NFκB1, PTEN* and *RPS6KA2*, were associated with risk among women with more European ancestry (0–28% Native American ancestry). Potentially meaningful differences in risk between ancestry groups (interaction adjusted *P* for multiple comparisons <0.15 by Holm's test) were observed for *IkBKB, mTOR, PIK3CA, PIK3CB, PRKAA1, PRKAG2* and *RPS6KA2*.

Next, we considered the associations of SNPs within strata defined by genetic ancestry, taking into account menopausal status. For several genes, associations differed by menopausal status (Table VI), including *mTOR, PDK1, PKD2, PIK3CA, PRKAA1, RPS6KA2* and *STK11*. For five SNPs, associations were limited to pre-/perimenopausal women. The TT genotype of *RPS6KA2* rs1220058 was associated with a significantly increased risk of breast cancer among those with more European ancestry; the AA genotype of *STK11* rs741765 showed a significant positive association with breast cancer risk among pre-/perimenopausal women with more Native American ancestry but associated with reduced risk of breast cancer among postmenopausal women. Similar associations were observed for the GG genotype of *STK11* rs811699. Among postmenopausal women with high Native American ancestry, *mTOR* rs2295080, *PDK1* rs11904366, *PDK2* rs1063647, *PRKAA1* rs1174943 and *PIK3CA* rs2699905 were associated with breast cancer risk.

Discussion

Breast cancer incidence rates differ by race and ethnicity. We found that women with higher Native American ancestry have considerably lower risk of breast cancer than do women with higher European ancestry. The reasons for these differences could be attributed to factors that differ between the groups, including BMI, physical activity, alcohol consumption, parity, age at first birth and education. However, adjustment for these factors only slightly attenuated the association between genetic ancestry and breast cancer. Furthermore, we observed differences in risk associated with genes in the CHIEF pathway by genetic ancestry, suggesting a biological basis for differences in breast cancer incidence rates in populations with different genetic admixture.

We assessed genetic admixture using 104 ancestral informative markers that were targeted at discriminating between European and Native American ancestry. These markers were selected from two sources; 58 markers were from Burchard (3) with a difference in minor allele frequency (ΔMAF) between Europeans and Native American Americans >0.50, and 46 were from Galanter (44) with a ΔMAF of >0.74. Although adjustment for genetic ancestry had minimal influence on odds ratio risk estimates, evaluation of breast cancer risk within genetic ancestry strata provided clues into unique breast cancer risk factors for women who had more Native American versus more European ancestry. The associations observed between genetic ancestry and breast cancer in this study support other data that suggest women with more Native American ancestry have lower breast cancer risk, independent of key non-genetic breast cancer risk factors (1). We observed the greatest differences in associations among postmenopausal women. At the population level, breast cancer incidence rates continue to steadily increase after menopause among NHW women, whereas they level off among Native American and Hispanic women (1). The association between genetic ancestry and breast cancer risk was slightly attenuated by factors such as BMI, parity, age at first birth, alcohol consumption and physical activity, factors known to influence breast cancer risk. A 20% overall reduction in risk was observed after adjustment for these factors; for postmenopausal women, a 30 to 40% reduction in risk was observed among those with highest Native American ancestry.

In the admixed population studied here, we evaluated the role of genetic variation in the CHIEF pathway, which comprises genes that may influence hormones, inflammation and energy homeostasis. We selected this pathway based on our understanding of the etiology of breast cancer that comes from epidemiology, laboratory and clinical studies. Our goal was to determine whether this key pathway influenced breast cancer differently in this genetically admixed population, thereby contributing to potential differences in breast cancer risk in admixed populations. We have shown that *IL6* SNPs had a greater influence on risk among Hispanic women than NHW women (45). Rates of diabetes are higher among Hispanic than NHW women, and IGF-1 levels also have been shown to vary in their association with breast cancer risk for these populations (46). Our previous assessment of insulin-related genes showed differences in association with breast cancer for NHW and Hispanic women (47). Dietary factors have been shown to contribute to breast cancer risk among both Hispanic and NHW women (48,49).

Components of the pathway that appear to be of importance include modest associations overall for *NFκB1, NFκB1A, PTEN, TSC1, TSC2, STK11* and *RPS6KA2*. Several components appear to be of greatest importance for those with more Native American ancestry, including *IkBKB, mTOR, PDK2, PIK3CA, PRKAA1, PRKAG2, RPS6KA2* and *TSC1*. *NFκB1* appears to influence risk among women with more European ancestry. Results suggest the importance of inflammation (*NFκB1, NFκB1A* and *IkBKB*), insulin signaling (*STK11, PTEN, Akt, TSC1* and *TSC2*), energy homeostasis (*PRKAA1, PRKAG2, PDK, PIK3CA* and *mTOR*) and cellular energy response (*RPS6KA2*) in defining breast cancer risk in this admixed population. *TSC1* and *TSC2* link energy homeostasis components of the pathway to inflammation via *NFκB*. This pathway appears most important for postmenopausal women, which is where the major divergence in breast cancer incidence rates is observed in admixed populations of European and Native American ancestry. These results suggest that inflammation, insulin and energy-related factors influence breast cancer risk and that these factors may have the greatest influence on more admixed and Native American populations.

Few prior studies have assessed genetic variation in these candidate genes and breast cancer risk, and those that have examined these associations have relied mainly on populations of European ancestry. Associations between variants in these genes and Native American ancestry have not been examined previously. Haiman and colleagues (50) used 17 SNPs to haplotype the *PTEN* gene, and they showed minor associations with a combined haplotype and risk of breast cancer. We saw a slight reduced risk of breast cancer overall for two of the SNPs they analyzed; individual SNP information was not provided in that manuscript. A study by Mehta and colleagues (29) reported associations between genetic variation in *TSC1* and *TSC2* and breast cancer risk by tumor ER/PR status and by menopausal status. Their

Table VI. Associations between candidate genes by menopausal status

% Native American ancestry		Pre-/Perimenopause							Postmenopause							
		0–28		29–70		71–100		Interaction *P* value[b]	0–28		29–70		71–100		Interaction *P* value	
		OR[a]	(95% CI)	OR	(95% CI)	OR	(95% CI)	2 way	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)	2 way	3 way
mTOR																
rs2295080[c]	TT	1.00		1.19	(0.91, 1.56)	1.00	(0.69, 1.44)	0.846	1.00		0.87	(0.71, 1.06)	0.54	(0.39, 0.74)	0.003	0.043
	TG	1.03	(0.80, 1.32)	1.04	(0.79, 1.38)	1.00	(0.66, 1.50)		0.90	(0.75, 1.06)	0.88	(0.71, 1.08)	0.65	(0.46, 0.91)	(0.008)	(0.127)
	GG	1.12	(0.73, 1.70)	1.05	(0.68, 1.63)	1.29	(0.59, 2.86)		0.85	(0.64, 1.14)	1.05	(0.73, 1.49)	1.36	(0.71, 2.58)		
PDK1																
rs11904366	GG	1.00		1.19	(0.95, 1.49)	1.04	(0.76, 1.43)	0.142	1.00		0.95	(0.81, 1.12)	0.62	(0.47, 0.81)	0.132	0.030
	GT/TT	1.09	(0.84, 1.41)	0.86	(0.62, 1.18)	0.88	(0.48, 1.61)		1.01	(0.84, 1.21)	0.92	(0.72, 1.17)	1.12	(0.66, 1.88)		(0.090)
PDK2																
rs1063647	TT	1.00		1.10	(0.79, 1.53)	1.11	(0.69, 1.78)	0.565	1.00		0.95	(0.75, 1.21)	0.33	(0.21, 0.52)	0.013	0.045
	TC/CC	1.02	(0.79, 1.31)	1.10	(0.83, 1.44)	0.97	(0.68, 1.39)		0.89	(0.74, 1.06)	0.84	(0.69, 1.03)	0.71	(0.53, 0.96)	(0.025)	(0.090)
PIK3CA																
rs2699905	GG/GA	1.00		1.11	(0.91, 1.37)	1.03	(0.76, 1.40)	0.016	1.00		0.94	(0.81, 1.10)	0.63	(0.49, 0.82)	0.391	0.019
	AA	1.74	(0.99, 3.06)	0.98	(0.48, 1.98)	0.21	(0.02, 1.73)	(0.114)	0.82	(0.57, 1.18)	0.44	(0.22, 0.89)	2.55	(0.65, 10.02)		(0.133)
rs6443624[d]	CC	1.00		1.05	(0.82, 1.35)	1.05	(0.74, 1.50)	0.937	1.00		1.12	(0.92, 1.35)	0.79	(0.59, 1.06)	0.011	0.101
	CA	0.95	(0.74, 1.22)	1.07	(0.81, 1.41)	0.76	(0.51, 1.15)		1.23	(1.04, 1.46)	0.94	(0.76, 1.15)	0.64	(0.45, 0.92)	(0.080)	
	AA	0.82	(0.50, 1.36)	0.96	(0.59, 1.57)	1.59	(0.61, 4.10)		1.01	(0.70, 1.45)	0.88	(0.61, 1.29)	0.52	(0.22, 1.21)		
PRKAA1																
rs11749437	TT	1.00		1.04	(0.84, 1.30)	1.00	(0.73, 1.37)	0.968	1.00		0.89	(0.76, 1.05)	0.62	(0.48, 0.81)	0.018	0.131
	TG/GG	0.92	(0.71, 1.20)	1.18	(0.82, 1.71)	0.53	(0.22, 1.33)		0.90	(0.75, 1.08)	1.04	(0.78, 1.38)	1.02	(0.49, 2.14)	(0.125)	
rs10035235	CC/CT	1.00		1.09	(0.88, 1.35)	0.82	(0.59, 1.14)	0.035	1.00		0.96	(0.82, 1.12)	0.69	(0.52, 0.91)	0.133	0.004
	TT	0.84	(0.53, 1.34)	0.99	(0.71, 1.38)	1.39	(0.93, 2.08)	(0.173)	1.21	(0.86, 1.71)	0.93	(0.72, 1.21)	0.65	(0.46, 0.91)		(0.030)
PRKAG2																
rs1001117	CC	1.00		1.01	(0.79, 1.31)	0.97	(0.68, 1.38)	0.569	1.00		1.04	(0.86, 1.25)	0.75	(0.56, 1.01)	0.027	0.107
	CT	0.93	(0.73, 1.20)	1.10	(0.83, 1.47)	0.89	(0.59, 1.34)		1.08	(0.91, 1.29)	1.00	(0.81, 1.25)	0.64	(0.45, 0.92)	(0.349)	
	TT	0.86	(0.57, 1.31)	0.91	(0.54, 1.54)	1.18	(0.44, 3.15)		1.35	(1.03, 1.77)	0.72	(0.47, 1.12)	0.61	(0.27, 1.38)		
RPS6KA2																
rs12200581	AA/AT	1.00		1.14	(0.93, 1.40)	1.03	(0.76, 1.39)	0.011	1.00		0.92	(0.80, 1.08)	0.66	(0.51, 0.85)	0.309	0.009
	TT	2.84	(1.40, 5.78)	0.40	(0.12, 1.28)	1.59	(0.10, 25.83)	(0.105)	0.78	(0.53, 1.14)	1.42	(0.66, 3.07)	Too few to analyze			(0.085)
rs12199759	AA	1.00		1.12	(0.88, 1.42)	1.00	(0.70, 1.44)	0.529	1.00		1.08	(0.91, 1.29)	0.79	(0.58, 1.08)	<0.001	0.118
	AG/GG	1.17	(0.91, 1.51)	1.16	(0.90, 1.51)	1.07	(0.75, 1.55)		1.28	(1.08, 1.53)	0.93	(0.76, 1.13)	0.65	(0.47, 0.88)	(0.008)	
STK11																
rs741765	GG/GA	1.00		1.04	(0.84, 1.30)	0.93	(0.67, 1.29)	0.038	1.00		0.91	(0.77, 1.06)	0.65	(0.49, 0.84)	0.235	0.011
	AA	0.94	(0.54, 1.64)	0.99	(0.60, 1.63)	2.68	(1.22, 5.89)	(0.076)	1.13	(0.78, 1.63)	1.00	(0.69, 1.46)	0.42	(0.21, 0.83)		(0.022)
rs8111699	CC/CG	1.00		1.05	(0.84, 1.31)	0.86	(0.62, 1.19)	0.049	1.00		0.94	(0.80, 1.11)	0.71	(0.54, 0.92)	0.323	0.024
	GG	1.02	(0.76, 1.37)	1.19	(0.88, 1.60)	1.62	(1.00, 2.64)	(0.076)	1.13	(0.92, 1.37)	1.06	(0.84, 1.33)	0.57	(0.37, 0.88)		(0.024)

[a]Odds ratios (OR) and 95% confidence intervals (CI) adjusted for age, study center, BMI, parity, age at first birth, alcohol intake and physical activity.
[b]Interaction *P* value for two-way (gene × admixture within menopausal group) and three-way interaction (gene × admixture × menopausal status); Holm's *P* value for adjustment for multiple comparison in parenthesis.
[c]Similar to rs1057079; r^2 is 0.78 for NHW and 0.90 for Hispanic women.
[d]rs7644648 similar to rs6643624; r^2 is 0.72 for NHW and 0.83 for Hispanic women.

cohort of 1121 cases included 78 Hispanic women although data were combined for analysis and presentation. They did not show significant differences in these characteristics in their case/case analysis. An analysis by Stevens *et al.* utilized data from several populations to assess associations of four SNPs in *PIK3CA* with breast cancer (51). In women of European ancestry, they observed a significant modest reduction in risk associated with rs1607237. In our study, the strongest associations with SNPs in the gene were observed for women with more Native American ancestry. A study by Curran and colleagues in Australia reported on breast cancer associations with common variants in *NFκB1* (CA repeat) and *NFκB1A*; they observed no significant associations in their small sample of 109 cases and an equal number of matched controls (52). Unfortunately, little is known about the functionality of these SNPs, so we are inferring the functionality based on the function of the gene itself.

Strengths of the present study include the large sample size that resulted from combining data from three population-based case-control studies conducted in the United States and Mexico. Our sample includes over 2100 breast cancer cases and 2500 controls who self-reported their ethnicity as Hispanic or Latina, completed study questionnaires and had DNA available for analysis. Using our genetic admixture data, we were able to further evaluate associations with genetic variants in women defined by genetic ancestry. Within our study population, we have a wide range of admixture that allows us to analyze individuals with highest Native American ancestry and those with more European ancestry. Although individually each of our three studies has previously examined ancestry with different marker sets (2,3,53), the combined data set allows for a much broader assessment of ancestry than has been possible previously. Given the difficulty in obtaining adequate samples of women of 100% Native American origin, by stratifying the population on genetic ancestry we were able to evaluate associations in that genetically defined population. We had adequate samples across this admixed population to evaluate differences in breast cancer risk. We also have harmonized extensive lifestyle data; the 4-Corner's Breast Cancer Study and the Mexico Breast Cancer Study used many components of the same questionnaire, which facilitated data harmonization.

The study is not without weaknesses, including the number of comparisons made to evaluate this candidate pathway. We have adjusted for multiple comparisons; however, we cannot exclude the possibility that associations could be spurious. Assessment of our findings in other large studies similar to ours is needed although replication in an existing study sample of this size with similar diverse genetic admixture may be difficult to find. Additionally, cutpoints set for genetic ancestry, although chosen based on the distribution in our population in order to have sufficient power to assess associations across the European/Native American ancestry spectrum, were arbitrary. Stronger associations with genetic ancestry would have been observed with more extreme cutpoints than those used. For instance if we used a cutpoint of 0.18 for the lower end of the distribution and 0.90 for the upper end of the distribution a 0.12 greater reduction in risk associated with genetic admixture was observed for women overall and for postmenopausal women.

The goal of this study is to obtain a better understanding of the biological basis for the racial/ethnic disparities in breast cancer risk. We have shown that genetic factors associated with insulin, inflammation and energetic factors have different associations with breast cancer risk among women classified by their European or Native American ancestry. Our results suggest that differences in breast cancer incidence rates between admixed populations could in part be from differences in biological factors. These findings need replication in other admixed populations. To further our understanding of the importance of these genes in breast cancer risk, it will be necessary to evaluate the influence of diet and lifestyle factors in conjunction with these genes.

Supplementary material

Supplementary Tables 1–4 can be found at http://carcin.oxfordjournals.org

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