5-Lipoxygenase and 5-Lipoxygenase-Activating Protein Gene Polymorphisms, Dietary Linoleic Acid, and Risk for Breast Cancer

Jun Wang,[1] Esther M. John,[2,3,4] and Sue Ann Ingles[5]

[1]Tufts Medical Center, Tufts University School of Medicine, Boston, Massachusetts; [2]Northern California Cancer Center, Fremont, California; [3]Department of Health Research and Policy, Stanford University School of Medicine; [4]Stanford Cancer Center, Stanford, California; and [5]Department of Preventive Medicine, University of Southern California, Los Angeles, California

Abstract

The n-6 polyunsaturated fatty acid 5-lipoxygenase pathway has been shown to play a role in the carcinogenesis of breast cancer. We conducted a population-based case-control study among Latina, African-American, and White women from the San Francisco Bay area to examine the association of the 5-lipoxygenase gene (*ALOX5*) and 5-lipoxygenase-activating protein gene (*ALOX5AP*) with breast cancer risk. Three *ALOX5AP* polymorphisms [poly(A) microsatellite, −4900 A>G (rs4076128), and −3472 A>G (rs4073259)] and three *ALOX5* polymorphisms [Sp1-binding site (-GGGCGG-) variable number of tandem repeat polymorphism, −1279 G>T (rs6593482), and 760 G>A (rs2228065)] were genotyped in 802 cases and 888 controls. We did not find significant main effects of *ALOX5* and *ALOX5AP* genotypes on breast cancer risk that were consistent across race or ethnicity; however, there was a significant interaction between the *ALOX5AP* −4900 A>G polymorphism and dietary linoleic acid intake (P = 0.03). Among women consuming a diet high in linoleic acid (top quartile of intake, >17.4 g/d), carrying the AA genotype was associated with higher breast cancer risk (age- and race-adjusted odds ratio, 1.8; 95% confidence interval, 1.2-2.9) compared with carrying genotypes AG or GG. Among women consuming ≤17.4 g/d of linoleic acid, *ALOX5AP* −4900 genotype was not associated with breast cancer risk (age- and race-adjusted odds ratio, 0.9; 95% confidence interval, 0.7-1.2). These results support a role for n-6 polyunsaturated fatty acids in breast carcinogenesis and suggest that epidemiologic studies on dietary fat and breast cancer should take into account genetic predisposition related to n-6 polyunsaturated fatty acid metabolism. (Cancer Epidemiol Biomarkers Prev 2008;17(10):2748–54)

Introduction

In animal studies, n-6 polyunsaturated fatty acids have a strong mammary tumor-enhancing effect (1). To exert their carcinogenic effects, the n-6 polyunsaturated fatty acids must first undergo oxidative metabolism, mainly through lipoxygenase and cyclooxygenase pathways, to form inflammatory compounds, eicosanoids, which influence cell signaling, structure, and metabolism (2, 3). The direct substrate of these oxidative pathways is arachidonic acid, mainly produced from dietary linoleic acid, the most common n-6 polyunsaturated fatty acid in American foods and cooking fats.

In the presence of 5-lipoxygenase-activating protein, 5-lipoxygenase converts arachidonic acid to leukotrienes, 5-hydroxy-6,8,11,14-eicosatetrenoic acid, and 5-oxo-6,8,11,14-eicosatetrenoic acid, all of which are inflammatory mediators in diseases such as asthma, cardiovascular diseases, and many other chronic inflammatory conditions (4). The 5-lipoxygenase pathway has also been implicated in carcinogenesis and tumor progression in tissues such as lung (5), colon (6, 7), prostate (8, 9), kidney (10), bladder (11), pancreas (12, 13), and esophagus (13-15). Blocking the 5-lipoxygenase pathway with 5-lipoxygenase-activating protein and/or 5-lipoxygenase-specific inhibitors is considered an important strategy for cancer intervention (16-18). In breast cancer, 5-lipoxygenase and 5-lipoxygenase-activating protein have been found to be overexpressed in tumor tissue (19) and have been related to cancer prognosis and survival (20). Disruption of the 5-lipoxygenase signaling pathway mediates growth arrest and apoptosis in breast cancer cells (21-23), and inhibits breast cancer metastases (24).

Earlier epidemiologic studies on dietary fat intake and breast cancer did not find positive association between n-6 polyunsaturated fatty acid and breast cancer risk (25). These analyses, however, did not take into account genetic predisposition related to n-6 polyunsaturated fatty acid metabolism. To determine whether 5-lipoxygenase-mediated dietary n-6 polyunsaturated fatty acids metabolism might influence breast cancer risk, we examined genetic variants in the 5-lipoxygenase gene (*ALOX5*) and 5-lipoxygenase-activating protein gene (*ALOX5AP*) in combination with dietary linoleic acid intake in a population-based multiethnic case-control

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Requests for reprints: Jun Wang, 750 Washington Street, Tufts Medical Center, Box 406, Boston, MA 02111. Phone: 617-638-9437; Fax: 617-636-1542. E-mail: jwang1@tuftsmedicalcenter.org

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study on breast cancer conducted in the San Francisco Bay area.

Materials and Methods

Study Population. Study subjects were participants in the San Francisco Bay Area Breast Cancer Study conducted from 1995 to 2003 and described elsewhere (26, 27). This analysis was based on breast cancer cases diagnosed between April 1, 1997, and April 30, 1999, and matched controls.

Cases. Latina, African-American, and White women with a 1st primary diagnosis of invasive breast cancer were identified through the population-based cancer registry of the Greater San Francisco Bay area, which ascertains all incident cancers as part of the Surveillance, Epidemiology and End Results program and the California Cancer Registry. Of 4,842 cases with the age of 35 to 79 years identified by the cancer registry, 630 (13%) could not be contacted (168 deceased, 71 physician refusal, 379 with wrong address or new residence outside of the Bay area, 12 refusals to participate in any study). A brief telephone screening interview that assessed study eligibility and self-reported race or ethnicity was completed by 90% of cases. All eligible Latina and African-American cases and a 10% random sample of White cases were selected for the study; 925 (89%) completed an in-person interview, including 323 Latinas (91% response), 290 African-Americans (90%), and 312 Whites (87%). A blood or mouthwash samples were collected for 814 (78%) cases. DNA was successfully extracted for 813 cases.

Controls. Controls were identified through random-digit dialing and frequency matched to cases on the expected race or ethnicity and 5-year age distribution. From the pool of eligible women (81% response to household enumeration), 1,479 were selected as controls. Of these, 103 (7%) could not be contacted (9 deceased, 94 moved or lost), and of the remaining controls, 93% completed the screening telephone interview. Of those eligible for the study, 1,046 (86%) completed the in-person interview, including 421 Latinas (88%), 305 African-Americans (82%), and 320 Whites (87%). Biospecimen samples were collected for 910 (75%) controls, and DNA was successfully extracted for 906 controls.

Data Collection. Professional bilingual interviewers administered a structured questionnaire on established and suspected breast cancer risk factors and took anthropometric measurements. A food frequency questionnaire, adapted from the 1995 106-item Block Health History and Habits Questionnaire (28, 29), was used to assess usual frequency of consumption and serving size during the reference year (defined as the year before diagnosis for cases or before selection into the study for controls) and to estimate intake of specific nutrients, including linoleic acid.

Selection of Polymorphisms. Polymorphisms for genotyping analysis were selected if they were both (1) common (at least 5% prevalence in any of the 3 racial or ethnic populations included in this study) and (2) potentially functionally significant. Potential functionality was based on nonsynonymous changes, previous reports of functional effects or association with relevant diseases, or location in the evolutionary conserved region in the 5′ regulatory areas [identified using evolutionary conserved region browser (http://ecrbrowser.dcode.org/; ref. 30)]. Three *ALOX5AP* polymorphisms, all upstream of the coding region of the gene, were included in this study: the −169 to −146 poly(A) microsatellite and 2 single-nucleotide polymorphisms that were located in the evolutionary conserved region, −4900A>G (rs4076128) and −3472A>G (rs4073259). Three *ALOX5* polymorphisms were selected, including the −176 to −147 Sp1-binding site 6-bp (-GGGCGG-) variable number of tandem repeat (VNTR) polymorphism, −1279G>T (rs6593482), and 760G>A (Lys254Glu; rs2228065). Our sequencing analysis showed that −1279G>T is also in complete linkage disequilibrium with 5 nearby polymorphisms, including -1700G>A (rs4986832), −1361(GTTAAA) deletion (rs10553050), −839G>A (rs7913948), −557T>C (rs12762303), and −59C>T.

Genotyping Methods. Genotyping of single-nucleotide polymorphisms was done with the Taqman assay using the TaqMan Core Reagent Kit (Applied Biosystems). The 15-μL reaction contained 1.5 μL 10× buffer, 1.2-μL internal standard, 1.5-μL stabilizer, 3.5 mmol/L $MgCl_2$, 0.2 mmol/L deoxynucleotide triphosphate, 300 nmol/L of each primer, 100 nmol/L of each probe, 0.3 unit Taq polymerase, and 20 ng genomic DNA. The conditions for real-time PCR were 10 min at 95°C, followed by 50 cycles of 95°C for 25 s and annealing temperature (58°C for *ALOX5* 760G>A and 60°C for *ALOX5AP* −4900A>G, −3472A>G, and *ALOX5* −1279G>T) for 60 s. The fluorescent signal was measured in the Applied Biosystems Sequence Detection System model 7900HT. Multicolor analysis was used to detect both alleles of the biallelic system. Samples were compared with nine previously sequenced controls (three of each genotype) to identify the three genotypes at each locus. Any sample outside of the parameters defined by the controls was identified as noninformative and was repeated.

Simple sequence length polymorphism analysis was used to analyze the *ALOX5AP* poly(A) microsatellite. The genomic region (−211 to −108) containing the poly(A) microsatellite was PCR amplified using primers end labeled with ^{33}P-γ-ATP. The 10 μL PCR mixture contained 1× PCR buffer, 0.25 mmol/L deoxynucleotide triphosphate, 4 pmol of each primer, 1.5 mmol/L $MgCl_2$, 1 unit Taq polymerase, and 15 ng genomic DNA. The PCR conditions were 94°C for 3 min, followed by 30 cycles of 94°C for 1 min, 61°C for 1 min, and 72°C for 30 s. Chain elongation was continued after the last cycle for 10 min. The PCR products were denatured at 95°C for 5 minutes and 3 μL of each sample was loaded on a 6% denaturing polyacrylamide gel and run at room temperature for 2 h at 80 W. Gels were then dried and exposed to X-ray films for 24 to 48 h. Alleles were sized by comparison with a previously sequenced control.

The GeneScan method was used to analyze the *ALOX5* VNTR polymorphism. The genomic region (−204 to −47) containing the polymorphism was PCR amplified with fluorescently labeled primers. The 10 μL PCR mixture contained 1× PCR buffer, 0.2 mmol/L deoxynucleotide triphosphate (7′deaza-dGTP:dGTP, 3:1), 5 pmol of each primer, 1.5 mmol/L $MgCl_2$, 0.5 μL of DMSO, 1.25 unit

Table 1. Primers and probes for the *ALOX5AP* and *ALOX5* gene genotyping

Polymorphism		Primers	Probes
ALOX5AP	−4900A>G	F: 5′CACACACAGATCAGTGCAGCAT3′ R: 5′CATCTGACCGTTAGCAAAGGTAACT3′	5′ CAGCGCTCTCCGTAA3′ 5′ CAGCGCCCTCCGTAA3′
	−3472A>G	F: 5′GCTGCAGCCAGAATCTTAATGTG3′ R: 5′CGCGCTTTAGAAGGTCGAAA3′	5′ CTGCGCGCTGGCT3′ 5′ CTGCGCACTGGCT3′
	Poly(A) microsatellite	F: 5′GGGACACACTGAACCACAGC3′ R: 5′GAAGATCCCCGGCACAATTA3′	N/A
ALOX5	−1279G>T	F: 5′CGCAGAAGCAATAAAATGTCTGT3′ R: 5′TTTGGAAAACTGGGCTATTTATCTTT3′	5′CATGTATCCGATTAGAGAC3′ 5′TCATGTATCCTATTAGAGACT3′
	VNTR	F: 5′CGTGAAGAGTGGGAGAGAAGTA3′ R: 5′TCCAGGTATCCGCATCTAGC3′	N/A
	760A>G	F: 5′GACCTGATGTTTGGCTACCAGTT3′ R: 5′CCAGGCTGCACTCTACCATCT3′	5′CTGCCCAAGAAGC3′ 5′CTGCCCGAGAAGC3′

Abbreviation: N/A, not applicable.

Taq polymerase, and 20 ng genomic DNA. The PCR conditions were 94°C for 6 min, followed by 35 cycles of 94°C for 30 s, 53°C for 30 s, and 72°C for 30 s. Chain elongation was continued after the last cycle for 5 min. The PCR products were 1:10 diluted and run on ABI 3700 Sequencer together with a ROX-labeled size standard (GeneScan 400HD Size Stand, PE ABI). Results were analyzed using the GeneScan program. Primers and probes for all genotyping reactions were listed in Table 1.

All genotyping assays included control samples that have had the genotype previously confirmed by sequencing. All PCR assays included a ''water blank'' to guard against contamination of the PCR. Five percent of samples were repeated to assure assay reproducibility.

Statistical Analysis. Genotype frequencies were examined separately for cases and controls and for each racial or ethnic group. χ^2 Tests were used to determine whether genotypic distributions among controls departed from Hardy-Weinberg equilibrium. Odds ratios and 95% confidence intervals (95% CI) for genotypes were estimated using unconditional logistic regression models. The *ALOX5* VNTR polymorphism was categorized

Table 2. Characteristics of study participants, by case-control status

	Cases (%; n = 802)	Controls (%; n = 888)	OR (95% CI)*	P
Age in reference year				
mean (SD)	57 (12)	56 (12)		
Race or ethnicity				
White	274 (34)	292 (33)		
African-American	243 (30)	250 (28)		
Latina	285 (36)	346 (39)		
Menopausal status				
Premenopausal	236 (29)	291 (33)	1.0 (ref)	
Postmenopausal	506 (63)	533 (60)	1.2 (0.9-1.6)	
Undetermined	60 (8)	64 (7)	1.2 (0.8-1.7)	0.5
Family history of breast cancer				
No	673 (84)	782 (88)	1.0 (ref)	0.02
Yes	129 (16)	106 (12)	1.4 (1.1-1.8)	
History of benign breast disease				
No	605 (75)	750 (85)	1.0 (ref)	
Yes	197 (25)	137 (15)	1.8 (1.4-2.3)	<0.0001
Age at menarche				
8-11	186 (23)	190 (22)	1.0 (ref)	
12-13	411 (52)	478 (54)	0.9 (0.7-1.1)	
>13	200 (25)	212 (24)	1.0 (0.7-1.3)	0.9
Age at first full-term pregnancy				
<20	161 (20)	211 (24)	1.0 (ref)	
20-24	256 (32)	284 (32)	1.2 (0.9-1.6)	
25-29	135 (17)	161 (18)	1.2 (0.8-1.6)	
≥30	113 (14)	106 (12)	1.5 (1.0-2.1)	
Nulliparous	136 (17)	123 (14)	1.5 (1.1-2.1)	0.01
Body mass index (kg/m^2)				
Premenopausal				
<25	106 (36)	90 (25)	1.0 (ref)	
25-29	77 (26)	115 (32)	0.5 (0.3-0.8)	
≥30	113 (38)	150 (42)	0.6 (0.4-0.9)	0.01
Postmenopausal				
<25	122 (24)	119 (23)	1.0 (ref)	
25-29	158 (31)	180 (34)	0.9 (0.6-1.2)	
≥30	223 (44)	227 (43)	1.0 (0.7-1.3)	1.0

Abbreviation: OR, odds ratio; ref, reference.
*Odds ratio and 95% CI, adjusted for age and race or ethnicity.

into two groups: wild type (5 repeats) and variant allele (3, 4, 6, or 7 repeats), the same as in other studies of this polymorphism (31, 32). For the *ALOX5AP* poly(A) microsatellite, unlike the biallelic distribution in previous reports (31, 33), we found this microsatellite included continuous variation ranging from 15A to 26A, with most alleles being 23A or 19A. In the analysis, we chose the least frequent repeat, 20A, as the cut point and grouped the microsatellite into long (>20A) and short (≤20A) alleles.

All analyses were adjusted for age and race or ethnicity. Risk factors evaluated as potentially confounding variables included place of birth, education, family history of breast cancer, history of benign breast disease, menopausal status, age at menarche, age at first full-term pregnancy, body mass index, height, lifetime physical activity, and alcohol intake. Because adjustment for these variables did not change the odds ratio estimates, we only reported the results adjusted for age and race or ethnicity. We also tested the interactions between *ALOX5* and *ALOX5AP* with dietary linoleic acid intake.

Formal tests of effect modification were done by including the appropriate interaction terms in the logistic regression model. All analyses were done using SAS 9.0 (SAS Institute).

Results

Characteristics of Study Subjects. Of the DNA samples available from 1,719 subjects, 28 samples (1.6%; 12 cases and 16 controls) failed genotyping for ≥1 polymorphisms and 1 sample had important demographic data missing. The final analysis was therefore based on 1,690 subjects (802 cases and 888 controls), of whom 34% were White, 29% were African-American, and 37% were Latina (Table 2). Approximately 61% of women were postmenopausal. Cases and controls were of similar age within each racial or ethnic group and within groups defined by menopausal status.

All genotypes were in Hardy-Weinberg equilibrium among controls in each racial or ethnic group. The allele frequencies of *ALOX5AP* −3472A>G, *ALOX5* VNTR polymorphism, and 760G>A were similar to those from previous reports for the same racial or ethnic groups (31, 34, 35).

The *ALOX5AP* and *ALOX5* Polymorphisms and Breast Cancer Risk. For *ALOX5AP*, the −4900A>G A allele was marginally associated with increased risk for breast cancer among Whites (AA versus GG; age-adjusted odds ratio, 1.5; 95% CI, 0.8-2.7; *P* trend for numbers of A allele = 0.07; Table 3). However, no associations were seen among Latinas and African-Americans. The *ALOX5AP* −3472A>G polymorphism and the poly(A) microsatellite were not associated with breast cancer risk in any racial or ethnic group.

For *ALOX5*, Latinas carrying the nonwild-type allele for the VNTR polymorphism were at increased risk for breast cancer (5/non-5 versus 5/5; age-adjusted odds ratio, 1.4; 95% CI, 1.0-1.9; non-5/non-5 versus 5/5; odds ratio, 1.3; 95% CI, 0.6-2.9; *P* trend for number of non-5 alleles = 0.10), as were Latinas carrying the −1279 T allele (GT versus GG; age-adjusted odds ratio, 1.4; 95% CI, 0.9-2.0; TT versus GG, odds ratio, 2.1; 95% CI, 0.7-6.5; *P* trend for number of T alleles = 0.06). No significant

Table 3. Association between the *ALOX5AP* and *ALOX5* polymorphisms and breast cancer risk, by race/ethnicity

Gene	Genotype	All			Whites			Latinas			African-Americans		
		Case	Control	OR (95% CI)*	Case	Control	OR (95% CI)†	Case	Control	OR (95% CI)†	Case	Control	OR (95% CI)†
ALOX5AP	−4900 GG	206	215	1.0 (ref)	22	30	1.0 (ref)	40	46	1.0 (ref)	144	139	1.0 (ref)
	AG	311	368	0.9 (0.7-1.2)	94	116	1.1 (0.6-2.0)	129	158	0.9 (0.6-1.5)	88	94	0.9 (0.6-1.3)
	AA	285	305	1.0 (0.8-1.4)	158	146	1.5 (0.8-2.7)	116	142	0.9 (0.6-1.5)	11	17	0.6 (0.3-1.4)
	P_{trend}			0.7			0.07			0.9			0.3
	−3472 GG	286	301	1.0 (ref)	40	41	1.0 (ref)	90	98	1.0 (ref)	156	162	1.0 (ref)
	AG	326	382	0.9 (0.7-1.2)	111	135	0.8 (0.5-1.4)	140	171	0.9 (0.6-1.3)	75	76	1.0 (0.7-1.5)
	AA	190	205	1.0 (0.7-1.3)	123	116	1.1 (0.7-1.8)	55	77	0.8 (0.5-1.2)	12	12	1.0 (0.5-2.4)
	P_{trend}			0.9			0.4			0.2			0.9
	Poly(A) LL	440	464	1.0 (ref)	207	207	1.0 (ref)	170	187	1.0 (ref)	63	70	1.0 (ref)
	LS	258	332	0.8 (0.7-1.0)	59	77	0.8 (0.5-1.1)	98	138	0.8 (0.6-1.1)	101	117	1.0 (0.6-1.5)
	SS	104	92	1.1 (0.8-1.6)	8	8	1.0 (0.4-2.7)	17	21	0.9 (0.5-1.8)	79	63	1.4 (0.9-2.2)
	P_{trend}			0.8			0.3			0.2			0.2
ALOX5	-1279 GG	568	651	1.0 (ref)	191	203	1.0 (ref)	210	276	1.0 (ref)	167	172	1.0 (ref)
	GT	221	219	1.1 (0.9-1.4)	82	85	1.0 (0.7-1.5)	67	65	1.4 (0.9-2.0)	72	69	1.1 (0.7-1.6)
	TT	13	18	0.8 (0.4-1.7)	1	4	0.3 (0.03-2.4)	8	5	2.1 (0.7-6.5)	4	9	0.5 (0.1-1.5)
	P_{trend}			0.5			0.8			0.06			0.7
	Sp1 binding site 5/5	418	501	1.0 (ref)	185	193	1.0 (ref)	176	238	1.0 (ref)	57	70	1.0 (ref)
	5/Non-5	304	298	1.2 (1.0-1.5)	84	91	1.0 (0.7-1.4)	97	95	1.4 (1.0-1.9)	123	112	1.4 (0.9-2.1)
	Non-5/non-5	80	89	1.0 (0.7-1.5)	5	8	0.7 (0.2-2.0)	12	13	1.3 (0.6-2.9)	63	68	1.1 (0.7-1.9)
	P_{trend}			0.3			0.6			0.1			0.6
	760 G>A GG	761	850	1.0 (ref)	273	292		282	343		206	215	1.0 (ref)
	GA	39	37		1	0		3	3		35	34	
	AA	2	1		0	0		0	0		2	1	
	(AA+GA) vs GG	41	38	1.2 (0.7-1.9)	1	0		3	3		37	35	1.1 (0.7-1.8)

*Odds ratio and 95% CI, adjusted for age and race or ethnicity.
†Odds ratio, adjusted for age.

Table 4. Association between *ALOX5AP* genotype and haplotype and breast cancer risk, by dietary linoleic acid intake and race or ethnicity

Linoleic acid intake (g/d)*	*ALOX5AP* -4900 genotype	All			Whites			Latinas			African-Americans		
		Case	Control	OR (95% CI) †	Case	Control	OR (95% CI) ‡	Case	Control	OR (95% CI) ‡	Case	Control	OR (95% CI) ‡
Q1-Q3 (≤ 17.4)	AG+GG	375	419	1.0 (ref)	94	103	1.0 (ref)	123	160	1.0 (ref)	152	155	1.0 (ref)
	AA	212	247	0.9 (0.7-1.2)	119	115	1.1 (0.8-1.7)	80	121	0.9 (0.6-1.3)	6	12	0.5 (0.2-1.4)
Q4 (> 17.4)	AG+GG	142	164	1.0 (ref)	22	43	1.0 (ref)	46	44	1.0 (ref)	80	78	1.0 (ref)
	AA	73	58	1.8 (1.2-2.9) P_{int} = 0.03	39	31	2.5 (1.2-5.0) P_{int} = 0.06	36	21	1.7 (0.8-3.4) P_{int} = 0.1	5	5	1.0 (1.0-1.0) P_{int} = 0.4

*Quartiles of intake among controls.
†Odds ratio and 95% CI, adjusted for age and race or ethnicity.
‡Odds ratio, adjusted for age.

associations were found among Whites or African-Americans. The African-American-specific 760G>A polymorphism was not associated with breast cancer.

No consistent associations were found when the analyses were stratified on family history of breast cancer, menopausal status, or tumor characteristics (stage at diagnosis, histologic grade, and estrogen and progesterone receptor status; data not shown). No interactions were found between the two genes (data not shown).

Dietary Linoleic Acid Intake and the *ALOX5AP* -4900A>G Polymorphism—Possible Interaction for Breast Cancer Risk. Dietary linoleic acid is the major source for arachidonic acid, the direct substrate for 5-lipoxygenase metabolic pathway. We therefore stratified the analysis of *ALOX5* and *ALOXAP* by dietary linoleic acid intake. Among women in the top quartile of linoleic acid intake (>17.4 g/d), the *ALOX5AP* −4900 AA genotype was associated with a 1.8-fold increase in breast cancer risk (95% CI, 1.2-2.9), whereas the AA genotype was not associated with breast cancer among women consuming ≤17.4 g/d of linoleic acid (odds ratio, 0.9; 95% CI, 0.7-1.2, P_{int} = 0.03; Table 4). This finding was consistent for Whites and Latinas. No evidence of interaction was found among African-Americans; however, the AA genotype was very rare in this population. No significant effect modification was found for other *ALOX5AP* or *ALOX5* polymorphisms.

Examination of the haplotypes of these two genes, as main effects or as interactions with linoleic acid intake, did not provide additional information (data not shown).

Discussion

In this study we observed an interaction between dietary linoleic acid intake and *ALOX5AP* genotype. The *ALOX5AP* -4900 AA genotype was associated with an 80% increase in breast cancer risk only among women with high intake (>17.4 g/d) of linoleic acid. Among women with lower linoleic acid intake, *ALOX5AP* genotype had no influence on breast cancer risk.

Dietary intake of n-6 polyunsaturated fatty acids has been found in animal models to have a strong promoting effect on breast cancer development (1). One possible mechanism involves the arachidonic acid 5-lipoxygenase pathway metabolites that have been found to promote tumorigenesis in breast tissue (21-23) and influence breast cancer prognosis and metastases (19, 24). Among humans, however, a meta-analysis of 45 epidemiologic studies did not support a positive association between n-6 polyunsaturated fatty acid and breast cancer risk (25). Similarly, in the larger study population of the San Francisco Bay Area Breast Cancer Study, which included the subjects of the current analysis (but was not limited to those with biospecimen collection), we did not find a significant association between dietary linoleic acid intake and breast cancer risk.[6] These analyses, however, did not take into account possible interindividual differences in linoleic acid metabolism and thus cannot rule out an effect of linoleic acid on breast cancer development.

The present study is the first to examine *ALOX5AP* and *ALOX5* polymorphisms with respect to breast cancer risk. Although we did not find significant main effects of 5-lipoxygenase pathway genes with breast cancer, our finding of an interaction between *ALOX5AP* and linoleic acid intake suggests that carrying a ''high-risk'' allele alone or high dietary n-6 polyunsaturated fatty acid intake alone may not be sufficient to increase breast cancer risk, whereas in women with both a high-risk allele (*ALOX5AP* -4900 A) and high dietary n-6 polyunsaturated fatty acids intake, risk may be increased nearly 2-fold. Although these findings need to be confirmed by other studies, our results suggest a role of the n-6 polyunsaturated fatty acid pathway in breast cancer etiology.

Whether *ALOX5AP* -4900A>G polymorphisms is the causal variant is not clear. The -4900A>G polymorphism is located in a region evolutionarily conserved across species, suggesting possible functional importance. Also in this region and in partial linkage disequilibrium with −4900A>G is the −3472A>G variant. We did not find a significant association between −3472 genotype and breast cancer risk. In an Icelandic study, the −3472 A allele was associated with increased risk for myocardial infarction (34); however, the -4900A>G polymorphism was not examined in that study. The only other variant that we identified in the proximate promoter region was the *ALOX5AP* poly(A) microsatellite, which has been

[6] J. Wang, E.M. John, P.L. Horn-Ross, S.A. Ingles. Dietary fat, cooking fat, and breast cancer risk in a multiethnic population, submitted for publication.

associated with asthma among Japanese (33) but not among Caucasians (31). We found no association between the poly(A) microsatellite and breast cancer risk, which is in agreement with the finding that this polymorphism has no functional significance (31).

In the present study, we did not find any significant association between *ALOX5* polymorphisms and breast cancer risk, although a weak association among Latinas could not be excluded. The *ALOX5* VNTR polymorphism is located in a Sp1-binding site. The functional studies on this polymorphism in general suggest diminished transcriptional activity of nonwild-type alleles (36, 37). Therefore, subjects carrying a nonwild-type allele should have diminished *ALOX5* transcription and consequently reduced production 5-hydroxy-6,8,11,14-eicosatetrenoic acid and leukotrienes. Because *in vitro* studies support a promotional effect of 5-hydroxy-6,8,11,14-eicosatetrenoic acid on breast cancer cell growth (21, 22), we would expect that subjects carrying the nonwild-type allele should have reduced risk for breast cancer. There was no evidence of an inverse association in this study; furthermore, among Latinas, the association was in the opposite direction. Interestingly, a study on the same polymorphism and atherosclerosis also found that the nonwild-type allele is associated with the enhanced intima media thickness and chronic arterial inflammation (32). The discrepancies between functional studies (36, 37) and association results from a previous study (32) and the current analysis suggest several possibilities. (*a*) The regulation of *ALOX5* is probably more complicated than what has been shown by *in vitro* studies on this single polymorphism. The combined functional effects of additional polymorphisms in the 5′ region remain to be studied. (*b*) This polymorphism might influence epigenetic regulation of *ALOX5* expression. Methylation at the Sp1-binding site has been found in the cell type–specific expression of *ALOX5* (3, 38). 5-Lipoxygenase is involved in the production of endogenous anti-inflammatory mediators such as lipoxins through mechanisms also involving other lipoxygenases and cyclooxygenases (39). It is possible that the effect of *ALOX5* on breast cancer is determined by the balance between pro- and anti-inflammatory mechanisms.

In our analyses, we also assessed other known breast cancer risk factors, including body mass index and other hormonal or reproductive factors, as potential confounders for the association between lipoxygenase pathway genes and breast cancer. None of these factors showed confounding effects, suggesting that the pathologic mechanisms underlying the lipoxygenase genes–breast cancer relationship is independent of the hormonal mechanism.

There are some limitations of this study. We only selected putatively functional polymorphisms in the *ALOX5* and *ALOX5AP* for the association analysis. We cannot exclude the possibility that other polymorphisms in these two genes may be associated with breast cancer risk because of the incomplete coverage of common genetic variations. It is also possible that other genes related to linoleic acid metabolic pathway or other metabolic pathways may directly or indirectly affect linoleic metabolism. Future studies on breast cancer and linoleic acid should also consider other relevant genes. Although we have sufficient power for detecting genetic main effects, the power to detect modest gene-environment interactions was limited, especially when stratifying on race or ethnicity. Further investigations are warranted to confirm the findings of our study.

In summary, the present study revealed a diet-gene interaction in the association between the 5-lipoxygenase metabolic pathway and breast cancer. This finding suggests that epidemiologic studies on dietary fat and breast cancer should take into account genetic predisposition related to n-6 polyunsaturated fatty acid metabolism.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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