POTENTIAL DIFFERENCES IN BREAST CANCER RISK FACTORS BASED ON CYP1A1 MSPI AND AFRICAN-AMERICAN-SPECIFIC GENOTYPES

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Objectives: Recent studies show that an MspI polymorphism in the 3′-noncoding region of the CYP1A1 gene is associated with breast cancer in African-American women but not in Caucasian women. In addition, an African-American-specific (AAS) polymorphism is located in intron 7 of this gene. We hypothesized that the AAS polymorphism may partially account for this race-specific association and that different environmental risk factor profiles are a function of genotype status. We studied both CYP1A1 polymorphisms to determine if African-American women with these variants have breast cancer risk factor profiles that are different from those of other African-American women.

Methods: A case-control analysis was conducted. Cases were 304 African-American patients pathologically diagnosed with breast cancer from 1995 to 1998 who lived in three Tennessee counties. Controls were 305 African-American women without breast cancer, selected through random-digit dialing and frequency matched to cases by age and county. Information on risk factors was collected through telephone interviews. Tumor tissue samples were collected for CYP1A1 genotyping. There were 215 and 188 cases with the MspI and AAS polymorphisms measured respectively.

Results: Our study results suggest that some risk factors for breast cancer are dependent upon CYP1A1 genotype. Specifically, low intakes of folate, methionine, vitamin C, and vitamin E appear to increase the risk of breast cancer in individuals with the AAS variant: the odds ratio (OR) estimates and 95% confidence intervals were 2.10 and 0.99–4.44 for folate, 1.96 and 0.91–4.23 for methionine, 2.13 and 1.00–4.53 for vitamin C, and 2.43 and 1.12–5.25 for vitamin E. Such associations are stronger for tumors with both AAS and MspI polymorphisms: the OR estimates increased to >6.00 for all these variables except for vitamin C.

Conclusions: This study found that methyl-deficient diets and antioxidant vitamins may be related to the risk of breast cancer as a function of the MspI and AAS genotpyes. Our results are preliminary because of a small number of cases with polymorphisms at both sites, but they indicate the need for large-scale epidemiologic studies of both African-American and Caucasian women that include genotype information from controls with more detailed information on risk factors. (*Ethn Dis*. 2006;16:207–215)

Key Words: African American, Breast Cancer, Case-Control Study, Polymorphism, Risk Factors

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INTRODUCTION

Studies on breast cancer risk factors in African-American women have been limited,[1–3] and most of the studies have been unable to determine whether risk factor profiles differ between African-American and Caucasian women. A noteworthy issue is that few of the studies that assess Black/White differences have investigated risk factors as a function of genetic background. Cancer usually results from the effects of both genetic and environmental factors. If African-American and Caucasian women differ in genotype frequencies at key loci, we must examine lifestyle and environmental factors in light of such genetic variation when studying Black/White differences.

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A recent study found that the MspI polymorphism (MspI [+]) in the 3′-noncoding region of the CYP1A1 gene is strongly associated with breast cancer in African Americans but not in Caucasians.[4] In this study, the risk of breast cancer was 2.3 (95% confidence interval [CI] 0.9–6.1) and 8.4 (95% CI 1.7–41.7) times higher for individuals heterozygous and homozygous for the variant allele, respectively, compared with individuals homozygous for the normal MspI restriction fragment length polymorphism (RFLP) (MspI [−]) in African Americans.[4] This association was not observed in Caucasians. The race-specific association suggests the impact of other factors unique to African Americans. The African-American-specific (AAS) polymorphism of the gene, which is in intron 7 of the gene[5] and has not been observed in Caucasians,[4] may be such a factor. The association with the MspI(+) allele in African Americans may be due to the concomitant presence of the AAS polymorphism (AAS [+]) in many of the affected individuals with MspI(+). In other words, those at risk for a particular risk factor exhibit linkage disequilibrium between these two polymorphic sites.

The CYP1A1 gene encodes a critical component of the P450 enzyme system that is important for the metabolism of estrogen and some known carcinogens.[6,7] Its polymorphisms may act in conjunction with other risk factors to affect breast cancer occurrence. Because many risk factors for breast cancer are linked to exposure to estrogens, it is important to examine the role of the CYP1A1 polymorphisms in the occurrence of breast cancer. The MspI and AAS polymorphisms may interact with each other in African Americans, conferring a different susceptibility to breast cancer, and such susceptibility further interacts with other risk factors, incurring breast cancer. Therefore, MspI/AAS genotype (both MspI[+] and AAS[+]) may represent a unique genetic susceptibility to breast cancer in African Americans. Because of the ethnic specificity of the genotype and because of likely gene-environment interaction, we hypothesize that African-American women with and without AAS polymorphisms may differ in risk factor profile. If this hypothesis is true, African-American women with these polymorphisms may possess risk factors different from those in Caucasian women because Caucasians do not have the AAS polymorphism. Our study examined risk factors for breast cancer in African-American women conditioned upon the MspI and AAS polymorphic states of the CYP1A1 gene.

MATERIALS AND METHODS

A case-control study was conducted. Cases were 304 African-American female patients diagnosed with breast cancer from 1995 to 1998 and who lived in Davidson, Shelby, and Hamilton counties, Tennessee. Controls (n=305) were African-American women without a history of breast cancer and frequency matched to cases by five-year age range and county.[8]

Cases were selected through the Tennessee Cancer Reporting System (TCRS). Eligible patients were contacted about the study and asked to participate after their doctors had given consent to contact them. Patients who completed a consent form were recruited as cases for the study. Controls were identified through random-digit telephone dialing.[9] For an identified eligible woman, the study purposes and procedures were described, and their willingness to participate in the study was asked. Women who agreed to participate and accepted an interview made up the control group. To compensate for the participants' time and effort for the study, we paid $25 for a completed interview and provided study subjects an opportunity to draw for $200. We also compensated cases $10 for agreeing to release their tumor tissue specimens. These procedures were approved by the institutional review board of Meharry Medical College.

Six hundred and seventy eligible women with a physician's name listed were identified from the TCRS. Thirty of the 670 patients were reported by their physicians to have died. Of the remaining 640 patients, physician's consent for contact was obtained for 478. Out of the 478 patients with physician's consent, 18 were deceased and 51 could not be located. Of the remaining eligible patients whom we were able to contact, 304 (64% of those with a doctor's consent or 74% of those whom we contacted) agreed to participate in the study and were subsequently interviewed. Two hundred and seventy-eight of these interviewed cases had tumor tissue specimens available.

The random digit dialing calls for control selection resulted in 5970 households that provided information on eligibility. Of these, 420 were identified with at least one eligible female. In households with more than one eligible female, one eligible woman was randomly chosen. For 33 eligible households, eligible women were never home. For another 11 households, eligible women would have participated but preferred to be interviewed at another time, which was not conducted subsequently for other reasons. Of the remaining 376 eligible women, 71 refused to participate in the study. As a result, 305 women (72.6%) were interviewed and used as controls.

Telephone interviews with a structured questionnaire were used to collect information on breast cancer risk factors. The interviews occurred generally one to three years after cancer diagnosis for cases. To reduce the potential effects of recall errors from delayed interviews, we used reference date. Information at or before the reference date was collected. The reference date for cases was defined as the date of diagnosis and for controls as the year corresponding to the diagnosis year of matched cases. Information we collected included data on reproductive and menstrual factors, history of benign breast diseases and sexually transmitted diseases, family history of breast cancer, personal habits and medication use, dietary intake, anthropometric measures, and demographic variables. Dietary intake was estimated using the Block-NCI Health Habits and History Questionnaire.[10] Dietary data were analyzed to calculate nutrient estimates with the Dietary Analysis Personal Computer System.

Paraffin-embedded tumor tissue samples were collected from hospitals where cases were pathologically diagnosed and were sent to the research team. Tissue slides were made. A pathologist reviewed slides for each sample to confirm the diagnosis of breast cancer. Tissue samples without identified tumors were excluded. The DNA from the tissue samples was extracted according to a method described originally by Sukpanichnant et al,[11] with some modifications.

The MspI and African-American-specific polymorphisms were measured by using a polymerase chain reaction (PCR) method. The primers used for

the MspI polymorphism were 5′-CAGTGAAGAGGTGTAGCCGCT-3′ and 5′-AGAGGCTGAGGTGGGAGAAT-3′. The primer sequences for the AAS polymorphism were 5′-CCTGGGAACATCACATTCCT-3′ and 5′-AGTCCTGGTGCCTGGATATG-3′. For both assays, we amplified the products by using the above oligos, Qiagen MasterMix, and the DNA. We used an annealing temperature of 64°C and 30 cycles of amplification for both. After amplification, samples were cleaned for sequencing by using the Amersham ExoSapIt kit. Products and diluted primers were taken to the Vanderbilt Sequencing Core where Dye Terminator cycle sequencing reactions were carried out. Samples plus the appropriate loading buffer were run on a 96-capillary sequencer (the ABI 3700). The PCR products were scored to determine the genotype. For quality control in genotyping, we simultaneously measured some control samples with known genotypes. Blank samples were used simultaneously to exclude the possibility of potential contamination.

Tumor tissue samples were collected for 278 cases. However, laboratory results were not obtained for 63 and 90 cases for the MspI and AAS polymorphisms, respectively. Reasons for the lack of results for these samples vary but probably include degradation of DNA for some samples, exhaustion of existing DNA for some samples, or the effects of secondary structure or regions of homology elsewhere in the genome perturbing the balance of the oligonucleotides.

We calculated single-locus Hardy-Weinberg (H-W) analyses by using TFPGA (tools for population genetic analyses). Statistical significance for the above was determined by using Fisher exact test. Both sites were in H-W equilibrium. Normalized pairwise linkage disequilibrium (D′) and haplotype frequencies were calculated by using the 2LD software.[12] Significant deviations from random haplotypes were detected by using empirically derived *P* values. The two sites exhibited significant linkage disequilibrium with a D′ of .74 ($P=.03$).

We analyzed the data for each of the polymorphisms individually as well as in combination (ie, MspI/AAS genotypes). Because the polymorphisms were not measured for controls, two steps were taken to estimate the role of risk factors on breast cancer according to genetic status. We first compared all cases and controls to estimate odds ratios (ORs) for different risk factors. We then divided cases into subgroups based on genotypes. The subgroups were divided into those individuals who had one or more variant alleles versus those who had none. This is a dominant model of risk. We estimated ORs for each subgroup. A higher OR for a factor in a case subgroup (such as cases with the AAS polymorphism) than that in the whole case group suggests that cases with a genetic variant are more susceptible to the effect of the factor.

The data were analyzed by using logistic regression.[13] We included in the models demographic variables such as age, marital status, educational level, and annual family income to control for possible confounding effects. We then added each potential risk factor to the model containing the demographic variables. Because of the small number of subjects in each case subgroup that resulted from dividing cases by genetic status, particularly MspI(+)/AAS(+) genotype, we did not conduct dose-response analysis (in which a factor is divided into multiple exposure levels) and analysis by menopausal status or adjust for many variables in the model. This study had a small number of subjects in each subgroup and was intended to provide initial data. Therefore, we only tested dichotomized risk factors while controlling for demographic variables. The ORs for risk factors and their 95% CIs were calculated.

Genetic status was defined as a dichotomous variable based on an individual's having no variant alleles compared to having one or two variant alleles (homozygous normal vs all other genotypes). Because this was a study to generate initial data, we examined all potential risk factors investigated in the original study. These factors included body mass index (BMI; weight in kilograms divided by height in meters squared), antioxidant vitamin usage (Vitamins A, E, and C), smoking, alcohol consumption, medical history of benign breast disease and cancer, family history of breast cancer, menstrual history (age at menarche, menopausal status, age at menopause, time from menarche to menstrual regularity, average cycle length, and average length of period), reproductive and steroid hormone-related factors (parity, age at first birth, number of pregnancies, history of infertility, miscarriage, age at first intercourse, use of birth control pills, and exogenous estrogen/progesterone use), exercise, use of electric bedding devices, and nutrient intake (fat, carbohydrate, protein, fiber, folate, methionine, etc). For continuous variables such as BMI and nutrient consumption, we assessed the variable dichotomized by the median value in controls. Sixteen cases and 14 controls were excluded from analysis based on nutrient intake variables because they reported unusually low or high dietary kilocalories (<500/day or >4000 per day) or reported a relatively large number of foods with missing data or consumption of >30 foods per day. Nutrient information might not be accurate for these subjects.

RESULTS

Demographic characteristics of the study subjects are presented in Tables 1–3. Table 1 shows the characteristics when cases were defined by the MspI status. Cases without the MspI poly-

Table 1. Demographic characteristics of controls and cases defined by the CYP1A1 MspI polymorphic status

Variable	Controls (%)	Cases	
		MspI+ (%)	MspI− (%)
Age at interview			
20–39	33 (10.8)	8 (12.1)	19 (12.8)
40–49	105 (34.4)	23 (34.9)	54 (36.2)
50–59	109 (35.7)	24 (36.4)	47 (31.5)
≥60	58 (19.0)	11 (16.7)	29 (19.5)
Marital status at reference date			
Married	132 (43.6)	31 (47.0)	60 (40.3)
Separated	35 (11.6)	5 (7.6)	17 (11.4)
Divorced	61 (20.1)	13 (19.7)	41 (27.5)
Widowed	34 (11.2)	9 (13.6)	9 (6.0)
Never married	41 (13.5)	8 (12.1)	22 (14.8)
Employment at reference date			
No	95 (31.4)	17 (25.8)	44 (29.5)
Yes	208 (68.7)	49 (74.2)	105 (70.5)
Education level			
≤High school	140 (46.5)	25 (37.9)	43 (29.1)
Vocational school	29 (9.6)	6 (9.1)	18 (12.2)
Some college	77 (25.6)	20 (30.3)	46 (31.1)
College, graduate, or professional school	55 (18.3)	15 (22.7)	41 (27.7)
Religion			
None	11 (3.6)	1 (1.5)	7 (4.7)
Protestant	275 (90.5)	57 (86.4)	130 (87.8)
Catholic	7 (2.3)	3 (4.6)	4 (2.7)
Other	11 (3.6)	5 (7.6)	7 (4.7)
Household income (dollars)			
<15,000	107 (36.5)	17 (26.6)	35 (24.6)
15,000–29,999	88 (30.0)	14 (21.9)	32 (22.5)
30,000–44,999	56 (19.1)	18 (28.1)	32 (22.5)
≥45,000	42 (14.3)	15 (23.4)	43 (30.3)

morphism (MspI[−]) were more likely to be divorced and less likely to be widowed compared to cases with the polymorphism (MspI[+]) and controls. Regardless of genetic status, cases were more likely to have had some college education or more than controls. Cases also tended to have a higher household income than controls. The distributions of age, employment status, and religion were similar between the comparison groups. When cases were defined by AAS status, cases with the variant (AAS[+]) were more likely to be divorced or never married and to be employed at the reference date than cases without the polymorphism (AAS[−]) and controls (Table 2). They were also more likely to have received a college degree and/or higher education, although they were less likely to have had only some college education. Both case groups had a higher household income than controls. When cases were divided according to both the MspI and AAS polymorphic states, fewer cases were in each case subgroup and frequencies were more variable (data not shown). Cases with MspI(−)/AAS(+) were more likely to be divorced or never married at reference date than other subgroups. Cases with either MspI(+) or AAS(+) or both were more likely to be employed than MspI(−)/AAS(−) cases and controls. All case subgroups, regardless of genetic status, tended to have more education and household income than controls. These demographic variables were adjusted for when assessing the relationship between risk factors and breast cancer by genetic status.

Table 3 presents the OR estimates of potential risk factors for breast cancer with cases divided by MspI or AAS status. Data are presented only when the 95% CI of an OR comparing all cases and controls does not include one. Factors that increase risk of breast cancer as a whole are lower daily intakes of folate, methionine, vitamins C and E, older age at first intercourse, family history of breast cancer, history of benign breast disease, and higher BMI. On the other hand, some of the factors associate with a decreased risk. These are longer menstrual cycle length, history of infertility test, history of smoking, history of alcohol consumption, and physical activity. When data were analyzed according to genotype, no significant differences between genotypes at the same site were shown for menstrual cycle length, history of infertility, family history of breast cancer, history of benign breast disease, smoking, physical activity, and BMI for both MspI and AAS. However, low intakes of vitamins C and E and folate increase the risk of breast cancer only with the AAS polymorphism while daily dietary calorie intake was not related to the disease (data not shown). Methionine intake is also close to significance for the AAS(+) individuals only. On the contrary, decreased risk related to alcohol consumption seemed more obvious for this group of individuals. Increased BMI might increase breast cancer risk only for the AAS(−)group. For the MspI variant, being ≥17 years of age at the first intercourse had a stronger association with the disease with the MspI(+) polymorphism.

We also analyzed the effects of the two polymorphic sites simultaneously (Table 4). When AAS(+) tumors were further subdivided by the MspI polymorphic status, the OR estimates were increased to 6.9–9.6 for low intakes of folate, methionine, and vitamin E for MspI(+)/AAS(+) tumors, although the CIs included the unity. However, these ORs fall outside of the corresponding

Table 2. Demographic characteristics of controls and cases defined by the CYP1A1 AAS polymorphic status

Variable	Controls (%)	Cases	
		AAS+ (%)	AAS− (%)
Age at interview			
20–39	33 (10.8)	7 (17.5)	18 (12.2)
40–49	105 (34.4)	13 (32.5)	55 (37.2)
50–59	109 (35.7)	14 (35.0)	51 (34.5)
≥60	58 (19.0)	6 (15.0)	24 (16.2)
Marital status at reference date			
Married	132 (43.6)	15 (37.5)	62 (41.9)
Separated	35 (11.6)	3 (7.5)	20 (13.5)
Divorced	61 (20.1)	13 (32.5)	34 (23.0)
Widowed	34 (11.2)	0 (.0)	12 (8.1)
Never married	41 (13.5)	9 (22.5)	20 (13.5)
Employment at reference date			
No	95 (31.4)	9 (22.5)	47 (31.8)
Yes	208 (68.7)	31 (77.5)	101 (68.2)
Education level			
≤High school	52 (35.4)	14 (35.0)	52 (35.4)
Vocational school	18 (12.2)	6 (15.0)	18 (12.2)
Some college	45 (30.6)	7 (17.5)	45 (30.6)
College, graduate, or professional school	32 (21.8)	13 (32.5)	32 (21.8)
Religion			
None	11 (3.6)	2 (5.0)	6 (4.1)
Protestant	275 (90.5)	33 (82.5)	130 (88.4)
Catholic	7 (2.3)	2 (5.0)	2 (1.4)
Other	11 (3.6)	3 (7.5)	9 (6.1)
Household income (dollars)			
<15,000	107 (36.5)	12 (31.6)	41 (28.9)
15,000–29,999	88 (30.0)	6 (15.8)	35 (24.6)
30,000–44,999	56 (19.1)	11 (28.9)	33 (23.2)
≥45,000	42 (14.3)	9 (23.7)	33 (23.2)

CIs from all of the other subgroups. There might be other variations in risk factor/breast cancer relationship depending on genetic status of the patient. History of alcohol consumption was negatively associated with MspI(−)/AAS(+) tumors. Although variation between two ORs seemed to exist for some other variables such as age at the first intercourse, history of benign breast disease, and BMI, the OR for one subgroup is included by the CI of the other subgroup for these variables.

DISCUSSION

A number of limitations of this study have to be made explicit to put the findings in their proper context. First, we were unable to assess the exact risk estimate for each polymorphism because information on the genotypes was unavailable for controls. Nevertheless, based on the same control group for each genetic state and based on the risk estimates from all cases as a whole, we are able to determine whether the risk for a specific factor is higher or lower for individuals with versus those without the polymorphism.

The other potential limitation was that a relatively high proportion of eligible cases were not included in the study due to failure to obtain some doctors' response/consent, nonresponse/relocation/death of the subjects or unavailability of tumor tissue samples. Considering difficulties in recruiting African Americans into a study, the participation rate of eligible women whom we were able to contact was not low. However, a synthesis of various factors, including doctors' refusal, inability to locate patients, and death, lessened the response rate. Unavailability of some tumor samples further decreased the number of cases in the analyses. If the unavailable women were different from the study participants, the study results would be biased; however, we have no reason to assume this is the case. For this project, the primary research interests were understanding differences in disease risk between the case subgroups as defined by genetic status. It is unlikely that any differences in risk factors between nonparticipating (or unavailable) and participating (or available) cases would be different depending upon the polymorphic status. Therefore, the relative differences between cases with and without the AAS polymorphism might not be materially affected.

Inaccuracy in estimating dietary intakes is also a concern. Errors in recalling dietary intakes from years earlier could have occurred. Such errors might have influenced the study results. However, we used the reference date to minimize the potential differences in recalling dietary intakes between the comparison groups. Recall errors were not likely to be different among genotypes.

Because of the failure to get genotype results for a substantial number of cases, the study had a relatively small number of patients with the polymorphisms. A small number of cases carried both polymorphisms (n=10). The small number of cases with both polymorphisms led to low power for the study and prevented us from conducting further relevant analysis for combined MspI and AAS polymorphisms. For example, it was difficult to analyze dose-effect relations or do analysis for premenopausal and postmenopausal women separately. It also made it difficult to control for many potential

Table 3. Odds ratio estimates of risk factors in relation to breast cancer by the CYP1A1 MspI status

	Controls	All cases		MspI(−) cases		MspI(+) cases		AAS(−) cases		AAS(+) cases	
Factor	*n**	*n*	OR†(95%CI‡)	*n*	OR (95%CI)	*n*	OR (95%CI)	*n*	OR (95%CI)	*n*	OR (95%CI)
Amount of daily folate intake (μg/day)											
>451.35	141	128	Reference	62	Reference	23	Reference	69	Reference	13	Reference
≤451.35	140	158	1.4 (1.0–1.9)	79	1.5 (1.0–2.3)	35	1.6 (.9–3.0)	70	1.1 (.7–1.7)	23	2.1 (1.0–4.4)
Amount of daily methionine intake (g/day)											
>.81	139	120	Reference	55	Reference	27	Reference	69	Reference	12	Reference
≤.81	142	166	1.3 (1.0–1.9)	86	1.5 (1.0–2.3)	31	1.1 (.6–1.9)	70	1.0 (.6–1.5)	24	2.0 (.9–4.2)
Amount of daily vitamin C intake (mg/day)											
>233.34	142	118	Reference	58	Reference	22	Reference	62	Reference	13	Reference
≤233.34	139	168	1.6 (1.1–2.3)	83	1.7 (1.1–2.6)	36	1.7 (.9–3.1)	77	1.4 (.9–2.1)	23	2.1 (1.0–.5)
Amount of daily vitamin E intake (mg/day)											
>67.17	141	122	Reference	60	Reference	24	Reference	68	Reference	12	Reference
≤67.17	140	164	1.5 (1.0–2.1)	81	1.5 (1.0–2.3)	34	1.5 (.9–2.8)	71	1.1 (.7–1.7)	24	2.4 (1.1–5.3)
Cycle length (days)											
<28	82	109	Reference	51	Reference	29	Reference	49	Reference	18	Reference
≥28	184	157	.6 (.4–.9)	80	.6 (.4–1.0)	30	.4 (.2–.8)	78	.7 (.4–1.1)	21	.5 (.2–1.0)
History of infertility test											
No	218	247	Reference	116	Reference	58	Reference	122	Reference	32	Reference
Yes	48	29	.5 (.3–.9)	11	.5 (.3–1.1)	6	.4 (.2–1.1)	10	.4 (.2–.7)	3	.4 (.1–1.5)
Age at 1st intercourse (years)											
<17	114	89	Reference	41	Reference	13	Reference	41	Reference	9	Reference
≥17	156	198	1.6 (1.1–2.3)	100	1.6 (1.0–2.6)	47	3.1 (1.5–6.6)	95	1.8 (1.1–2.9)	30	2.5 (1.0–6.0)
Family history of breast cancer											
No	241	196	Reference	94	Reference	44	Reference	97	Reference	28	Reference
Yes	52	103	2.4 (1.6–3.6)	53	2.6 (1.6–4.2)	21	2.3 (1.2–4.2)	48	2.3 (1.4–3.7)	12	1.8 (.9–4.0)
History of benign breast disease											
No	212	198	Reference	92	Reference	43	Reference	93	Reference	25	Reference
Yes	70	104	1.4 (1.0–2.1)	55	1.5 (1.0–2.4)	23	1.4 (.8–2.6)	53	1.6 (1.0–2.5)	15	1.6 (.8–3.4)
History of smoking											
No	158	188	Reference	89	Reference	45	Reference	91	Reference	26	Reference
Yes	137	114	.7 (.5–1.0)	58	.8 (.5–1.2)	21	.5 (.3–1.0)	55	.7 (.5–1.1)	14	.6 (.3–1.3)
History of alcohol consumption											
No	173	206	Reference	101	Reference	43	Reference	91	Reference	32	Reference
Yes	122	95	.6 (.4–.9)	45	.6 (.4–.9)	23	.8 (.4–1.4)	55	.8 (.5–1.2)	8	.3 (.1–.8)
Physical activity											
No	37	60	Reference	25	Reference	19	Reference	32	Reference	11	Reference
Yes	256	241	.5 (.3–.8)	121	.6 (.3–1.0)	47	.3 (.1–.6)	114	.4 (.2–.7)	29	.3 (.1–.7)
BMI											
≤25	118	100	Reference	49	Reference	25	Reference	45	Reference	17	Reference
>25	176	200	1.4 (1.0–2.0)	98	1.5 (.9–2.3)	40	1.2 (.7–2.1)	99	1.6 (1.2–2.5)	23	1.0 (.5–2.0)

* The number of study subjects, which may vary between models because of missing values of the variables in the model and excluded subjects for nutrient analysis.
† Adjusted for age, employment status, marital status, educational level, income, number of people in household, and religion.
‡ 95% confidence interval.

confounders. Low study power and possible effects of potential confounders should be kept in mind as a limitation of the study. Therefore, our results are preliminary but suggestive.

Despite these limitations, our study showed that low intakes of folate, methionine, vitamin C, and vitamin E might increase the risk of breast cancer in individuals with at least one copy of the AAS polymorphism. When AAS(+) tumors were further divided by the MspI polymorphic status, the OR estimates for these nutrients (except vitamin C) were substantially increased for tumors with the MspI(+)/AAS(+) genotype (although the confidence intervals included the unity). Such consistent increases, which may be partly related to the correlation among these nutrients, were also shown in case-only analyses comparing cases with the polymorphism(s) to MspI(−)/AAS(−) cases (data not shown). There was also other variation in the relationship between risk factors and breast cancer as a function of genotype. Alcohol consumption was less likely to be associated with AAS(+) tumors while BMI was more likely to be a risk factor for AAS(−) cancer. The association between alcohol consumption and breast cancer was further found for the MspI(−)/AAS(+) tumors.

These results need to be confirmed by studies with more subjects and

Table 4. Odds ratio estimates of risk factors in relation to breast cancer by CYP1A1 MspI and AAS genotypes

	Controls	MspI−/AAS− cases		MspI+/AAS− cases		MspI−/AAS+ cases		MspI+/AAS+ cases	
Factor	*n*[*]	*n*	OR[†] (95%CI[‡])	*n*	OR (95%CI)	*n*	OR (95%CI)	*n*	OR (95%CI)
Amount of daily folate intake (μg/day)									
>451.35	141	40	Reference	21	Reference	9	Reference	1	Reference
≤451.35	140	42	1.2 (.7–2.0)	19	1.0 (.5–1.9)	15	2.1 (.9–5.1)	6	7.4 (.8–66.8)
Amount of daily methionine intake (g/day)									
>.81	139	40	Reference	23	Reference	8	Reference	1	Reference
≤.81	142	42	1.0 (.6–1.7)	17	.6 (.3–1.3)	16	1.9 (.8–4.9)	6	6.9 (.8–61.8)
Amount of daily vitamin C intake (mg/day)									
>233.34	142	37	Reference	19	Reference	10	Reference	2	Reference
≤233.34	139	45	1.4 (.8–2.4)	21	1.1 (.6–2.2)	14	1.8 (.7–4.3)	5	2.7 (.5–15.4)
Amount of daily vitamin E intake (mg/day)									
>67.17	141	39	Reference	22	Reference	9	Reference	1	Reference
≤67.17	140	43	1.3 (.8–2.1)	18	.8 (.4–1.7)	15	1.9 (.8–4.7)	6	9.6 (.9–100.8)
Cycle length (days)									
<28	82	25	Reference	20	Reference	13	Reference	3	Reference
≥28	184	49	.8 (.4–1.4)	19	.4 (.2–.9)	11	.4 (.2–.9)	7	.8 (.2–3.4)
History of infertility test									
No	218	69	Reference	40	Reference	18	Reference	9	Reference
Yes	48	5	.3 (.1–.9)	3	.3 (.1–1.1)	2	.5 (.1–2.5)	1	.5 (.1–4.8)
Age at 1st intercourse (years)									
<17	114	23	Reference	10	Reference	6	Reference	1	Reference
≥17	156	56	1.8 (1.0–3.3)	31	3.0 (1.3–6.9)	19	2.6 (.9–7.9)	8	5.4 (.6–50.4)
Family history of breast cancer									
No	241	53	Reference	31	Reference	18	Reference	6	Reference
Yes	52	31	2.7 (1.5–4.7)	13	2.0 (1.0–4.1)	7	1.5 (.6–4.0)	4	3.2 (.8–12.7)
History of benign breast disease									
No	212	52	Reference	30	Reference	14	Reference	7	Reference
Yes	70	32	1.6 (.9–2.8)	15	1.5 (.7–3.0)	11	2.3 (.9–5.7)	3	.8 (.2–3.7)
History of smoking									
No	158	52	Reference	31	Reference	17	Reference	7	Reference
Yes	137	32	.7 (.4–1.2)	14	.6 (.3–1.1)	8	.6 (.2–1.5)	3	.4 (.1–1.8)
History of alcohol consumption									
No	173	55	Reference	26	Reference	23	Reference	6	Reference
Yes	122	29	.7 (.4–1.2)	19	1.1 (.6–2.2)	2	.1 (.0–.4)	4	1.0 (.3–4.0)
Physical activity									
No	37	17	Reference	12	Reference	4	Reference	4	Reference
Yes	256	67	.5 (.2–.9)	33	.3 (.1–.7)	21	.6 (.2–2.1)	6	.2 (.0–.6)
BMI									
≤25	118	23	Reference	16	Reference	10	Reference	4	Reference
>25	176	61	2.0 (1.1–3.5)	28	1.3 (.6–2.5)	15	1.2 (.5–2.9)	6	1.1 (.3–4.4)

* The number of study subjects, which may vary between models because of missing values of the variables in the model and excluded subjects for nutrient analysis.
† Adjusted for age, employment status, marital status, educational level, income, number of people in household, and religion.
‡ 95% confidence interval.

genotype information for controls. However, they suggest that risk factor profiles may vary depending on MspI or/and AAS polymorphic states. While we do not have ready explanations for other results, our findings on methyl-deficient diets (folate and methionine) and antioxidant vitamins are consistent with our study hypothesis, a stronger association for MspI(+)/AAS(+) tumors. Previous studies have found that breast cancer risk is higher among women with insufficient intakes of folate or food rich in methionine,[14–17] and low intakes of vitamins A, C, and E increase the risk of the disease[18–24] although results have not been consistent.[25–28] The relation of such associations to the CYP1A1 MspI/AAS polymorphisms had not been studied previously. How variation in a metabolic gene interacts with these nutrient factors and affects the risk of cancer is unknown and the mechanisms involved may be complex. Therefore, any statements about potential mechanisms are conjectural at present time.

The modification of risk factors in the context of a genetic polymorphism can be explained by two hypotheses. Either the variant being studied encodes a functional difference that responds to a particular metabolic or environmental risk or it is in linkage disequilibrium with functional variants. Fully understanding this hypothesis would require a functional relationship between

. . . our study showed that low intakes of folate, methionine, vitamin C, and vitamin E might increase the risk of breast cancer in individuals with at least one copy of the AAS polymorphism.

CYP1A1 and the various nutrients, which are mostly unknown and outside the scope of the present study. However, some studies suggest possible effects of CYP1A1 on the metabolism of some of the studied nutrients (for example, CYP1A1 may be involved in the metabolism of retinoid receptors and may play a role in ascorbate peroxidation that may further influence metabolism of vitamins A and C).[29,30] Moreover, the genotype-phenotype relationship has not been clearly ascertained for CYP1A1 MspI(+) or AAS polymorphism,[31,32] although the impact of the MspI(+) allele on the function of the enzyme has been suggested in Taiwanese[33] and African Americans (but not in Caucasians).[4]

Another, slightly modified, hypothesis may also explain the effect of MspI(+)/AAS(+) polymorphisms on the relationship between these nutrients and breast cancer risk. This hypothesis is based on interactions between intragenic polymorphisms and gene-gene interactions. The MspI(+) and AAS(+) may work together to affect function. Such a MspI(+)/AAS(+) genotype may be further associated with some other loci related to breast cancer or in linkage disequilibrium with various gene polymorphisms[34] related to the metabolisms of the nutrients. While gene-gene interactions have not been fully elucidated, studies have found that the mutation frequency of both p53, a tumor suppressor gene, and Ki-ras, an oncogene, was increased when MspI (+) of the CYP1A1 gene was present: the patients homozygous for MspI variant possessed a five-fold higher risk of having a mutation in P53 or Ki-ras than patients without MspI variant.[35] We are not aware of interactions between the CYP1A1 MspI/AAS polymorphisms and genes related to the metabolism of folate, methionine, and antioxidant vitamins. However, there may be interactions between the polymorphisms within the gene, either because of functional interaction or linkage disequilibrium between these two variants and another functional site we did not assay, and interactions between these polymorphisms and other genes that may confer a certain susceptibility to breast cancer. Such susceptibility further interacts with some nutrient factors, resulting in increased risk of breast cancer.

Despite the speculative nature of explanations for our findings, the results suggest that variants in the CYP1A1 gene can affect the role some nongenetic risk factors have on disease. This dependency of risk factors on genotype will need to be more carefully assessed in terms of differences in prevalence among ethnic groups. Group-specific polymorphisms such as the AAS variant of CYP1A1 may play a role in disease risk. Our study suggests that such genetic effects will need to be incorporated into studies of cancer risk. Although our results are preliminary in nature, they suggest that large-scale epidemiologic studies are needed, in which both African-American and Caucasian women are enrolled as study subjects and genotype information from controls and more detailed information on risk factors are collected.

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REFERENCES

1. Mayberry RM, Stoddard-Wright C. Breast cancer risk factors among Black women and White women: similarities and differences. *Am J Epidemiol*. 1992;136:1445–1456.
2. Palmer JR, Rosenberg L, Rao RS, et al. Oral contraceptive use and breast cancer risk among African-American women. *Cancer Causes Control*. 1995;6:321–331.
3. Kinney AY, Millikan RC, Lin YH, Moorman PG, Newman B. Alcohol consumption and breast cancer among Black and White women in North Carolina (United States). *Cancer Causes Control*. 2000;11:345–357.
4. Taioli E, Bradlow HL, Garbers SV, et al. Role of estradiol metabolism and CYP1A1 polymorphisms in breast cancer risk. *Cancer Detect Prev*. 1999;23:232–237.
5. Taioli E, Trachman J, Chen X, Toniolo P, Garte SJ. A CYP1A1 restriction fragment length polymorphism is associated with breast cancer in African-American women. *Cancer Res*. 1995;55:3757–3758.
6. Coughlin SS, Piper M. Genetic polymorphisms and risk of breast cancer. *Cancer Epidemiol Biomarkers Prev*. 1999;8:1023–1032.
7. Kawajiri K. CYP1A1. *IARC Sci Publ*. 1999; 148:159–172.
8. Zhu K, Hunter S, Payne-Wilks K, Roland CL, Forbes DS. Use of electric bedding devices and risk of breast cancer in African-American women. *Am J Epidemiol*. 2003;158:798–806.
9. Harlow BL, Davis S. Two one-step methods for household screening and interviewing using random digit dialing. *Am J Epidemiol*. 1988;127:857–863.
10. National Cancer Institute (NCI). *DIETSYS Version 3.0 User's Guide. Health Habits and History Questionnaire: Diet History and Other Risk Factors (Dietary Analysis System)*. Bethesda, Md: NCI; 1994.
11. Sukpanichnant S, Vnencak-Jones CL, McCurley TL. Detection of clonal immunoglobulin heavy chain gene rearrangements by polymerase chain reaction in scrapings from

archival hematoxylin and eosin-stained histologic sections: implications for molecular genetic studies of focal pathologic lesions. *Diagn Mol Pathol*. 1993;2:168–176.
12. Zapata C, Carollo C, Rodriguez S. Sampling variance and distribution of the D′ measure of overall gametic disequilibrium between multiallelic loci. *Ann Hum Genet*. 2001;65:395–406.
13. Hosmer D, Lemeshow S. *Applied Logistic Regression*. New York, NY: John Wiley and Sons, Inc; 1989.
14. Sharp L, Little J, Schofield AC, et al. Folate and breast cancer: the role of polymorphisms in methylenetetrahydrofolate reductase (MTHFR). *Cancer Lett*. 2002;181:65–71.
15. Shrubsole MJ, Jin F, Dai Q, et al. Dietary folate intake and breast cancer risk: results from the Shanghai Breast Cancer Study. *Cancer Res*. 2001;61:7136–7141.
16. Franceschi S, Favero A, La Vecchia C, et al. Influence of food groups and food diversity on breast cancer risk in Italy. *Int J Cancer*. 1995; 63:785–789.
17. van't Veer P, Dekker JM, Lamers JW, et al. Consumption of fermented milk products and breast cancer: a case-control study in The Netherlands. *Cancer Res*. 1989;49:4020–4023.
18. Katsouyanni K, Willett W, Trichopoulos D, et al. Risk of breast cancer among Greek women in relation to nutrient intake. *Cancer*. 1988;61: 181–185.
19. Willett WC, Hunter DJ. Vitamin A and cancers of the breast, large bowel, and prostate: epidemiologic evidence. *Nutr Rev*. 1994;52: S53–S59.
20. Levi F, Pasche C, Lucchini F, La Vecchia C. Dietary intake of selected micronutrients and breast-cancer risk. *Int J Cancer*. 2001;91:260–263.
21. Gandini S, Merzenich H, Robertson C, Boyle P. Meta-analysis of studies on breast cancer risk and diet: the role of fruit and vegetable consumption and the intake of associated micronutrients. *Eur J Cancer*. 2000;36:636–646.
22. Ronco A, De Stefani E, Boffetta P, Deneo-Pellegrini H, Mendilaharsu M, Leborgne F. Vegetables, fruits, and related nutrients and risk of breast cancer: a case-control study in Uruguay. *Nutr Cancer*. 1999;35:111–119.
23. Favero A, Parpinel M, Franceschi S. Diet and risk of breast cancer: major findings from an Italian case-control study. *Biomed Pharmacother*. 1998;52:109–115.
24. Negri E, La Vecchia C, Franceschi S, et al. Intake of selected micronutrients and the risk of breast cancer. *Int J Cancer*. 1996;65:140–144.
25. Longnecker MP, Newcomb PA, Mittendorf R, Greenberg ER, Willett WC. Intake of carrots, spinach, and supplements containing vitamin A in relation to risk of breast cancer. *Cancer Epidemiol Biomarkers Prev*. 1997;6:887–892.
26. Graham S, Zielezny M, Marshall J, et al. Diet in the epidemiology of postmenopausal breast cancer in the New York State Cohort. *Am J Epidemiol*. 1992;136:1327–1337.
27. Richardson S, Gerber M, Cenee S. The role of fat, animal protein, and some vitamin consumption in breast cancer: a case control study in southern France. *Int J Cancer*. 1991;48:1–9.
28. Potischman N, Swanson CA, Coates RJ, et al. Intake of food groups and associated micronutrients in relation to risk of early-stage breast cancer. *Int J Cancer*. 1999;82:315–321.
29. Lampen A, Meyer S, Arnhold T, Nau H. Metabolism of vitamin A and its active metabolite all-trans-retinoic acid in small intestinal enterocytes. *J Pharmacol Exp Ther*. 2000;295:979–985.
30. Lambert N, Chambers SJ, Plumb GW, Williamson G. Human cytochrome P450's are pro-oxidants in iron/ascorbate-initiated microsomal lipid peroxidation. *Free Radic Res*. 1996;24:177–185.
31. Cosma G, Crofts F, Currie D, Wirgin I, Toniolo P, Garte SJ. Racial differences in restriction fragment length polymorphisms and messenger RNA inducibility of the human CYP1A1 gene. *Cancer Epidemiol Biomarkers Prev*. 1993;2:53–57.
32. Garte S, Ganguly S, Taioli E. Effect of genotype on steady-state CYP1A1 gene expression in human peripheral lymphocytes. *Biochem Pharmacol*. 2003;65:441–445.
33. Wu MT, Huang SL, Ho CK, Yeh YF, Christiani DC. Cytochrome P450 1A1 MspI polymorphism and urinary 1-hydroxypyrene concentrations in coke-oven workers. *Cancer Epidemiol Biomarkers Prev*. 1998;7:823–829.
34. Garte S. The role of ethnicity in cancer susceptibility gene polymorphisms: the example of CYP1A1. *Carcinogenesis*. 1998;19: 1329–1332.
35. Kawajiri K, Eguchi H, Nakachi K, Sekiya T, Yamamoto M. Association of CYP1A1 germ line polymorphisms with mutations of the p53 gene in lung cancer. *Cancer Res*. 1996;56: 72–76.

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