Estrogen Metabolism–Related Genes and Breast Cancer Risk: The Multiethnic Cohort Study

Loïc Le Marchand,[1] Timothy Donlon,[1] Laurence N. Kolonel,[1] Brian E. Henderson,[2] and Lynne R. Wilkens[1]

[1]Etiology Program, Cancer Research Center of Hawaii, University of Hawaii, Honolulu, Hawaii and [2]Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, California

Abstract

Common polymorphisms in genes that affect estrogen levels may be associated with breast cancer risk. We investigated the associations between breast cancer and sequence variants in several genes in the estradiol/estrone metabolism pathway (*CYP1A1*2A*, *CYP1A2*1F*, *CYP1B1* Leu^{432}Val, *CYP3A4*1B*, *COMT* Val^{158}Met, *SULT1A1* Arg^{213}His) as well as the Arg^{554}Lys variant in *AHR* (a transcription factor for *CYP1A1*, *CYP1A2*, and *CYP1B1*) in a case-control study of 1,339 breast cancer cases and 1,370 controls nested in the Multiethnic Cohort Study. The Multiethnic Cohort Study is a large prospective study of men and predominantly postmenopausal women of Japanese, White, African American, Latino, and Native Hawaiian ancestry, residing in Hawaii and Los Angeles. We found no association between breast cancer and these polymorphisms, except for *CYP1A2*1F* which was inversely associated with risk. The odds ratio (95% confidence interval) for the AA, AC, and CC genotype was 1.0, 0.9 (0.7-1.0), and 0.7 (0.5-1.0), respectively (*P* for gene dosage effect = 0.03). This association seemed somewhat stronger for estrogen receptor (ER)/progesterone receptor (PR)–negative tumors than for ER/PR-positive tumors, and no statistically significant interaction with estrogen-related risk factors was detected. The findings provide no evidence for a role of *COMT* Val^{58}Met, *CYP1A1*2A*, *CYP3A4*1B*, *CYP1B1* Leu^{432}Val, *SULT1A1* Arg^{213}His, and *AHR* Arg^{554}Lys in breast cancer etiology. They also provide support for an inverse association between *CYP1A2*1F* and breast cancer, which is consistent with the observation of lower circulating estrogen levels in premenopausal women with the CC genotype in a previous study. (Cancer Epidemiol Biomarkers Prev 2005;14(8):1998–2003)

Introduction

The major susceptibility genes that have been identified for breast cancer, including *BRCA1* and *BRCA2*, have been most helpful in determining who is at risk in multicase families. However, mutations in these genes are rare and only explain a small percent of all breast tumors. Another area of research is focusing on common polymorphisms in genes that affect the synthesis or metabolism of estrogens because these hormones increase cell proliferation in the breast and because their circulating levels have been associated with breast cancer incidence (1-4). The risk associated with each of these polymorphisms is expected to be low. However, because they are common, they have the potential of explaining a large proportion of cases in the population. Past studies of such polymorphisms and breast cancer have been quite inconsistent (1). This may be due to the fact that these studies have typically focused on single genes, whereas multiple competing enzymes are involved. In addition, for the most part, these past studies have not considered modifying effects. It is possible that these associations are particularly strong in the subgroups of women who, due to their obesity, reproductive history, and/or hormone replacement therapy use, are exposed to high levels of estrogens.

We investigated the associations between breast cancer and functional polymorphisms in several genes in the estradiol/estrone metabolism pathway (*CYP1A1*, *CYP1A2*, *CYP1B1*, *CYP3A4*, *COMT*, and *SULT1A1*), as well as in *AHR* (a transcription factor for *CYP1A1*, *CYP1A2*, and *CYP1B1*), in a large case-control study nested in the Multiethnic Cohort Study.

Materials and Methods

The design and baseline characteristics of the Multiethnic Cohort Study were described in detail elsewhere (4). In short, participants are Hawaii and California (primarily Los Angeles) residents who entered the cohort from 1993 to 1996 by completing a 26-page mail questionnaire including demographic and lifestyle factors (such as diet and smoking), medical history, medication use, family history of common cancers and, for women, reproductive history and hormone use. The cohort included 118,441 women and 96,810 men ages 45 to 75 years at cohort creation in 1993. Among the women, 25% were Japanese American, 22% White, 21% Latino, 19% African American, 7% Hawaiian, and 6% of other ethnic/racial origin. Breast cancer cases were identified through the Rapid Reporting System of the Hawaii Tumor Registry and through quarterly linkages to the Los Angeles County Cancer Surveillance Program, two cancer registries that are members of the Surveillance, Epidemiology and End Results program of the National Cancer Institute. This was complemented by annual linkages to the State of California's cancer registry. A nested case-control study of breast cancer was initiated in 1995 among women of the five main ethnic groups (5). A sample of cohort participants was randomly selected to serve as controls; the selection was stratified by sex and ethnicity. Incident breast cancer cases occurring since January 1995, and controls were contacted for donation of a blood sample. Samples were collected at the subjects' homes, processed within 8 hours and stored at 80°C. The participation rate among cases was 74% and varied from 70% in African

Received 1/27/05; revised 5/4/05; accepted 5/18/05.

Grant support: Department of Defense grant DAMD 17-00-1-0283 (L. Le Marchand) and National Cancer Institute, U.S. Department of Health and Human Services grants R01 CA 63464 (B.E. Henderson) and CA54281 (L.N. Kolonel).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: The content of this article is solely the responsibility of the authors and does not necessarily represent the official views or policies of these institutions.

Requests for reprints: Loïc Le Marchand, Etiology Program, Cancer Research Center of Hawaii, University of Hawaii, Suite 407, 1236 Lauhala Street, Honolulu, HI 96813. Phone: 808-586-2988; Fax: 808-586-2082. E-mail: loic@crch.hawaii.edu

doi:10.1158/1055-9965.EPI-05-0076

Americans to 81% in Latinos. The corresponding rate for controls was 66% and varied from 60% in African Americans to 71% in Whites.

DNA was purified from whole blood using Midi Kits (Qiagen, Valencia, CA). The DNA samples were analyzed by PCR/RFLP for the *COMT* Val^{158}Met, *CYP1A1*2A*, *CYP1A2*1F* (A-154C), and *CYP1B1* Val^{432}Leu polymorphisms, as reported previously (6, 7). Samples were also genotyped for *CYP3A4*1B* using the following allele-specific primers: CYP3A4FC (wild type 5′-ATGAGGACAGCCATAGAGACAAGGGCTA-3′), CYP3A4FV (variant 5′-ATGAGGACAGCCATAGAGACAAGGGCTG-3′), and CYP3A4R (5′-CTTTCCTGCCCTGCACAG-3′). The PCR conditions are as follows: 94°C for 4 minutes, 94°C for 30 seconds, 60°C for 10 seconds, 72°C for 1 minute × 30 cycles, and 72°C for 7 minutes. The reaction product was subjected to electrophoresis and the 338-bp fragment identified on a 2% SeaKem (FMC BioProducts, Rockland, ME) agarose gel.

Genotyping for the *SULT1A1* G638A (Arg^{213}His) polymorphism was done using a modification of the method of Coughtrie et al. (8) using primers SULT-F 5′-GTTGGCTCTG-CAGGGTTTCTAGGA-3′ and SULT-R 5′-CCCAAACCC-CCTGCTGGCCAGCACCC-3′. Amplification consists of a 4-minute denaturation at 94°C followed by 40 cycles at 94°C for 30 seconds, 63°C for 30 seconds, and 72°C for 30 seconds. An incubation step at 72°C is added at the end of the reaction for 7 minutes. PCR products were digested with 5 units *Hae*II at 37°C for 3 hours and separated on a 3.0% SeaKem ME in 1× TAE buffer. The PCR product is 333 bp in length and is digested by *Hae*II to168- and 165-bp fragments for the wild-type allele and is undigested for the variant allele.

The Lys^{554}Arg polymorphism (G1721A) in exon 10 of the *AHR* gene (9) was genotyped by allele-specific PCR. The forward primers were 5′-AAACCTAGGCATTGATTTTG-AAGACATCCG-3′ and 5′-AAACCTAGGCATTGATTTTGAA-GACATCCA-3′ and the common reverse primer was 5′-ACGAATTGGTTAGAGTTCCAATTTTTAAAC-3′. The PCR conditions consisted of an initial denaturation at 94°C for 4 minutes followed by 25 cycles of 94°C for 30 seconds, 65°C for 30 seconds, and 72°C for 30 seconds followed by 28 cycles of 94°C for 30 seconds, 60°C 30 seconds, and 72°C for 30 seconds with a final extension at 72°C for 10 minutes. The reaction product was subjected to electrophoresis and the 348-bp fragment identified on a 2% SeaKem agarose gel.

Out of the 1,425 cases and 1,452 controls genotyped, 168 subjects were excluded because of missing information on covariates. The remaining 1,339 cases and 1,370 controls were available for data analysis. A race-adjusted comparison of these subjects with those who did not provide genotype data because of not donating blood or inconclusive results, revealed no difference in age, age at menarche, age at first birth, and parity. The statistical analysis used unconditional logistic regression to compute odds ratios and 95% confidence intervals for exposures of interest (10). Genotypes were modeled as two dummy variables representing the three levels, or as a gene dosage effect variable assigned a value of 1, 2, or 3 according to the number of variant alleles (zero, one, and two variant alleles, respectively). The final models were adjusted for age at blood draw, race/ethnicity, age at menarche, parity, and age at first birth. The likelihood ratio test was used to determine the significance of the interaction among certain variables with respect to breast cancer. The test compares a main effects, no interaction model with a fully parameterized model containing all possible interaction terms for the variables of interest. A Wald test from a polytomous logistic regression model was used to compare genetic trends for *CYP1A2*1F* between estrogen receptor (ER)/progesterone receptor (PR)–positive tumors and ER/PR-negative tumors. Genotype frequencies were tested for deviation from the Hardy Weinberg equilibrium with the χ^2 test.

Results

The baseline characteristics of the cases and controls have been described previously (5). Eighty-seven percent of cases and 82% of controls were postmenopausal at baseline. The mean age at blood draw was 66 years for cases and 63 years for controls. On the average, cases tended to be older at baseline, to have had their first child at an older age, and to have had fewer children. They also more often had a positive family history of breast cancer and used hormone replacement therapy. Cases tended to consume less folate but drank somewhat more alcohol.

The distributions of the genotypes were consistent with Hardy-Weinberg equilibrium within each ethnic/racial group for all genes, except for *AHR* in Caucasians (P = 0.006) and Latinos (P = 0.002). The allele frequencies (Table 1) showed much variation across ethnic/racial groups and were similar to those reported in past studies (6-9). Table 2 shows the odds ratios and 95% confidence intervals for each genotype, after adjusting for age at blood draw, ethnicity/race, age at menarche, parity, and age at first birth. No association was observed, except for *CYP1A2*1F* that was inversely associated with breast cancer. The odds ratio (95% confidence interval) for the AA, AC, and CC genotype was 1.0, 0.9 (0.7-1.0), and 0.7 (0.5-1.0), respectively (P for gene dosage effect = 0.03). This association was suggested for three of the five ethnic/racial groups studied (Table 3), although some of the groups (in particular the Hawaiians) were quite small. Exclusion of the Caucasians and Latinos from the *AHR* model in Table 2 did not change the results.

No effect modification was found for *CYP1A2*1F* by tertile of age at menarche, body mass index (BMI), HRT use, or pack-years at baseline (Table 4), or by age at first birth or meat doneness preference (data not shown). Similarly, no difference in effect for *CYP1A2*1F* was found by *CYP1A1*, *CYP1B1*, *AHR*, *CYP3A4*, *COMT*, or *SULT1A1* genotype (Table 5) or by stage at diagnosis. The effects of *CYP1A1*, *CYP1A2*, *CYP1B1*,

Table 1. Frequency for variant alleles by race and genotype

		Japanese (n = 249)	Caucasian (n = 199)	African American (n = 389)	Latino (n = 371)	Native Hawaiian (n = 162)
*CYP1A1*2A*	T3801C	0.36	0.11	0.21	0.32	0.42
*CYP1A2*1F*	A-154C	0.35	0.28	0.39	0.30	0.21
CYP1B1	Leu^{432}Val	0.20	0.43	0.76	0.34	0.20
AHR	Arg^{554}Lys	0.45	0.12	0.43	0.15	0.17
*CYP3A4*1B*	A-290G	0.00	0.03	0.56	0.07	0.02
COMT	Val^{158}Met	0.35	0.47	0.34	0.41	0.28
SULT1A1	Arg^{213}His	0.15	0.35	0.26	0.31	0.14

NOTE: Based on the data for the controls.

Table 2. Breast cancer odds ratios (95% confidence intervals) by genotype

Genotype	*n**	Odds ratio† (95% confidence interval)	Odds ratio‡ (95% confidence interval)
*CYP1A1*2A*			
TT	743/722	1.0	1.0
TC	493/530	1.0 (0.9-1.2)	1.0 (0.8-1.2)
CC	103/118	1.0 (0.8-1.4)	1.0 (0.6-1.4)
P		0.78§	0.85
*CYP1A2*1F*			
AA	640/617	1.0	1.0
AC	608/627	0.9 (0.7-1.0)	0.9 (0.7-1.0)
CC	91/125	0.8 (0.6-1.0)	0.7 (0.5-1.0)
P		0.03	0.03
CYP1B1 Leu^{432}Val			
LL	581/516	1.0	1.0
LV	495/525	1.0 (0.8-1.1)	0.9 (0.8-1.1)
VV	263/329	0.9 (0.7-1.2)	0.9 (0.7-1.1)
P		0.47	0.32
AHR Arg^{554}Lys			
RR	721/756	1.0	1.0
RL	463/456	1.1 (0.9-1.3)	1.1 (0.9-1.3)
LL	155/158	1.0 (0.8-1.3)	1.0 (0.7-1.3)
P		0.62	0.75
*CYP3A4*1B*			
AA	1,080/994	1.0	1.0
AG	164/254	0.9 (0.7-1.2)	0.9 (0.7-1.2)
GG	95/122	1.1 (0.6-1.6)	1.1 (0.8-1.7)
P		0.66	0.68
COMT Val^{158}Met			
Val/Val	519/550	1.0	1.0
Val/Met	624/614	1.0 (0.9-1.2)	1.0 (0.8-1.2)
Met/Met	196/206	0.9 (0.7-1.2)	0.9 (0.7-1.2)
P		0.71	0.64
SULT1A1 Arg^{213}His			
Arg/Arg	801/782	1.0	1.0
Arg/His	424/484	0.9 (0.7-1.0)	0.9 (0.7-1.0)
His/His	114/104	1.1 (0.8-1.5)	1.1 (0.8-1.5)
P		0.63	0.63

*No. cases/No. controls.
†Odds ratios adjusted for age at blood draw and race/ethnicity.
‡Odds ratios further adjusted for age at menarche, parity, and age at first birth.
§*P* value for gene dosage term assigned 1, 2, and 3 for 0, 1, and 2 variant alleles, respectively.

and *CYP3A4* did not vary by *AHR*, *COMT*, or *SULT1A1* genotype (data not shown).

Information on ER and PR status of the tumor was available from our two cancer registries on 68% and 65% of the breast cancer cases, respectively. Cases with and without ER and PR information did not differ by geographic site (Hawaii or California), race/ethnicity, age at diagnosis, stage, year of diagnosis, years of education, or *CYP1A2* genotype. Table 6 presents the association of *CYP1A2*1F* with, separately, ER-negative, ER-positive, PR-negative, PR-positive, ER/PR-negative, and ER/PR-positive breast cancers. The inverse association with *CYP1A2*1F* seemed stronger for ER/PR-negative breast cancer; however, the *P* value for comparison of gene dosage effects between the ER/PR-negative and ER/PR-positive groups was not statistically significant ($P = 0.13$).

Discussion

In this large case-control study of estrogen metabolism-related genes in a multiethnic population of predominantly postmenopausal women, we found no association with the *COMT* Val^{58}Met, *CYP1A1*2A*, *CYP3A4*1B*, *CYP1B1* Leu^{432}Val, *SULT1A1* Arg^{213}His, and *AHR* Arg^{554}Lys polymorphisms and breast cancer. However, we observed an inverse association with the presence of the *CYP1A2*1F* allele which was not modified by the classic risk factors for breast cancer or the other genes under study and seemed somewhat stronger for ER/PR-negative tumors.

Multiple enzymes are involved in the metabolism of estradiol and estrone, the most biologically active forms of estrogen. A number of inherited polymorphisms in genes controlling these enzymes are being studied because they are common in the population and have been associated with a change in the gene product or enzyme activity. They may serve as surrogate markers for long-term exposure to altered hormonal tissue levels and thus as biomarkers of individual breast cancer risk, especially when considered as part of a multigene model that take into account metabolic pathways as they become better characterized.

The hydroxylation of estradiol and estrone to water-soluble metabolites (hydroxyestrones, HE) is an important elimination step. Oxidation occurs via two main competing and mutually exclusive pathways resulting in either 16α-HE or formation of catechol estrogens, 2-HE or 4-HE (11, 12). Whereas 2-HE binds only weakly to the ER, 4-HE and 16α-HE retain a potent estrogenic activity. Catechol estrogens are inactivated by *O*-methylation catalyzed by catechol-*O*-methyltransferase (COMT). If this inactivation process is incomplete, reactive species, such as 3,4-semiquinones, can react with DNA to form adducts which, if not repaired, may cause mutations (12-14). Increased formation of 16α-HE and 4-HE has been associated with an elevated breast cancer risk (15-17).

Although the enzymes responsible for the 16α-hydroxylation have not been well elucidated, CYP1A2, CYP1A1, CYP3A4, and CYP1B1 have been shown to catalyze the formation of catechol metabolites (11, 18). Polymorphisms in the genes coding for these enzymes are suspected to result in altered estrogen metabolism. Two linked polymorphisms in *CYP1A1*, one at the 3′ end of the gene giving rise to an *Msp*I restriction site (*CYP1A1*2A*), the other in exon 7 resulting in a valine for leucine substitution (*CYP1A1*2B*), have been associated with an elevated CYP1A1 activity (19, 20), and with much inconsistency among studies, an increased breast cancer risk (21). A recent case-control study conducted in North Carolina (698 cases, 702 controls) found a 2.1-fold increased breast cancer risk among the subgroup of women with the *Msp*I variant who smoked for >20 years (22). Our data did not show a similar modifying effect of pack-years and are consistent with most past results in showing no association of *CYP1A1* with breast cancer.

In an *in vitro* study that tested 15 cytochrome *P*450 isoforms (including CYP1A2, CYP1A1, CYP1B1, and CYP3A4), CYP1A2 had the highest activity for the 2-hydroxylation of both 17β-estradiol and estrone and it also had a considerable activity for their 4-hydroxylation (23). The activity of this P450 enzyme has been shown to vary considerably among individuals, due to inducers (e.g., coffee, smoking, and contaminants) and presumably genetics (24). However, no common polymorphism has been found in the coding region of the *CYP1A2* gene that could be shown to contribute to this intraindividual

Table 3. Ethnic-specific breast cancer odds ratios (95% confidence intervals) by *CYP1A2*1F* genotype

	*n**	AA	AC	CC	*P*†
Japanese	395/249	1.0	1.1 (0.8-1.5)	0.6 (0.3-1.0)	0.30
Caucasian	336/199	1.0	0.8 (0.5-1.1)	1.0 (0.4-2.3)	0.35
African American	259/389	1.0	1.0 (0.7-1.4)	0.7 (0.4-1.2)	0.29
Latino	252/371	1.0	0.7 (0.5-1.0)	1.0 (0.6-1.9)	0.26
Hawaiian	97/162	1.0	1.0 (0.5-1.7)	0.6 (0.2-2.6)	0.64

NOTE: Adjusted for age at blood draw, age at menarche, parity, and age at first birth.
*No. cases/no. controls.
†*P* value for gene dosage term assigned 1, 2, and 3 for 0, 1, and 2 variant alleles, respectively.

variability in activity (25). Nevertheless, a common C → A polymorphism in intron 1 (*CYP1A2*1F*) has been associated with a lower CYP1A2 inducibility (26). Although our observation of an inverse association between this variant and breast cancer may be due to chance, it is also consistent with some recent findings. *CYP1A2*1F* was found to be associated with a lower mean mammographic density among 267 premenopausal women in Hawaii (27). In another study of 220 premenopausal women sampled on the fifth day after ovulation an average of 4.4 times over a 2-year period, the *CYP1A2*1F* TT genotype was also associated with a 26% lower mean circulating estradiol level (7). Based on the model proposed by Pike et al. (28), a 20% difference in circulating estrogens may result in a halving in lifetime breast cancer risk, an estimate which is similar to what we observed in this study. However, it is not clear in our data why the association with *CYP1A2*1F* seemed somewhat stronger for ER/PR-negative tumors, and not for ER/PR-positive tumors, as one may expect if the underlying mechanism were through an ER-related pathway. However, this mechanism cannot be excluded because breast tumors that do not express hormone receptors may have initially been ER/PR positive and subsequently transformed into ER/PR-negative tumors via epigenetic or genetic events (29). If reproduced, our findings might suggest that the *CYP1A2* variant would have a role early in the natural history of the disease, perhaps through lower circulating estrogens and/or protection against initiation by catechol estrogens. Another possibility is that ER-negative tumors (and especially those that are PR negative) express the newly discovered ERβ, making them differently responsive to the proliferative effect of estrogens (30).

A C → G transversion at position 1666 in exon 3 of the *CYP1B1* gene, which results in an amino acid substitution of Leu^{432} to Val, has been shown using human lung microsomes to result in a high enzyme activity and thus may contribute to the interindividual differences observed in CYP1B1 activity (31). Past data on this polymorphism and breast cancer have been inconsistent, with two studies finding an increased risk for the Val/Val genotype (32, 33) and one study showing no association (34). We failed to find an association between this polymorphism and breast cancer in our sample of predominantly postmenopausal women.

The expression of *CYP1A1*, *CYP1A2*, and *CYP1B1* is regulated by the aryl hydroxylase receptor (Ahr), a ligand-activated transcription factor (35). A G1721A polymorphism within the coding region of the *AHR* gene, which results in replacement of Arg^{554} by Lys, has been observed in a recent study using ethoxyresofurin *O*-deethylase to be associated with a 3-fold increase in CYP1A1 inducibility (36). We are not aware of any other published report on the *AHR* Arg^{554}Lys polymorphism and breast cancer to which our null results could be compared.

Finally, a variant allele containing an A → G mutation in a 5′ regulatory element of *CYP3A4* has recently been identified (37). This polymorphism was found to be associated with a lower risk of chemotherapy-induced leukemia observed after treatment with drugs metabolized by CYP3A4 (38), suggesting that the variant allele results in a decreased activity. Only one other study (39) has examined *CYP3A4*1B* and breast cancer. In agreement with our data, no association was found, although Kadlubar et al. (40) reported that girls with the variant allele had earlier menarche.

Conjugation of parent estrogens and catechol estrogens, involving methylation or sulfation, are important detoxification pathways. A G → A transition at codon 158 of the *COMT* gene, which leads to a substitution of methionine for valine, has been linked to a reduced COMT activity (41). At least eight studies (reviewed in ref. 21) have examined this variant in relation to breast cancer risk. Associations were found in subgroups based on age or other risk factors. However, there is no consistency across studies as to the subgroup in which the association was found. We failed to find any association with breast cancer, overall or in any of the subgroups examined in previous studies.

A common G-to-A transition at nucleotide 638 of the *SULT1A1* gene, resulting in an arginine-to-histidine substitution at codon 213, has been associated with a decreased sulfotransferase activity, as measured in platelets (42). Zheng et al. (43) recently found in a case-control study in Iowa that breast cancer risk increased with the number of His alleles

Table 4. Joint effects of *CYP1A2*1F* and age at menarche, body mass index, hormone replacement therapy use, and pack-years on breast cancer risk

	AA		AC		CC	
	*n**	OR† (95% CI)	*n*	OR (95% CI)	*n*	OR (95% CI)
Age at menarche (y)						
<12	347/320	1.0	336/318	0.9 (0.7-1.1)	46/65	0.6 (0.4-1.0)
13-14	217/214	0.7 (0.3-1.5)	208/240	0.6 (0.2-1.2)	34/43	0.6 (0.2-1.5)
>14	66/71	0.5 (0.1-2.7)	59/62	0.5 (0.1-2.5)	9/14	0.4 (0.1-2.3)
$P_{interaction}$				0.20‡		
Body mass index (kg/m^2)						
<23.8	244/203	1.0	247/207	0.9 (0.7-1.2)	30/31	0.8 (0.5-1.4)
23.9-28.2	213/194	1.1 (0.8-1.4)	185/215	0.8 (0.6-1.0)	32/43	0.7 (0.4-1.2)
>28.2	173/208	1.0 (0.7-1.3)	171/198	1.0 (0.7-1.3)	27/48	0.7 (0.4-1.2)
$P_{interaction}$				0.51		
Hormone replacement therapy use (y)						
Never	270/325	1.0	255/314	0.9 (0.7-1.2)	41/59	0.9 (0.6-1.4)
<5	186/154	1.3 (1.0-1.7)	171/183	0.9 (0.7-1.2)	26/36	0.8 (0.4-1.3)
>5	167/109	1.3 (1.0-1.8)	162/108	1.2 (0.9-1.7)	19/24	0.7 (0.4-1.4)
$P_{interaction}$				0.51		
Pack-years						
0	346/329	1.0	339/360	0.8 (0.7-1.0)	48/74	0.6 (0.4-1.0)
0.1-12.0	150/155	1.0 (0.8-1.3)	136/146	1.0 (0.7-1.3)	20/28	0.8 (0.4-1.5)
>12.0	134/121	1.1 (0.8-1.4)	128/114	1.0 (0.8-1.4)	21/20	1.1 (0.6-2.1)
$P_{interaction}$				0.76		

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval.

*No. of cases/no. of controls. Numbers of subjects vary due to missing information.

†ORs (95% CIs) adjusted for age at blood draw, age at menarche, parity, and age at first birth.

‡*P* value derived from a likelihood ratio test comparing a model with main effects for the *CYP1A2*1F* genotype and the risk factor, and a model with main effects and interaction terms.

Table 5. Joint effects of *CYP1A2*1F* and *CYP1A1*2A*, CYP1B1 Leu^{432}Val, *AHR*Arg^{554}Lys, *CYP3A4*1B*, *COMT*Val^{158}Met and *SULT1A1*Arg^{213}His on breast cancer risk

	AA		AC		CC	
	*n**	OR† (95% CI)	*n*	OR (95% CI)	*n*	OR (95% CI)
*CYP1A1*2A*						
TT	279/224	1.0	375/376	0.8 (0.6-1.0)	82/112	0.7 (0.5-1.0)
TC	259/274	0.9 (0.7-1.1)	220/236	0.8 (0.6-1.1)	6/10	0.5 (0.2-1.3)
CC	92/107	0.8 (0.6-1.1)	8/8	0.7 (0.3-2.0)	1/0	—
$P_{interaction}$				0.64‡		
CYP1B1 Leu^{432}Val						
LL	274/237	1.0	267/237	0.8 (0.6-1.1)	35/33	0.9 (0.5-1.5)
LV	240/247	0.9 (0.7-1.1)	211/225	0.8 (0.6-1.1)	35/47	0.6 (0.4-1.1)
VV	116/121	0.9 (0.7-1.3)	125/158	0.8 (0.6-1.1)	19/42	0.5 (0.3-0.9)
$P_{interaction}$				0.75		
AHR						
RR	368/375	1.0	303/318	0.9 (0.7-1.1)	40/54	0.8 (0.5-1.2)
RL	206/168	1.3 (1.0-1.7)	223/226	1.0 (0.7-1.3)	30/54	0.6 (0.4-1.0)
LL	56/62	0.8 (0.6-1.3)	77/76	0.9 (0.7-1.4)	19/14	1.4 (0.7-2.8)
$P_{interaction}$				0.13		
*CYP3A4*1B*						
AA	537/461	1.0	469/439	0.8 (0.7-1.0)	63/76	0.7 (0.5-1.0)
AG	67/106	0.8 (0.5-1.1)	81/120	0.8 (0.6-1.2)	13/26	0.6 (0.3-1.2)
GG	26/38	0.9 (0.5-1.6)	53/61	1.1 (0.7-1.8)	13/20	0.8 (0.4-1.8)
$P_{interaction}$				0.54		
COMT Val^{158}Met						
Val/Val	242/248	1.0	239/245	0.9 (0.7-1.2)	33/49	0.7 (04.-1.1)
Val/Met	300/264	1.1 (0.8-1.4)	273/281	0.9 (0.7-1.1)	41/59	0.7 (0.5-1.1)
Met/Met	88/93	0.9 (0.6-1.3)	91/94	0.8 (0.6-1.2)	15/14	1.0 (0.5-2.2)
$P_{interaction}$				0.71		
SULT1A1 Arg^{213}His						
Arg/Arg	376/335	1.0	364/356	0.9 (0.7-1.1)	51/82	0.6 (0.4-0.9)
Arg/His	195/219	0.8 (0.6-1.1)	192/216	0.8 (0.6-1.0)	30/36	0.8 (0.5-1.4)
His/His	59/51	1.0 (0.7-1.6)	47/48	0.8 (0.5-1.3)	8/4	2.1 (0.6-7.2)
$P_{interaction}$				0.22		

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval.
*No. cases/no. controls. Numbers of subjects vary due to missing information.
†OR (95% CIs) adjusted for age at blood draw, age at menarche, parity and age at first birth.
‡*P* value derived from a likelihood ratio test comparing a model with main effects for the *CYP1A2*1F* genotype and the risk factor, and a model with main effects and interaction terms.

among all women and with red meat doneness level among women with the Arg/Arg genotype. The latter association was not observed in women with the His/His genotype. A second study also found a direct association between breast cancer and the His/His genotype that was of borderline significance (44). In the present study, we failed to find any association between *SULT1A1* and breast cancer, overall or in subgroups defined by doneness level or smoking.

Compared with previous studies, the present investigation presents a number of important strengths, including its large size, information on hormone receptors, and the prediagnostic assessment of breast cancer risk factors. Its approach was also more comprehensive since past studies almost uniformly examined single genes. Although not all cohort members were genotyped due to the case-control study design, participation was relatively high and no major difference was found between respondents and nonrespondents. In addition, only <8% of eligible patients died before contact and no modifying effect of stage at diagnosis was observed, making a survival bias unlikely. A number of subgroup analyses were conducted on the data, increasing the risk of chance findings, but these analyses addressed hypotheses that were formulated a priori. Limitations to this study include that information on ER/PR status was missing for a third of the cases and that the current sample size does not yet allow for an adequate test of heterogeneity of effects across ethnic groups.

Table 6. Breast cancer odds ratios (95% confidence intervals) by ER and PR status and *CYP1A2*F* genotype

	*n**	AA	AC	CC	*P*†
ER negative	205/1,370	1.0	0.8 (0.6-1.1)	0.7 (0.4-1.2)	0.08
ER positive	703/1,370	1.0	0.9 (0.8-1.1)	0.8 (0.6-1.2)	0.26
PR negative	269/1,370	1.0	0.9 (0.7-1.1)	0.6 (0.4-1.1)	0.08
PR positive	605/1,370	1.0	0.9 (0.7-1.1)	0.9 (0.6-1.3)	0.41
ER/PR negative	165/1,370	1.0	0.8 (0.5-1.1)	0.5 (0.3-1.1)	0.03
ER/PR positive	562/1,370	1.0	0.9 (0.8-1.2)	0.9 (0.6-1.3)	0.41

NOTE: Adjusted by logistic regression for age at blood draw, race/ethnicity, age at menarche, parity and age at first birth.
*No. cases/no. controls; 431 cases had missing ER status and 465 cases had missing PR status.
†*P* value for gene dosage term assigned 1, 2, and 3 for 0, 1, and 2 variant alleles, respectively. The *P* value for comparison of gene dosage effects between the ER/PR-negative and ER/PR-positive groups by polytomous logistic regression was not statistically significant ($P = 0.13$).

In conclusion, the present study suggests that *COMT* Val^{58}Met, *CYP1A1*2A*, *CYP3A4*1B*, *CYP1B1* Leu^{432}Val, *SULT1A1* Arg^{213}His, and *AHR* Arg^{554}Lys are unlikely to play a significant role in breast cancer etiology. It also provides evidence for an inverse association between *CYP1A2*1F* and breast cancer, which may be related to the observation of lower circulating estradiol levels in premenopausal women with the TT genotype. Confirmation of these findings and a systematic characterization of the association between breast cancer and single nucleotide polymorphisms and haplotypes recently identified in *CYP1A2* seem warranted.

Acknowledgments

We thank Jana Koerte for assistance with data analysis and Wendy Chang, Annette Lum-Jones, and Ann Seifried for their help with genotyping.

References

1. Dunning AM, Healey CS, Pharoah PDP, Teare MD, Ponder AJ, Easton DF. A systematic review of genetic polymorphisms and breast cancer risk. Cancer Epidemiol Biomarkers Prev 1999;8:843–54.
2. Toniolo PG, Levitz M, Zeleniuch-Jacquotte A, et al. A prospective study of endogenous estrogens and breast cancer in postmenopausal women. J Natl Cancer Inst 1995;87:190–7.
3. Hankinson SE, Willett WC, Manson JE, et al. Plasma sex steroid hormone levels and risk of breast cancer in postmenopausal women. J Natl Cancer Inst 1998;90:1292–9.
4. Kolonel LN, Henderson BE, Hankin JH, et al. A multiethnic cohort in Hawaii and Los Angeles: baseline characteristics. Am J Epidemiol 2000;151:346–57.
5. Le Marchand L, Haiman CA, Wilkens LR, Kolonel LN, Henderson BE. *MTHFR* polymorphisms, diet, HRT and breast cancer risk: the Multiethnic Cohort Study. Cancer Epidemiol Biomarkers Prev 2004;13:2071–7.
6. Goodman MT, McDuffie K, Kolonel LN, et al. Case-control study of ovarian cancer and polymorphisms in genes involved in catecholestrogen formation and metabolism. Cancer Epidemiol Biomarkers Prev 2001;10:209–16.
7. Lurie G, Maskarinec G, Kaaks R, Stanczyk F, Le Marchand L. Polymorphisms associated with serum estrogens measured multiple times during a two-year period in premenopausal women. Cancer Epidemiol Biomarkers Prev 2005;14:1521–7.
8. Coughtrie MWH, Gilissen RAHJ, Shek B, et al. Phenol sulphotransferase *SULT1A1* polymorphism: molecular diagnosis and allele frequencies in Caucasian and African populations. Biochem J 1999;337:45–9.
9. Kawajiri K, Watanabe J, Eguchi H, et al. Polymorphisms of human Ah receptor gene are not involved in lung cancer. Pharmacogenetics 1995;5:151–8.
10. Breslow NE, Day NE. Statistical methods in cancer research. Vol 1. The analysis of case-control studies. IARC Scientific Pub. No. 32. Lyon: IARC; 1980.
11. Nebert DW. Elevated estrogen 16α-hydroxylase activity: is this a genotoxic or nongenotoxic biomarker in human breast cancer risk? J Natl Cancer Inst 1993;85:1888–91.
12. Cavalieri EL, Stack DE, Devanesan PD, et al. Molecular origin of cancer: catechol estrogen-3,4-quinones as endogenous tumor initiators. Proc Natl Acad Sci U S A 1997;94:10937–42.
13. Yager JD, Liehr JG. Molecular mechanisms of estrogen carcinogenesis. Annu Rev Pharmacol Toxicol 1996;36:203–32.
14. Zhu BT, Conney AH. Functional role of estrogen metabolism in target cells: review and perspectives. Carcinogenesis 1998;19:1–27.
15. Kabat GC, Chang CJ, Sparano JA, et al. Urinary estrogen metabolites and breast cancer: a case-control study. Cancer Epidemiol Biomarkers Prev 1997;6:505–9.
16. Bradlow L, Telang NT, Osborn MP. Estrogen metabolites as bioreactive modulators of tumor initiators and promoters. Adv Exp Med Biol 1996;387:285–96.
17. Liehr JG, Ricci MJ. 4-Hydroxylation of estrogens as marker of human mammary tumors. Proc Natl Acad Sci U S A 1996;93:3294–6.
18. Hayes CL, Spink DC, Spink BC, Cao JQ, Walker NJ, Sutter TR. 17 β-Estradiol hydroxylation catalyzed by human cytochrome *P*450 1B1. Proc Natl Acad Sci U S A 1996;93:9776–81.
19. Petersen DD, McKinney CE, Ikeya K, et al. Human CYP1A1 gene: cosegregation of the enzyme inducibility phenotype and an RFLP. Am J Hum Genet 1991;48:720–5.
20. Landi MT, Bertazzi PA, Shields PG, et al. Association between CYP1A1 genotype, mRNA expression and enzymatic activity in humans. Pharmacogenetics 1994;4:242–6.
21. Mitrunen K, Hirvonen A. Molecular epidemiology of sporadic breast cancer. The role of polymorphic genes involved in oestrogen biosynthesis and metabolism. Mutat Res 2003;544:9–41.
22. Li Y, Millikan RC, Bell DA, et al. Cigarette smoking, cytochrome P4501A1 polymorphisms, and breast cancer among African-American and White women. Breast Cancer Res 2004;6:R460–73.
23. Lee AJ, Cai MX, Thomas PE, Conney AH, Zhu BT. Characterization of the oxidative metabolites of 17β-estradiol and estrone formed by 15 selectively expressed human cytochrome P450 isoforms. Endocrinology 2003; 144:3382–8.
24. Le Marchand L, Franke AA, Custer L, Wilkens LR, Cooney RV. Lifestyle and nutritional correlates of cytochrome CYP1A2 activity: inverse associations with plasma lutein and α-tocopherol. Pharmacogenetics 1997;7:11–9.
25. Nakajima M, Yokoi T, Miutani M, Shin S, Kadlubar FF, Kamataki T. Phenotyping of CYP1A2 in Japanese population by analysis of caffeine urinary metabolites: absence of mutations prescribing the phenotype in the CYP1A2 gene. Cancer Epidemiol Biomarkers Prev 1994:3:413–21.
26. Sachse C, Brockmoller J, Bauer S, Roots I. Functional significance of a C→A polymorphism in intron 1 of the cytochrome P450 *CYP1A2* gene tested with caffeine. Br J Clin Pharmacol 1999;47:445–9.
27. Maskarinec G, Lurie G, Williams AE, Le Marchand L. An investigation of mammographic density and gene variants in healthy women. Int J Cancer 2004;112:683–8.
28. Pike MC, Krailo MD, Henderson BE, Casarande JT, Hoel DG. ''Hormonal'' risk factors, ''breast tissue age'' and the age-incidence of breast cancer. Nature 1983;303:767–70.
29. Zhu K, Bernard LJ, Levine RS, Williams SM. Estrogen receptor status of breast cancer: a marker of different stages of tumor or different entities of the disease? Med Hypotheses 1997;49:69–75.
30. Russo J, Russo IH. Genotoxicity of steroidal estrogens. Trends Endocrinol Met 2004;15:211–4.
31. Shimada T, Watanabe J, Kawajiri K, et al. Catalytic properties of polymorphic human cytochrome P450 1B1 variants. Carcinogenesis 1999;20:1607–13.
32. Bailey LR, Roodi N, Dupont WD, Parl FF. Association of cytochrome P450 1B1 (*CYP1B1*) polymorphism with steroid receptor status in breast cancer. Cancer Res 1998;58:5038–41.
33. Zheng W, Xie D-W, Jin F, et al. Genetic polymorphism of cytochrome *P*450-1B1 and risk of breast cancer. Cancer Epidemiol Biomarkers Prev 2000;9:147–50.
34. De Vivo I, Hankinson SE, Li L, Colditz GA, Hunter DJ. Association of *CYP1B1* polymorphisms and breast cancer risk. Cancer Epidemiol Biomarkers Prev 2002;11:489–92.
35. Swanson HI, Bradfield CA. The AH-receptor: genetics, structure and function. Pharmacogenetics 1993;3:213–30.
36. Smart J, Daly AK. Variation in induced CYP1A1 levels: relationship to CYP1A1, Ah receptor and GSTM1 polymorphisms. Pharmacogenetics 2000;10:11–24.
37. Rebbeck TR, Jaffe JM, Walker AH, et al. Modification of clinical presentation of prostate tumors by a novel genetic variant in CYP3A4. J Natl Cancer Inst 1998;90:1225–9.
38. Felix CA, Walker AH, Lange BJ, et al. Association of *CYP3A4* genotype with treatment-related leukemia. Proc Natl Acad Sci U S A 1998;95:13176–81.
39. Spurdle AB, Goodwin B, Hodgson E, et al. The CYP3A4*1B polymorphism has no functional significance and is not associated with risk of breast or ovarian cancer. Pharmacogenetics 2002;12:355–66.
40. Kadlubar FF, Berkowitz GS, Delongchamp RR, et al. The CYP3A4*1B variant is related to the onset of puberty, a known risk factor for the development of breast cancer. Cancer Epidemiol Biomarkers Prev 2003;12:327–31.
41. Lachman HM, Papolos DF, Saito T, Yu YM, Szumlanski CL, Weinshilboum RM. Human catechol-*O*-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. Pharmacogenetics 1996;6:243–50.
42. Raftogianis RB, Wood TC, Otterness DM, Can Leon JA, Weinshilboum RM. Phenol sulfotransferase pharmacogenetics in humans: association of common SULT1A1 alleles with TS PST phenotype. Biochem Biophys Res Commun 1997;239:298–304.
43. Zheng W, Xie D, Cerhan JR, Sellers TA, Wen W, Folsom AR. Sulfotransferase 1A1 polymorphism, endogenous estrogen exposure, well-done meat intake, and breast cancer risk. Cancer Epidemiol Biomarkers Prev 2001;10:89–94.
44. Tang D, Rundle A, Mooney L, et al. Sulfotransferase 1A1 (SULT1A1) polymorphism, PAH-DNA adduct levels in breast tissue and breast cancer risk in a case-control study. Breast Cancer Res Treat 2003 Mar; 78:217–22.