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Cigarette Smoking, Cytochrome *P450 1A1* Polymorphisms, and Breast Cancer Risk in the Nurses' Health Study[1]

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ABSTRACT

Environmental exposure to carcinogens may contribute to increasing breast cancer rates and geographic variation in breast cancer incidence in the United States. One class of chemicals that has received much attention are the polyaromatic hydrocarbons that are ubiquitous in the environment and occur in cigarette smoke. The cytochrome P450 1A1 (*CYP1A1*) gene codes for an enzyme that contributes to aryl hydrocarbon hydroxylase activity, which is involved in the metabolism of polyaromatic hydrocarbons. Genotypic variants of *CYP1A1* have been associated with increased aryl hydrocarbon hydroxylase activity, and some epidemiological studies suggest that women with the variant genotype(s) are at increased risk for breast cancer.

We prospectively evaluated the associations between the *CYP1A1* polymorphisms and breast cancer risk, as well as the potential modification of these associations by cigarette smoking, in a case-control study nested within the Nurses' Health Study. We analyzed the T→C transition at nucleotide 6235 (*MspI*) and the A→G transition at nucleotide 4889 (*exon 7*) in *CYP1A1* by PCR-RFLP assays among 466 incident breast cancer cases and 466 matched controls. Relative risks (RRs) and 95% confidence intervals (CIs) were used to quantify the risk of breast cancer among subjects who had at least one variant allele relative to subjects who were homozygous for the wild-type allele, using conditional logistic regression.

No overall increase in breast cancer risk with the variant *CYP1A1* genotypes was apparent (RR_{MspI}, 1.05; 95% CI, 0.74–1.50 and $RR_{exon\ 7}$, 0.88; 95% CI, 0.58–1.33). However, a suggestive increase in breast cancer risk was observed among women who had commenced smoking before the age of 18 and had the *CYP1A1-MspI* variant genotype compared to nonsmokers who were homozygous wild type for the polymorphism (RR, 5.65; 95% CI, 1.50–21.3; percentage of all breast cancer cases attributable to this risk factor, 2.5%). A similar gene-environment association was observed for the *exon 7* polymorphism (RR, 3.61; 95% CI, 1.11–11.7; percentage of all breast cancer cases attributable to this risk factor, 2.2%). These data are compatible with the hypothesis that cigarette smoking early in life is a modifiable cause of breast cancer in a subpopulation of genetically susceptible women. However, the proportion of breast cancer attributable to cigarette smoking at a young age among Caucasian women with the variant form of the *CYP1A1* polymorphisms is low.

INTRODUCTION

Concern has grown that environmental exposure to carcinogens may contribute to increasing breast cancer rates and geographic variation in breast cancer incidence (1). One class of chemicals that has received attention are the PAHs,[3] which are ubiquitous in the environment. In animal models, exposure to PAHs such as benzo(*a*)pyrene and 7,12-dimethylbenz(*a*)anthracene induce mammary tumors (2–4). A direct carcinogenic effect has been seen in animal studies, particularly if exposure occurs before first pregnancy (5), and in *in vitro*, PAHs cause transformation in breast epithelial cell lines (6). However, the relevance of these observations to human breast cancer remains unclear (7, 8).

Although epidemiological studies of cigarette smoking and breast cancer risk have consistently shown no overall association (8–11), biomarker studies in humans have reported findings similar to those in animals. Tobacco-related mutagens have been isolated from breast fluid of smokers (12, 13), and in a recent study, DNA adducts characteristic of tobacco smoke exposure were found in four of seven breast tumors from smoking women, but not in tumors from eight nonsmokers (14). A larger study also has reported the detection of benzo(*a*)pyrene-like adducts in the breast tissue of 41% of breast cancer patients, whereas in controls, these adducts were absent (15). These observations provide compelling evidence that constituents of cigarette smoke reach breast tissue and suggest the potential for PAHs to be involved in human breast carcinogenesis.

Most chemicals to which individuals are exposed undergo biotransformation *in vivo*. Levels of biotransformation enzymes vary substantially between individuals due to both genetic and environmental influences. These differing rates of metabolism may be important in modifying the risk of developing environmentally based diseases, and failure to account for such genetic factors may limit our ability to investigate the role of exogenous chemicals in human cancer.

An enzyme that has been studied extensively and is known to be polymorphic is the cytochrome *P450 1A1* (*CYP1A1*) gene product. This enzyme contributes to AHH activity (16) and metabolizes PAHs found in cigarette smoke, particularly in extrahepatic tissues (17). Although the functional significance of the *CYP1A1* genotypes remains uncertain (18–25), an increase in AHH enzymatic activity among the variant *exon 7* genotypes has been reported (18). This increase in enzymatic activity does not appear to be limited to those who are homozygous for the variant allele (18). Furthermore, a gene-dose effect for the *CYP1A1-MspI* polymorphism has been observed; *p53* mutation frequencies were highest among individuals who were homozygous variant for the *MspI* polymorphism and intermediate in subjects who were heterozygous for the polymorphism when compared with homozygous wild-type individuals (26). Individuals who are heterozygous for the *CYP1A1* polymorphisms are at increased risk of developing lung cancer (27, 28). Moreover, AHH activity has been observed in both normal and neoplastic human breast epithelium (29–31), and phenolic products and epoxides created by the monooxygenation of PAHs via AHH activity also have been found in breast cancer tissue (32).

To date, three investigations have examined associations between *CYP1A1* polymorphisms and breast cancer risk (33–35), but the results have been conflicting. Some of these inconsistencies may be attributable to variations in study population or to differences in which of the polymorphisms were genotyped. Rebbeck *et al.* (33) observed no association of breast cancer risk with the *exon 7* polymorphism among 96 incident breast cancer cases and 146 controls, whereas Ambrosone and

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[3] The abbreviations used are: PAH, polyaromatic hydrocarbon; AHH, aryl hydrocarbon hydroxylase; NHS, Nurses' Health Study; RR, relative risk; CI, confidence interval; PAR, percentage of all breast cancer cases attributable to the risk factor(s) in question.

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colleagues reported a significant increase in risk among postmenopausal women with the variant *exon 7* allele (based on 216 cases and 282 controls; Ref. 34). In addition, an interaction was observed in the latter study with smoking, suggesting that this polymorphism may be important in increasing breast cancer risk among light smokers (defined as <30 pack-years). More recently, Taioli *et al.* (35) observed an increase in breast cancer risk among African-American women (20 cases and 81 controls), but not among Caucasian women (29 cases and 175 controls), who have the *Msp*I variant genotype.

In this study, we evaluate both the associations between *CYP1A1* polymorphisms and breast cancer risk and the potential modification of these associations by smoking behavior in a case-control study nested within the prospective Nurses' Health Study (NHS).

MATERIALS AND METHODS

Study Population. In 1976, more than 121,000 female United States registered nurses between the ages of 30 and 55, residing in 11 larger states, completed a self-administered questionnaire, forming the basis of the NHS. The baseline questionnaire collected information on potential risk factors for breast cancer, as well as information on smoking habits early in life. Information on potential risk factors and identification of new cases of disease have been ascertained biennially through mailed questionnaires.

In 1989 and 1990, blood samples from 32,826 NHS cohort members were collected. Participants were sent a blood collection kit containing all necessary supplies for drawing blood. Each woman arranged to have a blood sample drawn and returned on ice via overnight courier. Approximately 97% of the samples were received within 24 h of blood collection. Upon arrival, the samples were centrifuged and aliquoted into plasma, buffy coat, and RBC components. The samples have been archived in continuously monitored liquid nitrogen freezers since collection.

Women who returned a blood sample and who had not been diagnosed with cancer (except for non-melanoma skin cancer) at the time of blood draw comprised the subcohort for this nested case-control study of breast cancer. Eligible cases were women who had a confirmed diagnosis of breast cancer anytime after blood collection up to May 31, 1994. During this period, 466 eligible cases of incident breast cancer were identified. Four hundred sixty-six controls were randomly selected from all women who had provided a blood sample and matched to cases on year of birth, menopausal status, postmenopausal hormone use, month of blood return, time of day of blood collection, and fasting status at blood drawing.

Laboratory Analysis. All analyses were conducted with laboratory personnel blinded to case status. DNA was obtained from buffy coat either by use of Chelex solution as described by Walsh *et al.* (36) or by use of a DNA extraction kit (Qiagen, Inc., Chatsworth CA). For analysis of the *CYP1A1-Msp*I RFLP, a modified version of the original method, which was described previously, was used (37). For the *exon 7* polymorphism, a modified version of the PCR/restriction digestion assay was used as described previously (38). The modification of the assay involved changes in buffer conditions and in the restriction enzyme used for RFLP analysis. The buffer concentrations used were as follows: 100 mM KCl, 100 mM $(NH_4)_2SO_4$, 200 mM Tris (pH 8.8), 70 mM $MgSO_4$, and 1% Triton X-100. The PCR product was digested overnight at 37°C with *Nco*I (New England Biolabs, Inc., Beverly MA).

Statistical Analysis. RRs and 95% CIs were used to quantify the risk of breast cancer among subjects who had at least one variant allele relative to subjects who were homozygous for the wild-type allele. RRs adjusted for matching variables and other potential confounders were estimated using conditional regression models. Separate analyses were conducted for each of the polymorphisms. Age at menarche (<12, 12–13, or >13 years), family history of breast cancer among mother or sisters, parity, age at first birth, body mass index (<22, 22–25, 25–29, >29 kg/m^2), and history of benign breast disease were considered as potential confounders. Current smoking status was defined based on the questionnaire immediately prior to diagnosis in the cases. Smoking status was similarly defined in controls using the questionnaire prior to the date of diagnosis of each control's matched case. Smoking status 10 years prior to diagnosis was defined as that reported in the questionnaire returned 10 years before the date of diagnosis of the case in each case-control pair.

Unconditional logistic regression models, including terms for the matching variables and other potential confounders, were used to assess the association of each of the *CYP1A1* polymorphisms with breast cancer characterized by histological type, stage of disease, and estrogen receptor status. Unconditional models allowed for the use of information from all control subjects when cases were limited to a specific histological type and receptor status.

To assess the presence of interactions between each of the *CYP1A1* polymorphisms and exposure to other substances, we compared the risk of breast cancer for subjects in each category of joint exposure to that of subjects who were homozygous wild type for the *CYP1A1* polymorphism and who had the lowest exposure level. Estimates for the individual and joint associations were calculated from logistic regression models using indicator variables created for each category, omitting the hypothesized low-low risk category. For categorical variables with more than two categories, the interaction was evaluated using the likelihood ratio test, comparing the model with indicator variables for the cross-classified variables with a reduced model containing indicator variables for the main effects only. Interactions with smoking status at diagnosis, smoking status 10 years prior to diagnosis, pack-years, smoking before first pregnancy, and age started smoking were assessed.

PARs were calculated using the following formula (39):

$$PAR = \frac{p_j(RR_j - 1)}{RR_j}$$

where p_j is the proportion of all cases that are in stratum j (*i.e.*, $p_j = x_j/x$, where x_j is the number of cases in stratum j and x is the total number of cases) and RR_j is the RR in stratum j for the risk factor in question.

RESULTS

Characteristics of Study Subjects. Among 466 case-control pairs, greater than 97% of the women self-reported to be Caucasian. Approximately 75% of cases and controls were postmenopausal with mean age of 60.7 years (±5.5). Differences in established breast cancer risk factors were mostly in the expected direction (Table 1).

Overall Association between *CYP1A1* Polymorphisms and Breast Cancer Risk. Neither of the polymorphisms was independently associated with overall breast cancer risk. The adjusted RRs (and 95% CIs) for the two polymorphisms were: $RRMsp_I$, 1.05 (95% CI, 0.74–1.50), and RRexon 7, 0.88 (95% CI, 0.58–1.33). Analyses by histological subtype were similar (Table 2). Analyses by menopausal status and stage of disease also did not alter the lack of association (data not shown).

Table 1 *Descriptive characteristics of cases of breast cancer (n = 466) and controls (n = 466)*

Variable	Cases	Controls	P^a
Body mass index (mean)	25.4	25.4	0.87
Age at menopause (mean)	48.5 years	47.9 years	0.18
Age at first birth[b] (mean)	25.2 years	24.9 years	0.48
Age at menarche (mean)	12.4 years	12.5 years	0.22
Parity			
Nulliparous	6%	9%	
≤2	36%	31%	
>2	58%	60%	0.16
Mother's history of breast cancer			
No	90%	94%	
Yes	10%	6%	0.04
Sister's history of breast cancer			
No	93%	96%	
Yes	7%	4%	0.05
History of benign breast disease			
No	43%	61%	
Yes	57%	39%	0.001
Smoking status at diagnosis			
Never	42%	47%	
Past	46%	42%	
Current	12%	11%	0.28

[a] Signed rank test for continuous variables; McNemar's test for categorical variables.
[b] Restricted to parous women.

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Table 2 *Association between breast cancer risk and CYP1A1-MspI and exon 7 polymorphisms by histological type and estrogen receptor status*

	N	% variant[a]	Adjusted[b] RR (95% CI)	Adjusted[c] RR (95% CI)
MspI				
Controls	466	(17.2%)	1.00 (reference)	1.00 (reference)
All tumors[d]	466	(18.7%)	1.10 (0.79–1.54)	1.05 (0.74–1.50)
Invasive[e,f]	392	(19.2%)	1.15 (0.81–1.64)	1.13 (0.79–1.62)
Ductal[e]	333	(19.9%)	1.20 (0.84–1.73)	1.18 (0.81–1.71)
Lobular[e]	42	(19.1%)	1.14 (0.50–2.57)	1.10 (0.48–2.56)
In situ[e]	73	(15.1%)	0.82 (0.41–1.65)	0.87 (0.42–1.81)
ER$^+$ [e,g]	264	(18.6%)	1.12 (0.75–1.67)	1.11 (0.74–1.66)
ER$^-$ [e,g]	66	(13.6%)	0.70 (0.33–1.49)	0.72 (0.33–1.57)
Exon 7				
Controls	466	(14.0%)	1.00 (reference)	1.00 (reference)
All tumors[d]	466	(13.1%)	0.93 (0.63–1.36)	0.88 (0.58–1.33)
Invasive[e,f]	392	(13.8%)	0.99 (0.67–1.47)	0.94 (0.63–1.41)
Ductal[e]	333	(13.9%)	0.99 (0.66–1.49)	0.93 (0.61–1.42)
Lobular[e]	42	(11.9%)	0.86 (0.32–2.30)	0.77 (0.28–2.11)
In situ[e]	73	(9.6%)	0.58 (0.25–1.35)	0.51 (0.21–1.22)
ER$^+$ [e,g]	264	(13.3%)	0.96 (0.61–1.49)	0.90 (0.57–1.42)
ER$^-$ [e,g]	66	(6.1%)	0.38 (0.13–1.09)	0.38 (0.13–1.12)

[a] Variants include subjects who are either heterozygous or homozygous for the variant allele.
[b] Logistic regression adjusted for the matching variables age, menopausal status, postmenopausal hormone use, date of blood draw, time of blood draw, and fasting status.
[c] Logistic regression model adjusted for the matching variables, body mass index, benign breast disease, age at menarche, parity, age at first birth, and family history of breast cancer.
[d] Conditional logistic regression model.
[e] Unconditional logistic regression model.
[f] Numbers add to more than 375, because there is a separate category for tumors classified as having both lobular and ductal involvement.
[g] ER, estrogen receptor; cases missing information on estrogen receptor status were deleted from analysis.

Interactions between Smoking Exposure Up to the Time of Diagnosis, Polymorphisms in CYP1A1, and Breast Cancer Risk. Interaction between the polymorphisms and smoking status at diagnosis and breast cancer risk was evaluated (Table 3). An increase in breast cancer risk among women who were variant for the *CYP1A1-MspI* genotype and who were current smokers at the time of diagnosis was observed (RR, 7.36; 95% CI, 1.39–39.0). When this interaction was assessed taking cumulative cigarette smoking dose up until the time of diagnosis (as estimated by lifetime pack-years smoked) into account, a moderate, nonsignificant increase in breast cancer risk among women who were variant for the *CYP1A1-MspI* genotype and had smoked greater than 30 pack-years was apparent (RR, 2.01; 95% CI, 0.91–4.42; Table 4).

Interactions between Smoking Exposure in Early Life, Polymorphisms in *CYP1A1*, and Breast Cancer Risk. Recently, smoking at any early age was reported to be associated with an increased risk of breast cancer (40). We, therefore, investigated the potential importance of smoking early in life in conjunction with the *CYP1A1* gene. There was a suggestion of an increase in cancer risk among women who had commenced smoking at less than 18 years of age, particularly among individuals who were variant for the polymorphisms compared with women who had never smoked and were wild type for the polymorphism. The adjusted RRs for the *MspI* and *exon 7* sites among these women who had started to smoke as adolescents were 5.65 (95% CI, 1.50–21.3; PAR, 2.5%) and 3.61 (95% CI, 1.11–11.7; PAR, 2.2%). Risk of breast cancer was not elevated for women with the variant genotypes who had started to smoke at a later age (Table 5). The tests for interaction between genotype and age at onset of smoking were marginally statistically significant ($P = 0.04$ for the *MspI* site and $P = 0.08$ for the *exon 7* site).

DISCUSSION

Despite considerable research, the association of cigarette smoking with breast cancer risk remains controversial. *A priori* hypotheses suggest that cigarette smoking could either increase (by its direct carcinogenic effect) or decrease (by its antiestrogenic effect) breast cancer risk. In general, there is little epidemiological evidence of an overall association between cigarette smoking and breast cancer risk (7–10). Yet, the importance of timing of exposure to tobacco smoke in mammary carcinogenesis is still under debate (40). Due to the role of *CYP1A1* in PAH metabolism, epidemiological studies investigating the role of *CYP1A1* and breast cancer risk have been conducted (33–35). However, the results have been inconsistent, and only one study assessed potential interactions with smoking (34). In this prospective study of 466 cases and an equal number of controls in predominantly Caucasian women, an overall increase in breast cancer risk with the variant *CYP1A1* genotypes was not observed. There was, however, a suggestion of a role of the variant alleles in breast cancer development in combination with both current and early onset of smoking.

Table 3 *RRs and 95% CIs between CYP1A1 and breast cancer risk by smoking status at diagnosis*

CYP1A1 genotype	Smoking status at diagnosis	Cases	Controls[a]	Adjusted[b] RR (95% CI)	Adjusted OR (95% CI)[c]
	Nonsmoker	198	218	1.0 (reference)	1.0 (reference)
	Past	213	197	1.18 (0.90–1.56)	1.25 (0.93–1.67)
	Current	55	49	1.24 (0.80–1.91)	1.44 (0.90–2.32)
MspI					
WT/WT	Nonsmoker	160	174	1.0 (reference)	1.0 (reference)
WT/WT	Past	173	164	1.14 (0.84–1.56)	1.21 (0.87–1.69)
WT/WT	Current	46	47	1.08 (0.68–1.71)	1.24 (0.75–2.03)
Variants[d]	Nonsmoker	38	44	0.95 (0.59–1.52)	0.89 (0.54–1.46)
Variants	Past	40	33	1.30 (0.78–2.17)	1.28 (0.75–2.20)
Variants	Current	9	2	4.75 (1.01–22.3)	7.36 (1.39–39.0)
LRT[e]				χ^2 (*df*, 2) = 4.22 $P = 0.12$	χ^2 (*df*, 2) = 5.61 $P = 0.06$
Exon 7					
WT/WT	Nonsmoker	178	189	1.0 (reference)	1.0 (reference)
WT/WT	Past	182	171	1.12 (0.83–1.52)	1.19 (0.86–1.59)
WT/WT	Current	45	10	1.18 (0.73–1.92)	1.32 (0.78–2.22)
Variants	Nonsmoker	20	29	0.75 (0.42–1.36)	0.66 (0.35–1.26)
Variants	Past	31	26	1.26 (0.72–2.20)	1.17 (0.64–2.11)
Variants	Current	10	9	1.14 (0.45–2.86)	1.51 (0.55–4.13)
LRT[e]				χ^2 (*df*, 2) = 0.90 $P = 0.64$	χ^2 (*df*, 2) = 1.09 $P = 0.58$

[a] There are five cases and three controls for whom "age started smoking" information is missing.
[b] OR, odds ratio; conditional logistic regression.
[c] Conditional logistic regression adjusted for the matching variables and body mass index, benign breast disease, age at menarche, parity, age at first birth, and family history of breast cancer.
[d] "Variants" are all women who are either heterozygous or homozygous for the variant allele.
[e] LRT, likelihood ratio test for the interaction between genotype and smoking status at diagnosis.

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Table 4 *RRs and 95% CIs between CYP1A1 and breast cancer risk by smoking in pack-years*

CYP1A1 genotype	Lifetime pack-years of smoking	Cases	Controls[a]	Adjusted[b] RR (95% CI)	Adjusted[c] RR (95% CI)
	Never smoker	200	220	1.0 (reference)	1.0 (reference)
	<30	164	149	1.23 (0.91–1.66)	1.38 (1.00–1.91)
	≥30	102	95	1.20 (0.85–1.69)	1.25 (0.86–1.81)
MspI					
WT/WT	Never	162	175	1.0 (reference)	1.0 (reference)
WT/WT	<30	136	126	1.16 (0.84–1.61)	1.30 (0.92–1.85)
WT/WT	≥30	81	84	1.05 (0.71–1.54)	1.08 (0.72–1.62)
Variants[d]	Never	38	45	0.89 (0.56–1.43)	0.81 (0.49–1.35)
Variants	<30	28	23	1.34 (0.73–2.47)	1.43 (0.74–2.77)
Variants	≥30	21	11	1.96 (0.93–4.13)	2.01 (0.91–4.42)
LRT[e]				χ^2 (*df*, 2) = 2.64 *P* = 0.27	χ^2 (*df*, 2) = 2.95 *P* = 0.23
Exon 7					
WT/WT	Never	179	190	1.0 (reference)	1.0 (reference)
WT/WT	<30	141	128	1.20 (0.87–1.66)	1.32 (0.93–1.87)
WT/WT	≥30	85	81	1.12 (0.76–1.64)	1.16 (0.77–1.75)
Variants	Never	21	30	0.77 (0.43–1.38)	0.67 (0.36–1.26)
Variants	<30	23	21	1.16 (0.62–2.19)	1.35 (0.68–2.69)
Variants	≥30	17	14	1.29 (0.61–2.74)	1.18 (0.52–2.66)
LRT[e]				χ^2 (*df*, 2) = 0.71 *P* = 0.70	χ^2 (*df*, 2) = 0.96 *P* = 0.62

[a] There are two controls for whom there is no pack-year information.
[b] Conditional logistic regression.
[c] Conditional logistic regression adjusted for the matching variables and body mass index, benign breast disease, age at menarche, parity, age at first birth, and family history of breast cancer.
[d] "Variants" are all women who are either heterozygous or homozygous for the variant allele.
[e] LRT, likelihood ratio test for the interaction between genotype and pack-years smoked.

Recently, smoking at an early age was reported to be associated with an increased risk of breast cancer (40). In a previous analysis of data from the overall NHS cohort, no increase in breast cancer risk was observed; however, the CIs did not exclude a modest association with smoking at an early age (10). We observed an association between breast cancer and an early age at onset of smoking in combination with the variant *CYP1A1* genotypes. The data suggest that any adverse effect of smoking exposure may occur during the period of early maturation of the breast tissue.

The data reported here are broadly consistent with the data of Ambrosone *et al.* (34), but different in specific aspects. In their case-control study of 216 cases and 282 controls, an increase in breast cancer risk was limited to "light" smokers (defined as <29 pack-years) who were variant for the *CYP1A1-exon 7* polymorphism. Although we also observed an increase in breast cancer risk among smokers, the risk was limited to those who were variant for the *CYP1A1-MspI* polymorphism and who had smoked greater then 29 pack-years. The fact that we examined multiple potential interactions between these genotypes and smoking characteristics (age at onset, duration, pack-years, and so on) suggests that these results should be interpreted with caution. Clearly, additional data will be required (*a*) to determine which of these polymorphisms in the *CYP1A1* gene, if any, is a better predictor of breast cancer risk; (*b*) to determine possible interaction with other metabolizing enzymes, such as *NAT2* and *GSTM1;* and (*c*) to characterize the interrelationships between breast cancer, age at onset of smoking, and subsequent intensity and duration of smoking.

The strengths of this study include the prospective collection of data in a cohort with a high follow-up rate and the largest sample size to date among studies assessing the potential role of *CYP1A1* in breast cancer

Table 5 *RRs and 95% CIs between CYP1A1 and breast cancer risk by age started smoking*

CYP1A1 genotype	Age started smoking (yr)	Cases[a]	Controls	Adjusted[b] RR (95% CI)	Adjusted[c] RR (95% CI)
	Nonsmoker	200	220	1.0 (reference)	1.0 (reference)
	<18	51	38	1.53 (0.94–2.46)	1.52 (0.90–2.58)
	≥18	210	205	1.13 (0.85–1.47)	1.24 (0.93–1.67)
MspI					
WT/WT	Nonsmoker	162	176	1.0 (reference)	1.0 (reference)
WT/WT	<18	37	35	1.20 (0.71–2.03)	1.11 (0.62–1.98)
WT/WT	≥18	175	173	1.08 (0.80–1.46)	1.19 (0.86–1.64)
Variants[d]	Nonsmoker	38	44	0.93 (0.60–1.49)	0.87 (0.53–1.43)
Variants	<18	14	3	5.01 (1.42–17.7)	5.65 (1.50–21.3)
Variants	≥18	35	32	1.22 (0.73–2.04)	1.26 (0.73–2.19)
LRT[e]				χ^2 (*df*, 2) = 5.43 *P* = 0.07	χ^2 (*df*, 2) = 6.63 *P* = 0.04
Exon 7					
WT/WT	Nonsmoker	180	191	1.0 (reference)	1.0 (reference)
WT/WT	<18	37	33	1.22 (0.71–2.10)	1.13 (0.62–2.05)
WT/WT	≥18	185	175	1.11 (0.83–1.49)	1.21 (0.88–1.66)
Variants	Nonsmoker	20	29	0.77 (0.42–1.40)	0.70 (0.37–1.33)
Variants	<18	14	5	3.19 (1.03–9.88)	3.61 (1.11–11.7)
Variants	≥18	25	30	0.87 (0.49–1.54)	0.90 (0.48–1.68)
LRT[e]				χ^2 (*df*, 2) = 3.92 *P* = 0.14	χ^2 (*df*, 2) = 5.13 *P* = 0.08

[a] There are five cases and three controls for whom "age started smoking" information is missing.
[b] Conditional logistic regression.
[c] Conditional logistic regression adjusted for the matching variables and BMI, benign breast disease, age at menarche, parity, age at first birth, and family history of breast cancer.
[d] "Variants" are all women who are either heterozygous or homozygous for the variant allele.
[e] LRT, likelihood ratio test for the interaction between genotype and age started smoking.

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risk. However, it should be noted that even with more than 900 subjects, the CI estimates for the odds ratios are wide when assessing interactions, because the variant allele frequency is quite low among Caucasians.

These observations are compatible with the overall null association between cigarette smoking and breast cancer risk that has repeatedly been reported. Because these polymorphisms in *CYP1A1* are relatively uncommon among Caucasians, any increased risk of smoking limited to *CYP1A1* heterozygotes would not translate into a detectable increase in risk associated with smoking in the overall population without taking genotype into account (41).

In our data, only 2.5% of the breast cancer cases that occurred in the NHS during this time period may be attributable to the combination of *CYP1A1* genotype and early smoking exposure. However, this gene-environment interaction could potentially be more important in populations in which the variant allele frequency is high. For example, the variant allele frequency for the *CYP1A1-MspI* polymorphism is approximately 0.33 in Asians compared with 0.11 in Caucasians (37, 42). Breast cancer incidence rates among women are highest for non-Hispanic whites (119 per 100,000), moderate in African-Americans (99 per 100,000), and lower in Hispanics (69 per 100,000) and Asians (60 per 100,000; Ref. 43). With the increase in cigarette smoking observed in recent decades among Asian women and other behavioral changes associated with a more Western lifestyle, smoking could have a substantial future impact on breast cancer incidence.

In summary, the *CYP1A1* gene was not independently associated with overall breast cancer risk in a predominantly Caucasian population in the Nurses' Health Study. However, the findings suggest that women who have the variant *CYP1A1* allele may constitute a subpopulation who are at increased risk for breast cancer in the presence of cigarette smoking exposure, particularly early in life. Further study is clearly warranted (particularly in populations with higher variant allele frequencies) to confirm and extend these observations.

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REFERENCES

1. Davis, D. L., Bradlow, H. L., Wolff, M., Woodruff, T., Hoel, D. G., and Anton-Culver, H. Medical hypothesis: xenoestrogens as preventable causes of breast cancer. Environ. Health Perspect., *101:* 372–377, 1993.
2. Cavalieri, N., Rogan, E., and Sinha, D. Carcinogenicity of aromatic hydrocarbons directly applied to rat mammary gland. J. Cancer Res. Clin. Oncol., *114:* 3–9, 1988.
3. Chatterjee, M., and Banerjee, M. R. Selenium mediated dose-inhibition of 7,12-dimethylbenz[a]anthracene-induced transformation of mammary cells in organ culture. Cancer Lett., *17:* 187–195, 1982.
4. Furr, B. J., and Jordan, V. C. The pharmacology and clinical uses of tamoxifen. Pharmacol. Ther., *25:* 127–205, 1984.
5. Russo, J., and Russo, I. H. Biological and molecular basis of mammary carcinogenesis. Lab. Invest., *57:* 112–137, 1987.
6. Calaf, G., and Russo, J. Transformation of human breast epithelial cells by chemical carcinogens. Carcinogenesis (Lond.), *14:* 483–492, 1993.
7. John, E. M., and Kelsey, J. L. Radiation and other environmental exposures and breast cancer. Epidemiol. Rev., *15:* 7–16, 1993.
8. Palmer, J., and Rosenberg, L. Cigarette smoking and the risk of breast cancer. Epidemiol. Rev., *15:* 145–156, 1993.
9. Baron, J. A., Newcomb, P. A., Longnecker, M. P., Mittendorf, R., Storer, B. E., Clapp, R. W., Bogdan, G., and Yuen, J. Cigarette smoking and breast cancer. Cancer Epidemiol. Biomarkers Prev., *5:* 399–403, 1996.
10. London, S. J., Colditz, G. A., Stampfer, M. J., Willett, W. C., Rosner, B. A., and Speizer, F. E. Prospective study of smoking and the risk of breast cancer. J. Natl. Cancer Inst., *81:* 1625–1631, 1989.
11. MacMahon, B. Cigarette smoking and cancer of the breast. *In:* N. Wald and J. Baron (eds.), Smoking and Hormone Related Disorders, pp. 154–66. Oxford, United Kingdom: Oxford University Press, 1990.
12. Petrakis, N. L., Maack, C. A., Lee, R. E., and Lyon, M. Mutagenic activity in nipple aspirates of human breast fluid. Cancer Res., *40:* 188–189, 1980.
13. Petrakis, N. L., Gruenke, L. D., Beelen, T. C., Castagnoli, N., Jr., and Craig, J. C. Nicotine in breast fluid of nonlactating women. Science (Washington DC), *199:* 303–304, 1978.
14. Perera, F. P., Estabrook, A., Hewer, A., Channing, K., Rundle, A., Mooney, L. A., Whyatt, R., and Phillips, D. H. Carcinogen-DNA adducts in human breast tissue. Cancer Epidemiol. Biomarkers Prev., *4:* 233–238, 1995.
15. Li, D., Wang, M., Dhingra, K., and Hittelman, W. N. Aromatic DNA adducts in adjacent tissues of breast cancer patients: clues to breast cancer etiology. Cancer Res., *56:* 287–293, 1996.
16. Pelkonen, O., and Nebert, D. W. Metabolism of polycyclic aromatic hydrocarbons: etiologic role in carcinogenesis. Pharmacol. Rev., *34:* 189–222, 1982.
17. Law, M. R. Genetic predisposition to lung cancer. Br. J. Cancer, *61:* 195–206, 1990.
18. Crofts, F., Taioli, E., Trachman, J., Cosma, G. N., Currie, D., Toniolo, P., and Garte, S. J. Functional significance of different human *CYP1A1* genotypes. Carcinogenesis (Lond.), *15:* 2961–2963, 1994.
19. Cosma, G., Crofts, F., Taioli, E., Toniolo, P., and Garte, S. Relationship between genotype and function of the human *CYP1A1* gene. J. Toxicol. Environ. Health, *40:* 309–316, 1993.
20. Jacquet, M., Lambert, V., Baudoux, E., Muller, M., Kremers, P., and Gielen, J. Correlation between P450 *CYP1A1* inducibility, *MspI* genotype, and lung cancer incidence. Eur. J. Cancer, *32A:* 1701–1706, 1996.
21. Wedlund, P. J., Kimura, S., Gonzalez, F. J., and Nebert, D. W. I462V mutation in the human *CYP1A1* gene: lack of correlation with either *MspI* 1.9 kb (*m2*) allele or *CYP1A1* inducibility in a three-generation family of east Mediterranean descent. Pharmacogenetics, *4:* 21–26, 1994.
22. Zhang, Z. Y., Fasco, M. J., Huang, L., Guengerich, F. P., and Kaminsky, L. S. Characterization of purified human recombinant cytochrome *P4501A1-Ile462* and *-Val462:* assessment of role for the rare allele in carcinogenesis. Cancer Res., *56:* 3926–3933, 1996.
23. Kawajiri, K., Nakachi, K., Imai, K., Watanabe, J., and Hayashi, S. I. The *CYP1A1* gene and cancer susceptibility. Crit. Rev. Oncol. Hematol., *14:* 77–87, 1993.
24. Landi, M. T., Bertazzi, P. A., Shields, P. G., Clark, G., Lucier, G. W., Garte, S. J., Cosma, G., and Caporaso, N. E. Association between *CYP1A1* genotype, mRNA expression and enzymatic activity in humans. Pharmacogenetics, *4:* 242–246, 1994.
25. Persson, I., Johansson, I., and Ingelman-Sundberg, M. *In vitro* kinetics of two human *CYP1A1* variant enzymes suggested to be associated with interindividual differences in cancer susceptibility. Biochem. Biophys. Res. Commun., *231:* 227–230, 1997.
26. Kawajiri, K., Eguchi, H., Nakachi, K., Sekiya, T., and Yamamoto, M. Association of *CYP1A1* germ line polymorphisms with mutations of the *p53* gene in lung cancer. Cancer Res., *56:* 72–76, 1996.
27. Xu, X., Kelsey, K. T., Wiencke, J. K., Wain, J. C., and Christiani, D. C. Cytochrome *P450 1A1 MspI* polymorphism and lung cancer susceptibility. Cancer Epidemiol. Biomarkers Prev., *5:* 687–692, 1996.
28. Sugimura, H., Hamada, G. S., Suzuki, I., Iwase, T., Kiyokawa, E., Kino, I., and Tsugane, S. *CYP1A1* and *CYP2E1* polymorphism and lung cancer, case-control study in Rio de Janeiro, Brazil. Pharmacogenetics, *5:* S145–S148, 1995.
29. Greiner, J. W., Malan-Shibley, L. B., and Janss, D. H. Detection of aryl hydrocarbon hydroxylase activity in normal and neoplastic human breast epithelium. Life Sci., *26:* 313–319, 1980.
30. Pyykko, K., Tuimala, R., Aalto, L., and Perkio, T. Is aryl hydrocarbon hydroxylase activity a new prognostic indicator for breast cancer? Br. J. Cancer, *63:* 596–600, 1991.
31. McKay, J. A., Murray, G. I., Ah-see, A. K., Greenlee, W. F., Craig, B. M., Burke, M. D., and Melvin, W. T. Differential expression of *CYP1A1* and *CYP1B1* in human breast cancer. Biochem. Soc. Transact., *24:* 327S, 1996.
32. Nebert, D. W. Role of genetics and drug metabolism in human cancer risk. Mutat. Res., *247:* 267–281, 1991.
33. Rebbeck, T. R., Rosvole, E. A., Duggan, D. J., Zhang, J., and Buetow, K. H. Genetics of *CYP1A1:* coamplification of specific alleles by polymerase chain reaction and association with breast cancer. Cancer Epidemiol. Biomarkers Prev., *3:* 511–514, 1994.
34. Ambrosone, C. B., Freudenheim, J. L., Graham, S., Marshall, J. R., Vena, J. E., Brasure, J. R., Laughlin, R., Nemoto, T., Michalek, A. M., Harrington, A., and Shields, P. G. Cytochrome *P450IA1* and glutathione *S*-transferase (M1) genetic polymorphisms and postmenopausal breast cancer risk. Cancer Res., *55:* 3483–3485, 1995.
35. Taioli, E., Trachman, J., Chen, X., Toniolo, P., and Garte, S. J. A *CYP1A1* restriction fragment length polymorphism is associated with breast cancer in African-American women. Cancer Res., *55:* 3757–3758, 1995.
36. Walsh, P. S., Metzger, D. A., and Higuchi, R. Chelex® 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques, *10:* 506–513, 1991.
37. Kawajiri, K., Nakachi, K., Imai, K., Yoshii, A., Shinoda, N., and Watanabe, J. Identification of genetically high risk individuals to lung cancer by DNA polymorphisms of the cytochrome *P450 1A1* gene. FEBS Lett., *263:* 131–133, 1990.
38. Cheng, T. J., Christiani, D. C., Xu, X., Wain, J. C., Wiencke, J. K., and Kelsey, K. T. Glutathione *S*-transferase M1 genotype, diet, and smoking as determinants of sister chromatid exchange frequency in lymphocytes. Cancer Epidemiol. Biomarkers Prev., *4:* 535–542, 1995.
39. Miettinen, O. S. Proportion of disease caused or prevented by a given exposure, trait or intervention. Am. J. Epidemiol., *99:* 325–332, 1974.
40. Palmer, J. R., Rosenberg, L., Clarke, E. A., Stolley, P. D., Warshauer, M. E., Zauber, A. G., and Shapiro, S. Breast cancer and cigarette smoking: a hypothesis. Am. J. Epidemiol., *134:* 1–13, 1991.
41. Rothman, K. J. Causal inference in epidemiology. *In:* Modern Epidemiology, pp. 11–13. Boston: Little, Brown, and Co., 1986.
42. Tefre, T., Ryberg, D., Haugen, A., Nebert, D. W., Skaug, V., Brogger, A., and Borresen, A. L. Human *CYP1A1* (cytochrome P_1 450) gene: lack of association between the *MspI* restriction fragment length polymorphism and incidence of lung cancer in a Norwegian population. Pharmacogenetics, *1:* 20–25, 1991.
43. Horn-Ross, P. L. Phytoestrogens, body composition, and breast cancer. Cancer Causes Control, *6:* 567–573, 1995.

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