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A prospective study of *NAT2* acetylation genotype, cigarette smoking, and risk of breast cancer

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Polymorphisms in the *N*-acetyltransferase 2 (*NAT2*) gene are determinants of the rate of metabolic activation of carcinogenic compounds such as aryl aromatic amines. Homozygosity for any combination of three variant alleles in Caucasians defines 'slow' acetylators; presence of one or two wild-type alleles characterizes 'rapid' acetylators. Although most previous studies have not observed an overall elevation in risk of breast cancer among slow acetylators, a recent study observed that cigarette smoking was associated with a large increase in risk of breast cancer among slow acetylators. We assessed the relation between *NAT2* acetylation status and breast cancer risk, and its interaction with smoking, in a prospective study of mainly Caucasian US women. Four hundred and sixty-six incident cases who were diagnosed with breast cancer after giving a blood specimen in 1989–90 were matched to 466 controls in a nested case-control study. *NAT2* genotype was determined using PCR-RFLP assays. The multivariate relative risk (RR) comparing slow with rapid acetylators was 0.9 (95%CI 0.7–1.2). Among slow acetylators, current smoking immediately prior to diagnosis was not associated with a significant elevation in risk compared with never smoking rapid acetylators (RR = 1.4, 95% CI 0.7–2.6). No significant association was seen between pack-years of smoking and risk of breast cancer among either slow or fast acetylators. A non-significant elevation in risk was observed among women who smoked for ⩾5 years prior to first pregnancy and were rapid acetylators, compared with never smoking rapid acetylators (RR = 1.5, 95% CI 0.9–2.6). In analyses limited to 706 post-menopausal women, the elevated risks for current smokers immediately prior to diagnosis who were slow acetylators compared with never smokers who were fast acetylators were slightly stronger but still not statistically significant. In summary, we observed little evidence of an association between *NAT2* genotype and breast cancer. In this prospective study, cigarette smoking was not appreciably associated with breast cancer among either slow or fast *NAT2* acetylators.

***Abbreviations:** *NAT2*, *N*-acetyltransferase 2; PAH, polycyclic aromatic hydrocarbon; *NAT1*, *N*-acetyltransferase 1; p450, cytochrome p-450.

Introduction

Breast cancer accounts for almost one-third of incident cancers among US women and the incidence rate of breast cancer has increased ~1% each year over the last 50 years (1). Controversy exists over how much of the international variation in incidence rates and the secular increase in the US can be explained by known breast cancer risk factors such as age at menarche, age at first birth, and parity, with estimates ranging from 30–50% (2,3). Environmental factors such as air pollution, pesticides, electromagnetic fields, cigarette smoking, and ionizing radiation, have all been invoked as possible explanations for the increasing rates (4); only ionizing radiation has been unequivocally associated with increased breast cancer risk (5).

Polycyclic aromatic hydrocarbons (PAH*) and aryl aromatic amine carcinogens are common exposures in modern life, and cause mammary tumors in some animal models [particularly if exposure occurs before first pregnancy (6)] bind to DNA in breast epithelial cells (7), and cause transformation of breast epithelial cell lines (8). Cigarette smoking is a major route of exposure to many carcinogens including polycyclic aromatic hydrocarbons and aryl aromatic amines (9). Certain of these carcinogens form reaction products which can adduct to DNA, potentially causing mutations. In a recent study, DNA adducts characteristic of PAH and cigarette smoke exposure were found in four out of seven breast tumors from smoking women, but were absent in tumors from eight non-smokers (10). In epidemiologic studies, cigarette smoking close to the time of diagnosis has not generally been associated with breast cancer, although few studies have addressed the possibility that onset of smoking in adolescence or before first pregnancy may increase risk (11).

Most xenobiotics are activated, or can be detoxified, by metabolic pathways for which genetic variation exists between individuals. For example, hemoglobin adduct levels of the aromatic amine 4-aminobiphenyl are higher among smokers who are slow *N*-acetylators, than among smokers who are rapid acetylators (12); *N*-acetylation is thus a detoxification step for some aromatic amines. A meta-analysis of 12 studies suggested that risk of bladder cancer was increased by 50% among slow acetylators (13). Aryl aromatic amines present in cigarette smoke (9,14–16), such as 4-aminobiphenyl, may be responsible for the increased risk of bladder cancer among slow acetylators, who are less able to detoxify this and other aromatic amines. Heterocyclic amines may be activated by *N*-acetylation through *O*-acetylation of *N*-hydroxy intermediates *N*-oxidized by CYP1A2 (17). Heterocyclic amines such as 2-amino-α-carboline and 2-amino-1-methyl-6-phenylimidazo [4,5-6] pyridine (PhIP) are present in cigarette smoke (14–16) and are potential substrates for *NAT2* activation.

N-acetylation phenotype is determined by the *NAT1* and *NAT2* genes (18). *NAT2* has four major alleles in Caucasian populations; individuals homozygous for any combination of the three slow acetylator alleles are slow acetylators, those heterozygous or homozygous for the rapid acetylator allele are

rapid acetylators (19). *N*-Acetyltransferase may also metabolically activate *N*-hydroxy arylamines through *O*-acetylation creating an electrophilic product which may cause mutations (20). The fact that slow *NAT2* status may represent a risk factor for cancer due to diminished potential to detoxify aryl amine substrates, or be protective due to diminished activation of heterocyclic amine substrates, makes it difficult to specify *a priori* the relations between cigarette smoking, *NAT2* genotype, and risk of breast cancer.

Several case-control studies have compared the prevalence of slow acetylators in breast cancer case series, with the prevalence in controls, with mixed results (21–26). Recently, in a case-control study of 185 postmenopausal cases and 213 controls, no overall association was observed with acetylation status, but an odds ratio of 6.6 (95%CI 1.7–25.4) was observed for slow acetylators who smoked 20 or more cigarettes per day, 20 years prior to diagnosis, compared with rapid acetylators (27). We assessed these relations in a prospective study of US women.

Materials and methods

Study population

In 1976, 121 700 married, registered nurses from 11 US states were enrolled in the Nurses' Health Study and have been subsequently followed. The cohort is followed by questionnaire every two years and self-reported diagnoses of breast cancer are confirmed by medical record review (28); follow-up as a proportion of potential person-years through 1994 is over 90%. Information on breast cancer risk factors such as family history and reproductive history is obtained by questionnaire and updated periodically. Age of onset of smoking, number of cigarettes per day after initiation of smoking, and age at quitting for past smokers were ascertained on the baseline questionnaire in 1976. Every two years subsequent to 1976, smoking status has been ascertained along with number of cigarettes per day among smokers. Current smoking status was defined at the questionnaire immediately prior to diagnosis in the cases, and the equivalent questionnaire for each matched control. Menopausal status was defined in response to a question on whether a woman's periods had ceased permanently. Women who had a hysterectomy with one or more ovaries left intact were classified as premenopausal until the age at which 10% of the cohort had undergone natural menopause (age 46 for smokers and 48 for non-smokers), postmenopausal at the age at which 90% of the cohort had undergone natural menopause (age 54 for smokers and 56 for non-smokers); in the intervening years these women were classified as uncertain menopausal status and excluded from menopause-specific analyses.

In 1989–90, 32 826 women sent us a blood sample which was separated into aliquots of plasma, red blood cells and buffy coat. Women who sent a blood sample were very similar to women who did not with respect to reproductive risk factors for breast cancer such as age at menarche, parity and age at first birth. The proportion of women who were current smokers was lower (14.4%) among women who gave a blood specimen than among those who did not (25.0%), and women who gave a blood specimen were slightly more likely to have a history of benign breast disease or a family history of breast cancer. These differences should not influence the internal validity of comparisons between cases and controls in the subcohort of women who gave a blood specimen, although the lower proportion of smokers in this subcohort does reduce the power of the analyses of smoking.

We defined cases as women who did not have a diagnosis of cancer (other than non-melanoma skin cancer) when they sent in the blood specimen, and who were subsequently diagnosed with breast cancer prior to June 1, 1994; 466 eligible cases occurred (392 invasive, 73 *in situ*, 1 uncertain histology). For each case we matched a control on year of birth, menopausal status, month of blood return, and postmenopausal hormone use. The study protocol was approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital.

Laboratory methods

Genotyping for *NAT2* was carried out using the PCR-RFLP method of Bell *et al.* (29) which detects the three *NAT2* slow acetylator alleles (30) (*NAT2**5A [M1], *NAT2**6A [M2], and *NAT2**7A [M3]) most commonly found in Caucasian-Americans. One case sample could not be genotyped. Laboratory personnel were blind to case-control status.

Table I. Descriptive characteristics of cases of breast cancer (n = 466) and controls (n = 466)

Variable	Cases	Controls	P value[a]
Age at blood draw (mean)	57.6 yrs	57.7 yrs	–
BMI (mean)	25.4	25.4	0.87
Age at menopause (mean)	48.5 yrs	47.9 yrs	0.18
Age at first birth (mean)[b]	25.2 yrs	24.9 yrs	0.48
Age at menarche (mean)	12.4 yrs	12.5 yrs	0.22
Parity nulliparous			
Nulliparous	6%	9%	0.16
1-2	36%	31%	–
>2	58%	60%	
Mother's history of breast cancer			
No	90%	94%	0.04
Yes	10%	6%	
Sister's history of breast cancer			
No	93%	96%	0.05
Yes	7%	4%	
History of benign breast disease			
No	43%	61%	0.001
Yes	57%	39%	
Current smoking status			
Never	42%	47%	0.28
Past	46%	42%	
Current	12%	11%	

[a]Signed rank test for continuous variables, McNemar's test for categorical variables.
[b]Among parous women.

Statistical analysis

We calculated odds ratios (hereafter referred to as relative risks) and 95% confidence intervals for the association of *NAT2* genotype with breast cancer using conditional logistic regression to assess whether the association with *NAT2* genotype was modified by past or recent smoking. Estimates for the effect of smoking for slow and rapid acetylators were calculated by including indicator variables for each category of smoking for each genotype in multivariate models including established breast cancer risk factors; the hypothesized low-low risk category (e.g. rapid acetylator non-smokers) was the reference category and was omitted from the model. Statistical significance of the interactions was assessed by using a likelihood ratio test (LRT) to compare the goodness of fit of the model with these interaction terms, with the reduced model containing indicator variables for the main effects of genotype and exposure (i.e. without interaction terms). For continuous exposure variables (e.g., pack-years of smoking), an interaction term in the multivariate model was included and the significance of the interaction was assessed with the Wald test. To assess effect modification by the matching variables age and menopausal status, we constructed separate unconditional models for different levels of these variables.

Results

The mean age of cases was 57.6 (±7.2) years (Table I); 76% of women were postmenopausal. Differences in established breast cancer risk factors were mostly in the expected direction; cases had a slightly higher age at first birth, and were more likely to have a history of benign breast disease and a family history of breast cancer. 97% of cases, and 95% of controls were Caucasian. The frequency of the three slow acetylator alleles among controls was very similar to that observed in previous studies of Caucasian-Americans (29). The frequency of slow acetylators was similar in cases and controls; the overall multivariate relative risk for the slow acetylation genotype was 0.9 (95%CI 0.7–1.2) (Table II). The relative risk (RR) for slow acetylation for women <60 years of age was 0.9 (95%CI 0.5–1.6), for women ⩾60 it was 1.1 (95%CI 0.7–1.6). Among 164 premenopausal women, the RR for slow acetylation was 0.7 (95%CI 0.3–1.6); among 706 postmenopausal women this RR was 1.0 (95%CI 0.7–1.4). We did not

observe evidence of significant interactions of *NAT2* genotype with family history of breast cancer, age at menarche, parity, age at first birth, history of benign breast disease, or body mass index. The relation of slow acetylation to breast cancer risk was similar for ductal carcinomas (332 cases) RR = 1.0 (95%CI 0.7–1.3), lobular carcinomas (42 cases) RR = 1.1 (95%CI 0.6–2.0); for invasive cases (391 cases) RR = 1.0 (95%CI 0.7–1.3) and *in-situ* cases (73 cases) RR = 0.9 (95%CI 0.5–1.4).

We observed a small and non-significant elevation in risk associated with current smoking immediately prior to diagnosis (multivariate RR = 1.4, 95%CI 0.7–2.6) among slow acetylators compared with never smoking rapid acetylators (*P*, interaction 0.79). When we further categorized by number of cigarettes smoked per day, there was minimal evidence of a dose-response relation among slow acetylators (Table III); (multivariate RR among smokers of 1-14 cigarettes per day = 1.1, 95%CI 0.4–3.0, multivariate RR among women smoking 15 or more = 1.5, 95%CI 0.7–3.2). The relative risk for slow acetylators smoking 15 or more cigarettes per day was reduced when examining smoking status 10 years prior to diagnosis, and there was no overall evidence for an interaction with genotype for smoking defined in this way (*P*, interaction 0.13) (Table IV); similar estimates were obtained for smoking status defined at enrollment in 1976 (13-16 years prior to diagnosis) (data not shown).

In analyses limited to the 164 premenopausal women we observed no indication of an increased risk among smokers immediately prior to diagnosis who were slow acetylators, however, the data were sparse with wide confidence intervals. Among 706 postmenopausal women the test for interaction between recent smoking and *NAT2* acetylation status was non-significant (*P* = 0.62). The elevated risk among current smokers who were slow acetylators compared with never smokers who were fast acetylators (multivariate RR = 1.8, 95%CI 0.9-3.6) was slightly stronger than in the data including premenopausal women, although the confidence interval was wide.

We observed little evidence of an interaction between genotype and total pack-years of smoking (*P* = 0.15) (Table V). Among slow acetylators, risks were non-significantly elevated in the highest categories of exposure compared with never smoking rapid acetylators; however, there was no strong evidence of a trend of increasing risk with increasing exposure among the slow acetylator group.

To examine the specific hypothesis that smoking prior to first pregnancy is a risk factor, we conducted analyses among parous women with complete information on early life smoking (19 parous cases, and 11 parous controls were missing this information) (Table VI). Among the rapid acetylator group, ever smokers before first pregnancy were at marginally significantly increased risk compared with never smokers (multivariate RR = 1.7, 95%CI 1.0–2.6), however, there was no evidence of a dose-response relation with number of years of smoking prior to first pregnancy. Similarly, among slow acetylators risk was increased among women who smoked for 1-5 years prior to first pregnancy, but was not elevated among women who smoked for 5 or more years. Average number of cigarettes smoked per day at initiation of smoking was not different for women in the two categories of duration of smoking before

Table II. *NAT2* genotype[a] and breast cancer risk among 466 cases and matched controls in the Nurses' Health Study

	Cases	Controls	RR (95% CI) Matched[b]	RR (95% CI) Multivariate[c]
Rapid	203	197	1.0	1.0
Slow[a]	262	269	1.0 (0.7–1.2)	0.9 (0.7–1.2)

[a]Participants were classified as slow acetylators if they were homozygous for any combination of the three slow acetylator alleles (*NAT2**5A, *NAT2**6A, *NAT2**7A); rapid acetylators were heterozygous or homozygous for the rapid acetylator allele. One case sample could not be genotyped.
[b]Conditional logistic regression model from case-control pairs matched on year of birth, menopausal status, month of blood return, and post-menopausal hormone use, fasting status at blood draw, time of day of blood draw.
[c]Conditional logistic regression model included terms for age at menarche (<12, 12–14, ⩾15 years), parity (0, 1–2, >2), age at first birth (⩽24, >24 years), BMI (<21, 21–<25, 25 <30, ⩾30 kg/m^2), family history of breast cancer in mother or sister(s) (yes, no), and history of benign breast disease (yes,no).

Table III. Relative risks and 95% CI for breast cancer risk stratified by *NAT2* genotype and smoking status immediately prior to diagnosis

Acetylation genotype		Smoking status			
		Never	Past	Current 1-14 cig. daily	Current 15+ cig. daily
Combined	Cases	198	213	21	34
	Controls	220	197	21	28
	Matched[a] RR	1.0 (ref.)	1.2 (0.9–1.6)	1.1 (0.6–2.1)	1.3 (0.8–2.3)
	Multivariate[b] RR	1.0 (ref.)	1.3 (0.9–1.7)	1.3 (0.6–2.5)	1.6 (0.9–2.8)
Rapid	Cases	88	95	10	10
	Controls	93	82	10	12
	Matched[a] RR[c]	1.0 (ref.)	1.2 (0.8–1.9)	1.1 (0.4–2.7)	0.9 (0.3–2.1)
	Multivariate[b] RR[c+]	1.0 (ref.)	1.2 (0.8–1.9)	1.1 (0.4–3.1)	1.2 (0.5–3.3)
Slow	Cases	109	118	11	24
	Controls	127	115	11	16
	Matched[a] RR[c]	0.9 (0.6–1.4)	1.1 (0.7–1.6)	1.1 (0.5–2.7)	1.6 (0.8–3.1)
	Multivariate[b] RR[c]	0.8 (0.6–1.3)	1.1 (0.7–1.7)	1.1 (0.4–3.0)	1.5 (0.7–3.2)

LRT for interaction: χ^2(3 df) = 0.43, *P* = 0.93.
[a]Conditional logistic regression analysis on case-control pairs matched as in footnote to Table 1.
[b]Conditional logistic regression analysis with terms for additional variables as described in footnote to Table I.
[c]All RR's are relative to never-smoking rapid acetylators.

Table IV. Relative risks and 95% CI for breast cancer risk stratified by *NAT2* genotype and smoking status 10 years prior to diagnosis

Acetylation status		Smoking status			
		Never	Past	Current 1–14 cig. daily	Current 15+ cig. daily
Combined	Cases	198	159	35	74
	Controls	221	154	34	57
	Matched[a] RR	1.0 (ref.)	1.2 (0.9–1.6)	1.1 (0.7–1.9)	1.5 (1.0–2.2)
	Multivariate[b] RR	1.0 (ref.)	1.2 (0.9–1.7)	1.3 (0.8–2.2)	1.6 (1.0–2.4)
Rapid	Cases	88	71	14	30
	Controls	93	61	21	22
	Matched[a] RR[c]	1.0 (ref.)	1.3 (0.8–2.0)	0.7 (0.3–1.4)	1.5 (0.8–2.8)
	Multivariate[b] RR[c]	1.0 (ref.)	1.3 (0.8–2.0)	0.7 (0.3–1.5)	1.8 (0.9–3.5)
Slow	Cases	109	88	21	44
	Controls	128	93	13	35
	Matched[a] RR[c]	0.9 (0.6–1.3)	1.1 (0.7–1.6)	1.8 (0.8–3.7)	1.4 (0.8–2.4)
	Multivariate[b] RR[c]	0.8 (0.6–1.3)	1.0 (0.7–1.6)	2.1 (0.9–4.8)	1.3 (0.7–2.3)

LRT for interaction: $\chi^2(3\ df) = 5.65$, $P = 0.13$.
[a]Conditional logistic regression analysis on case-control pairs matched as in footnote to Table 1.
[b]Conditional logistic regression analysis with terms for additional variables as described in footnote to Table I.
[c]All RR's are relative to never-smoking rapid acetylators.

Table V. Relative risks and 95% CI for breast cancer risk stratified by *NAT2* genotype and pack-years at diagnosis

Acetylation status		Pack-years[a]			
		0	0–<20	20–<30	⩾30
Combined	Cases	198	110	55	101
	Controls	220	112	38	94
	Matched[b] RR	1.0 (ref.)	1.1 (0.8–1.5)	1.6 (1.0–2.6)	1.2 (0.8–1.7)
	Multivariate[c] RR	1.0 (ref.)	1.2 (0.9–1.7)	1.8 (1.1–3.0)	1.3 (0.9–1.9)
Rapid	Cases	88	58	24	33
	Controls	93	44	16	42
	Matched[b] RR[d]	1.0 (ref.)	1.4 (0.9–2.3)	1.5 (0.8–3.1)	0.8 (0.5–1.4)
	Multivariate[c] RR[d]	1.0 (ref.)	1.6 (0.9–2.7)	1.6 (0.8–3.3)	0.8 (0.5–1.5)
Slow	Cases	109	52	31	68
	Controls	127	68	22	52
	Matched[b] RR[d]	0.9 (0.6–1.3)	0.9 (0.5–1.3)	1.5 (0.8–2.9)	1.4 (0.8–2.2)
	Multivariate[c] RR[d]	0.8 (0.5–1.3)	0.8 (0.5–1.4)	1.5 (0.8–3.1)	1.4 (0.8–2.3)

Wald test for interaction, $\chi^2(1\ df) = 2.07$, $P = 0.15$.
[a]Information on pack years of smoking was missing for 4 women.
[b]Conditional logistic regression analysis on case-control pairs matched as in footnote to Table I.
[c]Conditional logistic regression analysis with terms for additional variables as described in footnote to Table I.
[d]All RR's are relative to never-smoking rapid acetylators.

first pregnancy, suggesting the association with duration was unlikely to be confounded by intensity of smoking. Results were slightly stronger in analyses limited to postmenopausal women who were rapid acetylators (multivariate RR = 1.8, 95%CI 1.0–3.2 for ⩾5 years of smoking compared with never smoking prior to first pregnancy). When we limited the analyses involving the interactions of smoking and genotype to the 391 cases of invasive cancer and their controls, results were very similar to those including *in situ* cancers.

Discussion

In this prospective study *NAT2* acetylation status was not independently related to risk of breast cancer. The lack of association was similar within strata of age, menopausal status, and other breast cancer risk factors, suggesting that *NAT2* genotype does not interact with these established risk factors.

Previous studies of this association have been case-control studies mostly of smaller size than the current prospective study. In a study of 41 patients with advanced breast cancer, Bulovskaya *et al.* (21) reported that the prevalence of rapid acetylators was higher among these cases than among controls. In subsequent studies including 45–304 cases (22–27), the prevalence of rapid acetylators was similar in cases and controls. However, some previous reports have studied case series of prevalent rather than incident breast cancers; if acetylation status was associated with survival of breast cancer this would bias the estimate of genotype prevalence among the cases (31). Furthermore, genotyping methods were used in only two (26,27) of the previous studies; the other studies used phenotyping assays which may be influenced by breast cancer or chemotherapy (26). However, our prospective results are in good concordance with results from the case-control studies and suggest that any independent association of breast cancer with acetylation status is likely to be very weak.

Most previous studies did not assess whether associations with suspected breast cancer risk factors were modified by *NAT2* genotype. In a case-control study of 185 postmenopausal women in New York State, Ambrosone *et al*. (27) observed a

Table VI. Relative risks and 95% CI for breast cancer risk stratified by *NAT2* genotype and years smoked before pregnancy among parous women

Acetylation status		Years smoked before pregnancy		
		Never	1–<5 years	⩾5 years
Combined	Cases	181	87	149
	Controls	208	66	138
	Matched[a] RR	1.0 (ref.)	1.6 (1.1–2.4)	1.2 (0.8–1.6)
	Multivariate[b] RR	1.0 (ref.)	1.9 (1.2–2.8)	1.1 (0.8–1.6)
Rapid	Cases	75	37	66
	Controls	97	31	48
	Matched[a] RR[c]	1.0 (ref.)	1.5 (0.8–2.8)	1.6 (1.0–2.7)
	Multivariate[b] RR[c]	1.0 (ref.)	1.9 (1.0–3.6)	1.5 (0.9–2.6)
Slow	Cases	105	50	83
	Controls	111	35	90
	Matched[a] RR[c]	1.2 (0.8–1.8)	1.9 (1.1–3.4)	1.0 (0.7–1.6)
	Multivariate[b] RR[c]	1.1 (0.7–1.7)	2.0 (1.1–3.8)	0.9 (0.6–1.5)

Wald test for interaction: $\chi^2(1\ df) = 0.76$, $P = 0.38$.
[a]Conditional logistic regression analysis on case-control pairs matched as in footnote to Table I.
[b]Conditional logistic regression analysis with terms for additional variables as described in footnote to Table I.
[c]All RR's are relative to never-smoking rapid acetylators.

significant association between smoking >15 cigarettes per day and breast cancer risk limited to slow acetylators; the risk increased as the reference period for smoking increased from 2 years prior to diagnosis, to 10 and 20 years. In our study, the relative risk was only slightly increased among slow acetylators for current smokers compared with never smokers, and this increase was not statistically significant. The overall test for interaction of smoking immediately prior to diagnosis and *NAT2* genotype was not significant ($P = 0.93$). When smoking status was defined at 10 years prior to diagnosis to allow for latency of the effect of smoking, no substantial interaction was observed. In the analysis of pack-years smoked, the absence of strong evidence of a trend among slow acetylators of increasing risk with increasing exposure to cigarettes suggests that there is unlikely to be a substantial excess risk among long-term smokers who are slow acetylators. Over 20 epidemiologic studies have examined risk of breast cancer associated with current smoking at diagnosis and many of these have also assessed risk according to pack-years of smoking; the overall results indicate no association of cigarette smoking defined in these ways with breast cancer risk (11). As ~55% of Caucasians are slow acetylators, a substantial positive association in this subgroup should give rise to an attenuated, but still positive, result in studies which did not stratify by *NAT2* genotype, unless the increased risk among slow acetylators was balanced by a decreased risk among fast acetylators. We did not observe any evidence of this decreased risk for smokers who were fast acetylators.

Only a few epidemiologic studies have examined adolescent smoking behavior, or smoking prior to first pregnancy, with respect to breast cancer, and some have suggested increased risk (32,33), while others have been null (34–37). Although we observed a suggestion of an increase in risk associated with smoking prior to first pregnancy among rapid acetylators, the absence of a duration-response relation with increasing years of smoking argues against the biologic plausibility of the finding. Similarly, a significant positive association among slow acetylators who were smokers for 1–5 years prior to first pregnancy is likely to be a chance finding given that results were null for the larger group of women who smoked for >5 years.

Strengths of our study include its relatively large number of incident cases and its prospective design which should eliminate any recall bias in self-reported exposures such as cigarette smoking. Power to examine interactions of *NAT2* genotype and current smoking was somewhat limited however, by the lower proportion of current smokers in the subcohort of women who gave a blood specimen, leading to wide confidence intervals in some analyses, particularly those examining number of cigarettes smoked per day. Even although the study is relatively large, we were still limited in our power to examine interactions between genotype and smoking; the sample size needed to detect an interaction is typically at least four times larger than that needed to detect the main effect of a single variable (38). We had even less power to analyze these relations among premenopausal women separately, although there was no evidence of any positive associations or interactions among the 164 premenopausal women we did assess.

In summary, we observed little evidence of an association between *NAT2* genotype and breast cancer in a prospective study. Although we observed a modest increased risk among women who smoked prior to first pregnancy, the association was similar for both slow and rapid acetylators, and no duration–response association was apparent. We did not observe evidence of any substantial positive association of recent cigarette smoking with breast cancer risk among either slow or fast acetylators. These data suggest that *NAT2* acetylation status is not a major determinant of breast cancer risk, and that cigarette smoking is not a major risk factor for breast cancer among either slow or fast acetylators.

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