Cigarette Smoking, *N-Acetyltransferase 2* Genotypes, and Breast Cancer Risk: Pooled Analysis and Meta-analysis

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Abstract

Approximately 10 years ago, it was noted that smoking increased risk of breast cancer among women with *N-acetyltransferase 2* (*NAT2*) slow acetylation genotypes. This report was followed by a number of studies to address this question. We pooled data from 10 existing studies and also conducted a meta-analysis of 13 studies published from 1996 to October 2006 that were conducted among women, were published in English, and had adequate information on smoking and *NAT2* genotyping. Raw data were requested from authors. Unconditional logistic regression was done for pooled analysis, and random effect models was done for meta-analysis. Study heterogeneity was assessed, and sensitivity tests were done when subgroups were excluded from the analysis. In the pooled analysis, there was a significant interaction between smoking, *NAT2* genotype, and risk of breast cancer [pack-years (continuous variable, $P_{interaction}$ = 0.03)], with higher pack-years significantly associated with an increased risk of breast cancer among women with *NAT2* slow genotypes (pooled analysis relative risk, 1.49; 95% confidence interval, 1.08-2.04). These findings were supported by the meta-analysis including all studies; pack-years were significantly associated with risk among slow acetylators in a dose-dependent fashion (meta-analysis relative risk, 1.44; 95% confidence interval, 1.23-1.68 for ≥20 pack-years versus never smokers), but not among rapid acetylators. Similar relationships were noted for smoking status (ever, never) and duration of smoking. Our results show that cigarette smoking is associated with an increase in breast cancer risk among women with *NAT2* slow acetylation genotypes. Because slow *NAT2* genotypes are present in 50% to 60% of Caucasian populations, smoking is likely to play an important role in breast cancer etiology. (Cancer Epidemiol Biomarkers Prev 2008;17(1):15–26)

Background

Tobacco smoke constituents cause mammary tumors in rodents and affect carcinogenic pathways in human breast epithelial cells (1-5). Smoking-related DNA damage has been found in human breast tissue (6), and a number of studies have detected nicotine metabolites, mutagenic activity, and carcinogen-DNA adducts in human breast fluid, milk, tissue, and epithelial cells (7). However, the bulk of epidemiologic studies do not support an association between smoking and breast cancer risk (8). We hypothesized that the antiestrogenic effects of smoking may override potential carcinogenic effect and that associations with smoking may only be noted among women who are less capable of detoxifying tobacco smoke carcinogens (9). In 1996, we published the first report of results from an investigation of potential modification of associations between smoking and breast cancer risk by genotypes for *N-acetyltransferase 2* (*NAT2*; ref. 10). NAT2 is involved in the metabolism of aromatic amines, a major class of tobacco smoke carcinogens, and variant alleles in *NAT2* result in slow clearance of aromatic amines. In that case-control study, neither smoking nor *NAT2* genotype was independently associated with breast cancer risk, and there were no clear patterns of increased risk associated with smoking by *NAT2* genotypes among premenopausal women. However, among postmenopausal women, *NAT2* genotypes greatly modified the association of smoking with breast cancer risk. For slow acetylators, smoking 2, 10, and 20 years before the interview was associated with increased breast cancer risk in a dose-dependent manner, with more than a 4-fold increase in risk with smoking more than one pack of cigarettes per day 20 years before the interview. For rapid acetylators, there was a reduction in risk associated with smoking. Since those initial findings, a number of studies have been conducted to investigate associations between smoking, *NAT2* genotypes, and breast cancer risk. Because *NAT2* slow acetylation genotypes are present in ~50% to 60% of Caucasian populations (11), increased risk of breast cancer with smoking among this subset of the population would have a large public health effect. Herein, we report on a review of all epidemiologic studies conducted on this topic in the past 10 years and results from both pooled analysis and meta-analysis based on the data gathered from the majority of the original studies.

Received 6/30/07; revised 9/19/07; accepted 9/25/07.

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doi:10.1158/1055-9965.EPI-07-0598

Materials and Methods

Study Selection. We did a comprehensive literature search through Medline of all publications from 1996 to October 2006, using various combinations of the following keywords: *N-acetyltransferase 2, NAT2*, cigarette smoking, tobacco smoke, breast cancer, polymorphism, and genotype. References from relevant papers were also thoroughly examined for additional studies. We also contacted authors of papers that were in preparation at the time of analysis for inclusion of results. We confined the search to studies that investigated the relationship between cigarette smoking and breast cancer risk modified by *NAT2* genotypes, were conducted among women, and were published in English. Studies that only investigated smoking or *NAT2* in relation to breast cancer risk or studies without adequate information on cigarette smoking and *NAT2* genotypes were not included. In situations wherein multiple studies were published using the same data source, we included only the original study in our analysis. We also contacted corresponding authors of all identified eligible papers and requested raw data on age, smoking variables, menopausal status, *NAT2*, and case-control status. The study selection process is outlined in Fig. 1.

The initial search identified 16 studies, which included 2 hospital-based (12, 13) and 10 population-based case-control studies (10, 14-22), as well as four nested case-control studies in cohorts (23-26). Among these 16 studies, two (12, 21) had inadequate numbers of smokers in the case ($n \leq 4$) and control ($n \leq 1$) groups and were excluded from the analysis. The study by Delfino et al. (13) was excluded from analysis due to the inclusion of patients with benign breast disease in the control group and the lack of data on smoking exposure by *NAT2* status, resulting in 13 studies eligible for our analysis. Published data on associations between breast cancer risk and smoking variables stratified by *NAT2* and menopausal status were extracted and entered into meta-analysis. For the pooled analysis, raw data from 9 of 13 studies were provided, and one study contributed cross-tabulations of data. These data provision allowed pooled analysis at the individual level to evaluate potential associations between smoking and breast cancer risk by *NAT2* status, with adjustment for age and study design.

Data Analysis

Classification of NAT2*, Smoking, and Menopausal Status.* For genotyping of *NAT2*, all 13 studies ascertained three common variant alleles in the *NAT2* gene as slow acetylator alleles, i.e., *NAT2*5, NAT2*6*, and *NAT2*7*. Three studies also examined *NAT2*14* as a slow acetylator allele (16, 20, 25). *NAT2*4* was the common allele associated with rapid acetylation; other variants were also ascertained in several studies, including *NAT2*11, NAT2*12*, and *NAT2*13*, and classified as rapid acetylators (18, 19, 22, 24, 26). The frequencies for additional alleles included in some studies (*NAT2*14, NAT2*11, NAT2*12, NAT2*13*), however, are very low and are likely to have little effect on genotype classification. In all studies, rapid acetylators were defined as carriers of at least one of the rapid acetylator alleles. Three studies used more stringent criteria wherein only *NAT2*4* homozygotes were classified as rapid acetylators (16, 23, 25). In the studies by Millikan et al. (16) and Alberg et al. (25), women homozygous for *NAT2*4* were classified as rapid acetylators, and in the study by Lissowska et al. (22), women homozygous for *NAT2*4, NAT2*11, NAT2*12,* or *NAT2*13* were classified as rapid acetylators. In these three studies, intermediate acetylators were combined with rapid acetylators for data analysis. In our analysis, we defined rapid acetylators as carriers of at least one of the rapid acetylator alleles, consistent with the definition in most studies. For the pooled analysis, we adopted this same classification so that all studies had the same characterization of acetylation status.

Smoking status in all 13 studies was categorized as current, former, and never smokers, or as ever and never smokers. Age at smoking initiation, cigarettes smoked per day, years of smoking, and pack-years of smoking were also reported in most studies. In addition, seven studies measured passive smoke exposure, although different measurement instruments were used (13, 15-17, 19, 20, 25). In this analysis, we evaluated the effects of smoking status as ''never active versus ever active'' regardless of passive smoke exposure or as ''never active/passive versus ever active/passive'' for those studies wherein information on passive smoke exposure was available. In addition, we evaluated pack-years of smoking in association with breast cancer risk by categorizing a woman as never smoking, low pack-years (<20), or high pack-years (≥20) and evaluated duration by categorizing a woman as never smoking, fewer years smoking (<15 years), or longer duration smoking (≥15 years). Pack-years was defined as the average packs per day during smoking years times the number of years smoking; all studies used the same classification. Information on pack-years and duration of smoking as continuous variables was also available from studies that contributed raw data and was entered into pooled analysis. Other smoking measurements were not included in this analysis due to the limited data availability.

Natural or artificial menopause was used as the major criteria for the definition of menopausal status in all 13 studies, except in those by Krajinovic et al. (18), Egan et al. (14), and Alberg et al. (25). Age at menopause, as reported in four studies, ranged from 48 to 50 years for cases and 47 to 50 years for controls (10, 23, 24, 26). The similarity in defining menopausal status and minor fluctuation in the reported mean age at menopause made it reasonable to merge the raw data on menopausal status from the original studies.

Statistical Methods. Funnel plots with SE on the vertical axis as a function of effect size on the horizontal axis were used to explore the presence of publication bias (27). The degree of the funnel plot asymmetry was measured by one-sided Egger *P* values (28). Fail safe N was reported to depict the number of missing studies, which would be necessary to nullify the observed significant effects (29). All relative risks from meta-analysis (meta-RR) were computed from random effect models to account for heterogeneity in subgroup analysis (30). We applied random effect models in the meta-analysis, which account for both between-study and within-study variability, with the assumption that the included studies are a random sample from all possible

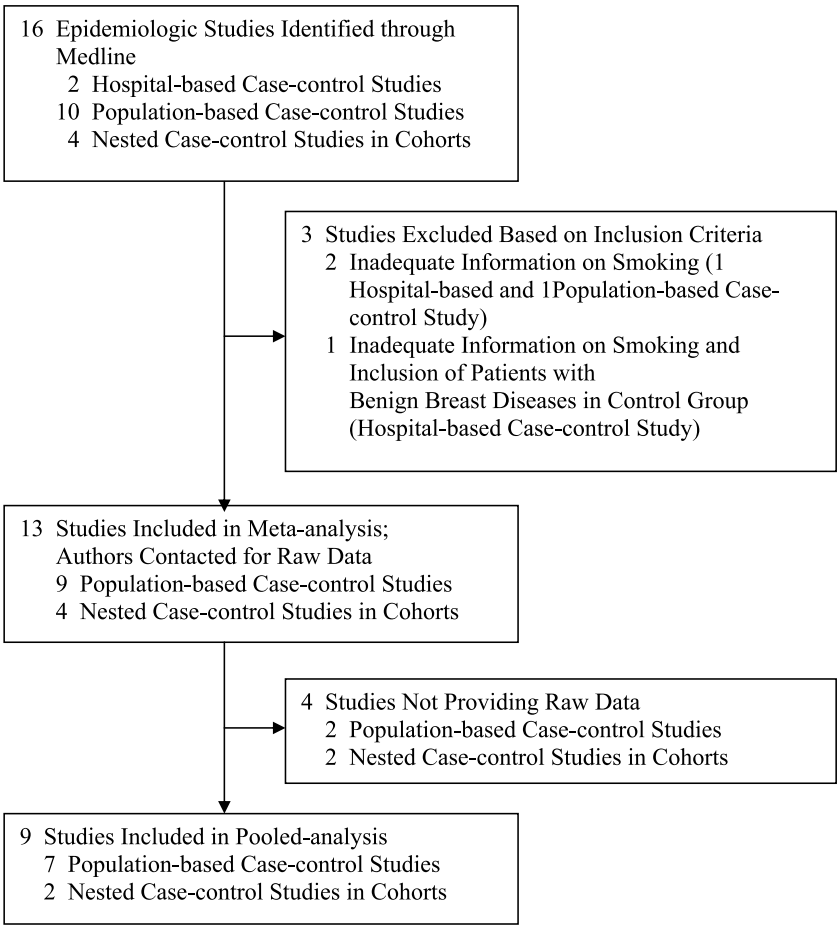


Figure 1. Study selection process.

studies. The extent of heterogeneity was examined by the Cochran's χ^2 test or Q test. Sensitivity analyses for the association between ever smoking and *NAT2* genotypes and breast cancer risk were done by evaluating changes in the meta-RRs in instances wherein studies without specific data availability were excluded, i.e., the exclusion of four studies without data for smoking exposure in pack-years (16, 18, 19, 24) and the exclusion of four studies not providing data for pooled analysis (19, 20, 23, 25). All meta-analyses were conducted using the Comprehensive Meta-analysis Version 2 (Biostat). For pooled analyses, we used unconditional logistic regression (PHREG procedure, SAS 9.1, SAS Institute) to calculate the pooled odds ratios (OR) and 95% confidence intervals (95% CI) based on the individual data, adjusting for age and study design. Interactions between *NAT2* genotypes and smoking were measured by including the multiplicative terms in the models and evaluated by the likelihood ratio test. In the pooled analysis, we also evaluated the joint effects of genotype and smoking on breast cancer risk by creating a new variable that incorporated data on both smoking and *NAT2* genotypes. For these analyses, we used never active smokers with rapid acetylator genotypes as the reference and contrasted other combinations of smoking and genotype against this reference group. Finally, population attributable risks were calculated as PAR = $p(r-1)/(p(r-1)+1)$, with p as prevalence of risk factor among controls and r as OR associated with risk factor (31). In addition, the population attributable risk related to interaction (PAR_i), defined as the proportional excess of disease attributed to the interaction of ever smoking and *NAT2* slow acetylation status over that which would have occurred if the susceptible genotype and exposure had acted independently according to a multiplicative model, was calculated (32).

Results

Meta-analysis. Table 1 shows the authors, location, study population, sample size, and variable definitions for menopausal status, cigarette smoking, and *NAT2* status, as well as the main findings of the 13 studies that were selected for analysis. In meta-analysis of these 13 studies, including 4,889 premenopausal and 7,033 postmenopausal women, there was no main effect of *NAT2* status on breast cancer risk (slow versus rapid acetylators: meta-RR 1.00, 95% CI 0.93-1.07). There was a slight increase in risk for ever active smoking versus never active smoking (meta-RR 1.17, 95% CI 1.07-1.27, fail-safe N 29), as well as a nonsignificant increase in risk for ever active/passive smoking versus never active/passive smoking (meta-RR 1.10, 95% CI 0.96-1.27). The funnel plot asymmetries for

Table 1. Epidemiologic studies on cigarette smoking, *NAT2* genotypes, and breast cancer risk included in the meta-analysis, 1996-2006

No.	Studies	Location, race, and case/control	Menopausal status	Smoking measures	*NAT2* definition	Main findings
1	Ambrosone et al., 1996*	USA, Caucasians, 304/327	Natural or artificial menopause if <50; cessation of menstruation if ≥50	(*a*) Daily smoking 2 and 20 y ago; (*b*) duration; (*c*) pack-years; (*d*) age at smoking initiation	(*a*) Rapid: ≥1 wt allele; (*b*) Slow: any combination of 2 of *NAT2**5, *NAT2**6, or *NAT2**7	Smoking associated with increased breast cancer among postmenopausal women with slow *NAT2* genotype
2	Hunter et al., 1997	USA, Caucasians, 466/466	Permanently ceased menstruation; hysterectomy and at the age that 90% cohort had natural menopause	(*a*) Smoking status (never, former, current); (*b*) smoking 10 y ago; (*c*) pack-years; (*d*) years smoked before pregnancy	(*a*) Rapid: two wt allele (homozygous); (*b*) Slow: any combination of two alleles of *NAT2**5A, *NAT2**6A, or *NAT2**7A	No significant associations; although suggestion of increased risk among postmenopausal smokers with *NAT2* slow genotype.
3	Millikan et al., 1998*	USA, Caucasians and African Americans, 498/473	As defined by Ambrosone et al., 1996	(*a*) Smoking status (never, former current); (*b*) cigarettes per day; (*c*) duration; (*d*) years of cessation; (*e*) passive smoking	(*a*) Rapid: two wt alleles (homozygous); (*b*) Intermediate: heterozygous for wt alleles; (*c*) Slow: any combination of two alleles of *NAT2**5, *NAT2**6, *NAT2**7, or *NAT2**14	*NAT2* rapid genotype was associated with an increased risk of breast cancer among postmenopausal women smoking within the past 3 y
4	Morabia et al., 2000*	Switzerland, Caucasians, 160/162	Last menses 1 y before interview, or bilateral oophorectomy	(*a*) Smoking status (never, ever passive, ever active, former, current); (*b*) cigarettes per day	(*a*) Rapid: ≥1 wt allele; (*b*) Slow: any combination of two alleles of *NAT2**5, *NAT2**6, or *NAT2**7	Passive and active smoking associated with increased breast cancer risk among women with rapid *NAT2* genotypes
5	Krajinovic et al., 2001	Canada, Caucasians, 149/207	Not defined	Smoking status (never and ever)	(*a*) Rapid: ≥1 wt in *NAT2**4 or *NAT2**12A; (*b*) Slow: any combination of two alleles of *NAT2**5, *NAT2**6A, or *NAT2**7B	No clear associations
6	Chang-Claude et al., 2002*	Germany, Caucasians, 422/887	Reported status 1 y before reference date; hysterectomy not accompanied by bilateral oophorectomy classified as unknown	(*a*) Smoking status (never, passive, former, current); (*b*) pack-years	(*a*) Rapid: ≥1 wt allele; (*b*) Slow: any combination of two alleles of *NAT2**5A, *NAT2**6A, or *NAT2**7B	Active smoking was associated with an increased risk of breast cancer among women with *NAT2* slow genotype, but passive smoke exposure was associated with an increased risk for those with *NAT2* rapid genotypes
7	Egan et al., 2003*	USA, Caucasians, 791/799	Not defined	(*a*) Smoking status (never, ever); (*b*) pack-years; (*c*) age at smoking initiation; (*d*) years of smoking before the first birth	(*a*) Rapid: ≥1 wt allele; (*b*) Slow: any combination of two alleles of *NAT2**5A, *NAT2**6A, or *NAT2**7A	Smoking associated with slightly increased risk of breast cancer among women with *NAT2* slow genotype
8	van der Hel et al., 2003*	The Netherlands, Caucasians, 229/264	Natural or artificial menopause	(*a*) Cigarettes per day; (*b*) duration of smoking	(*a*) Rapid: ≥1 allele of *NAT2**4, *NAT2**12 or *NAT2**13; (*b*) Slow: any combination of two of *NAT2**5A, *NAT2**6A, or *NAT2**7A; (*c*) Very slow: homozygous for *NAT2**5	Smoking associated with increased risk of breast cancer among women with *NAT2* slow genotype

(*Continued on the following page*)

Table 1. Epidemiologic studies on cigarette smoking, *NAT2* genotypes, and breast cancer risk included in the meta-analysis, 1996-2006 (Cont'd)

No.	Studies	Location, race, and case/control	Menopausal status	Smoking measures	*NAT2* definition	Main findings
9	Kocabas et al., 2004	Turkey, Turkish, 84/103	Natural menopause, or bilateral oophorectomy	Smoking status (never, active, passive)	(*a*) Rapid: ≥1 of *NAT2**4, *NAT2**12A, or *NAT2**12C; (*b*) Slow: any combination of two allele of *NAT2**5, *NAT2**6, or *NAT2**7	No associations
10	Alberg et al., 2004	USA, 110/113	Not defined	(*a*) Smoking status (never, passive, former, current); (*b*) pack-years	(*a*) Rapid: homozygous of wt alleles; (*b*) Intermediate: one low activity SNP; (*c*) Slow: any combination of two alleles of *NAT2**5, *NAT2**6, *NAT2**7, or *NAT2**14	Smoking associated with increased risk of breast cancer among women with *NAT2* slow genotype
11	Sillanpaa et al., 2005*	Finland, Caucasians, 483/482	Natural menopause, or bilateral oophorectomy; no menses and ≥51 y of age	(*a*) Smoking status (never active/passive, only passive, ex-active, current active); (*b*) pack-years	(*a*) Rapid: ≥1 wt allele; (*b*) Slow: any combination of two alleles of *NAT2**5, *NAT2**6, *NAT2**7, or *NAT2**14	Smoking associated with increased risk of breast cancer among women with *NAT2* slow genotype
12	van del Hel et al., 2005*	The Netherlands, Caucasians, 676/669	Natural or artificial menopause	(*a*) Smoking status (never, former, current); (*b*) cigarettes per day	(*a*) Rapid: ≥1 *NAT2**4, or *NAT2**12; (*b*) Slow: any combination of two alleles of *NAT2**5, *NAT2**6A, *NAT2**6C, or *NAT2**7B; (*c*) ery slow: homozygous for *NAT2**5	No associations
13	Lissowska et al., 2006*	Poland, Polish, 2386/2502	Premenopausal: <45 y Perimenopausal: 45-55 y Postmenopausal: >55 y	(*a*) Smoking status (never, former, current); (*b*) active and passive smoking	(*a*) Rapid: two of *NAT2**4, *NAT2**11, *NAT2**12, or *NAT2**13; (*b*) Intermediate: heterozygous for one rapid allele; (*c*) Slow: any combination of two alleles of *NAT2**5, *NAT2**6, or *NAT2**7	Ever smoking significantly associated with increased risk among women <45 y of age with *NAT2* slow genotype

*Studies that provided raw data for pooled analysis.

these associations were not statistically significant, with Egger *P* values of 0.18 and 0.24 for ever active smoking and ever active/passive smoking, respectively. When considering the potential modification of risk associated with smoking by *NAT2* status (Table 2), among slow acetylators, ever active smoking was significantly associated with increased breast cancer risk (meta-RR 1.27, 95% CI 1.16-1.39). There was no significant increase in risk associated with ever active smoking for those with rapid *NAT2* genotypes (meta-RR 1.05, 95% CI 0.95-1.17). These associations were not modified by menopausal status, and there was no apparent heterogeneity across all 13 studies. We also evaluated the risk of breast cancer associated with passive smoking by *NAT2* status from seven studies with this measurement (15-17, 19, 20, 22, 25) and did not observe modification by *NAT2* status (passive smoking versus never active/passive smoking: meta-RR 1.19, 95% CI 0.84-1.68 for rapid acetylators and meta-RR 1.13, 95% CI, 0.81-1.56 for slow acetylators). In these seven studies, however, there was considerable heterogeneity across the subgroups of rapid acetylators ($P = 0.03$ for rapid and $P = 0.18$ for slow acetylators; data not shown). This heterogeneity could, in part, explain the lack of associations for some studies and also precluded further stratified analysis by menopausal status.

In the 13 studies, pack-years of cigarette smoking were measured as a categorical variable in nine studies (10, 14, 15, 17, 20, 22, 23, 25, 26). A sensitivity analysis for risk associated with ever smoking by genotypes showed no differences between the estimates derived from these nine studies and all 13 studies; therefore, we used never active smokers as the reference group and dichotomized low and high pack-years at 20 for all studies except for that by Alberg and colleagues (25), which used 15 as the cutoff point. Exclusion of the study by Alberg et al. did not substantially change the estimates. The meta-RRs for low and high pack-years were 1.14 (95% CI, 1.03-1.25) and 1.26 (95% CI, 1.12-1.43), respectively, which were modified by *NAT2* genotypes. The meta-RRs for rapid acetylators were close to unity and nearly identical for low (meta-RR 1.06, 95% CI 0.93-1.21) and high (meta-RR 1.04, 95% CI 0.87-1.25) pack-years (Fig. 2). Among slow acetylators, however, there was an increased risk for both low and high pack-years, with a trend of dose-dependent

effect (meta-RR 1.21, 95% CI 1.08-1.35 for low pack-years and meta-RR 1.44, 95% CI 1.23-1.68 for high pack-years; Fig. 2). Further stratification of low pack-years into 1 to 9 and 10 to 19 categories among slow acetylators did not substantially change risk estimates (meta-RR 1.26, 95% CI 1.04-1.52 for 1 to 9 pack-years and meta-RR 1.24, 95% CI 0.99-1.54 for 10-19 pack-years, respectively).

Table 3 shows the risk of breast cancer associated with pack-years of smoking by *NAT2* status by menopausal status among the eight studies for which menopausal data were available (10, 14, 15, 17, 20, 22, 25, 26). Among *NAT2* slow acetylators, there was a significantly increased risk associated with pack-years in both premenopausal and postmenopausal women, and the risk estimates did not vary between these two groups (high pack-years versus never active smoking: meta-RR 1.47, 95% CI 1.08-2.01 for premenopausal women and meta-RR 1.41, 95% CI 1.15-1.72 for postmenopausal women). Among *NAT2* rapid acetylators, risk was not increased among either premenopausal or postmenopausal women for low or high pack-years. Similar to pack-years, duration of smoking was significantly associated with an increased risk of breast cancer among both premenopausal and postmenopausal women with *NAT2* slow genotypes, and risk estimates did not vary between these two groups (≥15 years versus never active smoking: meta-RR 1.35, 95% CI 1.11-1.65 for premenopausal women and meta-RR 1.40, 95% CI 1.11-1.76 for postmenopausal women). Among women with rapid *NAT2* genotypes, there were no associations between duration of smoking and breast cancer risk, regardless of menopausal status. In all subgroup analyses for pack-years and duration, no significant heterogeneity was observed.

Pooled Analysis. We also did pooled analysis to evaluate the risk of breast cancer associated with smoking status, pack-years, and duration by menopausal status and *NAT2* genotypes, based on 5,201 cases and 5,829 controls from nine studies that provided individual data (10, 14-18, 22, 24, 26). In the pooled analysis, there were significant interactions between *NAT2* genotypes and smoking status (ever active versus never active, $P_{interaction}$ = 0.02), pack-years (continuous variable, $P_{interaction}$ = 0.03), and duration (continuous variable, $P_{interaction}$ = 0.007) and significant dose-dependent effects for pack-years (continuous variable, P_{trend} = 0.0001) and duration (continuous variable, P_{trend} = 0.0001) among slow but not rapid acetylators.

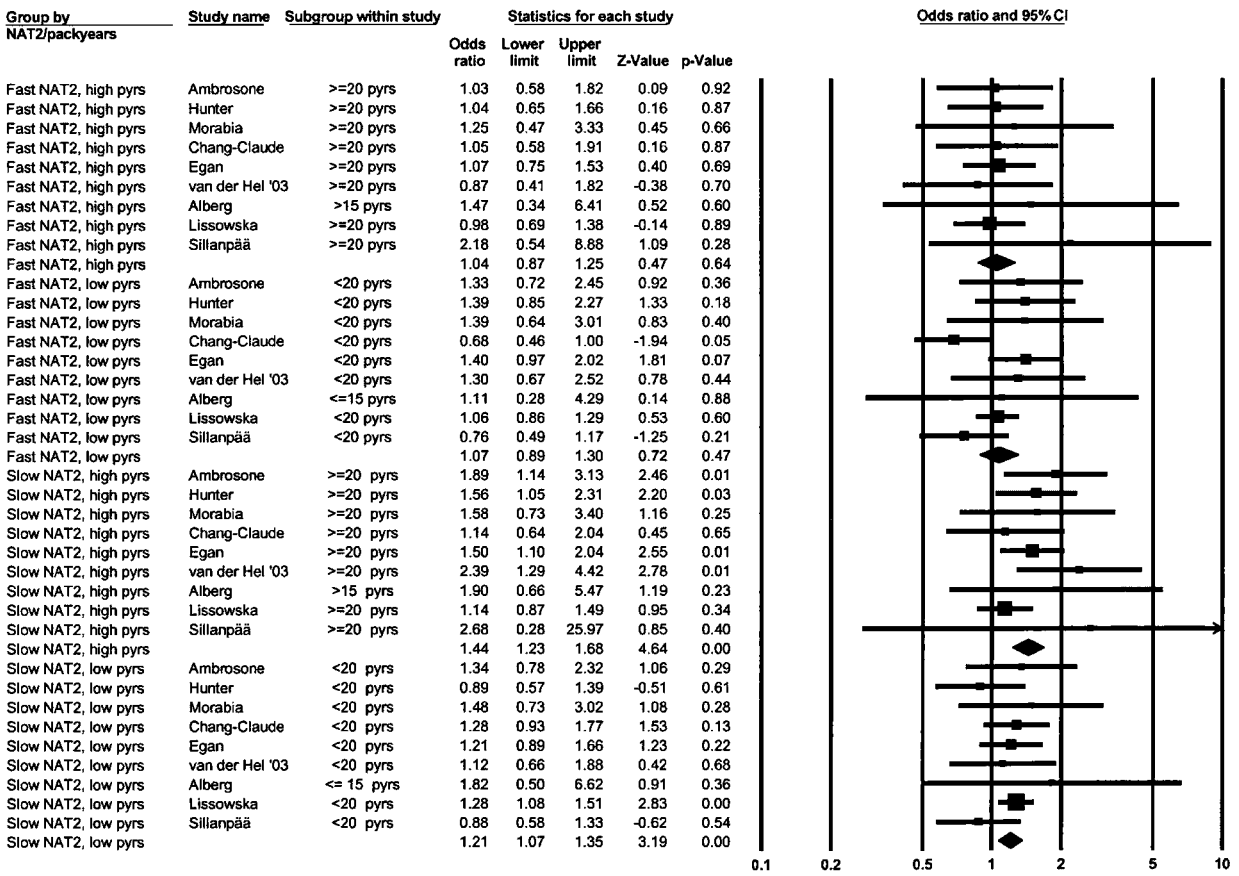
As shown in Table 4, the risk estimates from pooled analysis adjusting for age and study design were highly consistent with those from meta-analysis. Specifically, there was a significantly increased risk associated with ever active smoking among slow acetylators, regardless of menopausal status [relative risk from pooled analysis (pooled-RR) 1.18 (1.00-1.38) versus meta-RR 1.28 (1.09-1.50) for premenopausal women; pooled-RR 1.26 (1.11-1.44) versus meta-RR 1.34 (1.17-1.53) for postmenopausal women]. For pack-years, high pack-years were significantly associated with an increased risk of breast cancer among both premenopausal and postmenopausal women with *NAT2* slow genotypes [pooled-RR 1.49 (1.08-2.04) and meta-RR 1.47 (1.08-2.01) for premenopausal women; pooled-RR 1.42 (1.16-1.74) and meta-RR 1.41 (1.15-1.72) for postmenopausal women]. Longer duration of smoking (≥15 years) was significantly associated with an increased risk of breast cancer among both premenopausal and postmenopausal women with *NAT2* slow genotypes [pooled-RR 1.25 (1.02-1.54) versus meta-RR 1.35 (1.11-1.65) for premenopausal women; pooled-RR 1.31 (1.11-1.54) versus meta-RR 1.40 (1.11-1.76) for postmenopausal women]. Among women with *NAT2* rapid genotypes, no significant increase in risk was observed for ever active smoking, pack-years, or duration in either premenopausal or postmenopausal women.

We also created new variables combining *NAT2* genotypes with smoking status, pack-years, and duration of smoking and computed ORs using never smokers with rapid *NAT2* genotypes as the reference for each of these variables. As shown in Table 5, results from these analyses are consistent with those in the stratified analyses, with increased risk observed among women

Table 2. Meta-analysis of ever active smoking and breast cancer risk by *NAT2* genotypes and menopausal status

Study	*NAT2* slow genotype			*NAT2* rapid genotype		
	All	Premenopausal	Postmenopausal	All	Premenopausal	Postmenopausal
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Ambrosone, 1996	1.61 (1.04-2.47)	1.45 (0.70-2.98)	1.71 (1.0-2.92)	1.17 (0.73-1.89)	1.94 (0.84-4.48)	0.90 (0.50-1.61)
Hunter, 1997	1.26 (0.89-1.77)	—*	—*	1.17 (0.79-1.73)	—*	—*
Millikan, 1998	1.15 (0.81-1.65)	0.94 (0.56-1.57)	1.42 (0.86-2.35)	1.17 (0.81-1.68)	1.08 (0.63-1.84)	1.26 (0.77-2.06)
Morabia, 1999	1.52 (0.84-2.75)	1.16 (0.49-2.75)	2.05 (0.90-4.63)	1.29 (0.66-2.53)	1.56 (0.54-4.49)	1.16 (0.48-2.84)
Krajinovic, 2001	0.68 (0.33-1.42)	0.69 (0.16-2.96)	0.68 (0.29-1.60)	1.86 (0.75-4.60)	4.89 (0.46-51.87)	1.70 (0.60-4.84)
Chang-Claude, 2003	1.26 (0.92-1.72)	1.23 (0.86-1.75)	0.50 (0.13-1.91)	0.75 (0.52-1.07)	0.77 (0.51-1.15)	0.78 (0.19-3.19)
Egan, 2003	1.35 (1.05-1.74)	1.19 (0.78-1.82)	1.48 (1.05-2.07)	1.21 (0.90-1.63)	0.76 (0.45-1.29)	1.52 (1.04-2.24)
van der Hel, 2003	1.47 (0.92-2.37)	1.22 (0.62-2.38)	1.77 (0.90-3.47)	1.09 (0.60-1.97)	0.96 (0.44-2.09)	1.36 (0.54-3.46)
Kocabas, 2004	0.41 (0.15-1.09)	—*	—*	0.47 (0.15-1.52)	—*	—*
Alberg, 2004	1.87 (0.84-4.14)	0.50 (0.07-3.85)	2.41 (1.0-5.82)	0.85 (0.36-2.0)	4.67 (0.70-31.04)	0.49 (0.18-1.33)
Sillanpää, 2005	0.94 (0.63-1.41)	0.99 (0.56-1.75)	1.08 (0.58-1.99)	0.84 (0.55-1.28)	1.02 (0.54-1.90)	0.83 (0.46-1.51)
van der Hel, 2005	1.42 (1.09-1.85)	1.50 (0.98-2.30)	1.18 (0.83-1.68)	1.04 (0.76-1.42)	1.14 (0.68-1.89)	0.90 (0.59-1.36)
Lissowska, 2006	1.26 (1.07-1.48)	1.45 (1.05-2.0)	1.23 (1.02-1.48)	1.06 (0.88-1.29)	1.37 (0.94-2.0)	1.01 (0.80-1.27)
Meta-RR	1.27 (1.16-1.40)	1.28 (1.09-1.50)	1.34 (1.17-1.53)	1.05 (0.95-1.17)	1.08 (0.89-1.30)	1.07 (0.92-1.24)
$P_{heterogeneity}$	P = 0.30	P = 0.89	P = 0.39	P = 0.61	P = 0.26	P = 0.51

NOTE: Reference group: never active smoking.
*Data not available.



Note: Reference group comprises never smokers.

Figure 2. Packyears and breast cancer risk by NAT2 status.

with slow *NAT2* genotypes, but not among those with rapid acetylator genotypes.

Finally, we calculated attributable risks that smoking and *NAT2* slow acetylator genotypes contribute to breast cancer risk and could be eliminated with reduction of smoking. In the meta-analysis, 11 per 100 cases of breast cancer among *NAT2* slow acetylation genotypes could be explained by ever smoking. In contrast, smoking contributed only two breast cancers per 100 women among rapid acetylator genotypes. Results did not vary by menopausal status. Using data from the pooled analysis, for both premenopausal and postmenopausal women, the attributable risk for the interaction of smoking and *NAT2* slow acetylation genotype was five additional cases of breast cancer per 100 above what would have occurred if genotype and smoking acted independently.

Discussion

In the present meta-analysis that involved 4,889 premenopausal and 7,033 postmenopausal women, we found that smoking status, but not *NAT2* genotype status, was independently associated with breast cancer risk. When stratified by *NAT2* genotypes, ever active smoking was significantly associated with an increased risk among women with slow, but not rapid, acetylator genotypes. Furthermore, pack-years and duration of smoking were associated with an increase in breast cancer risk in a dose-dependent fashion among women with slow *NAT2* genotypes, whereas no increase in risk was observed among rapid acetylators. The associations between breast cancer risk and smoking status, pack-years, and duration were not modified by menopausal status. The pooled analysis, which adjusted for age and study design, confirmed the results from meta-analysis, that smoking was associated with increased risk of breast cancer among both premenopausal and postmenopausal women with *NAT2* slow acetylator genotypes, but not among women with *NAT2* rapid acetylator genotypes. When we created a variable combining genotypes and smoking variables, results were similar in direction to those in the stratified analysis. Because of the complex biochemical pathways that result in induction of

Table 3. Meta-analysis of pack-years and duration of smoking and breast cancer risk by *NAT2* genotypes and menopausal status

Smoking variable	*NAT2* slow genotype				*NAT2* rapid genotype			
	Premenopausal		Postmenopausal		Premenopausal		Postmenopausal	
	Cases/controls	Meta-RR (95% CI)	Cases/controls	Meta-RR (95% CI)	Cases/controls	Meta-RR (95% CI)	Cases/controls	Meta-RR (95% CI)
Pack-years*								
Never active	390/579	1.00	755/879	1.00	278/399	1.00	580/624	1.00
<20	435/583	1.21 (1.00-1.45)	495/474	1.28 (1.08-1.50)	297/443	1.00 (0.80-1.24)	359/353	1.12 (0.93-1.36)
≥20	113/107	1.47 (1.08-2.01)	303/257	1.41 (1.15-1.72)	89/86	1.34 (0.94-1.89)	170/206	0.98 (0.77-1.26)
Duration †								
Never active	460/636	1.00	792/926	1.00	338/453	1.00	630/678	1.00
<15 y	285/374	1.12 (0.91-1.37)	290/260	1.34 (1.09-1.65)	205/276	0.99 (0.72-1.36)	224/205	1.28 (0.87-1.88)
≥15 y	326/372	1.35 (1.11-1.65)	559/525	1.40 (1.11-1.76)	230/306	1.08 (0.85-1.35)	368/403	1.04 (0.87-1.26)

*Pack-years as a categorical variable were available from the following eight studies for meta-analysis: Ambrosone et al., 1996; Morabia et al., 2000; Chang-Claude et al., 2002; Egan et al., 2003; van der Hel et al., 2003; Alberg et al., 2004; Sillanpaa et al., 2005; Lissowska et al., 2006. The cutoff point for pack-years in the study by Alberg et al. was 15.

† Duration of smoking as a categorical variable was available from the following eight studies for meta-analysis: Ambrosone et al., 1996; Morabia et al., 2000; Chang-Claude et al., 2002; Egan et al., 2003; van der Hel et al., 2003; Millikan et al., 1998; Sillanpaa et al., 2005; Lissowska et al., 2006. The cutoff point for duration of smoking in years in the study by Millikan et al. was 20.

additional metabolic enzymes, the antiestrogenic effects of smoking among some women, and greater carcinogenic potential for others, risk estimates from stratified analysis may be more accurate than those using interaction variables, however. Specifically, it may be most appropriate to evaluate the effects of smoking separately among women by genotype, because mechanisms of carcinogenesis may be different based on *N*-acetylation detoxification capabilities.

In our analysis, we did not observe apparent publication bias or influence of missing studies nor major heterogeneity across the selected studies. Among studies, classification of genotypes and genotyping methods did not substantially differ for the more commonly occurring polymorphisms. Our findings are consistent with those observed in urinary bladder cancer studies, wherein cigarette smoking has been found to be significantly associated with increased risk of bladder cancer among slow *NAT2* acetylators (33-36).

In 2004, Alberg et al. conducted a meta-analysis using published data from nine studies (10, 12, 14-17, 23, 25, 26). They found that ever smoking was significantly associated with an increased risk of breast cancer among slow (meta-RR 1.37, 95% CI 1.19-1.58) but not rapid acetylators (meta-RR 1.15, 95% CI 0.97-1.35), consistent with what we observed in this larger analysis. Stratifying by menopausal status, which was available in six studies (10, 12, 14, 16, 25, 26), they found that the increased risk associated with slow genotypes was confined to postmenopausal women (meta-RR 1.61, 95% CI 1.29-2.01 for postmenopausal women versus meta-RR 1.10, 95% CI 0.83-1.46 for premenopausal women). However, our study now adds an additional five studies (18-20, 22, 24), but excluded one (12), and shows that smoking was associated with increased risk of breast cancer in both premenopausal and postmenopausal women with slow genotypes. We also provide consistency by using a pooled analysis.

The findings of associations among both premenopausal and postmenopausal women are counter to those from many earlier studies, including our own. We had initially hypothesized that among premenopausal women, with higher levels of circulating estrogens, the carcinogenic effects of slowly detoxified aromatic amines would be outweighed by the inducing effects of tobacco smoke on hormone metabolism. This mechanism may still be plausible among some groups of women, and results in the meta-analysis and pooled analysis could be weighted by a few of the more recent studies, primarily those undertaken in Europe. In these studies wherein associations were noted among premenopausal, as well as postmenopausal, women, it is possible that other factors that could modify relationships, such as oral contraceptive use, industrial pollution, etc., could contribute to the effects in women with putatively higher estrogen levels.

More recently, Terry et al. (37) conducted a meta-analysis based on data from 13 published studies and reported increased risk of breast cancer associated with smoking among women with both rapid and slow acetylator genotypes (meta-RR 1.2, 95% CI 1.0-1.5 for rapid and meta-RR 1.5, 95% CI 1.2-1.8 for slow genotypes), although the strength of association was stronger among slow acetylators. In stratified analysis by menopausal status, the increased risk was observed among postmenopausal women with slow *NAT2* genotypes (meta-RR 2.4, 95% CI 1.7-3.3) but not the other subgroups, in contrast to the Alberg meta-analysis (25). It is likely that methodologic differences, such as different criteria in including a study for analysis or in combining subgroups of women into a single group for comparison, may underlie the somewhat different observations across the meta-analysis studies. For example, both the studies by Alberg et al. and Terry et al. found that cigarette smoking was associated with breast cancer risk only among postmenopausal women with *NAT2* slow genotypes, which is likely due to the similar data inclusion in these two studies. Both studies included the same five studies, except that the study by Alberg et al. included one additional study by Morabia et al. (15). Also, the study by Huang et al. was included in Alberg's meta-analysis, but not in Terry's or our study. The prevalence of smoking was extremely low in the study by Huang et al., and the ORs were only computable for premenopausal rapid acetylators (12). In addition, the study by

Table 4. Pooled analysis of smoking status, pack-years, and duration and breast cancer risk by *NAT2* genotypes and menopausal status

Smoking variable	*NAT2* Slow Genotype				*NAT2* Rapid Genotype			
	Premenopausal		Postmenopausal		Premenopausal		Postmenopausal	
	Cases/ controls	Pooled-RR (95% CI)	Cases/ controls	Pooled-RR (95% CI)	Cases/ controls	Pooled-RR (95% CI)	Cases/ controls	Pooled-RR (95% CI)
Smoking status*								
Never active	501/653	1.00	865/1061	1.00	381/459	1.00	678/749	1.00
Ever active	697/776	1.18 (1.00-1.38)	937/881	1.26 (1.11-1.44)	482/587	1.0 (0.83-1.20)	660/663	1.08 (0.92-1.26)
Pack-years †								
Never active	314/490	1.00	575/701	1.00	222/323	1.00	437/484	1.00
<20	421/567	1.05 (0.86-1.28)	491/489	1.23 (1.03-1.46)	288/422	0.91 (0.72-1.16)	347/343	1.10 (0.89-1.35)
≥20	115/110	1.49 (1.08-2.04)	291/249	1.42 (1.16-1.74)	85/87	1.29 (0.89-1.86)	163/201	0.88 (0.69-1.13)
Duration ‡								
Never active	400/570	1.00	647/809	1.00	298/395	1.00	517/569	1.00
<15 y	266/353	1.03 (0.84-1.27)	279/252	1.36 (1.11-1.67)	192/256	0.97 (0.76-1.24)	215/192	1.23 (0.97-1.55)
≥15 y	310/354	1.25 (1.02-1.54)	542/512	1.31 (1.11-1.54)	216/286	1.00 (0.78-1.27)	352/387	1.01 (0.83-1.22)

NOTE: Adjusted for age and study design.
*Smoking status was available from the following nine studies for pooled analysis: Ambrosone et al., 1996; Millikan et al., 1998; Morabia et al., 2000; Krajinovic et al., 2001; Chang-Claude et al., 2002; Egan et al., 2003; van der Hel et al., 2003; van der Hel et al., 2005; Lissowska et al., 2006.
† Pack-years as a categorical variable were available from the following six studies for pooled analysis: Ambrosone et al., 1996; Morabia et al., 2000; Chang-Claude et al., 2002; Egan et al., 2003; van der Hel et al., 2003; Lissowska et al., 2006.
‡ Duration of smoking as a categorical variable was available from the following seven studies for pooled analysis: Ambrosone et al., 1996; Millikan et al., 1998; Morabia et al., 2000; Chang-Claude et al., 2002; Egan et al., 2003; van der Hel et al., 2003; Lissowska et al., 2006. The cutoff point in the study by Millikan et al. was 20 y.

Terry et al. included two studies based on the same population (17, 38) and used smokers at the highest level of smoking duration to compare with never smokers. If smoking duration was not available, then pack-years, cigarettes smoked per day, current smokers, or ever smokers were used alternatively in that order of priority.

Our study builds upon these previous meta-analyses with a more comprehensive and thorough assessment of smoking variables and inclusion of a recent large study from Poland (>2,000 cases and 2,000 controls; ref. 22). Importantly, we also did a pooled analysis of raw data from >5,000 cases and 5,000 controls to corroborate the meta-analysis and found consistent results. For pack-years, point estimates and confidence intervals were very similar using both approaches, with ORs for the highest tertile of pack-years of 1.47 and 1.49 in the meta-analysis and pooled analysis, respectively, for premenopausal women and 1.41 and 1.42, respectively, for postmenopausal women. For years of smoking, ORs were slightly higher in the meta-analysis, 1.35 for premenopausal women and 1.40 for postmenopausal women, and ORs were 1.25 and 1.31, respectively, in the pooled. It is

Table 5. Pooled analysis of smoking status, pack-years, and duration and breast cancer risk by *NAT2* genotypes and menopausal status, using never smokers with rapid NAT2 genotype as the reference group

Smoking variable	Premenopausal				Postmenopausal			
	NAT2 slow genotype		*NAT2* rapid genotype		*NAT2* slow genotype		*NAT2* rapid genotype	
	Cases/ controls	Pooled-RR (95% CI)	Cases/ controls	Pooled-RR (95% CI)	Cases/ controls	Pooled-RR (95% CI)	Cases/ controls	Pooled-RR (95% CI)
Smoking status*								
Never active	501/653	0.95 (0.80-1.14)	381/459	1.00	865/1061	0.90 (0.79-1.03)	678/749	1.00
Ever active	697/776	1.12 (0.94-1.34)	482/587	1.00 (0.83-1.21)	937/881	1.15 (1.00-1.33)	660/663	1.06 (0.91-1.24)
Pack-years †								
Never active	314/490	0.89 (0.72-1.10)	222/323	1.00	575/701	0.88 (0.75-1.03)	437/484	1.00
<20	421/567	0.96 (0.78-1.19)	288/422	0.87 (0.69-1.10)	491/489	1.09 (0.91-1.30)	347/343	1.09 (0.89-1.33)
≥20	115/110	1.36 (0.98-1.89)	85/87	1.23 (0.86-1.77)	291/249	1.26 (1.02-1.56)	163/201	0.87 (0.69-1.11)
Duration ‡								
Never active	400/570	0.94 (0.77-1.15)	298/395	1.00	647/809	0.88 (0.75-1.03)	517/569	1.00
<15 y	266/353	0.98 (0.79-1.23)	192/256	0.96 (0.75-1.22)	279/252	1.21 (0.98-1.49)	215/192	1.23 (0.97-1.54)
≥15 y	310/354	1.18 (0.95-1.47)	216/286	0.99 (0.78-1.26)	542/512	1.16 (0.98-1.38)	352/387	1.00 (0.83-1.21)

NOTE: Adjusted for age and study design.
*Smoking status was available from the following nine studies for pooled analysis: Ambrosone et al., 1996; Millikan et al., 1998; Morabia et al., 2000; Krajinovic et al., 2001; Chang-Claude et al., 2002; Egan et al., 2003; van der Hel et al., 2003; van der Hel et al., 2005; Lissowska et al., 2006.
† Pack-years as a categorical variable were available from the following six studies for pooled analysis: Ambrosone et al., 1996; Morabia et al., 2000; Chang-Claude et al., 2002; Egan et al., 2003; van der Helet al., 2003; Lissowska et al., 2006.
‡ Duration of smoking as a categorical variable was available from the following seven studies for pooled analysis: Ambrosone et al., 1996; Millikan et al., 1998; Morabia et al., 2000; Chang-Claude et al., 2002; Egan et al., 2003; van der Hel et al., 2003; Lissowska et al., 2006. The cutoff point in the study by Millikan et al. was 20 y.

possible that relationships were stronger in the meta-analysis because of the larger numbers of cases and controls. Alternatively, adjustment for age in the pooled analysis may have weakened observed relationships between breast cancer risk and number of years smoked among slow acetylators. Nonetheless, the consistency of findings in pooled analysis and meta-analysis provides substantial support for the assertion that smoking is related to breast cancer among women with slow *NAT2* genotypes, which comprises ~60% of Caucasian populations.

There is a clear biological rationale for investigating modification of breast cancer risk associated with smoking by *NAT2* genotypes. Aromatic amines are a major class of tobacco carcinogens, which include 2-naphthylamine and 4-aminobiphenyl, two compounds known to cause cancer in both animals and humans (39, 40). Epidemiologic studies have provided consistent evidence for a causal relationship between aromatic amines and urinary bladder cancer (41-43), and experimental studies have shown that aromatic amines are mammary carcinogens in rodents and humans (44-47). The metabolism of aromatic amines includes the activation and detoxification by metabolic enzymes, such as cytochrome P450s and *N*-acetyltransferase, and the *N*-acetylation process by NAT2 is an important detoxification step for aromatic amines (48). Animal models have shown that, upon exposure to aromatic amines, DNA adducts in prostate and bladder tissues were significantly higher in animals with *NAT2* slow genotypes (48), and human bladder cancer studies have shown that concentrations of 4-aminobiphenyl-hemoglobin adducts in the blood are significantly higher in slow acetylators than in rapid acetylators, particularly at low levels of carcinogenic exposure (49). In a study of breast tissue, women who smoked and had slow *NAT2* genotypes had significantly higher levels of aromatic DNA adducts in breast tissue than women who never smoked and had rapid *NAT2* genotypes (50).

The lack of strong associations between cigarette smoking and increased risk of breast cancer in epidemiologic studies may be partly due to the potential antiestrogenic effect of smoking (51-55) and the low carcinogenic dose that may reach the breast tissue from tobacco smoke (2). Although the relationship between cigarette smoking and breast cancer risk may continue to be a subject of debate, increasing evidence from molecular epidemiologic studies, particularly meta-analyses and pooled analyses, indicates that subgroups of women defined by genetic predisposition may be at higher risk of breast cancer if they are exposed to tobacco smoke (37). Findings from this large meta-analysis and pooled analysis support a positive relationship between cigarette smoking and breast cancer risk among women with slow *NAT2* genotypes.

In this analysis, we included a large sample of premenopausal and postmenopausal women, but were not able to evaluate the study by Hunter et al. (23) by menopausal status due to lack of stratified analysis in the paper, although that study population was mostly postmenopausal women. The raw data from that study were also not available for the pooled analysis. However, the results from the meta-analysis, which included the Hunter study, were similar to those for the pooled analysis, increasing our confidence that pooled results were not biased by the lack of data from this study. Furthermore, due to the variability in the measurement of passive smoke exposure, we were not able to perform detailed analyses on the associations between passive smoking and breast cancer risk, although some studies indicated that passive smoke exposure might be important in breast cancer etiology (8).

In the pooled analysis and meta-analysis, we were not able to control for potential confounders of relationships between smoking, *NAT2* genotype, and breast cancer risk. However, the individual studies did control for confounders, and unadjusted and adjusted ORs were not significantly different. Thus, it is likely that relationships observed in our analyses would not be subject to extensive confounding.

There were some differences in classification of *NAT2* genotypes, with five studies genotyping for *NAT2*12* and *NAT2*13* as rapid acetylation alleles (18, 19, 22, 24, 26). These alleles occur in ~2% to 3% of the population, and in recent years, this classification has become a subject of debate in light of the mixed reports regarding their phenotypic activities (56-59). Although these three additional ''rapid acetylator'' alleles were not examined in most of the studies and so may introduce some misclassification of rapid acetylators as slow, the effect is likely minor because of the low prevalence of these alleles. Furthermore, there is no reason to believe that genotyping misclassification using additional alleles would be different by case-control status; thus, this nondifferential misclassification would likely bias the results to the null. Furthermore, some studies created three acetylator categories, examining a gene-dose effect, with those heterozygous for rapid alleles (*NAT2*4, NAT2*11, NAT2*12,* or *NAT2*13*) classified as intermediate acetylators. While conceptually appropriate, this is not consistent with classification by phenotyping studies. In our analysis, we defined rapid acetylators as carriers of at least one of the rapid acetylator alleles. We did not have individual SNP data for the pooled analysis to assess effects of intermediate acetylators; however, in most studies, intermediate acetylators were subsequently combined with rapid acetylators for analysis in relation to risk.

Nondifferential misclassification could also result from potential genotyping errors. Although some studies reported quality control approaches to genotyping, such as running duplicate samples for some or all of the specimens, this information was not available for all studies. However, similar to potential misclassification of acetylator status based on additional alleles as addressed above, it is likely that this misclassification, if any, would also be nondifferential, with bias equally possible for cases as for controls, leading the analysis to yield null results.

In addition, in our analysis, we could not distinguish between different types of cigarettes. It has been reported that air-cured (black) cigarettes contain higher levels of aromatic amines, and smokers of air-cured cigarettes have higher circulating levels of 4-aminobiphenyl-hemoglobin adducts than those who smoke flue-cured cigarettes (60). Clearly, a precise measurement of exposure to carcinogens from different types of cigarettes, as well as accurate classification of genotypes, may be helpful to understand the biological mechanisms when genes involved in metabolic pathways are assessed.

Conclusions

Findings from this meta-analysis and pooled analysis indicate that cigarette smoking is associated with an increase in breast cancer risk among women with *NAT2* slow acetylator genotypes. Because of the frequency of slow acetylator genotypes among non-Asian populations (50-60% in Caucasians, 35-40% in African-Americans), inferring a population attributable risk of five additional cases of breast cancer per 100 above what would have occurred if genotype and smoking acted independently, smoking cessation programs need to be further targeted to women as a means for preventing breast cancer.

Acknowledgments

We thank Sabine Behrens for her excellent technical assistance in data management and analysis and the investigators who contributed their data for pooled analysis.

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Cancer Epidemiol Biomarkers Prev 2008;17:15-26.