**Short Communication**

**NAT1*10 and NAT1*11 Polymorphisms and Breast Cancer Risk**

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**Abstract**
Several recent epidemiological studies examined the association of N-acetyltransferase (NAT) 1 and 2 genotypes and breast cancer risk. Taken together, these studies do not support a strong role for the most common NAT alleles in etiology of breast cancer. Only one study estimated odds ratios (ORs) for the relatively rare NAT1*11 allele: a strong positive association for the NAT1*11 allele and breast cancer was reported, as well as strong combined effects for NAT1*11-containing genotypes and two environmental factors, smoking and red meat consumption. To further address the association of NAT1*11 and breast cancer, an analysis was performed using previously collected data from the Carolina Breast Cancer Study, a population-based, case-control study conducted in North Carolina. The OR for NAT1*11-containing genotypes and breast cancer was 0.5 (95% confidence interval, 0.2–1.3) among white women; ORs were not calculated among African Americans because only one participant exhibited the NAT1*11 allele. There was no evidence for combined effects of NAT1*11 and smoking. Unfortunately, the results of both studies of NAT1*11 are imprecise and lack sufficient statistical power to address fully the potential contribution of NAT1*11 to breast cancer. These results illustrate that the limitations imposed by sample size, as well as incomplete knowledge of biological function, need to be considered when planning and interpreting studies of genetic polymorphisms and environmental exposures.

**Introduction**
A recent study by Zheng et al. (1) examined the role of NAT3 genetic polymorphisms and breast cancer risk. NAT1 and NAT2 are involved in detoxication of aryl amines found in tobacco smoke and in activation of heterocyclic amines found in cooked meat. The relationship between NAT genotype and breast cancer risk has been examined in several recent studies (2–7). Several of these studies reported interactions between NAT genotype and environmental factors (smoking or diet), but these interactions were observed among subgroups (e.g., pre- or postmenopausal women). The results are not consistent across studies, and, taken together, they do not support a strong role for NAT1 or NAT2 genotypes in risk of breast cancer.

Zheng et al. (1) reported a positive association between the NAT1*11 allele and breast cancer risk, as well as strong combined effects for NAT1*11 genotype and two environmental exposures, cigarette smoking and red meat consumption. NAT1 encodes a variety of alleles, including NAT1*3, NAT1*4, NAT1*10, and NAT1*11. Correlations between NAT1 genotype and metabolic phenotype are poorly understood (reviewed in Ref. 5). However, recent evidence suggests that the enzyme encoded by the NAT1*11 allele exhibits increased metabolic activation of N-hydroxy aromatic amines, relative to protein products of the NAT1*3 and NAT1*4 alleles (reviewed in Ref. 1). In the study of Zheng et al. (1), the OR for NAT1*11/any genotype (presence of one or more copy of the NAT1*11 allele) compared to NAT1*3- or NAT1*4-containing genotypes (the more common alleles) was 3.9 (95% CI, 1.5–10.5). This OR is based on 11 cases and 7 controls. The OR for the combination of NAT1*11/any genotype and ever smoking (compared with the combination of NAT1*3- or NAT1*4-containing genotypes and never smoking) was 13.2 (95% CI, 1.5–116.0), based on five cases and one control with both exposures. The OR for the combination of NAT1*11/any genotype and the highest tertile of red meat consumption (compared with the combination of NAT1*3- or NAT1*4-containing genotypes and the lowest tertile of meat consumption) was 6.1 (95% CI, 1.1–33.2), based on five cases and two controls with both exposures. In contrast, the OR for NAT1*10/any genotype (compared with NAT1*3- or NAT1*4-containing genotypes) was 1.3 (95% CI, 0.8–1.9). The OR for the combination of NAT1*10/any genotype and smoking (compared with the combination of NAT1*3- or NAT1*4-containing genotypes and never smoking) was 1.4 (95% CI, 0.7–2.9), and the OR for the combination of NAT1*10/any genotype and high levels of red meat consumption was 1.6 (95% CI, 0.6–3.1).

This study is the first to report a positive association between the NAT1*11 allele and risk of cancer of any site and is also the first to investigate interactions with environmental exposures.

To further examine the association of NAT1*11 and NAT1*10 genotypes and breast cancer, previously collected data from the Carolina Breast Cancer Study, a population-based, case-control study of breast cancer, were used (5).

**Materials and Methods**
The study design of the Carolina Breast Cancer Study and methods for genotyping of NAT1 and NAT2 have been described previously (5). ORs for breast cancer and 95% CI were calculated using unconditional logistic regression models to examine associations for NAT1*10 and NAT1*11 alleles. PROC GENMOD of the software package SAS (version 6.11; SAS Institute, Cary, NC) was used to incorporate offset terms derived from sampling probabilities used to identify eligible participants and to adjust for age (as an 11-level ordinal vari-

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3 The abbreviations used are: NAT, N-acetyltransferase; CI, confidence interval; OR, odds ratio.


Results

ORs for NAT1*10/any and NAT1*11/any genotypes and breast cancer, adjusted for age and family history of breast cancer using logistic regression, are presented in Table 1. There was no association for NAT1*10/any genotype and breast cancer in African Americans or whites, and an inverse association for NAT1*11/any genotype and breast cancer was seen in whites. The latter estimate was very imprecise due to the low frequency of the NAT1*11 allele. ORs for the joint effects of genotype and smoking are presented in Table 2. An inverse association with breast cancer for the combination of NAT1*11/any genotype and smoking was observed among whites, although the estimate was imprecise. The role of red meat intake was not addressed because this information was not collected in our study.

Discussion

Our results do not support a role for NAT1*10 or NAT1*11 alleles in breast cancer risk. Both our study and the study of Zheng et al. (1) observed ORs close to the null value for NAT1*10/any genotype and for the combined effects of NAT1*10/any genotype and ever smoking. However, in contrast to Zheng et al. (1), we did not observe strong main effects for NAT1*11/any genotype or evidence of combined effects of smoking and NAT1*11/any genotype.

There are several possible explanations for the differences between our results and those of Zheng et al. (1). First, there may be differences in the populations studied. Our study population included African Americans (n = 391) and whites (n = 563). We categorized as “white” seven Native Americans, three Asian Americans, and three women who listed their race as “multiracial.” ORs for whites did not differ after excluding these 13 individuals. The study population of Zheng et al. (1) was reported as “virtually all” Caucasian. In our study, frequencies of the NAT1*10 allele differed by race, but ORs for NAT1*10 genotype and breast cancer were similar in African Americans and whites (Table 1). The NAT1*11 allele was observed in only one African American. Our study population was approximately half premenopausal and half postmenopausal (5), whereas that of Zheng et al. (1) was entirely postmenopausal. We conducted additional analyses stratifying on the basis of menopausal status. ORs did not differ substantially in premenopausal versus postmenopausal women, although estimates were extremely imprecise. For example, in postmenopausal white women, the adjusted OR for NAT1*11/any genotype (compared with NAT1*4-containing genotypes) was 0.6 (95% CI, 0.1–2.7), and the OR for the combination of NAT1*11/any genotype and ever smoking (compared with the combination of NAT1*3- or NAT1*4-containing genotypes and never smoking) was 0.4 (95% CI, 0.04 – 4.41). Distributions of most traditional risk factors and associations with breast cancer were similar in the two studies (1, 5), and the time period for case ascertainment in the study of Zheng et al. (Ref. 1; 1992–1994) overlapped that of our study (1993–1996).

A second source of potential differences in results is that the methods for genotyping NAT1 differed in the two studies. Both studies used PCR/RFLP-based methods, but the assay of
Zheng et al. (1) detected several alleles not detected by our study: (a) NAT1*14; (b) NAT1*15; (c) NAT1*17; and (d) NAT1*22. These four alleles were quite rare in the study of Zheng et al. (1), and failure to include them in our analysis would not affect ORs for NAT1*10/any genotype and NAT1*11/any genotype. Allele frequencies (q) for NAT1*10 and NAT1*11 did not differ significantly among controls across the two studies. For NAT1*10, q = 0.17 for controls in Zheng et al. (1) and 0.21 for white controls in our study (P = 0.18, $\chi^2$ test). For NAT1*11, q = 0.01 for controls in Zheng et al. (1) and 0.02 for white controls in our study (P = 0.28). The similarity in allele frequencies among controls suggests that the laboratory methods were comparable for detecting these alleles. Methods for classifying dose and duration of smoking differed in the two studies, but similar definitions for “ever” and “never” smoking were used. Odds in both studies were adjusted for age and family history (defined in both studies as “one or more first-degree relatives with breast cancer”).

The most plausible explanation for the difference in results between our study and that of Zheng et al. (1) is random error. The difference in ORs for NAT1*11/any genotype is due to different estimates of allele frequency for NAT1*11 among cases [q = 0.04 for cases in Zheng et al. (1), and q = 0.01 among white cases in our study (P = 0.01)]. Because NAT1*11 alleles are rare, differences in allele frequencies among cases could have arisen due to chance. Assuming random error as an explanation for differences in results, homogeneity $P$s (8) were calculated comparing CIs derived from the study of Zheng et al. (1) and postmenopausal whites in our study. These tests provide strong evidence for heterogeneity: the $P$ comparing CIs for the OR for NAT1*11/any genotype (compared with NAT1*3- or NAT1*4-containing genotypes) was 0.06; and the $P$ comparing CIs for the OR for NAT1*11/any genotype and smoking (compared with NAT1*3- or NAT1*4-containing genotypes and never smoking) was 0.03. The sample sizes [308 cases and 656 controls for Zheng et al. (1) and 290 white cases and 273 white controls in our study] yield roughly 80% power to detect an OR of 3.0 or greater (0.33 or less) for genotypes with a frequency of 4% at a significance level of 0.05 (9). However, neither study had adequate power to estimate combined effects for NAT1*11 genotype and environmental factors. Several methods for estimating sample size for case-control studies of gene-environment interaction have been developed (10–12). Even the most optimistic of these methods suggest that over 2000 cases and 2000 controls would be needed to investigate interactions between NAT1*11/any genotype and smoking or red meat consumption. In addition, Rothman et al. (13) showed that the presence of even small amounts of genotype or exposure misclassification can increase sample size requirements substantially.

The need for large sample sizes is an important challenge for epidemiologists studying gene-environment interaction. The advent of high throughput techniques will allow investigators to conduct genotyping assays on large numbers of participants and increase power to estimate gene-environment interactions (15). Empirical Bayes’ methods may prove useful for addressing data sparseness and associations arising due to chance (16, 17). However, for loci such as NAT1, where knowledge of biological function and toxicokinetics is incomplete, studies of gene-environment interaction will continue to present significant challenges in interpretation. Repetition in multiple study populations may contribute little to causal inference. Thus, in addition to the problems of sample size and statistical power, the limitations imposed by incomplete knowledge of biological function need to be considered when planning and interpreting studies that estimate joint effects for genetic polymorphisms and environmental exposures.

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