The Association between Glutathione *S*-Transferase M1 Genotype and Polycyclic Aromatic Hydrocarbon-DNA Adducts in Breast Tissue

Andrew Rundle,[1] Deliang Tang, Jingzi Zhou, Stan Cho, and Frederica Perera

Division of Environmental Health Sciences, Joseph L. Mailman School of Public Health, Columbia University, New York, New York 10032

Abstract

A major goal in molecular epidemiology is to identify preventable environmental risk factors and susceptible subpopulations. In a hospital-based molecular epidemiological case-control study of breast cancer, we investigated the relationship between DNA damage from exposure to polycyclic aromatic hydrocarbons (PAHs) and susceptibility attributable to inherited deletion of the xenobiotic detoxifying gene, glutathione *S*-transferase M1 (*GSTM1*). Prior to breast surgery, women ($n = 227$) were enrolled and interviewed and donated a blood sample. PAH-DNA adduct levels were measured by immunohistochemistry in breast tissue samples retrieved from pathology blocks, and *GSTM1* genotype was determined by PCR using WBC DNA. The *GSTM1* analysis included 95 cases and 87 benign breast disease controls. *GSTM1* genotype was not associated with breast cancer case-control status (odds ratio = 0.73; 95% confidence interval, 0.37–1.44). However, the *GSTM1* null genotype predicted PAH-DNA adduct levels in malignant ($\beta = 0.407$; $P = 0.003$) and nonmalignant ($\beta = 0.243$; $P = 0.05$) breast tissue from cases. This relationship was not seen in tissue from controls ($\beta = 0.095$; $P = 0.341$). When tissue from controls was compared with tumor tissue from cases, there was a significant case-control difference in PAH-DNA adduct levels among women who were *GSTM1* null. There was no such case-control difference among women who were homozygous or heterozygous for *GSTM1*. There was an interaction between *GSTM1* and case-control status on adduct levels in breast tissue ($P = 0.002$). The results suggest that genetic susceptibility to the formation of PAH-DNA adducts in breast tissue may play a role in breast cancer development.

Introduction

In recent decades, the incidence of breast cancer has increased ~1% per year in the United States (1, 2). In the United States, one of nine women will receive a diagnosis of breast cancer by the age of 85, with an estimated 178,700 new cases and 43,500 deaths in 1998 (3). Because known risk factors account for only an estimated 40–50% of breast cancer cases in the United States (4), there is growing interest in the hypothesis that environmental contaminants may be playing a causative role (5–10). In the past, environmental cancer epidemiology has been hampered by difficulties in obtaining accurate data on individual exposures and on individual variation in response to carcinogens. The development of biomarkers has provided a tool that can circumvent these problems by providing individual measurements of the biological dose of carcinogens, preclinical effects, and susceptibility to cancer (11).

The subjects in the current study were participants in a molecular epidemiological hospital-based case-control study involving breast cancer cases, BBD[2] controls, and healthy controls. The parent study was designed to test the hypothesis that exposure to PAHs is associated with breast cancer and that the risk of exposure is modulated by genetic susceptibility factors. PAHs are known human carcinogens and are proven mammary carcinogens in experimental animals (5). We and others have shown that aromatic- and PAH-DNA adducts are present in breast tumor tissue and nontumor tissue from breast cancer cases as well as tissue from women with BBD and women who underwent reduction mammoplasty (6, 12). In results published elsewhere, we have shown that PAH-DNA adduct levels measured by immunohistochemistry in breast tumor tissue from cases and benign tissue from controls were associated with breast cancer case-control status, that adducts in tumor tissue were significantly correlated with those in nontumor tissue from cases (13), and that PAH-DNA adduct levels in tumor tissue were positively associated with ER expression. These findings were consistent with prior studies that found aromatic-DNA adducts measured by the ^{32}P post-labeling method to be higher in normal tissue from cases than in breast tissue from reduction mammoplasty controls (6, 12).

The *GSTM1* genotype was evaluated as a marker of individual susceptibility to PAH-DNA adduct formation and breast cancer development. The GSTs are a family of enzymes that are, in part, responsible for the detoxification of reactive electrophiles, including the PAH epoxide intermediates, through conjugation of the electrophile with glutathione. Among the several classes of GSTs, GSTμ enzyme activity has been found to vary substantially between individuals because of an inherited deletion of the *GSTM1* gene. Individuals with the null genotype are unable to detoxify PAHs through this particular glutathione pathway. Several studies have shown that individuals with the *GSTM1* null genotype are at increased risk for lung cancer, particularly if they are cigarette smokers (14–16).

Received 10/28/99; revised 6/6/00; accepted 7/13/00.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] To whom requests for reprints should be addressed, at Molecular Epidemiology Program, Columbia School of Public Health, 60 Haven Avenue, Room B-109, New York, New York 10032.

[2] The abbreviations used are: BBD, benign breast disease; PAH, polycyclic aromatic hydrocarbon; ER, estrogen receptor; GSTM1, glutathione *S*-transferase M1; CPMC, Columbia-Presbyterian Medical Center; DCIS, ductal carcinoma *in situ*; OR, odds ratio; CI, confidence interval; ETS, environmental tobacco smoke.

Several studies have looked at the relationship between *GSTM1* genotype and breast cancer risk (17–23) and between *GSTM1* and prognosis (17, 24), with inconsistent results. Most suggest that *GSTM1* by itself is not a risk factor for breast cancer.

The impact of *GSTM1* genotype on PAH-DNA adduct levels in breast tissue and on breast cancer risk is of interest given the prior detection of adducts in breast tissue and the observed association between carcinogen-DNA adducts and breast cancer status (12, 13, 17). Therefore, we assessed the relationship between *GSTM1* genotype and PAH-DNA adduct levels in breast tumor tissue, nontumor tissue from cases, and tissue from controls with BBD. The relationships between *GSTM1* genotype and case-control status, and between *GSTM1* genotype and various prognostic variables, were also examined.

Materials and Methods

Study Population. From 1994 to 1998, women referred for breast surgery at CPMC were enrolled in a hospital-based case-control study. Patients without prior cancer or prior treatment who were referred for breast surgery were eligible for the study. They were recruited prior to surgery through private practices and the breast clinic. A screening mammogram or participation in a breast cancer screening program was not an eligibility criterion. After informed consent had been obtained, during their preoperative tests patients took part in a structured interview covering established reproductive breast cancer risk factors, active and passive smoking, dietary practices, other environmental and occupational exposures, and vitamin consumption. Patients whose confirmed diagnosis was DCIS or invasive ductal or invasive lobular cancer were defined as cases. The cases had predominantly early-stage cancer (17% DCIS, 46% stage I, 34% stage II, and 3% stage III). Patients with rare tumors were not included because the small numbers precluded analyses by histological type. Patients diagnosed with BBD or BBD with hyperplasia were classified as controls. BBD patients whose diagnoses were other than these categories (*e.g.*, BBD with atypia or lobular carcinoma *in situ*) were excluded from analysis because of their increased risk of breast cancer. Thus, the control group represented women at average or only slightly increased risk for future breast cancer. Breast cancer patients seen at CPMC for follow-up surgery (*e.g.*, mastectomy or re-excisions) after an initial surgical biopsy at another hospital were excluded from the study. Additional exclusion criteria included a prior history of cancer at any site except basal skin cancer, current pregnancy, recent bone fractures, or recent breast-feeding. The last two exclusion criteria were included because these factors were thought to interfere with biomarkers used in other aspects of this study.

Laboratory Methods. To assess individual *GSTM1* genotype, DNA was extracted from blood leukocytes and analyzed by PCR as described previously (25). The primers used in the PCR mixture were G5 (5′-GAA CTC CCT GAA AAG CTA AAG C-3′) and G6 (5′-GTT GGG CTC AAA TAT ACG GTG G-3′; Ref. 25). Women who were homozygous (+/+) or heterozygous (−/+) for *GSTM1* were classified as *GSTM1* positive, and those who were homozygous deleted (−/−) were classified as *GSTM1* null.

The immunohistochemical assay for PAH-DNA adducts was carried out as described previously (26). The advantages of this assay are that it can be used with small tissue samples, that adduct levels in adjacent morphological structures in the same tissue can be evaluated, that it has good sensitivity and specificity for measuring DNA adducts, and that it can be applied to fixed paraffin-embedded samples (27). Briefly, using standard immunohistochemical techniques, tissue slides were incubated with anti-benzo[*a*]pyrene-diolepoxide-DNA monoclonal antibody 5D11, kindly provided by Dr. Regina Santella (Mailman School of Public Health, New York, NY). Staining was accomplished using a biotinylated antimouse secondary antibody (Vector Laboratories, Burlingame, CA), ABC reagents, and diaminobenzidine. Methyl green was used as a counter stain. Nuclear staining was quantified by absorbance image analysis with the Cell Analysis System 200 microscope (Becton Dickinson, San Jose, CA) running the Cell Measurement software. A total of 50 cells (five fields, with 10 cells per field scored) were measured on each tissue slide. Results are reported in absorbance units as described previously (26). The scored cells were selected to be representative, in terms of intensity, of the cells in the field. Serial tissue slices from laboratory control breast tissue specimens previously shown to have low and high staining for adducts were used as negative- and positive-control samples, respectively, and were run with every batch. As an additional negative control, in each batch a laboratory control sample was run without the primary antibody.

Statistical Methods. *GSTM1* was first evaluated as a predictor of case-control status. ORs and 95% CIs were calculated using logistic regression analysis, controlling for age, ethnicity, age at menarche, parity, age at first birth, history of breast feeding, family history of breast cancer, and alcohol consumption. ORs were also calculated separately in pre- and postmenopausal women.

GSTM1 was then evaluated as a predictor of PAH-DNA adduct levels in breast tissue. Data on PAH-DNA adduct levels were natural log-transformed to generate a normal distribution. Where natural log-transformed data were used, both arithmetic and geometric means are provided. Student's *t* test analysis was used to determine whether adduct levels were associated with *GSTM1* genotype status. Linear regression analyses evaluated the relationship between genotype and adduct levels (as a dependent variable), controlling for other potential predictors of adduct levels, including age, ethnicity, active smoking status, ETS exposure, charred food consumption, parity, and history of breast feeding. Initially, analyses were performed separately with data from tissue samples from BBD controls, and with data from tumor and nontumor tissue from cases. Linear regression analyses were also performed with the combined PAH-DNA adduct data from tumor and benign tissue as the dependent variable. In addition to a variable for *GSTM1* genotype, this model included independent variables for case-control status and for the interaction between case-control status and *GSTM1* genotype. A second model assessed this interaction while controlling for known breast cancer risk factors (age, ethnicity, early age at menarche, parity, age at first birth, history of breast feeding, alcohol consumption, and a family history of breast cancer) and PAH exposure variables (current smoking status, current exposure to ETS at work and/or home, and high consumption of charred meats).

Data on several prognostic factors (tumor size, stage, and erbB-2 and ER expression) were available in the pathology reports prepared by the CPMC Department of Pathology. *t* Tests were used to determine whether tumor size, erbB-2 expression, or ER expression differed by *GSTM1* genotype. ER expression was measured as the percentage of cells in a tissue section expressing ER. To dichotomize tumors into ER-positive or -negative groups, a cutoff of >10% of the cells staining positive was used. This is the same cutoff used by the Pathology Department for clinical purposes. The χ^2 test was used to

Table 1 PAH-DNA adducts in breast tissue by *GSTM1* genotype

	GSTM1 +/+, +/−		*GSTM1* −/−		
	Mean[a] (SD)	Geometric mean (SD)	Mean (SD)	Geometric mean (SD)	
Cases					
Tumor	0.39 (0.21) $n = 43$	0.33 (1.74)	0.61 (0.37) $n = 40$	0.50 (1.93)	$P = 0.003$[b]
Nontumor	0.37 (0.18) $n = 40$	0.33 (1.60)	0.50 (0.30) $n = 37$	0.43 (1.82)	$P = 0.050$
Benign controls	0.37 (0.18) $n = 41$	0.34 (1.57)	0.41 (0.18) $n = 43$	0.37 (1.59)	$P = 0.341$
		$P = 0.96$[c]		$P = 0.02$[c]	

[a] In absorbance units.
[b] P for t tests based on ln-transformed data (geometric means), comparing adduct levels in subjects who were *GSTM1* +/+, +/− *vs.* those who were *GSTM1*−/−.
[c] P for t tests based on ln-transformed data (geometric means), comparing cases (tumor tissue) *vs.* controls.

determine whether there was an association between tumor stage (0, 1, 2+) and *GSTM1* genotype.

Results

An extensive report on enrollment and patient demographics in the parent study has been published elsewhere (13). The BBD controls had a breast cancer risk profile that was similar to what would be expected for a control group drawn from the general population of women at risk for breast cancer. Because BBD without atypia is associated with a small increased risk of future breast cancer, the BBD control may have shared more risk factors with the case group than a representative group of healthy women not undergoing breast surgery. One apparent similarity between the BBD control group and the cases was the prevalence of women with a family history of breast cancer (18% in BBD controls and 20% among cases). However, to the extent that cases and controls shared risk factors (measured and unmeasured), if these risk factors were associated with adduct levels or *GSTM1* status, there would be a tendency to bias the results to the null, underestimating the true effects.

With respect to the current study, which was restricted to surgical subjects, a total of 274 subjects were enrolled prior to breast surgery. Forty-seven of the women were excluded because of a final diagnosis of atypia, lobular carcinoma *in situ*, or a rare breast cancer type (*e.g.*, cystosarcoma phyloides). Of the remaining subjects, 119 and 108 women had confirmed diagnoses corresponding to the case and control definitions, respectively. Data on *GSTM1* genotype were available from 95 cases and 87 controls, as were data on both *GSTM1* status and PAH-DNA adduct levels from 84 controls and 83 cases. In univariate analysis, *GSTM1* genotype was not associated with case-control status (OR = 0.92; 95% CI = 0.51–1.64). After controlling for age, ethnicity, breast feeding status, parity, age at menarche, age at first birth, family history of breast cancer, and current alcohol consumption, *GSTM1* genotype remained unassociated with breast cancer case-control status (OR = 0.73; 95% CI = 0.37–1.44). This was true after stratification by menopausal status (for premenopausal women, OR = 0.52, 95% CI = 0.20–1.4; for postmenopausal women, OR = 1.03, 95% CI = 0.37–2.89).

As reported separately (13), increasing adduct levels measured in tumor tissue and tissue from controls were associated with case-control status ($P = 0.04$), and adducts in tumor and nontumor tissue from cases were significantly correlated ($P < 0.001$). The association between adducts measured in nontumor tissue and tissue from controls and case-control status was positive but not statistically significant.

The *GSTM1* null genotype was associated with increased PAH-DNA adduct levels in both tumor and nontumor tissue from cases, but not in benign tissue from controls (Table 1 and Fig. 1). The difference in adduct levels by genotype was strongest in tumor tissue. Linear regression analysis showed that the *GSTM1* null genotype was associated with adducts in tumor tissue ($\beta = 0.407$; $P = 0.003$) and with adduct levels in nontumor tissue ($\beta = 0.243$; $P = 0.05$). *GSTM1* and adducts were not associated in benign tissue ($\beta = 0.095$; $P = 0.341$). After control for age, ethnicity, current smoking status, current ETS exposure, consumption of charred food, parity, and breast feeding, *GSTM1* remained significantly associated with adduct levels in tumor tissue ($\beta = 0.447$; $P = 0.002$) but not in nontumor tissue ($\beta = 0.220$; $P = 0.095$) or benign tissue ($\beta = 0.060$; $P = 0.580$).

The relationship between *GSTM1* genotype and adduct levels in cases was further analyzed excluding women with diagnoses of DCIS. Compared with the full analysis, in the subset of cases with invasive cancer the differences in adduct levels associated with *GSTM1* genotype were more pronounced in both tumor and nontumor tissue but, possibly because of the reduced sample size, were of only borderline statistical significance. Because women with a family history of breast cancer were overrepresented in the BBD control group, the relationship between adducts and *GSTM1* was reanalyzed in the controls, adjusting for family history, and in the subset of controls with no family history. However, in neither of these analyses did *GSTM1* genotype predict adduct levels in benign tissue, suggesting that the increased enrollment among the BBD controls of women with a family history of breast cancer did not bias the results.

Among women who were *GSTM1* +/+ or +/−, there was no case-control difference in adduct levels. However, among the *GSTM1*-null women, adduct levels in tumor tissue from cases were significantly higher than in breast tissue from controls ($P = 0.02$; Table 1). In a multiple linear regression model with adducts (in tumor and benign tissue) serving as the dependent variable and case-control status and *GSTM1* genotype as independent variables, the β for the interaction term for the joint effects of *GSTM1* and case-control status was of borderline significance ($\beta = 0.31$; $P = 0.06$). In a multiple linear regression model that also controlled for known breast cancer risk factors and PAH exposure, the interaction term for the joint effects of *GSTM1* and case-control status was significant ($\beta = 0.25$; $P = 0.002$).

The associations between *GSTM1* genotype status and prognostic factors recorded in the pathology reports were also

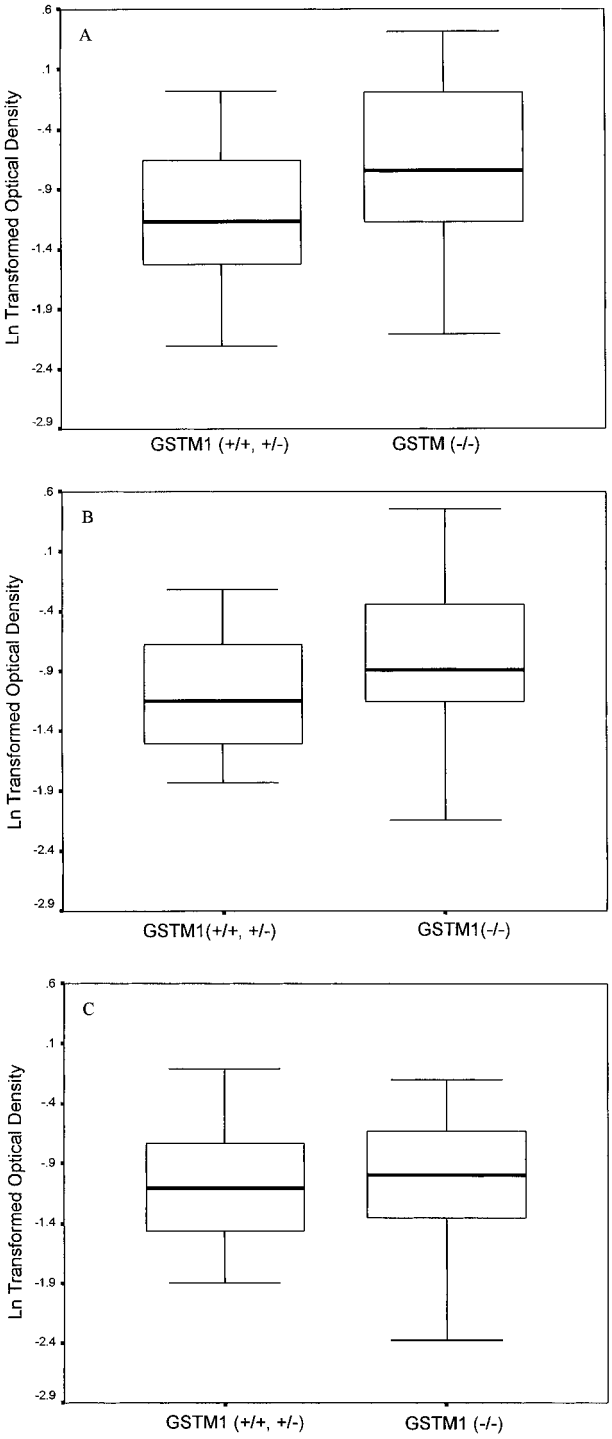

Fig. 1. PAH-DNA adduct levels in breast tissue by *GSTM1* genotype. *A*, adduct levels in tumor tissue from cases; *B*, adduct levels in nontumor tissue from cases; *C*, adduct levels in benign tissue from controls.

assessed. *GSTM1* genotype was not associated with tumor size, stage, or erbB-2 expression (Table 2). However, the *GSTM1* null genotype was associated with increased ER expression (Table 2). This relationship was explored further to determine whether *GSTM1* genotype was associated with case-control status in the subset of cases with ER-positive tumors compared with controls. However, in this subset, there was no association between *GSTM1* genotype and case-control status (OR = 0.94; 95% CI = 0.48–1.84). ER expression, measured as the percentage of cells in a tumor that expressed ER, was associated with both *GSTM1* genotype and adduct staining, potentially acting as a confounder in the observed relationship between *GSTM1* genotype and adduct staining. Because ER is not generally measured in tissue sections from DCIS cases and ER data were missing for several subjects with invasive tumors, ER data were available only for 57 subjects with adduct and *GSTM1* data. In this smaller subset of individuals, *GSTM1* genotype was not significantly associated with adduct levels before (β = 0.29; P = 0.09) or after (β = 0.23; P = 0.17) controlling for ER expression. However, subjects missing ER data had significantly higher levels of PAH-DNA staining in their tumor sections and were nonsignificantly more likely to be *GSTM1* null (63% *versus* 42%). Therefore, the exclusion of subjects (31% of the cases) with no ER data appears to have introduced a selection bias into the analyses of ER as a confounder and complicates the interpretation of the results. However, the similar association between *GSTM1* and adduct levels in nontumor tissue suggests that ER expression does not explain all of the association between these two factors seen in tumor tissue.

Table 2 *GSTM1* genotype and pathologic variables (ER expression, tumor size, erbB-2, and stage)

	Mean (SD); n^a	
	GSTM1 +/+, +/−	*GSTM1* −/−
ER expression[b] (% of cells)	46.80 (31.98); 38	61.55 (22.74); 29
Tumor size[c] (cm)	1.17 (1.23); 44	1.45 (0.90); 49
ErbB2[d] (pg/cell)	0.16 (0.31); 21	0.11 (0.22); 34
Stage[e]		
0	10 (22%)	4 (9%)
1	21 (45%)	26 (55%)
2–3	15 (33%)	17 (36%)

[a] Results for stage given as *n* (%).
[b–d] *t* Test: [b] P = 0.03; [c] P = 0.20; [d] P = 0.49.
[e] P = 0.20, χ^2 test.

The relationships between adduct levels in tumor tissue and tumor stage, size, and erbB-2 expression were also assessed. Adduct levels were not associated with any of these pathological features before or after control for *GSTM1* genotype. Furthermore, in separate analyses of *GSTM1*-null cases and *GSTM1* +/+, +/− cases, there were no associations between adduct levels in tumor tissue and tumor size, stage, or erbB2 expression. The relationship between adduct levels in tumor tissue and ER expression appears to be restricted to the cases who were *GSTM1* +/+, +/− (β = 0.006, P = 0.07, n = 33 *versus* β = 0.002, P = 0.76, n = 23 among *GSTM1*-null subjects). However, the small numbers of subjects and the large proportion of subjects with missing ER data limit the interpretation of these results.

Discussion

In the current study, *GSTM1* genotype alone was not associated with case-control status; however, there appeared to be an interaction among *GSTM1*, case-control status, and PAH-DNA adducts. The finding of no association between *GSTM1* and risk of breast cancer when *GSTM1* was considered alone is consistent with several recently published studies (17, 20–22), although a few studies have seen an association with the null

genotype in older or postmenopausal women (18, 19, 23). Results from the Nurses' Health Study have linked the null genotype with better survival (17), whereas another study found the opposite (24). In our population, *GSTM1* genotype was associated with ER expression, suggesting that *GSTM1*-null individuals might have a better prognosis. However, *GSTM1* showed no association with tumor size, stage, or erbB-2 expression.

The data show a strong association between *GSTM1* deletion and increased PAH-DNA adduct levels in breast tumor tissue, and a borderline significant association in nontumor tissue from cases, but no association in breast tissue from controls. This suggests that the *GSTM1* genotype plays a role in preventing the accumulation of genetic damage in breast tumor and nontumor tissue. This finding stands in contrast to much of the literature, which has shown that in other tissues *GSTM1*, as a single biomarker of metabolism, is a poor predictor of adduct levels (15, 16, 28–32). It is possible that *GSTM1* plays a more important role in PAH metabolism in breast compared with other tissues.

The possibility that ER expression may play a confounding role in the association between *GSTM1* genotype and PAH-DNA levels in tumor tissue could not be fully explored in this study because of the substantial number of cases for whom ER data were not available. Although control for ER expression modestly altered the β for *GSTM1*, subjects for whom ER data were missing tended to be *GSTM1* null and to have higher adduct levels. This bias obscures the relationship between *GSTM1* and PAH-DNA seen in the full case group and makes it difficult to interpret the relationships among these three variables. However, the similar association between *GSTM1* and adduct levels in nontumor tissue suggests that ER expression does not explain all of the association between these two factors seen in tumor tissue.

Our data indicate that case-control status and *GSTM1* genotype interact, such that case-control differences in adduct levels are seen only in those women who are *GSTM1* null. The finding of an association between *GSTM1* and adduct levels in cases only and of no association between *GSTM1* and case-control status may be interpreted in several ways. The first relates to inherent susceptibility to breast cancer and, therefore, to the etiology of the disease. It is possible that among women who are *GSTM1* deleted, there is a subpopulation who are constitutively more susceptible to developing DNA damage from xenobiotic exposures and that these are the women who go on to develop breast cancer. This susceptible subpopulation might have PAH metabolism pathways that yield more adducts, or they might have impaired DNA repair, so that adducts are less likely to be removed. Because several other enzymes are involved in metabolizing PAH, the increased susceptibility might be the result of greater activation via the phase I metabolic genes such as *CYP1A1* or *1B1*, or less competent detoxification through the sulfotransferase and UDP-glucuronosyltransferase pathways (33–36).

A second possible interpretation is consistent with the observations that the case-control differences were stronger in tumor tissue than in normal tissue from cases, and that no differences were seen in adduct levels by *GSTM1* genotype in controls. This interpretation is that there are acquired changes in the metabolic pathways of breast tumor cells that result in *GSTM1* genotype becoming an important determinant of PAH-DNA adduct levels. The UDP-glucuronosyltransferase and sulfotransferase phase II detoxification pathways are involved in detoxifying both estrogen and PAH, and a number of studies have reported alterations in these pathways in breast tumors (37–40). These changes in metabolism appear to allow estrogen-dependent tumors to produce high intracellular levels of estrogen, thus fueling their own growth (38–41). Such tumor-specific acquired alterations in metabolism may increase levels of the active PAH intermediates and alter the balance of PAH metabolism, such that *GSTM1* activity becomes a relatively more important determinant of genetic damage from PAHs and perhaps other carcinogens. Such acquired alterations in metabolism in tumor tissue might thus explain the elevated adduct levels seen in tumor tissue from *GSTM1*-null cases. Increased adduct levels may lead over time to increased mutations and genomic instability, further contributing to the cancerous phenotype and possibly to cancer progression. The hypothesis that estrogen dependence is associated with changes in PAH metabolism is supported by the correlation seen between PAH-DNA adduct levels and ER expression (13)

Under this latter scenario, our finding that PAH-DNA adduct levels in breast tumor tissue were associated with breast cancer case-control status (13) might be interpreted to reflect a late tumor effect rather than an etiological role for PAH. However, the observations that PAH-DNA adducts are also elevated in nontumor tissue and that *GSTM1* appears to be an important determinant of PAH-DNA adducts in nontumor as well as tumor tissue suggest that these metabolic changes occur prior to, or early, in tumor development (13). Moreover, in the study, most cases were early stage (DCIS or stages I and II) and adduct levels in tumor tissue were not associated with tumor size or stage overall or in either *GSTM1* subgroup, reducing the likelihood that the observed association reflects only cancer progression.

An advantage of this study, in contrast to prior investigations that evaluated adducts in breast tissue (6, 12), is the fact that the women in this study constitute a representative sample of the joint source population of women being seen for breast-related complaints at CPMC and that the control group was made up of women who would be treated at the same hospital if they had breast cancer. A disadvantage of the design, potentially biasing the study toward the null, is that the BBD patients without atypia have a slightly increased risk of future breast cancer (42) and may be expected to share more risk factors (both measured and unmeasured) with the cases than a control group of healthy women drawn from the source population. However, past studies comparing healthy women and women with BBD did not indicate that women with BBD were more likely to have risk factors for breast cancer (43–46). In the present study, it appears that women with a family history of breast cancer were overrepresented in the BBD control group, which is consistent with past studies of women with BBD (43, 44, 47). However, rather than signifying an etiological role in BBD, the increased prevalence of a family history among women with BBD is thought to be related to the fact that women with a family history are often more intensely screened, and that the threshold for a biopsy referral is lower for these women (43, 44, 47). In the present study, statistical analyses that focused on family history among the BBD controls suggested that this factor was not biasing the analyses of *GSTM1* genotype and adduct levels.

A limitation of the immunohistochemical assay is that results cannot be reported in adduct units; *e.g.*, as adducts per 10^8 nucleotides (48). Although the antibody was raised against benzo[*a*]pyrene-diolepoxide-DNA adducts, it cross-reacts with other PAHs that form diolepoxide adducts, and does so with varying affinities (48–50). Humans are exposed to complex mixtures of PAHs, and multiple adduct species are likely to be present. Because the composition of this mixture cannot be

predicted and probably varies from individual to individual, relevant standards cannot readily be derived from treated cell lines or experimental animals. However, immunohistochemical assays for PAH-DNA adducts, including several that have used the same antibody (5D11), previously have demonstrated the ability to reflect differences in environmental exposure to PAHs as a class of chemicals (26, 27, 51–58).

In conclusion, these results are consistent with the majority of the literature showing that the *GSTM1* null genotype as a single biomarker of metabolic susceptibility is not associated with breast cancer status. However, our data suggest that *GSTM1* does play a role in preventing the accumulation of environmentally induced genetic damage in both breast tumor and nontumor tissue from cases. These data also suggest that complex relationships exist between metabolism, *GSTM1* genotype, adduct formation, estrogen dependence, and risk. Further studies are warranted to confirm these relationships.

References

1. Glass, A. G., and Hoover, R. N. Rising incidence of breast cancer: relationship to stage and receptor status. J. Natl. Cancer Inst., *82:* 693–696, 1990.

2. Harris, J. R., Lippman, M. E., Veronesi, U., and Willett, W. Breast cancer. N. Engl. J. Med., *327:* 319–328, 1992.

3. American Cancer Society. Cancer Facts and Figures–1998. Atlanta: American Cancer Society, 1998.

4. Madigan, M. P., Ziegler, R. G., Benichou, J., Byrne, C., and Hoover, R. N. Proportion of breast cancer cases in the United States explained by well-established risk factors. J. Natl. Cancer Inst., *87:* 1681–1685, 1995.

5. El-Bayoumy, K. Environmental carcinogens that may be involved in human breast cancer etiology. Chem. Res. Toxicol., *5:* 585–590, 1992.

6. Perera, F. P., Estabrook, A., Hewer, A., Channing, K. M., Rundle, A., Mooney, L. A., Whyatt, R., and Phillips, D. H. Carcinogen-DNA adducts in human breast tissue. Cancer Epidemiol. Biomark. Prev., *4:* 233–238, 1995.

7. Morris, J. J., and Seifter, E. The role of aromatic hydrocarbons in the genesis of breast cancer. Med. Hypotheses, *38:* 177–184, 1992.

8. Falck, F., Ricci, A., Wolff, M. S., Godbold, J., and Deckers, P. Pesticides and polychlorinated biphenyl residues in human breast lipids and their relation to breast cancer. Arch. Environ. Health, *47:* 143–146, 1992.

9. Wolff, M. S., Toniolo, P. G., Lee, E. W., Rivera, M., and Dubin, N. Blood levels of organochlorine residues and risk of breast cancer. J. Natl. Cancer Inst., *85:* 648–652, 1993.

10. Gammon, M., Hibshoosh, H., Terry, M. B., Bose, S., Schoenburg, J., Brinton, L., Bernstein, J., and Thompson, W. D. Cigarette smoking and other risk factors in relation to p53 expression in breast cancer among young women. Cancer Epidemiol. Biomark. Prev., *8:* 255–263, 1999.

11. Perera, F. P. Environment and cancer: who are susceptible? Science (Washington DC), *278:* 1068–1073, 1997.

12. Li, D., Wang, M., Dhingra, K., and Hittelman, W. N. Aromatic DNA adducts in adjacent tissues of breast cancer patients: clues to breast cancer etiology. Cancer Res., *56:* 287–293, 1996.

13. Rundle, A., Tang, D., Hibshoosh, H., Estabrook, A., Schnabel, F., Cao, W., Grumet, S., Della Rocca, A., and Perera, F. Genetic damage in breast tissue from polycyclic aromatic hydrocarbons is associated with breast cancer. Carcinogenesis (Lond.), *21:* 1281–1289, 2000.

14. McWilliams, J. E., Sanderson, B. J. S., Harris, E. L., Richert-Boe, K. E., and Henner, W. D. Glutathione *S*-transferase M1 (GSTM1) deficiency and lung cancer risk. Cancer Epidemiol. Biomark. Prev., *4:* 589–594, 1995.

15. Rebbeck, T. R. Molecular epidemiology of the human glutathione *S*-transferase genotypes GSTM1 and GSTT1 in cancer susceptibility. Cancer Epidemiol. Biomark. Prev., *6:* 733–743, 1997.

16. Tang, D. L., Rundle, A., Warburton, D., Santella, R. M., Tsai, W-Y., Chiamprasert, S., Hsu, Y. Z., and Perera, F. P. Associations between both genetic and environmental biomarkers and lung cancer: evidence of a greater risk of lung cancer in women smokers. Carcinogenesis (Lond.), *19:* 1949–1953, 1998.

17. Kelsey, K. T., Hankinson, S. E., Colditz, G. A., Springer, K., Garcia-Closas, M., Spiegelman, D., Manson, J. E., Garland, M., Stampfer, M. J., Willet, W. C., Speizer, F. E., and Hunter, D. J. Glutathione *S*-transferase class μ deletion polymorphism and breast cancer: results from prevalent versus incident cases. Cancer Epidemiol. Biomark. Prev., *6:* 511–515, 1997.

18. Ambrosone, C. B., Freudenheim, J. L., Graham, S., Marshall, J. R., Vena, J. E., Brasure, J. R., Laughlin, R., Nemoto, T., Michalek, A. M., Harrington, A., Ford, T. D., and Shields, P. G. Cytochrome P4501A1 and glutathione *S*-transferase (M1) genetic polymorphisms and postmenopausal breast cancer risk. Cancer Res., *55:* 3483–3485, 1995.

19. Charrier, J., Maugard, C. M., Le Mevel, B., and Bignon, Y. J. Allelotype influence at glutathione *S*-transferase MI locus on breast cancer susceptibility. Br. J. Cancer, *79:* 346–353, 1999.

20. Bailey, L. R., Roodi, N., Verrier, C. S., Yee, C. J., Dupont, W. D., and Parl, F. F. Breast cancer and CYP1A1, GSTM1, and GSTT1 polymorphisms: evidence of a lack of association in Caucasians and African Americans. Cancer Res., *58:* 65–70, 1998.

21. Ambrosone, C. B., Coles, B. F., Freudenheim, J. L., and Shields, P. G. Glutathione-*S*-transferase (GSTM1) genetic polymorphisms do not affect human breast cancer risk, regardless of dietary antioxidants. J. Nutr., *129* (2S Suppl.)*:* 565s–568s, 1999.

22. Zhong, S., Wyllie, A. H., Barnes, D., Wolf, C. R., and Spurr, N. K. Relationship between the GSTM1 genetic polymorphism and susceptibility to bladder, breast and colon cancer. Carcinogenesis (Lond.), *14:* 1821–1824, 1993.

23. Helzlsouer, K. J., Selmin, O., Huang, H. Y., Strickland, P. T., Hoffman, S., Alberg, A. J., Watson, M., Comstock, G. W., and Bell, D. Association between glutathione *S*-transferase M1, P1 and T1 genetic polymorphisms and development of breast cancer. J. Natl. Cancer Inst., *90:* 512–518, 1998.

24. Nedelcheva Kristensen, V., Andersen, T. L., Erikstein, B., Geitvik, G., Skovlund, E., Nesland, J. M., and Borresen-Dale, A. L. Single tube multiplex polymerase chain reaction genotype analysis of GSTM1, GSTT1 and GSTP1: relation of genotypes to TP53 tumor status and clinicopathological variables in breast cancer patients. Pharmacogenetics, *8:* 441–447, 1998.

25. Bell, D. A., Taylor, J. A., Paulson, D. F., Robertson, C. N., Mohler, J. L., and Lucier, G. W. Genetic risk and carcinogen exposure: a common inherited defect of the carcinogen-metabolism gene glutathione *S*-transferase M1 (GSTM1) that increases susceptibility to bladder cancer. J. Natl. Cancer Inst., *85:* 1159–1164, 1993.

26. Zhang, Y. J., Weksler, B. B., Wang, L., Schwartz, J., and Santella, R. M. Immunohistochemical detection of polycyclic aromatic hydrocarbon-DNA damage in human blood vessels of smokers and non-smokers. Atherosclerosis, *140:* 325–331, 1998.

27. Romano, G., Sgambato, A., Boninsegna, A., Flamini, G., Curigliano, G., Yang, Q., La Gioia, V., Signorelli, C., Ferro, A., Capelli, G., Santella, R., and Cittadini, A. Evaluation of polycyclic aromatic hydrocarbon-DNA adducts in exfoliated oral cells by an immunohistochemical assay. Cancer Epidemiol. Biomark. Prev., *8:* 91–96, 1999.

28. Whyatt, R. M., Bell, D. A., Santella, R. M., Garte, S. J., Jedrychowski, W., Gladek-Yarborough, A., Cosma, G., Manchester, D. K., Young, T-L., Wahrendorf, J., Cooper, T. B., Ottman, R., and Perera, F. P. Polycyclic aromatic hydrocarbon-DNA adducts in human placenta and modulation by CYP1A1 induction and genotype. Carcinogenesis (Lond.), *19:* 1389–1392, 1998.

29. Schoket, B., Phillips, D. H., Kostic, S., and Vincze, I. Smoking-associated bulky DNA adducts in bronchial tissue related to CYP1A1 MspI and GSTM1 genotypes in lung patients. Carcinogenesis (Lond.), *19:* 841–846, 1998.

30. Peluso, M., Airoldi, L., Armelle, M., Martone, T., Coda, R., Malaveille, C., Giacomelli, G., Terrone, C., Casetta, G., and Vineis, P. White blood cell DNA adducts, smoking, and NAT2 and GSTM1 genotypes in bladder cancer: a case-control study. Cancer Epidemiol. Biomark. Prev., *7:* 341–346, 1998.

31. Pastorelli, R., Guanci, M., Cerri, A., Negri, E., La Vecchia, C., Fumagalli, F., Mezzetti, M., Cappelli, R., Panigalli, T., Fanelli, R., and Airoldi, L. Impact of inherited polymorphisms in glutathione *S*-transferase M1, microsomal epoxide hydrolase, cytochrome P450 enzymes on DNA, and blood protein adducts of benzo(*a*)pyrene-diolepoxide. Cancer Epidemiol. Biomark. Prev., *7:* 703–709, 1998.

32. Butkiewicz, D., Grzybowska, E., Hemminki, K., Ovrebo, S., Haugen, A., Motykiewicz, G., and Chorazy, M. Modulation of DNA adduct levels in human mononuclear white blood cells and granulocytes by CYP1A1 CYP2D6 and GSTM1 genetic polymorphisms. Mutat. Res., *415:* 97–108, 1998.

33. Larsen, M., Angus, W., Brake, P., Elton, S., Sukow, K., and Jefcoate, C. Characterization of CYP1B1 and CYP1A1 expression in human mammary epithelial cells: role of the aryl hydrocarbon receptor in polycyclic aromatic hydrocarbon metabolism. Cancer Res., *58:* 2366–2374, 1998.

34. Spink, D., Spink, B., Cao, J., DePasquale, J., Pentacost, B., Fasco, M., Li, Y., and Sutter, T. Differential expression of CYP1A1 and CYP1B1 in human breast epithelial cells and breast tumor cells. Carcinogenesis (Lond.), *19:* 291–298, 1998.

35. Merrick, B. A., Mansfield, B. K., Nikbakht, P. A., and Selkirk, J. K. Benzo[*a*]pyrene metabolism in human T47D mammary tumor cells: evidence for sulfate conjugation and translocation of reactive metabolites across cell membranes. Cancer Lett., *29:* 139–150, 1985.

36. Monteith, D. K., Michalopoulos, G., and Strom, S. C. Conjugation of chemical carcinogens by primary cultures of human hepatocytes. Xenobiotica, *20:* 753–763, 1990.

37. Albin, N., Massaad, L., Toussaint, C., Mathieu, M. C., Morizet, J., Parise, O., Gouyette, A., and Chabot, G. G. Main drug-metabolizing enzyme systems in human breast tumors and peritumoral tissues. Cancer Res., *53:* 3541–3546, 1993.

38. Santen, R. J., Leszczynski, D., Tilson-Mallet, N., Feil, P. D., Wright, C., Manni, A., and Santner, S. J. Enzymatic control of estrogen production in human breast cancer: relative significance of aromatase versus sulfatase pathways. Ann. NY Acad. Sci., *464:* 126–137, 1986.

39. Yue, W., Santner, S. J., Masamura, S., Wang, J. P., Demers, L. M., Hamilton, C., and Santen, R. J. Determinants of tissue estradiol levels and biologic responsiveness in breast tumors. Breast Cancer Res. Treat., *49* (Suppl. 1)*:* 1s–7s, 1998.

40. Pasqualini, J. R., Chetrite, G., and Netour, E. L. Control and expression of oestrone sulphatase activities in human breast cancer. J. Endocrinol., *150* (Suppl.)*:* 99s–105s, 1996.

41. Hobkirk, R. Steroid sulfotransferases and steroid sulfate sulfatases: characteristics and biological roles. Can. J. Biochem. Cell Biol., *63:* 1127–1144, 1985.

42. Dupont, W. D., and Page, D. L. Breast cancer risk associated with proliferative disease, age at first birth, and a family history of breast cancer. Am. J. Epidemiol., *125:* 769–779, 1987.

43. Goehring, C., and Moabia, A. Epidemiology of benign breast disease, with special attention to histologic types. Epidemiol. Rev., *19:* 310–327, 1997.

44. Berkowitz, G., Kelsey, J., LiVolsi, V., Holford, T., Merino, M., Ort, S., O'Connor, T. Z., and White C. Risk factors for fibrocystic breast disease and its histopathologic components. J. Natl. Cancer Inst., *75:* 43–50, 1985.

45. Hsieh, C., Walker, A., Trapido, E., Crosson, A., and MacMahon, B. Age at first birth and breast atypia. Int. J. Cancer, *33:* 309–312, 1984.

46. Duffy, S. W., Roberts, M. M., and Elton, R. Risk factors relevant to cystic breast disease: a case-control study. J. Epidemiol. Community Health, *37:* 271–273, 1983.

47. Pastides, H., Kelsey, J., Holford, T., and LiVolsi, V. An epidemiologic study of fibrocystic breast disease with reference to ductal epithelial atypia. Am. J. Epidemiol., *121:* 440–447, 1985.

48. Santella, R. Immunological methods for detection of carcinogen-DNA damage in humans. Cancer Epidemiol. Biomark. Prev., *8:* 733–739, 1999.

49. Weston, A., Manchester, D. K., Poirier, M. C., Choi, J. S., Trivers, G. E., Mann, D. L., and Harris, C. C. Derivative fluorescence spectral analysis of polycyclic aromatic hydrocarbon-DNA adducts in human placenta. Chem. Res. Toxicol., *2:* 104–108, 1989.

50. Santella, R. M., Gasparro, F. P., and Hsieh, L. L. Quantitation of carcinogen-DNA adducts with monoclonal antibodies. Prog. Exp. Tumor Res., *31:* 63–75, 1987.

51. Zenzes, M., Bielecki, R., and Reed, T. Detection of benzo(*a*)pyrene diol epoxide-DNA adducts in sperm of men exposed to cigarette smoke. Fertil. Steril., *72:* 330–335, 1999.

52. Zenzes, M., Puy, L., and Bielecki, R. Immunodetection of benzo[*a*]pyrene adducts in ovarian cells of women exposed to cigarette smoke. Mol. Hum. Reprod., *4:* 159–165, 1998.

53. Zenzes, M., Puy, L., Bielecki, R., and Reed, E. Detection of benzo[*a*]pyrene diol epoxide-DNA adducts in embryos from smoking couples: evidence for transmission by spermatozoa. Mol. Hum. Reprod., *5:* 125–131, 1999.

54. Zhang, Y. J., Hsu, T. M., and Santella, R. M. Immunoperoxidase detection of polycyclic aromatic hydrocarbon-DNA adducts in oral mucosa cells of smokers and nonsmokers. Cancer Epidemiol. Biomark. Prev., *4:* 133–138, 1995.

55. Hsu, T., Zhang, Y., and Santella, R. Immunoperoxidase quantitation of 4-aminobiphenyl- and polycyclic aromatic hydrocarbon-DNA adducts in exfoliated oral and urothelial cells of smokers and nonsmokers. Cancer Epidemiol. Biomark. Prev., *6:* 193–199, 1997.

56. Motykiewicz, G., Malusecka, E., Grzybowska, E., Chorazy, M., Zhang, Y. J., Perera, F. P., and Santella, R. M. Immunohistochemical quantitation of polycyclic aromatic hydrocarbon-DNA adducts in human lymphocytes. Cancer Res., *55:* 1417–1422, 1995.

57. Mancini, R., Romano, G., Sgambato, A., Flamini, G., Giovagnoli, M., Boninsegna, A., Carraro, C., Vecchione, A., and Cittadini, A. Polycyclic aromatic hydrocarbon-DNA adducts in cervical smears of smokers and nonsmokers. Gynecol. Oncol., *75:* 68–71, 1999.

58. Motykiewicz, G., Michalska, J., Pendzich, J., Malusecka, E., Strozyk, M., Kalinowska, E., Butkiewicz, D., Mielzynska, D., Midro, A., Santella, R., and Chorazy, M. A molecular epidemiology study in women from Upper Silesia, Poland. Toxicol. Lett. (Amst.), *96:* 195–202, 1998.