Sensitivity to Benzo(a)pyrene Diol-Epoxide Associated with Risk of Breast Cancer in Young Women and Modulation by Glutathione S-Transferase Polymorphisms: A Case-Control Study

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ABSTRACT

Mounting epidemiological evidence suggests that smoking may play a role in the etiology of breast cancer. Because smoking-related DNA adducts are detectable in both normal and malignant breast tissues, we hypothesized that breast cancer patients may be sensitive to tobacco-induced carcinogenesis, and this sensitivity could be modulated by variants of metabolic genes. To test this hypothesis, we evaluated benzo(a)pyrene diol-epoxide (BPDE)-induced mutagen sensitivity and polymorphisms of GSTM1 and GSTT1 in a pilot case-control study of breast cancer. Short-term cell cultures were established from blood samples of 100 female breast cancer patients and 105 healthy controls. After 5 h of in vitro exposure to 4 μM of BPDE, we harvested the lymphocytes for cytogenetic evaluation and recorded and compared the frequency of BPDE-induced chromatid breaks between cases and controls. We used a multiplex PCR-based assay to simultaneously detect polymorphisms of GSTM1 and GSTT1 from genomic DNA. We performed univariate and multivariate logistic regression analyses and calculated odds ratios (OR) and 95% confidence intervals (CIs). Cases had a significantly higher frequency of chromatid breaks than did controls (P < 0.0001). The level of chromatid breaks greater than the median value of controls was associated with a 3.0-fold increased risk of breast cancer [adjusted odds ratio (ORadj) = 3.11; 95% CI = 1.72–5.64]. The risk was more pronounced in those who were <45 years (ORadj = 4.79; 95% CI = 1.87–12.3), ever-smokers (ORadj = 5.55; 95% CI = 1.85–16.6), alcohol drinkers (ORadj = 4.64; 95% CI = 1.70–12.7), and those who had the GSTT1 null variant (ORadj = 8.01; 95% CI = 1.16–55.3). These data suggest that sensitivity to BPDE-induced chromosomal aberrations may contribute to the risk of developing breast cancer, and such sensitivity may be modulated by both genetic and environmental factors. Larger studies are needed to confirm our findings.

INTRODUCTION

Breast cancer is the most common incident cancer in women in the United States, with an estimated 192,000 new cases and 42,200 deaths in 2001 (1). Although the role of genetic factors in the etiology of breast cancer is well established in familial breast cancer (2), the contribution of environmental factors is still largely unknown. However, mounting epidemiological evidence suggests that cigarette smoking increases the risk for breast cancer in high-risk breast cancer families (3), that smoking during pregnancy increases breast cancer risk in very young women (4), and that smoking may be associated with risk in subsets of breast cancer patients such as hormone receptor-negative breast cancer (5).

A number of PAHs are widespread environmental contaminants known to cause mammary cancers in experimental animals (6). Benzo(a)pyrene is one of the PAHs found in tobacco smoke, and its metabolite BPDE is considered a classic DNA-damaging carcinogen (7). GSTs catalyze the detoxification of carcinogen metabolites including BPDE and ROS and, therefore, modulate the level of BPDE DNA adducts (8). Several genes that code for these enzymes are polymorphic, and their genetic polymorphisms are likely to be associated with tobacco-induced mutagenesis.

Although evidence is still lacking in supporting a direct link between smoking and risk of breast cancer, tobacco-related DNA adducts have been found in breast tumor and adjacent normal tissues (9–11). Tobacco smoke contains various chemicals including PAHs and ROS that induce various types of DNA damage including strand breaks, suggesting that these chemicals could be involved in breast carcinogenesis (6, 12). Therefore, genetic polymorphisms in enzymes that are involved in the metabolism of PAHs and ROS may also play a role in individual susceptibility to breast disease (13, 14). Chromosomal aberrations, particularly those induced by ionizing radiation and impaired repair of DNA strand breaks have been shown to increase the risk of breast cancer (15–18). Recently, some studies demonstrated that polymorphisms of GSTM1 and GSTT1 are associated with a moderately increased risk of breast cancer and act as modifiers of breast cancer risk (19, 20), whereas some other studies failed to find them as risk factors for breast cancer (21, 22). To examine the role of BPDE-induced mutagen sensitivity in the development of breast cancer and possible modulation by genetic polymorphisms that are involved in metabolism of BPDE, we conducted a pilot case-control study to investigate the role of BPDE-induced chromosomal aberrations in susceptibility to breast cancer and the role of null GSTM1 and GSTT1 genotypes as modifiers of the risk associated with BPDE-induced chromosomal aberrations.

MATERIALS AND METHODS

Study Subjects. Incident breast cancer cases (n = 100) were consecutively recruited from female patients who were undergoing breast surgery in the Department of Surgical Oncology at the University of Texas M. D. Anderson Cancer Center between August 1998 and March 2001. All of the cases had histopathologically confirmed breast cancer. To facilitate the study of genetic susceptibility, we limited our case subjects to those ≥50 years of age. Cancer-free controls (n = 105) were obtained from patients (n = 22) undergoing breast reduction surgery and spouses of the cancer patients (n = 83) unrelated to the cases. Each participant donated 10 ml of blood collected in heparinized tubes and completed a short questionnaire that elicited information on age, sex, ethnicity, smoking status, and alcohol consumption. Cases and controls were
frequency matched for age (±5 years), sex, ethnicity, and smoking status (ever- and never-smokers).

**Cytogenetic Analysis.** The BPDE-induced mutagen sensitivity assays were performed as described previously (23). Briefly, two primary lymphocyte cultures were established from each individual by inoculating 1 ml of blood into a T-25 plastic culture flask with 9 ml of RPMI 1640 (Life Technologies, Inc., Grand Island, NY) containing 112.5 μg/ml phytohemagglutinin (Murex Biotech Limited, Dartford, England). A dose-response curve was constructed, and the 4-μM BPDE was found to be adequate for in vitro treatment (24). After ~67 h of incubation at 37°C, the cultures were treated with BPDE for 5 h. The 5-h exposure period was chosen to avoid possible cytotoxic effects of longer exposure and because BPDE-DNA adduct levels appear to reach a steady-state after ~67 h of incubation at 37°C, the cultures were treated with BPDE for 5 h. The 5-h exposure period was chosen to avoid possible cytotoxic effects of longer exposure and because BPDE-DNA adduct levels appear to reach a steady-state.

**RESULTS**

The characteristics of these 100 cases and 105 controls are summarized in Table 1. The frequency matching on age and ethnicity resulted in a mean age of 43.2 (range = 23–50 years; SD = 6) and 42.2 (range = 28–55 years; SD = 7.9) years for both cases and controls with a median of 45 and 44 years, respectively (data not shown). Although there were more cases than controls ≥45 years of age, this difference was not statistically significant (P = 0.186). There was also no significant difference in the distribution of ethnic groups (P = 0.388); ~80% of the subjects were Caucasians in both cases and controls. These results suggest that frequency matching on age and ethnicity was adequate. There were more ever-smokers among the controls than the cases, but the difference was not statistically significant (P = 0.386). The proportion of ever-drinkers in the cases (43%) was nearly identical to that in the controls (43.8%).

There were no differences in the distribution of the GSTM1 and GSTT1 null genotypes between cases and controls (P = 0.743 and P = 0.690, respectively), and these null genotypes were not associated with risk of breast cancer (Table 2). However, 74% of the cases exhibited a mutagen sensitivity value >0.38 (the median of the control values) b/c (P < 0.0001). This elevated sensitivity to BPDE was associated with >3-fold increased risk of breast cancer (OR = 3.01; 95% CI = 1.67–5.43), which did not change after adjustment for age, ethnicity, smoking, and alcohol use (adjusted OR = 3.11; 95% CI = 1.72–5.64; Table 2).

The mean b/c value was also significantly higher in the 100 cases (0.52; median = 0.48) than in the 105 controls (0.40; median = 0.38; P < 0.001; Table 3). To evaluate whether the elevated sensitivity to BPDE is modulated by genetic and environmental factors listed in Table 1, we performed stratified analysis. In general, the mean mutagen sensitivity by GSTM1 and GSTT1 in breast cancer

**Table 1 Distribution of selected variables for breast cancer patients and healthy controls**

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td>100 (100)</td>
<td>105 (100)</td>
<td>0.186</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;45</td>
<td>46 (46.0)</td>
<td>58 (55.2)</td>
<td></td>
</tr>
<tr>
<td>≥45</td>
<td>54 (54.0)</td>
<td>47 (44.8)</td>
<td></td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td>0.388</td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>79 (79.0)</td>
<td>83 (79.0)</td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>8 (8.0)</td>
<td>13 (12.4)</td>
<td></td>
</tr>
<tr>
<td>Mexican American</td>
<td>13 (13.0)</td>
<td>9 (8.6)</td>
<td></td>
</tr>
<tr>
<td>Tobacco use</td>
<td></td>
<td></td>
<td>0.386</td>
</tr>
<tr>
<td>Never</td>
<td>64 (64.0)</td>
<td>61 (58.1)</td>
<td></td>
</tr>
<tr>
<td>Ever</td>
<td>36 (36.0)</td>
<td>44 (41.9)</td>
<td></td>
</tr>
<tr>
<td>Alcohol use</td>
<td></td>
<td></td>
<td>0.907</td>
</tr>
<tr>
<td>Never</td>
<td>57 (57.0)</td>
<td>59 (56.2)</td>
<td></td>
</tr>
<tr>
<td>Ever</td>
<td>43 (43.0)</td>
<td>46 (43.8)</td>
<td></td>
</tr>
</tbody>
</table>

* χ² test.
tagen sensitivity value of the cases in each subgroup was significantly higher than that of the controls, except among small subgroups of 8 African Americans and 13 Mexican Americans (Table 3).

To evaluate the modulation of the sensitivity by other factors, we additionally stratified the mutagen sensitivity values by the median of the control b/c values to calculate crude and adjusted ORs and 95% CI in logistic regression analyses (Table 4). Again, consistent with the results in Table 3, significantly increased ORs were observed in all but nonwhites for each stratum. These risk estimates were unchanged after adjustment for age, ethnicity, smoking, and alcohol use in multivariate logistic regression models (Table 4). However, the elevated risk associated with the sensitivity to BPDE was more pronounced in subgroups of <45 years (adjusted OR = 4.79; 95% CI = 1.87–12.3); ever-smokers (OR = 5.55; 95% CI = 1.85–16.6); alcohol ever-drinkers (OR = 4.64; 95% CI = 1.70–12.7), and the GSTT1 null genotype (OR = 8.01; 95% CI = 1.16–55.3). These data suggest that the risk for breast cancer associated with in vitro sensitivity to BPDE may be modulated by genetic and environmental factors. However, there were few observations in some of the cells for additionally testing possible interactions.

DISCUSSION

BPDE mutagen sensitivity has consistently been shown to be a susceptibility marker for tobacco-induced cancers including those of the lung and head and neck (26–28). In this pilot study, we demonstrated that the BPDE-induced mutagen sensitivity is also a risk factor for breast cancer. This risk appears to be modulated by young age, smoking status, alcohol use, and the GSTT1 null genotype, additionally suggesting a possible role of gene-environment interaction. These findings are consistent with previous reports on detectable tobacco-induced DNA adducts in breast tissues and the possible role of GSTM1 and GSTT1 null genotypes in the etiology of breast cancer (19, 20). Our findings suggest that combining genotypic and phenotypic biomarkers for studies of genetic susceptibility may be a more powerful approach than using either phenotypic or genotypic biomarkers alone for future studies of breast cancer etiology.

The exact mechanism of how BPDE induces chromosomal aberrations remains to be determined. Our early data suggested that with the BPDE dose (4 μM) used in this study, we could induce DNA adducts in vitro 15 min after treatment and that the level of adducts increased 100-fold over the background adduct levels as measured by the 32P-postlabeling method (24). BPDE-induced bulky DNA adducts are repaired by the NER pathway (29, 30). During the repair process, it is likely that delayed completion of initial nicking of DNA strands by NER may induce DNA strand breaks that eventually lead to chromatic breaks. However, no studies have shown that sensitivity to chemical carcinogen-induced adducts and chromosome aberrations in lymphocytes are directly correlated with those in breast tissue, although such a correlation has been established between lymphocytes and lung tissue (31).

It has been shown that the risk of developing breast cancer in women is associated with the frequencies of chromosomal aberrations induced by X-ray (9) or γ radiation (32, 33) in peripheral blood lymphocytes. Elevated risk has been reported in association with a polymorphism (Lys751Gln) in the XPD, a NER gene (34). These findings suggest that sensitivity to radiation-induced chromosomal aberrations in lymphocytes may contribute to genetic susceptibility to breast cancer. In breast tumors and normal tissues, DNA adducts induced by carcinogens characteristic of complex mixtures of aromatic compounds (such as PAHs) and tobacco smoke are found in some studies (9, 10, 35) but not in others (11, 36). The GST null genotypes may modulate PAH-DNA adduct levels in both malignant and nonmalignant breast tissue (37) and are associated with increased risk of breast cancer (38), particularly in those women who had more than one null genotype and heavy alcohol consumption (19). These results suggest a role of GST polymorphisms in the formation of PAH-DNA adducts that contribute to genetic susceptibility to breast cancer and a possible gene-environment interaction in the etiology of breast cancer.

However, results from several other studies do not support an association between any specific polymorphism of GSTM1, GSTT1, and GSTP1 in susceptibility to sporadic breast cancer (21, 22), either independently or in combination. Indeed, we did not observe any increased risk associated with the GSTM1 and GSTT1 null genotypes alone, but these null genotypes modulated the risk associated with sensitivity to BPDE in our study population. Therefore, our results

### Table 2 Logistic regression analysis for GST genotypes and the BPDE mutagen sensitivity associated risk of breast cancer

<table>
<thead>
<tr>
<th>GSTM1</th>
<th>Cases (n = 100)</th>
<th>Controls (n = 105)</th>
<th>Crude OR (CI)</th>
<th>Adjusted OR*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>52 (52.0)</td>
<td>57 (54.3)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Null</td>
<td>48 (48.0)</td>
<td>48 (47.5)</td>
<td>1.10 (0.65–1.72)</td>
<td>1.04 (0.85–1.81)</td>
</tr>
<tr>
<td>GSTT1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>84 (84.0)</td>
<td>86 (81.9)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Null</td>
<td>16 (16.0)</td>
<td>19 (18.1)</td>
<td>0.86 (0.41–1.78)</td>
<td>0.85 (0.40–1.75)</td>
</tr>
<tr>
<td>Mutagen sensitivity (b/c)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>By median</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤0.38</td>
<td>26 (26.0)</td>
<td>54 (51.4)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>&gt;0.38</td>
<td>74 (74.0)</td>
<td>51 (48.6)</td>
<td>3.01 (1.67–5.43)</td>
<td>3.11 (1.72–5.64)</td>
</tr>
</tbody>
</table>

* Adjusted for age (in yrs), ethnicity, smoking, and alcohol use in a logistic regression model.
emphasize the importance of studying phenotypic and genotypic biomarkers simultaneously.

In summary, our results from this pilot study suggest that the BPDE mutagen sensitivity phenotype may be a risk factor for genetic susceptibility to breast cancer, and the risk is modulated by the GSTM1 and GSTT1 null genotype. Although we did not have detailed information on alcohol consumption in our study population, we also observed a remarkable risk associated with the BPDE mutagen sensitivity in smokers. The high risk associated with such sensitivity in subgroups of young age and smoking additionally suggest a possible gene-environment interaction in the etiology of breast cancer. Although this pilot case-control study was hospital-based and may not provide generalizable findings to the general population, the difference in phenotype by genotypes suggests that the findings are biologically plausible and important for future studies. In addition, because this study had a relatively small sample and used multiple comparisons, some of the findings could have been attributable to chance. However, the consistently significant findings of a main effect and risk modulation existing in most subgroups examined exclude the possibility of the findings occurring by chance. Larger studies with more rigorous designs are warranted to confirm these findings as well as to investigate the possible risk modulation by genetic polymorphisms of DNA repair genes that are relevant to the phenotype.

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