Synergistic Effects of NAT2 slow and GSTM1 null Genotypes on Carcinogen DNA Damage in the Lung

Mi-Sun Lee¹, Li Su¹, and David C. Christiani¹,²

Abstract

Background: Polymorphisms in carcinogen detoxification enzymes, NAT2 and GSTM1, have been suggested as susceptibility factors for DNA damage and lung cancer. However, little information is available on DNA adduct burden in the lung tissue and polymorphisms in NAT2 and GST genes. We investigated the independent and combined effects of the metabolic gene polymorphisms of NAT2 and GSTs on DNA adduct formation in different tissues (lung and blood) in lung cancer patients.

Methods: DNA adducts were measured in lung and blood by the ³²P-postlabeling assay. Multiple regression models were used to assess adjusted percent change in DNA adduct levels associated with GST and NAT2 genotypes.

Results: After adjusting for potential confounders, as well as for other GST gene variants, lung adduct levels significantly increased by 150.3% [95% confidence interval (95% CI), 35.4-362.6%] for the GSTM1 null and by 73.9% (95% CI, 3.2%-212.4%) for the NAT2 slow acetylator genotype, respectively. No association was seen with polymorphisms of other GST genes such as GSTT1 and GSTP1. The high-risk group, the combined GSTM1 null plus NAT2 slow, had significantly enhanced levels of lung adducts by 295% (95% CI, 72.7%-803.5%) over those associated with single genes, suggesting a synergistic effect on DNA damage in the target lung tissue.

Conclusions: The increase in DNA adduct levels in lung is associated with the GSTM1 null and NAT2 slow genotypes alone or in combination.

Impact: These results suggest that GSTM1 and NAT2 genotypes play an independent and interactive role in the formation of carcinogen DNA adduct in the lung. Cancer Epidemiol Biomarkers Prev; 19(6); 1492–7. ©2010 AACR.

Introduction

DNA damage is considered an important step in the carcinogenic process (1–3). Exposure to mutagenic carcinogens such as tobacco-derived aromatic amines and polycyclic aromatic hydrocarbons (PAH) and their derivatives, nitro-PAHs, can cause DNA adduct formation after metabolic activation (4–8). These carcinogenic DNA adducts occur in the early stage of mutation and, when the damage is excessive and left unrepaired, may ultimately lead to induction of lung cancer (9, 10).

Inherited differences in the metabolic capacity of carcinogens can play a role in DNA damage and therefore increase the risk of lung cancer. Polymorphisms in two important carcinogen detoxification genes, NAT2 and GSTM1, have been suggested as susceptibility factors for lung cancer (11, 12). Epidemiologic evidence shows the associations of GSTM1 null genotype with increased carcinogen DNA adduct burden (13, 14) and risk of lung cancer (15). However, no information is available linking the polymorphisms in NAT2 and GST genes with the formation of DNA adducts in the lung tissue, nor is there information on the potential interaction of these factors. Furthermore, data suggesting the influence of NAT2 acetylator status in the lung cancer population have been conflicting (11, 16–18). Notwithstanding, we previously reported a correlation between DNA adduct levels in different tissues (10). Tissue-specific differences in DNA adduct patterns can be caused by the metabolic activation of tobacco-derived carcinogens in different tissues (19, 20).

Therefore, the present study was designed to examine the independent and combined effects of genetic variation in NAT2 and GSTM1 on carcinogen-induced DNA damage measured in two different tissues: lung and blood mononuclear cells (MNC).

Materials and Methods

Study population and collection of specimens

The study population was described previously (21). This study was approved by the Committees on the Use of Human Subjects in Research at the Massachusetts General Hospital and the Harvard School of Public Health. Lung cancer patients at Massachusetts General
Analysis of DNA adducts

We analyzed our previously reported data on DNA adducts in lung and blood MNC samples using the \(^{32}\)P-postlabeling assay (10, 21). In these prior studies, total relative DNA adducts were measured in the diagonal reactive zone plus discrete adducts (in nine regions) and were considered primarily to represent aromatic hydrophobic adducts, mainly PAH-DNA adducts (10). The DNA adducts level was expressed as relative adduct level as reported previously (10). As a validation analysis, each sample was repeated at least twice and average adduct levels were obtained from the combination of all experiments of the relative adduct levels. The coefficient of variation for the repeated measurements was 14% for the positive control.

Statistical analysis

Dependent variables, DNA adduct levels per \(10^{10}\) nucleotides, were transformed using natural logarithms to improve normality. For computational purposes, samples below the limit of detection, which is 1 adduct per \(10^{10}\) nucleotides, were assigned a value of half the limit of detection as in prior reports (12). All the results for adduct levels are expressed as geometric mean with the corresponding 95% confidence intervals (95% CI). Additionally, multivariate-adjusted geometric mean adduct levels were estimated by using linear regression models. Potential confounders such as age at diagnosis, sex, smoking status (ex-smoker and current smoker), and pack-years of smoking were included in the multivariate analysis. The reference group included patients with GSTM1 present, GSTTI present, GSTP1 common (AA), or NAT2 rapid type. Using this approach, we examined the independent effect of even a single at-risk genotype, compared with those who carried common alleles for the GSTs or NAT2. We estimated the percent changes in DNA adduct levels for the risk genotype compared with the common allele as \(e^{\beta - 1} \times 100\%\), with 95% CI \(e^{\beta \pm 1.96 \times SE} - 1\) \(\times 100\%\), where \(\beta\) and SE are the estimated regression coefficient and its SE. To examine the combined effects of GSTM1 and NAT2, we constructed a model that included all possible combinations between GSTM1 and NAT2 polymorphisms, with the low-risk combination of GSTM1 wild-type plus NAT2 rapid acetylation as a reference category. All statistical analyses were done using SAS version 9.1 (SAS Institute, Inc.).

Results

Baseline characteristics

To determine associations between GST and NAT2 polymorphisms and DNA adduct levels, we assessed a population of 135 lung cancer patients who completed genotyping and DNA adducts analysis. Demographic and clinical characteristics and DNA adduct levels in lung and blood MNC in the patient group are shown in Table 1. Overall, 59% of the subjects were former smokers who had not smoked for at least 1 year, leaving

![Table 1. Characteristics of patients with lung cancer and DNA adduct levels in the lung and blood MNC (n = 135)](https://example.com/table1)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n (%) or mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male)</td>
<td>78 (57.8)</td>
</tr>
<tr>
<td>Race (Caucasian)</td>
<td>131 (97.0)</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
</tr>
<tr>
<td>Current smokers</td>
<td>56 (41.5)</td>
</tr>
<tr>
<td>Ex-smokers</td>
<td>79 (58.5)</td>
</tr>
<tr>
<td>Histologic analysis</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>65 (48.1)</td>
</tr>
<tr>
<td>Squamous</td>
<td>44 (32.6)</td>
</tr>
<tr>
<td>Others*</td>
<td>26 (19.3)</td>
</tr>
<tr>
<td>Age at diagnosis (y)</td>
<td>66.3 ± 10.6</td>
</tr>
<tr>
<td>DNA adduct levels, adducts per (10^{10}) nucleotides†</td>
<td></td>
</tr>
<tr>
<td>Lung DNA adduct level (n = 135)</td>
<td>86.2 ± 4.7</td>
</tr>
<tr>
<td>Blood MNC DNA adduct level (n = 53)</td>
<td>36.4 ± 3.2</td>
</tr>
<tr>
<td>Pack-years‡</td>
<td>63.5 ± 40.6</td>
</tr>
</tbody>
</table>

*Others include small-cell, large-cell, and mixed cell types. †Geometric mean ± geometric SD. ‡Pack-years: number of packs per day × years of smoking.

Hospital were recruited between December 1992 and December 2000. Surgically resected noninvolved lung tissue was sampled from 142 patients who were undergoing surgical operation for histologically confirmed, newly diagnosed lung cancer. Among the participants, nonsmokers (n = 7) were excluded. Lung tissue specimens were frozen immediately on dry ice and stored deep-frozen at −70°C until DNA adduct analysis. Blood samples were collected into heparin-treated tubes from each subject and applied to Ficoll-Hypaque density gradients to separate MNC from erythrocytes and granulocytes. Among the cases, blood volumes ≥20 mL considered sufficient for DNA adduct analysis were available for 53 patients. Information on demographic factors, smoking history, and occupation was obtained by trained interviewers using a modified standardized American Thoracic Society respiratory questionnaire.

Genotyping of GST polymorphisms and NAT2

The details of the methods for GST polymorphisms and NAT2 genotyping have been previously described (15, 18). All of the genetic polymorphisms were analyzed using PCR-RFLP techniques. Individuals carrying wild-type alleles (i.e., two “rapid” alleles, NAT2 *4/*4) were defined as the “rapid acetylator” genotype and those with only one copy of a rapid allele (heterozygous variant genotype, NAT2 *4) or those with two slow alleles (homozygous variant genotypes; NAT2 *5A, *5B, *5C, *6, or *7) were labeled “slow acetylators.”
Association between individual GSTs, NAT2, and DNA adduct in lung and MNC

The genotype distribution of the polymorphisms and their association with DNA adduct levels are given in Table 2. The DNA adduct levels are expressed as covariate-adjusted geometric mean with the corresponding 95% CIs estimated by linear regression models. Regarding the difference in adduct levels in lung tissue according to genotype, the adjusted geometric mean of DNA adduct levels with the variant genotype of GSTM1 null and the slow NAT2 acetylator genotype were higher than those with presence of the wild-type GSTM1 genotype or rapid NAT2 acetylator, 173.9 versus 69.5 (P = 0.005) and 144.9 versus 83.3 (P = 0.068), respectively. We examined multiple regression models for the adjusted percent change in DNA adduct levels associated with each GSTs genotype and NAT2 activity. The DNA adduct levels in lung tissue significantly increased by 150.3% (95% CI, 35.4-362.6%) for GSTM1 null and by 73.9% (95% CI, −3.2% to 212.4%) for NAT2 slow acetylator after controlling for potential confounders and other GSTs genes. We also observed an increase in DNA adduct levels in blood MNC with variant homozygous deleted GSTM1 null by 267.4% (95% CI, 28.2-952.9%), but none of GSTP1 AG/GG, GSTT1 null, and NAT2 slow types were statistically significant.

Combined effects of GSTM1 and NAT2 on DNA adduct levels

Figure 1 illustrates the combined effects of GSTM1 genotype with NAT2, as they are thought to act as potential concurrent risk factors of individual genetic susceptibility to DNA damage. We constructed a model that included all of the possible combinations between GSTM1 and NAT2, with the low-risk combination of GSTM1 present and NAT2 rapid acetylator as a reference group. The high-risk group, defined as the combination of GSTM1 null plus NAT2 slow, was highly significantly associated with an increased level of DNA adducts in the lung of 295% (95% CI, 72.7-803.5%) compared with the low-risk combination. In the case of blood analysis, the sample number was small; thus, our combined analysis suffers from a lack of statistical power to find such associations.

Table 2. Estimated percent changes and 95% CIs in DNA adducts in lung and blood MNC per 10^{10} nucleotides by GSTs polymorphisms and NAT2 activity

<table>
<thead>
<tr>
<th>GSTM1</th>
<th>DNA adduct in lung</th>
<th>DNA adduct in blood MNC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>Adjusted GM (95% CI)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Null</td>
<td>56 (43.8)</td>
<td>173.9 (100.9-299.7)</td>
</tr>
<tr>
<td>Wild-type</td>
<td>72 (56.2)</td>
<td>69.5 (41.1-117.3)</td>
</tr>
<tr>
<td>GSTP1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AG or GG</td>
<td>57 (48.3)</td>
<td>115.1 (65.9-201.0)</td>
</tr>
<tr>
<td>AA</td>
<td>62 (51.7)</td>
<td>104.9 (63.0-174.8)</td>
</tr>
<tr>
<td>GSTT1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Null</td>
<td>17 (14.0)</td>
<td>119.7 (53.0-270.5)</td>
</tr>
<tr>
<td>Wild-type</td>
<td>104 (86.0)</td>
<td>100.9 (73.0-139.5)</td>
</tr>
<tr>
<td>NAT2 activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slow</td>
<td>48 (58.5)</td>
<td>144.9 (87.1-241.2)</td>
</tr>
<tr>
<td>Rapid</td>
<td>34 (41.5)</td>
<td>83.3 (48.5-143.2)</td>
</tr>
</tbody>
</table>

NOTE: Coefficients are expressed as percent changes in aromatic DNA adduct—associated GSTs polymorphisms and NAT2 activity adjusted for age at diagnosis, sex, smoking status, pack-years, and GSTM1, GSTT1, GSTP1, and NAT2 genes. Abbreviation: GM, geometric mean.

*Adjusted for age at diagnosis, sex, smoking status, pack-years, and GSTM1, GSTT1, GSTP1, and NAT2 genes.
†P < 0.05.
‡P < 0.1.

41% as current smokers. Geometric mean DNA adduct levels were 86.2 adducts per 10^{10} nucleotides in lung tissues and 36.4 adducts per 10^{10} nucleotides in MNC samples. As expected, the geometric mean of DNA adduct levels was higher in current smokers than in ex-smokers, 179.4 versus 51.3 adducts per 10^{10} nucleotides in lung (P < 0.001) and 60.0 versus 24.0 adducts per 10^{10} nucleotides in blood MNC (P = 0.005), respectively. Additional details of histologic and clinical stage data were published in another report (10).
Discussion

To date, there have been no studies examining the associations of independent and combined carcinogen-metabolizing gene polymorphisms with DNA adduct levels in the target organ, the lung. This study provides evidence for increased DNA adduct levels associated with the GSTM1 null and NAT2 slow genotypes alone or in combination. Moderate to strong associations for the GSTM1 null and NAT2 slow alleles were found, such that these polymorphisms could account for 150% and 74% increases in DNA adduct levels in the lung, respectively. The combination of both “at risk” genotypes, GSTM1 null/NAT2 slow, which are related to reduced phase II enzyme activity, could lead to a 295% (95% CI, 72.7-803.5%) increase in DNA adducts in the lung. Compared with the increases associated with single alleles alone, this indicates an estimated 71% synergism [295% − (150% + 74%)]. Despite the biological plausibility, none of other GSTs polymorphisms evaluated in this study was associated with DNA adduct levels.

The evidence for the independent role of metabolic genotypes and their combinations on carcinogen-DNA adducts in different tissues is insufficient. A few studies have reported a relationship between NAT2 and/or GSTM1 polymorphisms and DNA adducts. As we found in this study, individuals with the GSTM1 null genotype have a greater level of lung DNA adducts formed by benzo[a]pyrene diol epoxide in currently smoking lung cancer patients (13, 14). With regard to blood adducts, GSTM1 null was associated with increased benzo[a]pyrene diol epoxide-DNA adduct levels in leukocytes of smokers (22). Elevated DNA adduct levels in placental tissue were associated with GSTM1 null genotype among subjects living in a polluted area in the Czech Republic (23). Conversely, no association was seen between DNA adducts in total WBC and the GSTM1 null genotype among 89 cases of primary lung cancer (12) and 296 healthy adults (31). However, we measured DNA adducts in MNC, a longer-lived fraction of the WBC (10). Therefore, the DNA adducts in MNC are a better representation of long-term DNA damage, with relatively less variation over time, which may explain the difference between our results and those of earlier studies. NAT2 gene polymorphisms have been proposed as a cancer risk factor (24). The slow NAT genotype has been considered as the recessive trait and linked to a 2-fold increase in lung cancer (17) and an increased risk of bladder cancer (25). Although the evidence for the NAT2 acetylator status as a risk factor in lung cancer has been conflicting (11, 16–18), its effect on DNA damage in lung tissue has not been studied. We found that lung adducts with the NAT2 slow acetylation genotype increased by 74% (95% CI, −3.2% to 212.4%, P = 0.068), as compared with the NAT2 fast genotype, whereas no such association was seen in blood adducts. Our study was based on lung cancer patients who are former and current smokers. Thus, they might be exposed to tobacco-carcinogens at relatively high levels, overwhelming the relatively modest effects of common genetic variants (26). Other studies of smoking lung cancer populations support our findings on blood adducts (17, 27, 28).

Very few studies have reported associations of the combined NAT2 slow acetylator and GSTM1 null genotypes with increased susceptibility to DNA damage in peripheral blood among current smoking lung cancer cases (16) and with elevated risk of bladder cancer (29). We found that genetic factors influence individual susceptibility to DNA damage, and that the combination of NAT2 slow and GSTM1 null genotypes is significantly associated with increased lung adduct levels compared with those in low-risk genotypes (NAT2 rapid-GSTM1 null). This result indicates that carrying more than one of the risk polymorphisms may have synergistic effects on DNA damage in the target tissue and could potentially lead to an increase in carcinogenic potency.

Blood MNC, accounting for about 30% of total WBC, has been suggested as a surrogate tissue for estimating the burden of lung adducts (10). Positive correlations between blood and lung adduct levels (r = 0.77, P < 0.001) were reported in a lung cancer population (10, 21). However, we found independent effects of GSTM1 null on DNA adducts in both tissues, but the effect of NAT2 polymorphisms differed by tissue. Tissue-specific differences were also observed for combinations of these factors. Possibly, metabolic activation of PAHs by various cell types in various tissues leads to tissue-specific differences (19). The metabolism of benzo[a]pyrene differed among rat lung, liver, and peripheral blood lymphocytes (20) and among human samples from bronchus, esophagus, colon, and

![Figure 1. The adjusted geometric mean (GM) and 95% CIs of the DNA adducts levels in lung by all two-way combinations between GSTM1 and NAT2. W, GSTM1 wild-type; N, GSTM1 null; S, NAT2 slow acetylator; R, NAT2 rapid acetylator. *P < 0.1; **P < 0.05.](image-url)
duodenum (30). Differences in individual tissue repair capabilities and in cell division may also influence tissue-specific variations in expression of carcinogen-metabolizing enzymes and in the lifetime of adducts (20). These could explain the differential results of lung and blood MNC. Alternatively, our blood data were limited due to small sample sizes; thus, we acknowledge that our combined analyses suffer from lack of statistical power to detect some associations.

We measured DNA adducts by the nuclease P1 version of the $^{32}$P-postlabeling technique. This assay is generally applied to analyze a mixture of adducts, often referred to as aromatic or bulky adducts (2), which are induced mainly from the ubiquitous PAHs. However, the technique is also effective for the detection of DNA adducts from other aromatic compounds such as aromatic amines or heterocyclic amines (31). Further study is needed to address chemical characterization of tobacco carcinogen-induced DNA damage in smoking populations if the chemical nature of DNA adducts differs by smoking status (21).

In conclusion, this study suggests that GSTM1 null and NAT2 slow genotypes lead to enhanced DNA damage from mutagens in tobacco smoke. In particular, a synergistic deleterious effect was noted when these high-risk genotypes were considered together with the target lung tissue. Therefore, the assessment of a single polymorphic genotype may not be sufficient for evaluating individual receptivity to DNA damage (32). These results provide a better understanding for the identification of vulnerable populations to the DNA damage linked to lung cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

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