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Research Article

Cancer Epidemiology, Biomarkers & Prevention

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

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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 ^{32}P-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% to 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 carcinogen 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

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Table 1. Characteristics of patients with lung cancer and DNA adduct levels in the lung and blood MNC (n = 135)

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

*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."

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, *GSTT1* 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 β 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

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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

	DNA adduct in lung			DNA adduct in blood MNC		
	n (%)	Adjusted GM (95% CI)*	%Change (95% CI)	*n* (%)	Adjusted GM (95% CI)*	%Change (95% CI)
GSTM1						
Null	56 (43.8)	173.9 (100.9-299.7)	150.3 (35.4-362.6)†	24 (46.1)	88.2 (33.5-231.9)	267.4 (28.2-952.9)†
Wild-type	72 (56.2)	69.5 (41.1-117.3)	Reference	28 (53.9)	24.0 (8.9-64.4)	Reference
GSTP1						
AG or *GG*	57 (48.3)	115.1 (65.9-201.0)	9.7 (–40.7 to 103.0)	27 (54.0)	67.5 (22.5-202.7)	115.4 (–30.1 to 564.1)
AA	62 (51.7)	104.9 (63.0-174.8)	Reference	23 (46.0)	31.3 (12.9-76.1)	Reference
GSTT1						
Null	17 (14.0)	119.7 (53.0-270.5)	18.6 (–50.5 to 184.0)	5 (9.4)	57.2 (12.1-269.8)	54.6 (–68.2 to 652.1)
Wild-type	104 (86.0)	100.9 (73.0-139.5)	Reference	48 (90.6)	37.0 (21.8-62.7)	Reference
NAT2 activity						
Slow	48 (58.5)	144.9 (87.1-241.2)	73.9 (–3.2 to 212.4)‡	23 (67.7)	39.0 (16.6-91.8)	–28.2 (–70.5 to 74.8)
Rapid	34 (41.5)	83.3 (48.5-143.2)	Reference	11 (32.3)	54.3 (20.0-147.4)	Reference

NOTE: Coefficients are expressed as percent changes in aromatic DNA adduct–associated *GST* 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).

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.

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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 *GSMT1* 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

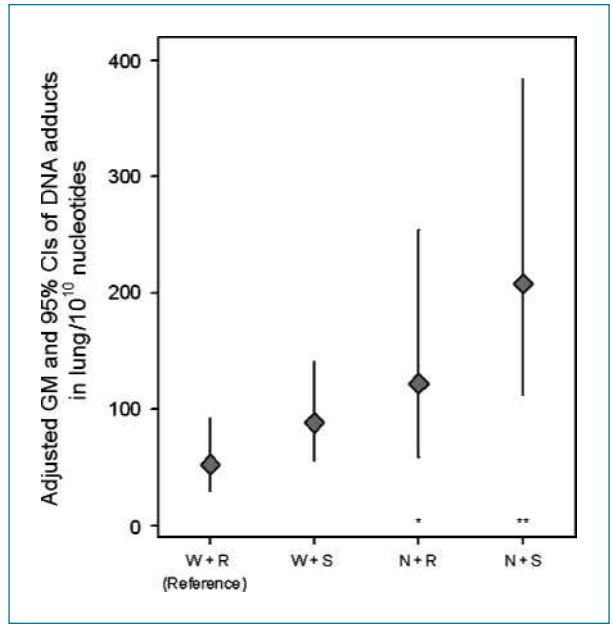

Figure 1. The adjusted geometric mean (GM) and 95% CIs of the DNA adducts levels in lung by all two-way combinations between *GSTM1* and *NAT2*. W, *GSTM1* wild-type; N, *GSTM1* null; S, *NAT2* slow acetylator; R, *NAT2* rapid acetylator. *, $P < 0.1$; **, $P < 0.05$.

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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 susceptibility 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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