Associations between both genetic and environmental biomarkers and lung cancer: evidence of a greater risk of lung cancer in women smokers


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This molecular epidemiologic case-control study of lung cancer incorporated three complementary biomarkers: the glutathione S-transferase M1 (GSTM1) null genotype, a potential marker of susceptibility, and polycyclic aromatic hydrocarbon–DNA adducts (PAH–DNA) and sister chromatid exchanges (SCE), both indicators of environmentally induced genetic damage. Associations between biomarkers and lung cancer were investigated, as were possible gene–environment interactions between the GSTM1 null genotype and tobacco smoke exposure. Subjects included 136 primary non-small cell lung cancer surgical patients and 115 controls at the Columbia Presbyterian Medical Center. Questionnaire and Tumor Registry data, pre-treatment blood samples and biomarker measurements on blood were obtained. Overall, GSTM1 null genotype was significantly associated with lung cancer [odds ratio (OR) = 2.04, 95% confidence interval (CI) = 1.13–3.68]. ORs for GSTM1 and lung cancer were significant in females (2.50, 1.09–5.72) and smokers (2.25, 1.11–4.54) and not significant in males (1.4, 0.58–3.38) and non-smokers (0.88, 0.18–4.33). However, ORs for males versus females and smokers versus non-smokers did not differ significantly. The OR for GSTM1 and lung cancer in female smokers was 3.03 (1.09–8.40), compared with 1.42 (0.53–4.06) in male smokers. In contrast to PAH–DNA adducts in leukocytes, SCE did not differ between cases and controls. Neither biomarker differed significantly between the two GSTM1 genotypes. The combined effect of elevated PAH–DNA adducts and GSTM1 genotype on case-control status (16.19, 1.2–115) appeared multiplicative. Results suggest that the effect of the GSTM1 null genotype is greatest in female smokers, which is consistent with other evidence that indicates women are at higher risk of lung cancer than males, given equal smoking. Persons with both the GSTM1 deletion and elevated PAH–DNA adducts may represent a sensitive subpopulation with respect to carcinogens in tobacco smoke and other environmental media.

Introduction

This study used several complementary biomarkers to investigate gene–environment interactions in lung cancer.

Abbreviations: CPMC, Columbia Presbyterian Medical Center; GSTM1, glutathione S-transferase M1; PAH, polycyclic aromatic hydrocarbon; PCR, polymerase chain reaction; SCE, sister chromatid exchanges.

Inherited deletion of the glutathione S-transferase M1 (GSTM1) gene is thought to be a cancer susceptibility biomarker, in that the GSTM1 protein product catalyzes the metabolic detoxification of a number of carcinogens in cigarette smoke and other environmental media, including the polycyclic aromatic hydrocarbons (PAHs), ethylene oxide and styrene (1–5). The GSTM1 gene is absent in 40–50% of the US population (6). The preponderance of epidemiological studies indicates a protective effect of GSTM1 against lung cancer (5,7–16), while others, possibly because of uncontrolled confounding by gender and ethnicity, or chance, do not (17). These data suggest that individuals lacking the GSTM1 detoxification pathway may be at increased risk of genetic damage and cancer from PAH and other environmental carcinogens.

Two well-studied biomarkers of environmentally induced genetic damage are carcinogen–DNA adducts and sister chromatid exchanges (SCE). Because of their ability to reflect individual variation in metabolism of carcinogens as well as repair of DNA damage, carcinogen–DNA adducts provide an informative individual biologic dosimeter for specific genotoxic pollutants (18–20). The biologic rationale for measuring adducts in studies of cancer also derives from the high correlation observed experimentally between carcinogenicity and adduct formation for a series of mutagens/carcinogens, including benzo[a]pyrene (B[a]P) (19–22). SCE represent reciprocal exchanges between sister chromatids at the same locus following breakage and recombination of the DNA strand during replication. SCE have been widely studied as a non-chemical-specific indicator of genetic damage from mutagens/carcinogens (23), although the health risk associated with this marker is unknown. An increase in SCE has been seen in smokers, in some but not all studies (24–26).

We have reported previously that PAH–DNA adducts were significantly associated with lung cancer in this case-control study (see ref. 18 for details). The present report describes the associations observed between three biomarkers (adducts, SCE and GSTM1), alone and in combination, and lung cancer.

Materials and methods

Subjects and specimen collection

A total of 136 primary pathology confirmed non-small cell lung cancer surgical patients and 115 controls at the Columbia Presbyterian Medical Center (CPMC) participated in the study (see Table I). The study population was 62% Caucasian. The controls were patients in the Department of Orthopedics at CPMC with no history of either cancer or benign lung disease. A sample of blood was obtained from each case upon admission to the hospital for surgery but prior to treatment. A sample of blood was also obtained from each control preoperatively. A total of 76% of the patients contacted participated in the study. Non-participating patients were those who missed interviews and had incomplete tumor registry data, or those who had an incomplete pathology report during the patient enrollment period of the study. Because of the inadequate quantity of DNA extracted from some samples, 86% of the subjects had results for adducts and 87% for GSTM1. Because of technical demands, only a subset of blood samples (from the first 66 subjects enrolled) was analyzed for SCE.

A detailed questionnaire was administered by trained interviewers to obtain...
information concerning cigarette smoke exposure (lifetime and during the past 2 years), dietary P AHs, workplace exposure to P AHs and caffeine consumption. The questionnaire also elicited family history of cancer. Specifically, smoking status was determined as current smoker (within the last 2 months), former smoker or non-smoker. Measures of active smoking included current smoking levels: cigarettes per day (CPD) and pack years (PY), or packs per day smoked. A dietary PAH exposure score was calculated for each subject that reflected average weekly intake of broiled or smoked meat and fish during the previous 2 years. Caffeine consumption was quantified in terms of average daily servings of caffeinated beverages over the preceding 2 years, since caffeine may influence both DNA adducts and cytogenetic markers (26). In addition, subjects, were characterized by area of residence (urban versus rural), other forms of smoking (e.g., pipes and marijuana) and occupational and other environmental exposures to P AHs. Information on alcohol, X-rays, medications and health status was collected as potential confounders for the cytogenetic analyses.

Among all subjects in the study, 211 (85%) were personally interviewed. Information about age, gender, cigarette smoking history, tumor histology (cases) and familial history of lung cancer in first degree relatives was ascertained from the CPMC Tumor Registry for the remaining 35 lung cancer cases and five controls. As previously reported, this secondary source of data is in good agreement with information obtained at interview (18). Therefore, subjects having information from either questionnaire or tumor registry were combined in the analysis. Lastly, pathology reports were reviewed from all of the cases.

Laboratory analyses

To assess individual GSTM1 genotype, DNA was extracted from blood leukocytes and analyzed by polymerase chain reaction (PCR) as previously described (6). The primers used in the PCR mix were G5, 5' GAA CTC CCT GAA AAG CTA AAG C; G6, 5'-GTT GGG CTC AAA TAT ACG GTG G. The SCE assay was performed on lymphocytes according to a variation of the method of Carrano and Moore (23). A total of 50 metaphases were examined for each individual; 25 cells were scored by each of two observers to mitigate technician variability. Results were reported as number of SCE per cell. P AH–DNA adducts were measured in leukocyte DNA by a competitive ELISA assay using a polyclonal antibody that recognizes structurally related PAH–diol epoxide–DNA adducts with fluorescence endpoint detection as previously described (27).

Statistical methods

Details on the statistical analysis of P AH–DNA adduct data are published elsewhere (18). A similar approach was taken for SCE analysis. The differences in SCE levels between cases and controls, as well as between smoking and GSTM1 strata were examined by Student's t-test. Univariate analysis was used to determine whether specific host factors (age, gender, ethnic history of lung cancer, type of lung cancer) and exposure variables (active, passive cigarette smoking, other forms of smoking, dietary P AHs and caffeine consumption, occupational exposure to P AHs, area of residence, alcohol consumption, X-rays, medications and health status) were independently associated (P < 0.1) with SCE. Similar to that for DNA adducts, the multiple regression model for analysis of the association between SCE and lung cancer included smoking status, age, gender and ethnicity. The dose–response relationships between biomarker levels and cigarette smoking (CPD, PY) were also assessed by multiple linear regression. Odds ratios (ORs) were calculated using logistic regression, adjusting for the same known or potential confounders. Because of the small percentage of African-American, Hispanic and Asian subjects in the study, ethnicity stratification is based on Caucasian versus non-Caucasian. Joint effects of GSTM1 and PAH–DNA adducts and GSTM1 and SCE on lung cancer (case status) were analyzed by logistic regression with both PAH–DNA adducts and SCE as categorical variables. As in previous analyses, adducts were dichotomized as high versus low, using a cut-off of two standard deviations (SDs) above the mean in controls. Interaction terms for the joint effects of all possible pairs of the following variables (GSTM1, PAH–DNA adducts, SCE, GST, gender and smoking) were not significant and were not included in the final model. Given the smaller number of subjects with SCE results, data were dichotomized at the mean in controls. To further analyze the joint effects of these risk factors, each subject was classified into one of four risk groups based on his/her results for each pair of biomarkers, GSTM1 and adducts, GSTM1 and SCE (28). ORs were calculated for each risk category and possible multiplicative relationships were examined. The limited number of subjects with SCE all did not permit similar analyses of SCE and adducts.

Results

Demographic and cigarette smoking data are summarized in Table I for cases and controls. The cases and controls were generally comparable in terms of age, season of sample collection, dietary P AH exposures, other forms of smoking, alcohol consumption and number of X-rays received during the preceding 2 years. However, the cases reported more smoking and had a higher proportion of males and Caucasians than the control group. A significantly higher percentage of lung cancer cases had the GSTM1 null genotype (57%, n = 105) than controls (44%, n = 81, OR = 2.04). As shown in Table II, after stratification by gender and smoking status, the ORs for GSTM1 were statistically significant in females (OR = 2.50) and in current smokers and ex-smokers (OR = 2.25), but not in males or non-smokers. The OR for GSTM1 and lung cancer among female smokers was significant (3.03), in contrast to that for male smokers (1.42). However, the ORs for females versus males, and smokers versus non-smokers were not significantly different; nor did the respective interaction terms (GST and gender, and GST and smoking) reach statistical significance (P ≤ 0.05). Since the distribution of GSTM1 varies by ethnic group, the same analysis was applied to the Caucasian subpopulation, and the results were very similar to those found in the entire population (Table III).

Results on P AH–DNA adducts as a single marker have been reported in detail (18). As previously discussed, P AH–DNA adduct levels in leukocytes were significantly higher in cases than in controls (P < 0.05), and adducts increased with the number of cigarettes smoked (CPD) among the 51 cases who were current smokers (P = 0.05) but not among the 22 current smokers in the control group (18).

Mean SCE levels were 11.3 ± 3.7 (mean ± SD) in cases and 10.0 ± 4.2 in controls, a difference that was not statistically significant before or after adjustment for age, gender, ethnicity and smoking (P = 0.49). SCE were increased in smokers and ex-smokers compared with non-smokers in the case group...
effect of PAH–DNA adducts and GSTM1 genotype combined. A total of increased across these four risk categories, but also showed a multiplicative to be in the highest risk category (IV). The ORs were not only significantly subjects who had high adduct levels and were GSTM1 null were considered served as a reference group (I) in the model. In contrast, those study environmental exposures. The purpose was to assess possible genetic damage from tobacco smoke and other biomarkers that might ultimately be useful in risk prediction at the group or individual level.

In this study, there was a significant association between the GSTM1 genotype and lung cancer (OR = 1.79), consistent with some, but not all previous studies (5,7–16). The effect was greater in smokers and ex-smokers, suggesting that the genotype exerts a deleterious effect only in more exposed individuals. A similarly designed bladder cancer case-control study has provided additional support for this theory (6).

The data suggest that the effects of GSTM1 deletion may be heightened among female smokers. The finding that the OR for the GSTM1 null genotype was significant in women (OR = 2.16) but not in men (OR = 1.6) and highest in female smokers is consistent with a prior report (29), which suggested gender differences in both the biology and genetic susceptibility to lung cancer. However, the ORs for men and women were not significantly different from each other. Several epidemiological studies, using different designs and methods of measurement, have reported that for a given level of cigarette exposure, women are at greater risk than men (30,31). Additionally, among lung cancer patients, carcinogen–DNA adduct levels and p53 mutation in lung tumors from smokers were higher in women than men for the same levels of smoking (32,33). The results of these studies suggest that women may have an increased risk of smoking-induced lung cancer (30–33).

Possible mechanisms for a gender-related difference in risk
associated with GSTM1 deletion, if one truly exists, include greater activity of CYP P450 enzymes leading to enhanced formation of DNA adducts and p53 mutation, and hormonal effects on tumor promotion (31). For example, there may be differences in the pattern or overall level of induced P450 enzymes in women compared with men, which results in women having higher levels of DNA-reactive carcinogenic intermediates that are detoxified by GSTM1.

Regarding the other biomarkers, SCE did not differ between cases and controls, which is consistent with prior studies (23,34). This finding is unlike that for PAH–DNA adducts in leukocytes, which were significantly associated with lung cancer (18). The insignificant difference between SCE in cases and controls may reflect the fact that a variety of chemical exposures causes SCE, but not all are lung carcinogens.

In this study, neither PAH–DNA adducts nor SCE were significantly modulated by GSTM1 genotype, before or after stratiﬁng on gender, ethnicity and smoking. These results are in agreement with some (34–36) but not all (38) prior studies of DNA adducts in leukocytes. The absence of a signiﬁcant effect of GSTM1 on PAH–DNA adducts may be caused by the fact that GSTM1 acts on multiple tobacco smoke carcinogens, in addition to PAHs, and that PAH–DNA adduct levels are also modulated by CYP1A1 activity and DNA repair, both of which vary between individuals. Other investigators have reported an effect of GSTM1 on SCE (39–41). This suggests that in the currently studied population, SCE may have been partially induced by exposures to xenobiotics that are not detoxified by GSTM1.

Finally, the combined effect of GSTM1 deletion and high PAH–DNA adducts on lung cancer appeared to be multiplicative. Conclusions are limited by the small number of subjects in some categories and by the large conﬁdence intervals around estimated ORs. Prospective analyses and larger studies will be required to deﬁnitively establish interacitive effects of exposure-related markers and genes in this disease. A multiplicative effect, if conﬁrmed, would be consistent with some overlap between the genetic damage accounted for each of the two biomarkers. Alternatively, these markers may act at different and/or multiple stages in the carcinogenic process, in which case a greater than additive effect might be expected.

The results are consistent with other evidence of a greater risk of lung cancer in female compared with male smokers. They support the use of complementary biomarkers to provide new insights into cancer etiology and prevention. Case-control studies are not able to establish the predictive value of the biomarkers. However, if conﬁrmed, especially in longitudinal or nested case-control designs, the results of this study could lead to new approaches in prevention through the early identiﬁcation of populations and individuals at greater risk of lung cancer.

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References

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