Indoor Coal Combustion Emissions, GSTM1 and GSTTI Genotypes, and Lung Cancer Risk: A Case-Control Study in Xuan Wei, China

Qing Lan, Xingzhou He, Debra J. Costa, Linwei Tian, Nathaniel Rothman, Guizhou Hu, and Judy L. Munford

Center for Environmental Medicine and Lung Biology, University of North Carolina, Chapel Hill, North Carolina 27599-7315 [Q. L., D. J. C.]; Chinese Academy of Preventive Medicine, Beijing, 100050 China [X. H.]; University of California, School of Public Health, Berkeley, California 94720 [L. T.]; Occupational Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Bethesda, Maryland 20892 [Q. L., N. R.]; Biomar International, Chapel Hill, North Carolina 27514 [G. H.]; and United States Environmental Protection Agency, Research Triangle Park, North Carolina 27711 [J. L. M.]

Abstract

The lung cancer mortality rate in Xuan Wei County, China, is among the highest in the country and has been associated with exposure to indoor smoky coal emissions that contain high levels of polycyclic aromatic hydrocarbons. This risk may be modified by variation in metabolism genes, including GSTM1, which encodes an enzyme known to detoxify polycyclic aromatic hydrocarbons. To investigate the relationship between GST genotypes and lung cancer risk in Xuan Wei County, we analyzed GSTM1 and GSTTI genotypes in a population-based case-control study. A total of 122 lung cancer patients and 122 controls, individually matched by age, sex, and home fuel type, were studied. Compared to subjects who used less than 130 tons of smoky coal during their lifetime, heavier users (≥130 tons) had a 2.4-fold (95% confidence interval, 1.3–4.4) increased risk of lung cancer. The GSTTI genotype was associated with a 2.3-fold (95% confidence interval, 1.3–4.2) increased risk of lung cancer. Furthermore, there was some evidence that smoky coal use was more strongly associated with lung cancer risk among GSTM1-null versus GSTTI-positive individuals. In contrast, the GSTTI genotype was not significantly associated with lung cancer risk. Our data suggest that the GSTM1-null genotype may enhance susceptibility to air pollution from indoor coal combustion emissions.

Introduction

In rural Xuan Wei County, Yunnan Province, China, the lung cancer mortality rate is five times the national average. Females in Xuan Wei County are almost all nonsmokers, yet they have the highest lung cancer rate in China (eight times the Chinese national average for females), and the rate among men is also among the highest in China. In general, male lung cancer mortality rates are usually higher than female rates, presumably because of their higher smoking rates. It is very unusual to find similar female and male lung cancer mortality rates (especially when the females are mostly nonsmokers), but this is the case in Xuan Wei (27.7 and 25.3 per 100,000 for males and females, respectively; Ref. 1).

Previous studies have shown an etiological link between lung cancer mortality and domestic smoky coal use. Smoky coal emissions contain high concentrations of PAHs (1–5). Indoor air concentration of particulate matter and extractable organic matter can reach as high as 24.4 and 17.6 mg/m³, respectively, during the burning of smoky coal for home cooking and heating (1). The corresponding concentrations of benzo(a)pyrene, an indicator of PAHs, can become as high as 14.7 µg/m³ (6) during cooking, comparable to exposure levels experienced by coke oven workers.

The role of genetic polymorphisms in relation to lung cancer risk has not been investigated previously in Xuan Wei. The polymorphic GST superfamily encodes enzymes that are known to catalyze the conjugation of xenobiotics and glutathione, and thus, these genes may play a significant role in the detoxification and occasional activation of xenobiotics. Normal or increased GST enzyme activity may protect susceptible tissues from somatic mutations in cellular DNA by facilitating the conjugation and subsequent elimination of electrophilic carcinogens. Carriers of homozygous deletions in the GSTM1 and GSTTI genes display a lack of GSTM1 and GSTTI enzyme activity, respectively (7). The GSTM1 enzyme detoxifies the reactive metabolites of PAHs and other electrophilic carcinogens, whereas the GSTTI enzyme metabolizes other potential carcinogens, such as halomethanes and ethylene oxide (8, 9). Variant forms of these susceptibility genes are common in the population, and based on their specific substrate specificities, they may interact only with particular components of an environmental exposures and thereby alter risk of developing lung cancer. Given that lung cancer risk in Xuan Wei County is in great part attributable to exposure to high levels of indoor air pollutants, including PAHs, we carried out a case-control study to determine whether the GSTM1 or GSTTI genotype was associated with increased lung cancer risk.

1 The abbreviations used are: PAH, polycyclic aromatic hydrocarbon; CI, confidence interval; COPD, chronic obstructive pulmonary disease; GST, glutathione S-transferase; OR, odds ratio.
Subjects and Methods

Study Design and Inclusion Criteria. This was a population-based case-control study. All residents of Xuan Wei were considered the target population. During the period from March 1995 to March 1996, 135 newly diagnosed lung cancer cases, based on a minimum of clinical symptoms and X-ray, were identified at Xuan Wei County Hospital, Yang Chang Mine Hospital, Lai Bin Mine Hospital, Yun Dan Hospital, and the Yunnan Provincial First People’s Hospital (located in Kunming). Almost all lung cancer cases diagnosed in Xuan Wei County are admitted to one of these five hospitals. Among the 135 cases, 133 cases (98%) agreed to participate in this study. The additional criteria for inclusion as a lung cancer case for this study were as follows: (a) confirmation of the clinical diagnosis of lung cancer based on the examination of tissue (14 cases; 10.5%), tracheal lavage and tracheal brushing (21 cases; 15.8%); or sputum sample (70 cases; 52.6%); or (b) the subject died within a 1-year period (17 cases; 12.8%). We chose the 1-year limit based on a report by Hu et al. (10) showing that the 1-year survival rate among Xuan Wei lung cancer patients was 43.2%. Thus, we used death within 1 year as a way to substantiate the diagnosis of lung cancer for those for whom we had only clinical symptoms and X-ray records available. A total of 122 lung cancer cases met the above inclusion criteria. Among them, cell type information on 35 cases was available including 24 non-small cell carcinoma, 3 small cell carcinoma, and 8 others.

One control was selected for each lung cancer case, matching by sex, age (±2 years), village, and type of fuel currently used for cooking and heating at home. Within 2 weeks after the diagnosis and recruitment of each lung cancer case, a control was selected randomly from the list of household registrations (which included age and sex) from the same villages in which the lung cancer patient lived. The participation rate of the controls was 100%. A standardized closed-question form of questionnaire was used to obtain demographic information, smoking history, family and personal medical history, and information on other variables. Each subject was asked to recall the total tonnage of smoky coal or the number of tractor loads (which can be equated with tonnage) that were purchased annually from the local coal distributor. The subjects were also asked about any changes in the rate of their consumption of smoky coal. The cumulative exposure to smoky coal use for a given individual was determined by multiplying the annual rate of smoky coal use times the number of years. Coal consumption was generally fixed for the households over the life cycle of the family. Trained investigators interviewed all cases at the hospital, whereas controls were interviewed in their homes. For the protection of the human subjects, this international research study was conducted according to the guidelines of the World Medical Association Declaration of Helsinki. All of the subjects in this study signed a consent form. The research protocol was approved by an EPA Human Subjects Research Review Official.

Buccal Cell Sample Collection. Each case and control provided a sample of buccal cells for genotyping. They used a toothbrush to loosen the buccal cells and then rinsed with a saline solution. The buccal cells were attached to the toothbrush and then washed into the saline solution; these were collected and stored in a second saline solution containing 50% ethanol at −20°C. The buccal cells were transported to the United States by air and immediately frozen at −20°C.

Genotyping. DNA was isolated from the buccal cells as described previously (11). Determination of GSTM1 and GSTT1 genotypes was conducted using a previously reported PCR-based method (12, 13). This method included using both GST-specific primer pairs and a third primer pair for β-globin in the same amplification mixture. The absence of either a GSTM1- or a GSTT1-specific fragment indicated the corresponding null genotype. A β-globin-specific fragment was used as a positive control for the adequacy of PCR. The buccal cell sample from each subject was assayed twice. Evaluation of genotypes was carried out blinded to case status.

Statistical Methods. Matched ORs and 95% CIs were used to estimate the association between lung cancer and GST genotypes and other risk factors by univariate and multiple conditional logistic regression using Statistical Analysis Software, version 6.12 (SAS Institute Inc.), and S-PLUS for Windows (MathSoft Inc., Cambridge, MA). The OR for smoky coal use was estimated for the mean lifetime cumulative exposure to smoky coal, based on the distribution in the controls. In addition, smoky coal use was modeled as a linear relationship between the total amount of smoky coal use and the log odds of lung cancer. The linear relationship was checked by adding a quadratic term to the regression model and there was no significant improvement in fit. Furthermore, nonparametric logistic regression models using generalized additive models (14) also indicated that a linear model was appropriate for these data.

Additional variables used in the analysis were pack-years of tobacco smoking (defined as cigarette packs smoked daily multiplied by years of smoking, gram equivalents of leaf tobacco, assuming 1 g per cigarette), COPD, and a family history of lung cancer. The first three variables were defined using two levels according to the approximate mean of the distribution in the control group. COPD includes chronic bronchitis and/or emphysema. History of COPD was based on the county or communal hospital record. Chronic bronchitis was defined as having a medical history of cough and sputum on most days for at least 3 months over 2 consecutive years. Emphysema diagnosis was based on clinical symptoms, X-ray, and/or other hospital records. The subject was defined as having a positive family history of lung cancer if there was at least one case of lung cancer among first or second-degree relatives. In this study, most of the subjects with a positive family history had at least one first-degree relative who had developed lung cancer, only one subject had a second-degree family relative with a history of lung cancer. An ever-smoker was defined as a smoker of at least one cigarette per day for 6 months or longer. The association between smoky coal and lung cancer was stratified by GSTM1 genotype, and tests were performed to assess departure from multiplicative effects. The analyses were also conducted both including and excluding the 17 cases who lacked tissue or cytological confirmation who had died within a year, and the results were very similar. The results presented here include these 17 cases.

Results

Table 1 shows the distribution of characteristics for both cases and controls. Age, sex, and type of fuel source were comparable. Eighty-eight % of the males were tobacco smokers, whereas only one female smoked. Therefore, the effect of tobacco smoking was evaluated only in male smokers. Ethnic group, educational level, household income, and dwelling type were comparable in cases and controls (not shown).

Table 2 shows association between several risk factors and the development of lung cancer in both unadjusted and adjusted analyses. A significant increase in the risk of lung cancer was found for the GSTM1-null genotype (OR, 2.3; 95% CI, 1.3–4.2). Compared to subjects who used less than 130 tons of smoky coal during their lifetime, heavier users had a 2.4-fold
logistic regression.

We carried out a population-based case-control study of lung cancer in Xuan Wei County, China, and found that high levels of smoky coal use and the GSTM1-null genotype were each associated with increased risk. Furthermore, there was some evidence that the smoky coal-lung cancer association was stronger among subjects who were GSTM1 null compared to individuals with a positive GSTM1 genotype. These findings are consistent with our previous report that PAHs are probably the major class of lung carcinogens present in smoky coal (1) and with the well-established role of GSTM1 in the detoxification of these compounds (9).

Discussion

We carried out a population-based case-control study of lung cancer in Xuan Wei County, China, and found that high levels of smoky coal use and the GSTM1-null genotype were each associated with increased risk. Furthermore, there was some evidence that the smoky coal-lung cancer association was stronger among subjects who were GSTM1 null compared to individuals with a positive GSTM1 genotype. These findings are consistent with our previous report that PAHs are probably the major class of lung carcinogens present in smoky coal (1) and with the well-established role of GSTM1 in the detoxification of these compounds (9).

Our study has several strengths. It was population based and had very high participation rates among both cases and controls. Furthermore, we were able to quantitatively estimate smoky coal use for each subject. In addition, genotyping was carried out twice on all samples to minimize genotype misclassification. Fifty-one percent of our controls carried the GSTM1-positive genotype. These findings are consistent with our previous report that PAHs are probably the major class of lung carcinogens present in smoky coal (1) and with the well-established role of GSTM1 in the detoxification of these compounds (9).

We also explored the association between smoky coal exposure as a continuous variable, which was consistent with a log-linear increase in lung cancer risk, and its potential interaction with GSTM1 genotype. When coal use was expressed as a continuous variable, the risk of lung cancer was increased by 1.7-fold per 100 tons (95% CI, 1.3–2.4) for all subjects, by 1.2-fold per 100 tons (95% CI, 0.8–1.9) among GSTM1-positive subjects, and by 2.4-fold per 100 tons (95% CI, 1.6–3.9) among GSTM1-null genotype subjects (test for multiplicative interaction, P = 0.05). Results were negligibly affected after conducting sensitivity analysis by excluding subjects at the high extreme of smoky coal use.

(95% CI, 1.3–4.4) increased risk of lung cancer. Smoking more than 25 pack-years was associated with a 1.5-fold (95% CI, 0.8–2.9) increased risk of lung cancer.

Compared to subjects exposed to low levels of smoky coal (<130 tons) who were GSTM1-positive, the OR was 1.8 (95% CI, 0.8–4.1) for GSTM1-null subjects exposed to low levels of smoky coal (<130 tons) and 1.8 (95% CI, 0.7–4.6) for the GSTM1-positive subjects exposed to high levels of smoky coal (≥130 tons; Table 3). The OR for the joint effect of high levels of smoky coal exposure and the GSTM1-null genotype was 5.2 (95% CI, 2.1–12.6), although the test for multiplicative interaction was not significant (P = 0.4).

We also explored the association between smoky coal exposure as a continuous variable, which was consistent with a
continuous variable, was more strongly associated with risk of lung cancer among subjects with the GSTM1-null genotype.

To our knowledge, there are no previous reports of GSTM1 genotype and lung cancer risk within a population in which lung cancer risk is caused primarily by environmental PAH exposure. A recently reported cross-sectional study of coke oven workers, who are exposed to high levels of PAHs, found that individuals with the GSTM1-null genotype had elevated PAH-DNA adduct levels in peripheral blood lymphocytes (20). This provides some biological support for our observation that the GSTM1-null genotype increases lung cancer risk in the presence of substantial environmental PAH exposure.

A recent meta-analysis of GSTM1 genotype and lung cancer risk reported that the GSTM1-null genotype was only weakly associated with lung cancer risk in the general population (21). However, the vast majority of lung cancer cases in these studies were most likely attributable to tobacco smoke, and there is still uncertainty about which of the many carcinogens in tobacco smoke are most important for lung cancer risk. In contrast, many of the lung cancer cases in our series were probably caused by exposure to PAHs. As such, they may represent a relatively unique population in which to study genetic modification of these compounds.

We found no association between the GSTT1-null genotype and lung cancer. This result could be attributable to the fact that GSTT1 is not important, or at least not rate-limiting, in the metabolism of PAHs (22). Alternatively, given our relatively small sample size, our null finding could represent a type II error.

In conclusion, we found that smoky coal use and the GSTM1-null genotype were associated with increased risk of lung cancer in Xuan Wei County. Furthermore, there was some evidence that the association between smoky coal and lung cancer was stronger among subjects with the GSTM1-null genotype. This study provides the first evidence, to our knowledge, that the GSTM1-null genotype may be associated with lung cancer risk attributable to environmental PAH exposure. This hypothesis should be evaluated in larger studies carried out within Xuan Wei County and other populations with substantial environmental exposure to PAHs.

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