Associations of CYPIA1, GSTM1, and CYP2E1 Polymorphisms with Lung Cancer Suggest Cell Type Specificities to Tobacco Carcinogens

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ABSTRACT

The dramatic shift in the pathological presentation of lung cancer [the proportional decrease in squamous cell carcinoma (SCC) and increase in adenocarcinoma (AC)] observed in the United States after the 1950s may have taken place as the result of the reduction in polycyclic aromatic hydrocarbons (PAHs) and the increase in N-nitrosamines in inhaled smoke from filtered low-yield cigarettes. The predominant mutation patterns of these tumors also suggest differences in their etiology. We tested the hypothesis that genetic susceptibility to PAHs, as determined by polymorphisms in CYPIA1 and GSTM1, predominantly causes lung SCCs, and susceptibility to nitrosamines, as determined by polymorphisms in CYP2E1, predominantly causes lung ACs. CYPIA1 and GSTM1 play a major role in the metabolic activation and detoxification of PAHs, respectively, and CYP2E1 plays a major role in the metabolic activation of nitrosamines. We conducted a population-based case-control study among 341 incident lung cancer cases and 456 controls of Caucasian, Japanese, or Hawaiian origin. In-person interviews collected detailed information on lifestyle risk factors, and DNA extracted from peripheral leukocytes was used in PCR-based genotyping assays. Logistic regression analyses were used to compute odds ratios and 95% confidence intervals (CIs) for each cell type, adjusting for smoking and dietary variables. The presence of at least one copy of the CYPIA1 Mspl variant allele was found to be associated with a 2.4-fold (95% CI, 1.2–4.7) increase in the risk of SCC when this gene was considered singly and a 3.1-fold (95% CI, 1.2–7.9) increase in the risk of SCC when combined with a GSTM1 deletion. No significant association was found between Mspl and all lung cancers or other cell types or with the CYPIA1 exon 7 polymorphism. In contrast, the CYP2E1 Rs1 and Dra1 polymorphisms were not clearly related to SCC risk, but these homozygous variant genotypes were associated with a 10-fold (95% CI, 0.0–0.5) decrease in the risk of overall lung cancer (Rs1 variant) and AC (Dra1 variant) compared to the homozygous wild-type genotypes. Inverse associations with these two closely linked CYP2E1 polymorphisms were also suggested for small cell carcinoma. In agreement with past experimental and epidemiological data, the associations found in this study between CYPIA1 and lung SCC and between CYP2E1 and lung AC suggest a certain specificity of tobacco smoke PAHs for lung SCC and tobacco-specific nitrosamines for lung ACs.

INTRODUCTION

A dramatic change in the histological presentation of lung cancer has taken place behind the shroud of the 20th century lung cancer epidemic. In the past 50 years, AC, a rare tumor type at the turn of the century, has replaced SCC as the most frequent lung cancer histological type in many developed countries (1). Indeed, for some subgroups of the United States population, such as white men in Connecticut, the incidence of lung SCC has now peaked, whereas the rate for lung AC is still increasing (2). It has been proposed that changes in cigarette composition and smoking behavior may have caused this histological shift (2, 3). Until the 1950s, cigarettes were predominantly unfiltered and high tar and produced smoke too irritating to permit deep inhalation. As a result, most smoke particulates were deposited on the epithelium at the branches of central bronchi, where SCCs predominantly occur (3, 4). Smokers of more recently manufactured cigarettes have compensated for the lower nicotine yield by inhaling more deeply and smoking more intensely, resulting in an increased exposure of the peripheral lung, the site of origin of most ACs, to tobacco carcinogens (3, 4). To achieve lower tar, tobacco ribs and stems and more burley varieties were incorporated into the tobacco blend of American cigarettes (3, 4). This reformulation has led to a reduction in PAHs and an increase in nitrogen oxides and N-nitrosamines in the inhaled smoke (3, 4). Thus, it is possible that the proportional decrease in lung SCCs may be related to a reduction in the PAH exposure of the central bronchi, and the dramatic rise in lung ACs may be related to an increased exposure of the peripheral lung to tobacco-specific nitrosamines.

A great deal of experimental data also suggest etiological differences between the two main lung cancer cell types. Mutations in the p53 tumor suppressor gene are twice as common in SCCs (65%) as in ACs (33%), with a greater proportion of these mutations being G to T transversions in SCCs (45%) than in ACs (23%; Ref. 5). Benzo-(a)pyrene, the most potent of the PAHs, has been shown to preferentially form adducts at major p53 hot spots for G to T transversions in lung tumors (6), providing a direct mechanistic link between a PAH in tobacco smoke and a mutation that occurs most frequently in SCCs. Tobacco-specific nitrosamines are known to induce lung ACs in rodents when injected systemically (7). Such tumors contain activated K-ras proto-oncogenes with a mutation in codon 12 (7). Similar DNA alterations are commonly found in the lungs of smokers and in lung ACs (7).

Both PAHs and nitrosamines require metabolic activation by cytochrome P450 enzymes to exert their genotoxic effects. CYPIA1 and CYP2E1 are of critical importance for the activation of PAHs and nitrosamines, respectively (8). GSTM1 plays a major role in the detoxification of PAH-activated intermediates (9). These enzymes exhibit wide interindividual variability in their activity. Genetic polymorphisms thought to be linked to functional changes in these enzymes have been associated with lung cancer in the past, but with great inconsistency across studies (10–25). Because past studies were often small, and all used convenience samples or hospital-based designs, these inconsistent findings may have been due to insufficient power and/or selection bias. We also postulated that they may have resulted from some underlying cell type specificity in these associations. We conducted a large population-based case-control study of genetic susceptibility to lung cancer to test the hypothesis that susceptibility to PAHs, as determined by polymorphisms in CYPIA1 and GSTM1, predominantly causes lung SCCs, and susceptibility to nitrosamines, as determined by polymorphisms in CYP2E1, predominantly causes lung ACs.
ASSOCIATIONS OF POLYMORPHISMS WITH LUNG CANCER

MATERIALS AND METHODS

The human subjects protocol for this study was reviewed and approved by the Committee on Human Studies of the University of Hawaii (Honolulu, HI) and by the institutional review board of each participating hospital. We also obtained written informed consent from all subjects.

Lung cancer patients were identified by the rapid-reporting system of the Hawaii Tumor Registry, a member of the Surveillance, Epidemiology, and End Results Program of the National Cancer Institute. Eligible cases were all patients with histologically confirmed primary lung cancer who were diagnosed between January 1, 1992 and March 31, 1997 in all main medical centers on the island of Oahu, Hawaii. Other eligibility criteria included age between 26 and 79 years, Oahu residency, no previous history of lung cancer, and appropriate ethnicity (>75% Japanese, >75% Caucasian, any Hawaiian/part-Hawaiian heritage). Histological information was directly abstracted from each patient’s pathology report. An interview was completed for 64% of the eligible cases. The main reasons for nonparticipation were patient refusal (17%), physician refusal (2%), and death with the absence of a suitable surrogate for interview (17%).

Controls were selected randomly from a list of Oahu residents interviewed by the State of Hawaii Department of Health as part of a health survey of a 2% random sample of state households. This source was supplemented with controls from Health Care Financing Administration participants on Oahu. One control was matched to each case on sex, ethnicity, and age (±2 years). The overall participation rate for the controls was 62%. Reasons for nonparticipation included refusal (25%), inability to locate (10%), serious illness (1%), and death (2%). The analysis presented here was conducted with 341 cases (76% of interviewed cases) and 456 population controls (80% of interviewed controls) who donated a blood specimen for the study.

In-person interviews were conducted at the subjects’ homes by trained interviewers. On average, cases were interviewed within 4 months of diagnosis. The questionnaire included detailed demographic information, including the ethnic origin of each grandparent, a lifetime history of tobacco and alcohol use, a quantitative food frequency questionnaire, and a personal history of second-hand smoke, various relevant medical conditions and occupational exposures, and a family history of lung diseases. Information was collected on the types (nonfiltered cigarettes, filtered cigarettes, cigars, and pipes) of tobacco product ever smoked daily for at least 6 months and, for each tobacco product, the usual amount per day, the age at which the subject started smoking, the overall duration of use, and, for ex-smokers, the age at which the subject stopped smoking. We also inquired about any periods of smoking cessation for each tobacco product during the subject’s life. Smokers were considered current smokers if they smoked up to 1 year before the date of diagnosis, former smokers if they smoked up to 5 years before the date of diagnosis for cases or up to the date of the interview for controls. For this analysis, cigarettes, pipes, and cigars were treated equivalently.

The food frequency questionnaire used in this study (26) has been previously validated in our population (27). Frequencies and the amounts consumed were sought for 242 food items or categories. The reference period for the dietary questionnaire was the year before diagnosis for cases or the year before the interview for controls. Colored photographs of most food items showing three different portion sizes, as well as measuring cups and spoons, were used in the interview to facilitate the quantification of intakes. Subjects were also questioned about the brand and dose of any vitamin supplements (including multivitamin pills) taken for a minimum of 3 months during the reference period. The food composition data were based primarily on the United States Department of Agriculture nutrient database (28) and supplemented with data from other research and commercial publications.

Laboratory personnel were blinded to the case-control status of the subjects. DNA was purified from peripheral blood lymphocytes by SDS/proteinase K treatment and phenol/chloroform extraction (29). The first of two CYP2E1 polymorphisms studied is a T to C transition 264 bp downstream from the polyadenylate signal that creates a Mspl restriction site. Genotyping for this polymorphism was carried out by PCR amplification using primers 5'-TCGTCAGTTCCTGAAAG-3' and 5'-GAAGAGCCAAGGACAGGTAC-3'. We modified the assay developed by Kim et al. (31) to investigate the polymorphism in intron 6 of CYP2E1 described by Uematsu et al. (19) with the restriction endonuclease Dral. Primers 5'-TCGTCAGTTCCTGAAAG-CAGG-3' and 5'-GAAGGCTCTGATGCAAGTACCGA-3' were used in a PCR reaction consisting of an initial denaturation step at 94°C for 2 min followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 2 min, with final annealing and extension steps at 60°C for 1 min and 72°C for 10 min. After digestion with Dral, the PCR product was resolved on an agarose gel.

CYP2E1 also exhibits several polymorphisms in the 5’ flanking region of the gene. One of them includes two distinct base substitutions that are in genetic disequilibrium with each other and creates RsuI and PstI restriction sites (32). We used primers 5'-TTTACCTGTTCCTTACATTG-3' and 5'-CCAGTTCGACTCTACATTGTC-3' to amplify a region containing the two different base substitutions as described in Ref. 36. To detect the deletion of the GSTM1 gene locus, we amplified exons 6–7 of the gene using primers 5'-GAACCTCCTGGAAAGCTAAAGC-3' and 5'-GGTTGGGTTCAAAATTACGGCTG-3' (33). We amplified a 268-bp fragment of the β-globin gene as an internal standard using primers 5'-CACTCTCCACTCCGAGCCC-3' and 5'-GAAGGACCCAAAGGCAATG-3'.

In the statistical analysis, we used χ² statistics for homogeneity to test for case-control differences in the distributions of the genotypes or other parameters under study. Correlations between categorical variables were measured by the coefficient (34). Unconditional logistic regression (35) was used to compute the ORs and 95% CIs, with adjustment for several covariates found to be associated with risk (age, sex, and race, indicator variables; saturated fat and total vegetable intakes, continuous variables). The matched pair design was not followed in this analysis, because often only one member donated a blood sample. Nutrient intakes were adjusted for caloric intake using the method of residuals (36). Other dietary variables, such as the intake of specific carotenoids, vitamin C, vitamin E, and fiber, were considered but were not retained in the final model because they were not as strongly associated with risk as that of total vegetables. Several ways of modeling the smoking effect were explored, including separate categorization for duration and amount, use of a pack-years and age-started term, logarithmic transformations of the variables, and the addition of higher polynomial terms. The best-fitting model was one that included an indicator variable for smoking status (ever, never smoked) and separate continuous terms for duration, amount and (duration)². The log-likelihood ratio test was used to test the statistical significance of modeled effects. We also used this test to determine the interaction among certain variables with respect to lung cancer risk. The test compared a main effects, no interaction model with a fully parameterized model containing all possible interaction terms for the variables of interest. Genetic trends were modeled by assigning a value of 1, 2, or 3 to the genotype variable according to the subject’s number of variant alleles (zero, one, and two rare alleles, respectively).

RESULTS

Table 1 presents relevant characteristics of the lung cancer cases and population controls. No significant differences were found in the age, sex, and ethnic distributions of cases and controls. As expected, smoking was strongly associated with lung cancer risk. In addition, and also in agreement with past studies in this population (37, 38), cases consumed more saturated fat and less vegetables than did controls.

Among population controls, the frequency of the CYP2E1 Mspl variant allele was 36.8% in Japanese, 8.9% in Caucasians, and 42.4% in Hawaiians. The frequency of the CYP1A1 Ile-Val polymorphism was 24.4% for Japanese, 3.4% for Caucasians, and 16.3% for Hawaiians. The frequency of the CYP2E1 Dral variant allele was 33.3% in Japanese, 6.6% in Caucasians, and 15.4% in Hawaiians. The frequency of the RsuI rare allele was 25.6% in Japanese, 2.0% in Caucasians, and 16.2% in Hawaiians. The frequency of the GSTM1

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deletion was 54.6% in Japanese, 60.0% in Caucasians, and 67.0% in Hawaiians. The frequencies of the CYPIA1 Mspl or exon 7 variant alleles by genotypes for the CYP1A1 and CYP2E1 polymorphisms, respectively. The frequencies of the CYP1A1 Mspl or exon 7 variant alleles for all cases, ACs, small cell carcinomas, and other cell types were similar to those for population controls. However, the Mspl variant allele was significantly more common in cases with SCC than in controls (P = 0.005). The frequency of the exon 7 variant allele was also elevated for SCC, but not significantly so. The Mspl and exon 7 polymorphisms were closely linked in this study (φ = 0.7; P = 0.001), as reported earlier for these ethnic groups (11, 13). In contrast, the variant alleles for the CYP2E1 Drai and Rsa1 polymorphisms and, in particular, the homozygote variant genotypes were less common in lung cancer overall, ACs, and, to a lesser extent, small cell carcinomas, as compared to controls. As previously found for these ethnic groups (19, 32), the Drai and Rsa1 polymorphisms were closely linked in our data (φ = 0.9; P = 0.001).

Table 4 presents adjusted ORs and 95% CIs for all lung cancer and site-specific cancers, as compared to controls. As previously found for these ethnic groups (11, 13). In contrast, the variant alleles for the CYP2E1 Drai and Rsa1 polymorphisms and, in particular, the homozygote variant genotypes were less common in lung cancer overall, ACs, and, to a lesser extent, small cell carcinomas, as compared to controls. As previously found for these ethnic groups (19, 32), the Drai and Rsa1 polymorphisms were closely linked in our data (φ = 0.9; P = 0.001).
ASSOCIATIONS OF POLYMORPHISMS WITH LUNG CANCER

Table 4 ORs (and 95% CIs) for lung cancer by CYP1A1 and CYP2E1 genotypes

<table>
<thead>
<tr>
<th>CYP1A1 Mspl</th>
<th>All (341/456)</th>
<th>SCC (74/456)</th>
<th>AC (162/456)</th>
<th>Small cell carcinoma (51/456)</th>
<th>Other (52/456)</th>
</tr>
</thead>
<tbody>
<tr>
<td>m1/m1</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>m1/m2</td>
<td>1.3 (0.9-1.9)</td>
<td>2.3 (1.1-4.7)</td>
<td>1.0 (0.6-1.7)</td>
<td>1.2 (0.5-2.7)</td>
<td>1.5 (0.7-3.2)</td>
</tr>
<tr>
<td>m2/m2</td>
<td>1.2 (0.6-2.2)</td>
<td>2.6 (1.0-6.7)</td>
<td>0.7 (0.3-1.6)</td>
<td>1.1 (0.4-3.5)</td>
<td>0.6 (0.2-2.5)</td>
</tr>
<tr>
<td>P = 0.34</td>
<td>P = 0.02</td>
<td>P = 0.60</td>
<td>P = 0.75</td>
<td>P = 0.94</td>
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</table>

<table>
<thead>
<tr>
<th>CYP2E1 Dra1</th>
<th>A/A</th>
<th>A/G</th>
<th>G/G</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>DD</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>DC</td>
<td>1.0 (0.7-1.6)</td>
<td>1.2 (0.6-2.4)</td>
<td>1.1 (0.7-1.8)</td>
<td>0.6 (0.3-1.4)</td>
<td>1.0 (0.5-2.1)</td>
</tr>
<tr>
<td>CC</td>
<td>0.2 (0.1-0.7)</td>
<td>0.7 (0.2-3.0)</td>
<td>0.1 (0.0-0.5)</td>
<td>0.3 (0.0-2.5)</td>
<td>0.3 (0.0-2.5)</td>
</tr>
<tr>
<td>P = 0.12</td>
<td>P = 0.92</td>
<td>P = 0.10</td>
<td></td>
<td></td>
<td>P = 0.43</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CYP2E1 Rus1</th>
<th>c1/c1</th>
<th>c1/c2</th>
<th>c2/c2</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>DD</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>DC</td>
<td>1.0 (0.5-1.3)</td>
<td>0.7 (0.3-1.5)</td>
<td>0.8 (0.4-1.3)</td>
<td>0.3 (0.1-0.9)</td>
<td>0.6 (0.3-1.4)</td>
</tr>
<tr>
<td>CC</td>
<td>0.8 (0.0-0.5)</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P = 0.03</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a ORs were adjusted for age, sex, ethnicity, smoking status, years of smoking, (years of smoking), number of cigarettes smoked/day, and saturated fat and total vegetable intakes.

10-fold decrease in the risk of overall lung cancer (Rsa1 variant) and AC (Dra1 variant) compared to the homozygous wild-type genotypes. Inverse associations with these two closely linked CYP2E1 polymorphisms were also suggested for small cell carcinomas.

Cigarette smoke contains hundreds of constituents that may play a role in carcinogenesis (4). Compounds that have received the most interest include PAHs, N-nitrosamines, active oxygen species, aldehydes, and metal. The exact contribution of these agents to lung carcinogenesis in humans is unknown. However, mutational spectrum analysis of lung tumors implicates certain patterns of p53 mutation with specific cell types. For example, many more lung SCCs present the p53 mutations are hallmarks of mutagenesis involving certain PAHs, such as benzo(a)pyrene, and other PAHs were administered in the lungs of rats, the tumors induced were almost exclusively SCCs (39).

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Cytochrome P450 1A1 and glutathione S-transferase μ are, respectively, phase I and phase II enzymes involved in the metabolic activation and detoxification of PAHs found in tobacco smoke. The activity of cytochrome P450 1A1 has been shown to vary greatly in the lung tissue of different individuals (40, 41) as an inheritable trait (42) and as the result of induction by tobacco smoke (43). Higher levels of aryl hydrocarbon hydroxylase activity (which is mediated by CYP1A1) have been reported in the lymphocytes of lung cancer patients than in those of controls (44, 45), but this finding has been difficult to replicate (46). Consequently, interest has focused on common genetic polymorphisms recently identified in the CYPIA1 and GSTM1 genes. The GSTM1 polymorphism is a deletion of the gene and results in a loss of enzymatic activity. The two linked CYP1A1 polymorphisms (Mspl and Ile-Val) have been associated with an increase in enzymatic activity as well as inducibility (47-50).

The association between CYP1A1 and lung cancer risk has been examined in a number of case-control studies. However, these studies have all used convenience samples or a hospital series and rarely have

Table 5 ORs (and 95% CIs) for lung cancer by CYPIA1 Mspl genotype and race

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Japanese</th>
<th>Caucasian</th>
<th>Hawaiian</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n OR (95% CI)</td>
<td>n OR (95% CI)</td>
<td>n OR (95% CI)</td>
</tr>
<tr>
<td>m1/m1</td>
<td>9/70</td>
<td>1.0</td>
<td>16/146</td>
</tr>
<tr>
<td>m1/m2</td>
<td>9/80</td>
<td>1.4 (0.4-4.3)</td>
<td>8/29</td>
</tr>
<tr>
<td>m2/m2</td>
<td>5/24</td>
<td>2.1 (0.5-8.4)</td>
<td>P = 0.29*</td>
</tr>
</tbody>
</table>

a ORs were adjusted for age, sex, ethnicity, smoking status, years of smoking, (years of smoking), number of cigarettes smoked/day, and saturated fat and total vegetable intakes.

b Number of cases/number of controls.

c OR and 95% CI.

d P for the genetic trend.

Table 6 ORs for the combined effect of GSTM1 and CYPIA1 Mspl on cell types of lung cancer

<table>
<thead>
<tr>
<th>CYP1A1 Mspl</th>
<th>GSTM1</th>
<th>SCC</th>
<th>OR (95% CI)</th>
<th>AC</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>m1/m1</td>
<td>+</td>
<td>11/101</td>
<td>1.0</td>
<td>39/101</td>
<td>1.0</td>
</tr>
<tr>
<td>m1/m1</td>
<td>-</td>
<td>17/147</td>
<td>1.1 (0.4-2.7)</td>
<td>59/147</td>
<td>0.9 (0.5-1.6)</td>
</tr>
<tr>
<td>m1/m2, m2/m2</td>
<td>+</td>
<td>16/81</td>
<td>1.9 (0.7-5.1)</td>
<td>34/81</td>
<td>1.2 (0.6-2.3)</td>
</tr>
<tr>
<td>m1/m2, m2/m2</td>
<td>-</td>
<td>30/121</td>
<td>3.1 (1.2-7.9)</td>
<td>29/121</td>
<td>0.7 (0.4-1.4)</td>
</tr>
</tbody>
</table>

a ORs were adjusted for age, sex, ethnicity, smoking status, years of smoking, (years of smoking), number of cigarettes smoked/day, and saturated fat and total vegetable intakes.

b +, at least one functional allele; -, no functional GSTM1 allele.

c Number of cases/number of controls.
carefully adjusted risk estimates for smoking variables. Overrepresentation of the variant Mspl and Rsal alleles in lung cancer patients was first reported in Japan, a population with a high frequency of these alleles (10, 11). This observation has since been confirmed in a second Japanese study (12). In the Japanese data, the association was clearly stronger for SCC than for AC (12, 51, 52). In contrast, studies of CYP1A1 and lung cancer conducted in Caucasians have been mostly inconsistent, with some early studies finding no association (13-15), and more recent ones reporting an increased risk with the variant alleles (16, 17). These discrepancies have been attributed to a lack of statistical power in some of these studies due to the rarity of the CYP1A1 polymorphisms in Caucasians. Interestingly, studies that have assessed polymorphisms in both phase I and phase II pathways have been more consistent, including those in Caucasians, in finding elevated ORs for lung cancer in individuals with both CYP1A1 and GSTM1 polymorphisms (53-57). Again, the evidence for such an association was strongest for SCC. Similarly, the evidence for an association between GSTM1 (alone) and lung cancer has been most suggestive for lung SCC, particularly at moderate levels of smoking, compared with any other cell types (58). Thus, overall, the findings from the present study, which is the first using a population-based design, are consistent with those of past studies in implicating the linked Mspl and Ile-Val polymorphisms in CYP1A1, especially when combined with a GSTM1 deletion, in determining the genetic predisposition to lung SCC.

Tobacco-specific nitrosamines are known to be strong inducers of lung ACs in rodents when administered systemically (7). They have limited effect when applied topically, because they need to be activated in the liver or in the target organ. Consequently, exposure to the ultimate carcinogens is expected to be greatest in highly vascularized areas, such as the bronchioles and alveoli, compared with the less vascularized large bronchi (3). The same DNA and hemoglobin adducts produced in rodents have also been detected at higher levels in smokers than in nonsmokers (59). These adducts have been associated with the development of lung AC in rodents, with frequent codon 12 mutations in the K-ras proto-oncogene (60). In humans, the same K-ras mutations are found in 30% of lung ACs and are rarely found in SCCs (61).

Cytochrome P450 2E1 plays an important role in the metabolic activation of various nitrosamines, including several potent tobacco-specific procarcinogens (8). In addition, it effectively reduces oxygen to radical species, thus contributing to lipid peroxidation and oxidative stress (62). Although the Rsal polymorphism in the CYP2E1 gene has been associated with increased transcription of a reporter gene in vitro (32), phenotyping studies using the drug chlorzoxazone as a metabolic probe have shown that individuals with the variant Rsal allele have a lower basal CYP2E1 activity, and that enzyme activity is less inducible by ethanol (63-64).

It is important to note that the observed associations between polymorphisms and lung cancer may not be representative of the general population. For example, the frequency of certain variants may differ between populations, and the effect of a variant may be modified by other genetic or environmental factors. Additionally, the limited sample size of the present study may have limited the statistical power to detect small associations. Therefore, further research is needed to confirm these findings and to better understand the role of genetic factors in the development of lung cancer.

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