Susceptibility to Lung Cancer in Light Smokers Associated with CYPIA1 Polymorphisms in Mexican- and African-Americans

Naoko Ishibe, John K. Wiencke, Zheng-fa Zuo, Alex McMillan, Margaret Spitz, and Karl T. Kelsey

Departments of Epidemiology and Environmental Health, Harvard School of Public Health, Boston, Massachusetts 02115 [N. I.]; Laboratory for Molecular Epidemiology, Departments of Epidemiology and Biostatistics, University of California-San Francisco, San Francisco, CA 94143-0560 [I. K. W.]; Department of Cancer Biology Harvard School of Public Health, Boston, Massachusetts 02115 [Z-f. Z., K. T. K.]; Department of Biostatistics, Department of Cancer Biology Harvard School of Public Health, Boston, Massachusetts 02115 [A. M.]; Department of Epidemiology, University of Texas M.D. Anderson Cancer Center, Houston, Texas 77030 [M. S.]; and Occupational Health Program, Harvard School of Public Health, Boston, Massachusetts 02115 [K. T. K.]

Abstract

The gene-environment associations between potential carcinogenic agents modified by polymorphisms in the cytochrome P450 1A1 (CYPIA1) gene and lung cancer risk were assessed in a hospital-based case-control study composed of African- and Mexican-Americans. The study involved 171 cases and 295 controls identified from the greater Houston and San Antonio metropolitan areas. Both the exon 7 and MspI polymorphisms were analyzed by RFLP of PCR-amplified DNA, and in addition, the African-American-specific polymorphism was assayed for subjects who reported that they were African-American. Logistic regression analysis was performed to assess the association between each of the CYPIA1 polymorphisms and lung cancer, adjusting for the matching variables (age, sex, ethnicity) and other potential risk factors. Interactions between pack-years smoked, CYPIA1 genotypes, and case status were also evaluated.

The variant allele frequencies did not differ by case status, but the distributions of genotypes were strikingly different by ethnicity. In addition, both the exon 7 and MspI polymorphisms, but not the African-American-specific polymorphism, were modified by the amount of cigarette consumption measured in pack-years. An approximate 2-fold increase in lung cancer risk among individuals with one or more of the variant alleles was observed among light smokers (defined as having smoked $\leq 30$ pack-years). The respective risk ratios for the exon 7 and MspI polymorphisms were $2.26$ (95% confidence interval, 0.82–6.26) and $2.03$ (95% confidence interval, 0.82–4.01) at low smoking dose. No such increase in risk was found among heavy smokers (>30 pack-years). This phenomenon at low smoking dose was also observed when the two common polymorphisms were combined, which resulted in a progressive increase in risk with an increasing number of variant alleles. These results indicate that at low smoking levels, the MspI and exon 7 CYPIA1 genetic polymorphisms confer susceptibility to lung cancer.

Introduction

Lung cancer is the leading cause of all cancer deaths in the United States. Approximately 150,000 individuals will succumb to this disease this year (1). Although environmental exposures such as cigarette smoke have consistently been found to be associated with the development of lung cancer, only a fraction of exposed individuals actually develop the disease, suggesting that there may be genetically determined factors that modify environmental exposures and result in variations in host susceptibility.

The AHH enzyme is responsible for activating the carcinogenic polyaromatic hydrocarbons found in tobacco (2, 3). Inherited variations in AHH have been observed, and consequently, there has been great interest in the CYPIA1 gene that contributes to AHH activity. Phenotypically, CYPIA1 inducibility has been reported to be associated with lung cancer development (4). In the human CYPIA1 gene, three polymorphisms have been reported: a MspI RFLP in the 3′-noncoding region (5); an adenine-to-guanine transition at nucleotide 4889 in exon 7 (6); a cytosine-to-adenine transversion at nucleotide 4887 (7), and another MspI RFLP, also in the 3′-noncoding region, that has only been observed among individuals of African descent (8).

Each locus has been investigated epidemiologically for any relationship with lung cancer development, with varying results. In the Japanese, where the MspI variant allele frequency is 0.31, the homozygous variant individuals have been observed to be overrepresented among lung cancer patients (5, 9). A similar association between the exon 7 polymorphism and lung cancer risk has also been reported for this ethnic group (6).

More interestingly, studies conducted among Japanese have consistently reported a stronger association between the variant genotypes and lung cancer risk among light smokers (10–12). To address this observation, several researchers have analyzed and presented their findings in various ways. Among individuals who develop lung cancer, cumulative smoking dose has been found to be lower among persons carrying the MspI variant alleles than for wild-type individuals (10). This phenomenon was observed to be stronger when the analysis was

1. The abbreviations used are: AHH, aryl hydrocarbon hydroxylase; CI, confidence interval.

Received 6/3/97; revised 8/25/97; accepted 8/27/97.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

$^1$ This study was made possible by Grants ES00002, ES06717, ES08357, ES04705 and T32ES07069 from the National Institute of Environmental Health Sciences.

$^2$ To whom requests for reprints should be addressed, at Department of Cancer Biology, 665 Huntington Avenue, Boston, MA 02215. Phone: (617) 432-3313; Fax: (617) 432-0107; E-mail: kelsey@hop.harvard.edu.
restricted to squamous cell carcinoma. In addition, risk ratios calculated within the smoking stratum have also been used to illustrate this low dose effect of the gene (11, 12).

In contrast, results from studies conducted among Caucasian populations have reported inconsistent findings (13-18). However, a German group observed a relationship between the exon 7 site and lung cancer (17), and a recent report from the United States also reported an association of the MspI variant with lung cancer risk (18). These discrepancies have been attributed primarily to the lower prevalence of the CYP1A1 variant traits among Caucasians, resulting in a reduction in statistical power to detect associations between the trait and lung cancer.

There are other potential differences between ethnicities that may account for some of these inconsistencies. For example, the expression of CYP1A1 is dependent on the aryl hydrocarbon signal transduction pathway, and if there are differences in the aryl hydrocarbon receptor and/or the aryl hydrocarbon nuclear translocator between ethnicities, these two could also affect the associations observed.

For other ethnic and racial groups, few data are available on the potential role of CYP1A1 in lung carcinogenesis. In a Brazilian study by Sugimura et al., although no association was observed with the MspI polymorphism (19-21), a statistically significant overrepresentation of variant exon 7 genotypes was observed among lung cancer cases (20). The effects of the variant genotypes were particularly enhanced among light smokers (defined as having smoked <40 pack-years). These researchers were unable, however, to look at this relationship by stratifying on race (i.e., African versus American Indian descent) due to the small number of subjects within these subgroups. An investigation in such mixed populations would be of interest due to reports that people of the Americas (American Indians) are of Asian descent (22, 23), as well as in light of the fact that an association between the MspI and exon 7 CYP1A1 polymorphisms has been observed with lung cancer among the Japanese.

To investigate whether the CYP1A1 gene could explain part of the differences in lung cancer incidence rates seen between racial groups, we examined the relationship of CYP1A1 polymorphisms to lung cancer risk in 171 lung cancer cases and 295 control subjects of Mexican-American and African-American descent.

Subjects and Methods

Study Subjects. The cases and controls included in this report are from an ongoing hospital-based case-control study of lung cancer in minority populations described previously (24, 25). A total of 466, 464, and 285 blood samples were genotyped for the MspI, exon 7, and African-American-specific polymorphisms, respectively. The following results were based on 171 cases and 295 controls for whom we have genotype information. The cases were newly diagnosed patients with histologically confirmed lung cancer who had not undergone radiotherapy or chemotherapy. Those who were enrolled also self-reported to be of African-American or Mexican-American ancestry. The patients were identified from The University of Texas M. D. Anderson Cancer Center and from county, community, and Veterans Affairs hospitals in the Houston and San Antonio metropolitan areas. Controls were identified from a convenience sample recruited from community centers, cancer-screening programs, churches, and employee groups. Only individuals without a history of cancer were eligible for participation as controls. The controls were frequency matched to the cases by sex, ethnicity, and age (±5 years).

Data Collection. After informed consent was obtained, interviews were conducted by trained interviewers/phlebotomists in English or Spanish. Data on sociodemographic characteristics, recent and past tobacco use, other lifestyle habits, occupational exposures, and family history of cancer were collected. Ten ml of blood were drawn into heparinized tubes for molecular genetic analyses.

CYP1A1 Genotyping. DNA was obtained from heparinized whole-blood specimens by use of Chelex solution as described by Walsh et al. (26). The samples were assayed without knowledge of case status. A modified method originally described by Kawajiri et al. was used to identify CYP1A1 MspI genotypes (5).

For the exon 7 and African-American-specific polymorphisms, PCR/restriction digestion genotyping methods were used as described previously (27, 28). As a quality-control measure, positive controls for each genotype were included in every PCR reaction.

Statistical Analysis. Logistic regression analysis was performed to assess the association between each of the CYP1A1 polymorphisms and lung cancer. This analysis adjusted for the matching variables (age, sex, and ethnicity) and other potential risk factors. For purposes of modeling the association between the CYP1A1 variant gene and lung cancer, the homozygous and heterozygous variant individuals were combined into one group and compared with the homozygous wild-type subjects who served as the reference group. In addition, subset analyses stratified by ethnicity were conducted.

For the combined genotype analysis, individuals were pooled as follows: the "high-risk" group comprised individuals who had at least one variant allele at both the MspI and exon 7 loci; the "intermediate-risk" group included subjects who were wild type at either polymorphism; and the "low-risk" group, which served as the reference, was made up of those people who were wild type at both sites. Interactions between pack-years smoked, CYP1A1 genotypes, and case status were evaluated by stratified analysis. Due to the small number of nonsmoking cases, all nonsmokers were excluded from the analysis (8 cases and 118 controls). Among smokers, light and heavy smokers were categorized by the 75th percentile pack-year value for controls (i.e., ≤30 pack-years and >30 pack-years). We, then, calculated the association between the variant polymorphism(s) and lung cancer risk (i.e., risk ratios) within the pack-year stratum to determine whether the polymorphisms behaved differently at different levels of smoking exposure.

All tests of statistical significance were two-sided. All analyses were performed with the software package SAS (SAS Institute Inc., Cary, NC).

Results

Sociodemographics and Smoking History. The results of this study are based on the 171 cases and 295 controls for whom there is genotype information. Except for a slight overrepresentation of men among biomarker participants, differences in variable distributions (i.e., age, education attainment, ethnic makeup, and smoking behavior) were minimal between participants and nonparticipants (56 controls and 68 cases). Table 1 describes the sociodemographic characteristics and tobacco use of the subjects, stratified by ethnicity.

As expected, there were significant differences in smoking history between cases and controls. Cases started smoking
earlier and quit later, giving them considerably greater exposure to cigarette smoke than controls (approximately 50 pack-years compared with 21 pack-years).

**CYP1A1 Polymorphisms and Lung Cancer.** None of the three polymorphic loci was associated with lung cancer risk (Table 2). Even when stratified by histological type, there were no meaningful differences observed. The odds ratios for squamous cell carcinoma with the MspI, exon 7, and African-specific polymorphisms were 0.67 (95% CI, 0.30–1.50), 0.52 (95% CI, 0.16–1.68), and 1.17 (95% CI, 0.35–3.86), respectively. The odds ratios when restricted to adenocarcinomas were 1.03 (95% CI, 0.53–2.02), 1.24 (95% CI 0.47–3.28), and 0.96 (95% CI 0.34–2.73), respectively. These numbers were based on 54 squamous cell carcinomas and 53 adenocarcinomas and were adjusted for the matching variables and pack-years smoked.

There was, however, a significant difference in the distributions of the exon 7 and MspI polymorphisms when stratified by ethnicity. Particularly striking is the fact that the prevalence of the exon 7 polymorphism and the MspI polymorphism were significantly different among African-Americans. However, even among Mexican-Americans, where the variant genotype prevalence was high, a direct association between either the exon 7 or MspI polymorphisms and lung cancer risk was not observed.

Table 3 displays the combined effect of the two common MspI and exon 7 genotypes. Individuals were classified as subjects who had no variant allele (i.e., "neither," WT/WT at both polymorphisms), one or two variant alleles in one of the two polymorphisms but not in the other ("either"), or having one or more variant alleles in both polymorphisms ("both"). Odds ratios were calculated by comparing subjects in the "both" or "either" categories to subjects in the "neither" group. For the African-American subpopulation, the odds ratios for the "either" and "both" categories were 1.31 (95% CI, 0.68–2.49) and 2.03 (95%, CI 0.17–23.0), whereas for the Mexican-Americans, the odds ratios were 0.76 (95% CI, 0.23–2.53) and 1.29 (95% CI, 0.60–2.81), respectively. These analyses were adjusted for the matching factors and for pack-years smoked.

**Interactions between Smoking, CYP1A1 Polymorphisms, and Lung Cancer.** Analyses to assess any potential interactions between CYP1A1 polymorphisms, smoking, and lung cancer risk were also performed by stratified analysis (Table 4). When the risk ratios were calculated within smoking categories, any increase in lung cancer risk associated with one or more of the variant alleles was limited to light smokers. The risk ratios for the exon 7 and MspI polymorphisms were 2.26 (95% CI, 0.82–6.26) and 2.03 (95% CI, 1.03–4.01), respectively. The estimates did not change much even when the cigarette dose cutoff was changed to 15 pack-years; however, the risk ratio for the MspI allele was no longer significant (data not shown).

Interestingly, this trend at low dose was also observed when the two common polymorphic genotypes were combined in the analysis (Table 5). There was a progressive increase in risk with an increase in the number of variant alleles for light smokers. The risk ratios between genotypes within this exposure level were 1.43 (95% CI, 0.64–3.21) and 2.95 (95% CI, 1.04–8.34). This progressive increase with increasing number of variant alleles was not observed for these three groups in the presence of high exposure. The risk ratios for this exposure
1.078 CYP1A1 and Lung Cancer Susceptibility

Table 4  Odds ratios and 95% confidence intervals between CYP1A1 and lung cancer by pack-years

<table>
<thead>
<tr>
<th>CYP1A1 genotype</th>
<th>Pack-yr</th>
<th>Cases</th>
<th>Controls</th>
<th>Risk ratioa,b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT/WT</td>
<td>≤30</td>
<td>38</td>
<td>89</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td>Variantsb</td>
<td>≤30</td>
<td>14</td>
<td>31</td>
<td>2.26 (0.82-6.26)</td>
</tr>
<tr>
<td>WT/WT</td>
<td>&gt;30</td>
<td>89</td>
<td>28</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td>Variantsb</td>
<td>&gt;30</td>
<td>20</td>
<td>12</td>
<td>0.61 (0.22-1.70)</td>
</tr>
<tr>
<td>Mspl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT/WT</td>
<td>≤30</td>
<td>21</td>
<td>67</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td>Variantsb</td>
<td>≤30</td>
<td>31</td>
<td>54</td>
<td>2.03 (1.03-4.01)</td>
</tr>
<tr>
<td>WT/WT</td>
<td>&gt;30</td>
<td>65</td>
<td>19</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td>Variantsb</td>
<td>&gt;30</td>
<td>45</td>
<td>21</td>
<td>0.68 (0.31-1.47)</td>
</tr>
<tr>
<td>African-American-specific</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT/WT</td>
<td>≤30</td>
<td>36</td>
<td>66</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td>Variantsb</td>
<td>≤30</td>
<td>5</td>
<td>11</td>
<td>0.68 (0.21-2.20)</td>
</tr>
<tr>
<td>WT/WT</td>
<td>&gt;30</td>
<td>74</td>
<td>23</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td>Variantsb</td>
<td>&gt;30</td>
<td>12</td>
<td>4</td>
<td>1.08 (0.29-4.08)</td>
</tr>
</tbody>
</table>

a Adjusted for matching variables of age, ethnicity, and sex.
b Risk ratio between variant group and wild-type group within smoking level.
c WT/Var and Var/Var combined.

Discussion

Epidemiological studies investigating the relationship between CYP1A1 and lung cancer risk have produced varying results. Furthermore, epidemiological studies have observed differences in age-adjusted incidence rates for lung cancer between races. One aim of this study was to determine whether the differences in lung cancer risk reported in African-Americans and Hispanic-Americans could be attributed to the CYP1A1 gene itself or in combination with smoking exposure.

The variant allele frequencies for the Mspl and exon 7 polymorphisms in the CYP1A1 gene were significantly different between Mexican-Americans and African-Americans. The former have similar distributions to those observed among subjects of Mongolian descent. This phenomenon has also been reported for other polymorphic metabolic traits, such as the NAT2 (N-acetyltransferase 2) gene. The 857A mutation constitutes 30–35% of slow acetylator alleles among Asians and Hispanics, but only 1–5% in Caucasians (29). In addition, Hartmann and colleagues (30) have found that Hispanic distributions of variable number of tandem repeats were closer to East Asians than to either blacks or whites, and Cerda-Flores et al. (31) have also reported a Texas sample of United States Southwest Hispanics to be an admixture of ~8% black, ~62% Caucasian, and ~30% American Indian (31). These findings potentially reflect the Bering Strait migrations of Asiatic people into North America 15,000–30,000 years ago (32). It should be noted, however, that such similarities between Asians and Hispanics were not observed for other polymorphic metabolic traits, such as the glutathione S-transferase T1 (33).

The data from this study are concordant with those consistently reported by Japanese researchers (5, 6, 11, 12). When we examined the gene-environment interaction between cigarette dose and genotype, we observed a low dose effect of smoking, in which subjects with the variant genotypes for the common polymorphisms, but not for the race-specific polymorphism, were at greater risk at lower exposure levels. Although the odds ratios of all genotypes increased with increasing levels of cigarette consumption (data not shown), an approximate 2-fold increase in risk was observed with both the Mspl and exon 7 variant genotypes when compared with the wild-type subjects at the lower smoking exposure. No increase in lung cancer risk of having the variant genotypes was observed within the “heavy” smoking stratum. A similar relationship has also been reported by Nakachi et al. (11) for the Mspl polymorphism and Sugimura et al. (20) for the exon 7 polymorphism. When the Mspl and exon 7 genotypes were combined, the low dose effect remained, where, interestingly, a stepwise increase in lung cancer risk among light smokers was observed with increasing number of variant alleles. This is in agreement with the increase in CYP1A1 inducibility and enzymatic activity reported by Crofts et al. (34) for subjects with either the exon 7 polymorphism or with both polymorphisms.

Although not all studies have demonstrated an interaction between cigarette dose and the CYP1A1 gene (14, 16), there are biochemical data to support this observation. Interaction between gene inducibility among variants in the exon 7 allele and smoke exposure has been reported by Crofts and coworkers (34). In fact, the numbers observed here are similar to the inducibility ratio found by this group (34). In addition, such low-dose effects have been reported for other metabolizing enzymes. Vineis et al. (35) reported a similar phenomenon with the NAT2 gene and smoking exposure (35). It appears that the gene plays a more important role at lower exposure levels.

Interestingly, this low-dose association between the CYP1A1 polymorphisms and lung cancer risk has been observed in two ethnic groups with relatively low lung cancer incidence (36, 37) that have similar relatively high allele frequency distributions. In contrast, we saw no association with either of the two common polymorphisms or with the race-specific polymorphism in African-Americans, who have a higher overall incidence of lung cancer (38). What is particularly striking is the extreme rarity of the exon 7 polymorphism. Consistent with Taioli et al. (39), who observed a variant allele frequency of 2.43%, we found the allele frequency of this polymorphism to be 1.84%. In contrast, the variant allele frequency among Hispanics was 33.3%. At the same time, the prevalence of the Mspl polymorphism is intermediate to those observed in Caucasians and Asians. Clearly, the linkage between the two loci is different in different racial groups.

Because of the report by Taioli et al. (40) that the race-specific polymorphism is a risk factor for the development of adenocarcinomas (40), we also analyzed the data by stratifying by histological type. Our results do not support an association between the African-American-specific polymorphism and adenocarcinoma risk. Rather, these results are in agreement with our earlier report (28) and that of London and colleagues (41).

Downloaded from cebp.aacrjournals.org on August 15, 2017. © 1997 American Association for Cancer Research.
Although the Japanese studies have found stronger associations, this may be in part attributable to the stratification of the analyses by histological type. Unfortunately, we do not have adequate numbers to assess the interaction between the polymorphisms and smoking after stratifying on histology. In addition, there are two potential sources of selection bias: namely, differences by participation. In both instances, these may be in part attributable to the stratification of observation may be important in light of the fact that Mexican-Americans have been observed to have significantly lower smoking rates when compared with Caucasians (42-44). Further research is needed to evaluate these associations in a larger sample of Mexican- and African-American populations, respectively. Additional study of minority populations is indicated to confirm and extend these observations.

Acknowledgments
We thank Drs. David J. Hunter, Howard Liber, and Donna Spiegelman for their critical review of the manuscript.

References


Susceptibility to lung cancer in light smokers associated with CYP1A1 polymorphisms in Mexican- and African-Americans.

N Ishibe, J K Wiencke, Z F Zuo, et al.


Updated version
Access the most recent version of this article at:
http://cebp.aacrjournals.org/content/6/12/1075

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.