p73 G4C14-to-A4T14 Polymorphism and Risk of Lung Cancer

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Abstract

Genetic variants in genes controlling cellular processes such as cell cycle, DNA repair, and apoptosis may modulate lung cancer risk. p73 has some p53-like activity and plays an important role in modulating these processes. The noncoding region of exon 2 of the p73 gene has two polymorphisms that are in complete linkage disequilibrium with one another, which may alter translation efficiency of the p73 protein. To test the hypothesis that this p73 polymorphism plays a role in the etiology of lung cancer, we conducted a hospital-based case-control study of 1054 patients newly diagnosed with lung cancer and 1139 cancer-free controls and evaluated the association between the p73 variant AT allele and risk of lung cancer. Cancer-free controls were frequency matched to the cases by age (≥5 years), sex, and smoking status, and all subjects were non-Hispanic whites. The variant AT allele and genotypes were more common among the cases than among the controls (P = 0.0007 and P < 0.001, respectively). Compared with the GC/GC genotype, the variant GC/AT and AT/AT genotypes were associated with a statistically significantly increased risk for lung cancer [adjusted odds ratio (OR) = 1.32, 95% confidence interval (CI), 1.10–1.59 and OR = 1.54, 95% CI, 1.05–2.26, respectively] in an allele dose-effect relationship (trend test: P < 0.001). The risk associated with the AT allele (GC/AT+AT/AT) was more pronounced in younger (≤50 years) individuals (OR = 1.53, 95% CI, 1.00–2.37), men (OR = 1.61, 95% CI, 1.26–2.06), light smokers (OR = 1.58, 95% CI, 1.17–2.14), and squamous cell carcinoma (OR = 1.79, 95% CI, 1.32–2.42). These results suggest that this p73 polymorphism may be a marker for susceptibility to lung cancer.

Introduction

Less than 20% of smokers develop lung cancer, suggesting an interindividually variable in genetic susceptibility to tobacco carcinogenesis in the general population (1). Therefore, polymorphisms in carcinogen metabolic (2), cell cycle control (3), and DNA repair pathways (1, 4) may modulate susceptibility to lung cancer. p73, a gene structurally similar to p53, is localized to the 1p36 chromosomal region (5). Functionally, p73 activates the promoters of several p53-responsive genes participating in cell-cycle control, DNA repair, and apoptosis and inhibits cell growth in a p53-like manner by inducing apoptosis or G1 cell-cycle arrest (6–9).

Although p73 mutations in both cell lines and primary tumors occurred in <2% of all cancers (10–12), loss of heterozygosity at the p73 locus is relatively common (12–15). Higher p73 expression levels in lung tumor tissues compared with adjacent normal tissues (16) are associated with p53 mutations (11, 17), suggesting a role of p73 in compensating for the loss of p53 function (11, 17, 18). However, overexpression of wild-type p73 may also have some p53-independent functions either as an oncogene (10) or as a tumor suppressor gene (16) in lung carcinogenesis. Therefore, p73 may play an important role in the development of lung cancer.

To date, there are at least 19 single nucleotide polymorphisms in p73 (5, 19, 20) as shown in Fig. 1. These single nucleotide polymorphisms either within introns (located ≥ 16 bp from the splice sites) or exons (not causing amino acid changes) are unlikely to be deleterious, except for three single nucleotide polymorphisms: two in exon 2 and one in promoter 3 (Fig. 1). The two common single nucleotide polymorphisms at positions 4 (G→A) and 14 (C→T) in the uncoding region of exon 2 of the p73 gene are in complete linkage disequilibrium with one another as one variant. The AT allele may affect p73 function, perhaps by altering the efficiency of translation initiation (5), whereas the P3 variant did not have a significant biological effect (20). Studies have investigated the role of the p73 G4C14-to-A4T14 polymorphism in risk of several types of cancer (21–24) but only one examined non–small-cell lung cancer. Therefore, the purpose of this study was to analyze the association of the p73 G4C14-to-A4T14 polymorphism with risk of lung cancer in a large hospital-based case-control study among non-Hispanic whites only.

Materials and Methods

Study Subjects. The subjects were recruited consecutively from an ongoing molecular epidemiologic study of lung cancer conducted in the Department of Epidemiology at The University of Texas M. D. Anderson Cancer Center (4). The 1056 case subjects included in this study had newly diagnosed, histopathologically confirmed, and previously untreated (by radiotherapy or chemotherapy) lung cancer without restrictions on age, sex, stage, or histology, and all cases were non-Hispanic whites. The 1140 control subjects were selected from a pool of cancer-free subjects recruited from the largest multispecialty physician practice, the Kelsey Seybold Foundation, with multiple clinics throughout the Houston metropolitan area. The controls were frequency-matched to the cases on age (≥5 years), sex, ethnicity, and smoking status (i.e., current, former, and never). The participation response rate was 77.4% for the cases and 73.3% for the controls. Those who had smoked <100 cigarettes in their lifetimes were defined as never smokers and others as ever smokers. The exclusion criteria included previous radiotherapy or chemotherapy, previous cancer, and recent (in last 6 months) blood transfusions. Each subject was scheduled for an interview after informed consent was obtained, and a structured questionnaire was administered by interviewers to collect information on demographic data and selected risk factors. The study was approved by the institutional review boards of M. D. Anderson Cancer Center and the Kelsey Seybold Foundation.

p73 Polymorphism Genotyping. We extracted genomic DNA from the buffy-coat fraction of the blood samples by using a Qiagen DNA Blood Mini kit (Qiagen, Inc., Valencia, CA) according to the manufacturer’s instructions. We typed for the p73 G4A genotype by PCR with the published sequences of the confronting two-pair primers, which makes genotyping possible by electrophoresis without restriction digestion (25). We performed the PCRs with a PTC-200 DNA Engine (Peltier Thermal Cycler; MJ Research, Inc., Water- town, MA) in 10 μL of PCR mixture. This PCR mixture included ~20 ng of genomic DNA, 0.1 mmol/L each deoxynucleotide triphosphate, 1× PCR buffer (50 mmol/L KCl, 10 mmol/L Tris HCl, and 0.1% Triton X-100), 1.5 mmol/L MgCl2, 0.5 units of Taq polymerase (Sigma-Aldrich Biotechnology, St. Louis, MO), and 2 pmol of each of four primers. The primers targeted three...
fragments of 193, 270, and 428 bp, depending on the polymorphic sites (i.e., A86885G and T86895C of GenBank accession no. AL136528; ref. 17). The modified amplification conditions were 10 minutes of initial denaturation at 95°C followed by 35 cycles of 1 minute at 95°C, 45 seconds at 62°C, and 1 minute at 72°C, and a 5-minute step at 72°C for the final extension. All PCR products were visualized on a 2% agarose gel containing a 0.25 μg/mL ethidium bromide. Because these two variant alleles are in complete linkage disequilibrium (5), only three genotypes are available: the homozygous GC/GC, the heterozygous GC/AT, and the homozygous AT/AT. PCR was performed under Hardy-Weinberg equilibrium (5), only three genotypes are available: the homozygous GC/GC, the heterozygous GC/AT, and the homozygous AT/AT. PCR was performed, and the results evaluated without knowledge of the subjects’ case-control status. At least 10% of the samples were retested, and the results were 100% concordant.

**Statistical Analysis.** Differences in select demographic variables, smoking, pack-years smoked, and p73 genotype frequencies between the case patients and control subjects were evaluated by using the χ² test. The association between the p73 variant genotypes and risk of lung cancer was estimated by computing the odds ratios (ORs) and their 95% confidence intervals (CIs) from both univariate and multivariate logistic regression analyses. Stratification analysis was used to estimate risk for subgroups by age, sex, smoking status, pack-years smoked, and tumor histology. All tests were two-sided, and all statistical analyses were performed with Statistical Analysis System software (version 8e; SAS Institute, Cary, NC).

### Results

Of the total 1056 case patients and 1140 control subjects recruited, DNA samples of 2 cases and 1 control failed to yield genotyping data on repeated experiments; therefore, the final data analysis included 1054 cases and 1139 controls (Table 1). There was no difference in the distributions of age, sex, and smoking status between the cases and controls as a result of frequency matching on these variables (P = 0.610 for age, 0.105 for sex, and 0.543 for smoking status). The median age was 62 years for both cases (mean 61.1, range 32–87) and controls (mean, 61.0 years; range, 32 to 91 years). There were more heavy smokers (pack-years ≥38) and fewer lighter smokers (pack-years <38) among the cases than among controls, although these differences were not statistically significant. However, the difference in pack-years smoked between the cases and controls was statistically significant (P < 0.01). Therefore, these variables were further adjusted for in the multiple logistic regression analyses. Of the 1054 cases, 505 (47.9%) were classified as adenocarcinomas, 224 (21.2%) as squamous cell carcinomas, 205 (19.5%) as non-small-cell carcinomas, 73 (6.9%) as small-cell carcinomas, and 47 (4.50%) as other carcinomas that included large and giant cell carcinomas.

The p73 AT variant allele frequency and genotype distributions of the case and control subjects are summarized in Table 2. The genotype distributions of controls were in agreement with Hardy-Weinberg equilibrium (χ² = 0.606, P = 0.436). The AT variant allele was more common among the cases (0.250) than among the controls (0.207), and this difference was statistically significant (χ² = 11.40, P < 0.001), suggesting that the AT allele may be the risk or marker.

### Table 1  Frequency distributions of selected variables in lung cancer cases and control subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Case subjects (N = 1054)</th>
<th>Control subjects (N = 1139)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y) ≤50</td>
<td>178 (16.9)</td>
<td>176 (15.4)</td>
<td>0.610</td>
</tr>
<tr>
<td>50–62</td>
<td>336 (31.9)</td>
<td>378 (33.2)</td>
<td></td>
</tr>
<tr>
<td>≥62</td>
<td>540 (51.2)</td>
<td>585 (51.4)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>552 (52.4)</td>
<td>557 (48.9)</td>
<td>0.105</td>
</tr>
<tr>
<td>Male</td>
<td>502 (47.6)</td>
<td>582 (51.1)</td>
<td></td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever smoker</td>
<td>877 (83.2)</td>
<td>955 (83.9)</td>
<td>0.543</td>
</tr>
<tr>
<td>Never smoker</td>
<td>177 (16.8)</td>
<td>184 (16.1)</td>
<td></td>
</tr>
<tr>
<td>Pack-years smoked</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1</td>
<td>180 (17.1)</td>
<td>196 (17.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1–37</td>
<td>293 (27.8)</td>
<td>469 (41.2)</td>
<td></td>
</tr>
<tr>
<td>≥38</td>
<td>581 (55.1)</td>
<td>474 (41.6)</td>
<td></td>
</tr>
</tbody>
</table>

* Two-sided χ² test.

### Table 2  Risk of lung cancer associated with the p73 genotypes and allele frequencies

<table>
<thead>
<tr>
<th>p73 Genotype</th>
<th>Case subjects (N = 1054)</th>
<th>Control subjects (N = 1139)</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR (95% CI)†</th>
</tr>
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<tr>
<td>G4C14-to-A4T14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GC/GC (ref.)</td>
<td>593</td>
<td>56.3</td>
<td>721</td>
<td>63.3</td>
</tr>
<tr>
<td>GC/AT</td>
<td>394</td>
<td>37.4</td>
<td>365</td>
<td>32.1</td>
</tr>
<tr>
<td>AT/AT</td>
<td>67</td>
<td>6.4</td>
<td>53</td>
<td>4.7</td>
</tr>
<tr>
<td>Trend test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AT allele</td>
<td>0.250</td>
<td>0.207</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Adjusted for age, sex, smoking status, square root of pack-years, and time since smoking cessation (in years) in a logistic regression model.
† χ² = 80.12, P = 0.0000 for genotype distributions; χ² = 11.40, P = 0.0007 for allele frequency; the observed genotype frequency in the control subjects was in agreement with Hardy-Weinberg equilibrium (p²+2pq+q² = 1) (χ² = 0.606, P = 0.436).
allele. Similarly, GC/AT heterozygotes and AT/AT homozygotes were more common among the cases than among the controls, and this difference was also statistically significant ($\chi^2 = 80.12, P < 0.001$).

Compared with GC/GC homozygotes, GC/AT heterozygotes had a $\sim 30\%$ increased risk (crude OR = 1.31; 95% CI, 1.10–1.57) for lung cancer, and the adjusted OR (1.32, 95% CI, 1.10–1.59) was nearly identical to the crude OR; moreover, AT/AT homozygotes was associated with an even higher risk (adjusted OR = 1.54, 95% CI, 1.05–2.26); and there appeared to be an “allele-dose” effect, i.e., the ORs increased as the number of variant AT alleles increased (trend test: $P = 0.0006$ for both crude and adjusted ORs; Table 2).

Because the AT/AT variant homozygotes were relatively uncommon, we combined them with the GC/AT variant heterozygotes for additional stratification analysis. The combined variant genotype (GC/AT/AT+AT) was associated with a $>30\%$ increased risk (adjusted OR = 1.35; 95% CI, 1.14–1.61) for lung cancer with adjustment for age, sex, smoking status, square root of pack-years smoked, and time since smoking cessation (age, sex, smoking, and possibly cell differentiation (5, 26–28)). Among the smokers (OR = 1.32, 95% CI, 1.10–1.59) and ever smokers (OR = 1.37, 95% CI, 1.17–1.65), the association was not statistically significant.

### Discussion

In this large case-control study in a non-Hispanic white population, we found an association between the $p73$ polymorphism and risk of lung cancer and a trend of increasing risk associated with an increased number of the $p73$ variant AT alleles. Our findings are biologically plausible because $p73$ has some functions similar to or independent of $p53$ (6, 26) and plays a role, particularly in compensation for loss of $p53$ function, in the regulation of the cell cycle, DNA repair, apoptosis, and possibly cell differentiation (5, 26–28). Although the exact functional relevance of the $p73$ AT variant allele remains unknown, one study has shown that the GC to AT change may lead to formation of a stem-loop structure and so may influence $p73$ translation efficiency (5). Another study suggested that the $p73$ variant AT allele may play a critical role in the development or progression of early cancerous lesions because it may lead to difference in $p73$ function, perhaps due to splice variant expression (21). Furthermore, this $p73$ polymorphism may be in linkage disequilibrium either with other functional polymorphisms, thereby altering the function of $p73$ or with adjacent susceptibility loci that affect either the expression or activity levels of enzymes involved in carcinogenesis (29). However, these hypotheses need to be tested in future studies.

Several studies, although with relatively small observations, have investigated the role of this $p73$ polymorphism in cancer susceptibility. In an Irish study of 84 case subjects of esophageal cancer and 152 healthy, age- and sex-matched normal population control subjects, the AT/AT homozygous genotype appeared to protect against risk for esophageal cancer (21). Early Japanese studies did not find evidence for an association with risk of cancer: one study included 192 histologically confirmed non–small-cell lung cancer cases and 241 controls (23); one included 200 breast cancer patients and 282 controls (24);
and another included 102 esophageal cancer patients, 144 stomach cancer patients, 147 colorectal cancer patients, and 241 outpatient controls (22). However, one recent Japanese study with 112 cervical cancer patients and 320 healthy women and 122 noncancer female outpatients found that this p73 polymorphism was associated with a borderline increased risk (OR = 1.57, 95% CI, 0.99–2.48; refs. 30).

To overcome the small sample problems in the previously published studies and to validate ethnic-related risk that may be associated with this p73 polymorphism, we conducted this lung cancer study with a much larger sample size. As a result, we were able to observe increased risk associated with both variant GC/AT and AT/AT genotypes. More importantly, we observed that the risk was in an allele-dose response manner. The greater risk associated with the combined p73 GC/AT+ AT/AT genotype in younger (≤50 years) subjects and light (compared with heavy) smokers suggests an early age of onset and lower levels of exposure characteristic of genetic susceptibility. We also observed a significantly higher risk in men than in women, particularly among smokers; however, the interaction between sex and the p73 polymorphism was only borderline significant, which warrants additional investigations with larger sample sizes.

In addition, we found that the risk was higher for patients with squamous cell lung cancer that was more likely induced by tobacco smoke (31). Indeed, the finding of a greater risk in ever smokers than in never-smokers suggests that this p73 polymorphism may have an impact on repair of DNA damage induced by polycyclic aromatic hydrocarbons in tobacco smoke. However, the significance of this finding may be limited due to the small number of patients with squamous cell lung cancer included in this study. Future studies with larger sample sizes of this subtype of lung cancer are needed to confirm this finding.

To understand the underlying molecular mechanisms, additional studies on the p73 functional relevance of the p73 variant AT allele are needed to provide biological plausibility for the findings of the present study.

Because this was a hospital-based case-control study and the controls were not selected from the same population from which the cases arose, we could not rule out possible selection bias. Also, we restricted our analysis to non-Hispanic white subjects, so it is uncertain whether these results are generalizable to other populations. However, by matching on age, sex, ethnicity, and smoking status, potential confounding factors might be minimized. To confirm the role of this p73 polymorphism in cancer risk requires additional larger studies in different populations and other types of cancer.

In conclusion, our study provides evidence that the p73 AT variant genotypes are significantly associated with an increased lung cancer risk in a non-Hispanic white population. The risk association is especially noteworthy in younger individuals, men, light smokers, and patients with squamous cell lung carcinomas. Although the exact mechanisms for the risk remain unknown, it is possible that the p73 polymorphism is functionally relevant itself or in linkage disequilibrium with alleles at other susceptibility loci. To further explore the role of the p73 gene in the etiology of lung cancer, studies on the p73 polymorphisms other than the AT variant may be warranted. We are currently testing other functional polymorphisms of the p53, p63, and MDM2 genes, which are members of the p53 family, to determine whether their variants interact in the etiology of lung cancer.

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