Null Results in Brief

Lack of Association between Polymorphisms in Inflammatory Genes and Lung Cancer Risk

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Introduction

Polymorphisms of key genes of inflammation pathways may be involved in lung carcinogenesis. Cigarette smoke stimulates airway epithelial cells to release proinflammatory cytokines, such as interleukin-1β (IL-1β). IL-1β triggers a cascade of inflammation reaction through the induction of inflammation-related substances including tumor necrosis factor-α and reactive oxygen species. This is known to result in activation of the nuclear factor κB transcription factor signal pathway, leading to up-regulation of various inflammation-related genes, including IL6 and COX2.

IL-6 is a major cytokine that is expressed in tumor-infiltrating cells. Lung cancer patients have been shown to have significantly higher serum levels of IL-6 compared with healthy controls (1).

Cyclooxygenase 2 (COX-2) is a key rate-limiting enzyme that converts arachidonic acid into proinflammatory prosta-glandins. COX2 is overexpressed in lung cancer, where its overexpression was reported as a poor prognostic factor in non–small cell lung cancer patients. Furthermore, several studies point to a chemopreventive effect of nonsteroidal anti-inflammatory drugs, of which COX-2 is a major target, in lung cancer patients (2).

The IL1B gene contains a single nucleotide polymorphism (SNP) in the promoter region at position -31 (rs1143627). This T/C transversion is 31 bp upstream of the transcription start site and the presence of the C allele causes disruption of a TATA box. Several SNPs in the COX2 gene have been identified. In particular, the COX2.8473 T > C (exon 10, 3′ untranslated region, rs5275) polymorphism may affect mRNA stability, thereby causing more inflammation. The promoter IL6 -174 G > C (rs1800795) SNP is associated with levels of IL-6 protein and the C-reactive protein (3).

We previously investigated the association between these polymorphisms and lung cancer in a case-control study based on a Norwegian population. We found an increased risk of non–small cell lung cancer for the carriers of T allele of the IL1B

-3IC > T polymorphism (4) and the C allele of the COX2.8473 C > T polymorphism (5), as well as increased risk of squamous cell carcinoma for carriers of the polymorphism of the IL6 promoter and risk of squamous cell carcinoma of the lung (5).

Hypothesis

In the present study, we have investigated the role of polymorphisms in the key inflammation-related genes IL1B, COX2, and IL6 as risk factors for lung cancer. The SNPs in each gene were selected on the bases of reported functional or biological relevance and of our previous results in a smaller case-control study done on a Norwegian population.

Materials and Methods

Study Subjects. The study includes 2,135 cases and 2,115 controls recruited in 15 centers of six countries in central and eastern Europe. Details on the study setup and on subject recruitment have been previously reported (6).

Briefly, the study population consists of 370 individuals from Romania (161 cases-209 controls), 623 from Hungary (335 cases-288 controls), 1370 from Poland (675 cases-695 controls), 727 from Russia (404 cases-323 controls), 490 from Slovakia (280 cases-390 controls), and 670 from Czech Republic (280 cases-390 controls). Most centers recruited hospital controls, whereas in Poland population controls were selected. Cases and controls underwent an identical interview with a standard questionnaire on consumption of alcohol and tobacco and occupational history. Both cases and controls were informed and gave written consent to participate in the study and to allow their biological samples to be genetically analyzed. Approval for the study was given by the relevant ethical committees.

Genotyping. The population used for the present study is smaller than the total of subjects recruited because it includes subjects for whom good-quality DNA was available. DNAs were extracted from whole blood samples or normal tissue by use of QiAamp Blood kit (Qiagen, Hilden, Germany).

DNAs from cases and controls were randomized and mixed on PCR plates to assure that an equal number of cases and controls could be simultaneously analyzed. Genotyping was carried out using the Taqman assay (Applied Biosystems, Foster City, CA). Primers and probes used for genotyping and all experimental conditions were identical to those previously reported (4, 5).
Table 1. Main effects of genetic polymorphisms stratified by smoking status

<table>
<thead>
<tr>
<th>Genotype</th>
<th>IL1B T-31C&lt;sup&gt;a&lt;/sup&gt;</th>
<th>IL6 G-174C</th>
<th>COX2 T8473C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases/controls OR (95% CI)</td>
<td>Cases/controls OR (95% CI)</td>
<td>Cases/controls OR (95% CI)</td>
</tr>
<tr>
<td>Total</td>
<td>Wt/Wt 674/653 1 Reference</td>
<td>629/615 1 Reference</td>
<td>855/805 1 Reference</td>
</tr>
<tr>
<td></td>
<td>Wt/Var 663/606 1.06 (0.90, 1.25)</td>
<td>954/993 0.89 (0.76, 1.04)</td>
<td>886/904 0.96 (0.83, 1.11)</td>
</tr>
<tr>
<td></td>
<td>Var/Var 157/161 0.90 (0.69, 1.18)</td>
<td>412/374 1.00 (0.82, 1.22)</td>
<td>224/228 0.97 (0.77, 1.22)</td>
</tr>
<tr>
<td>Smoking</td>
<td>Wt/Wt 663/606 1.06 (0.90, 1.25)</td>
<td>954/993 0.89 (0.76, 1.04)</td>
<td>886/904 0.96 (0.83, 1.11)</td>
</tr>
<tr>
<td>Never smokers&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Wt/Wt 47/192 1 Reference</td>
<td>50/231 1 Reference</td>
<td>62/257 1 Reference</td>
</tr>
<tr>
<td></td>
<td>Wt/Var 60/192 1.24 (0.84, 1.24)</td>
<td>72/311 0.96 (0.63, 1.48)</td>
<td>69/309 0.90 (0.60, 1.35)</td>
</tr>
<tr>
<td></td>
<td>Var/Var 14/46 1.20 (0.58, 2.50)</td>
<td>30/118 1.00 (0.58, 1.72)</td>
<td>19/80 0.90 (0.48, 1.68)</td>
</tr>
<tr>
<td>Ex smokers</td>
<td>Wt/Wt 110/184 1 Reference</td>
<td>115/161 1 Reference</td>
<td>159/238 1 Reference</td>
</tr>
<tr>
<td></td>
<td>Wt/Var 124/157 1.32 (0.92, 1.87)</td>
<td>186/291 0.91 (0.67, 1.25)</td>
<td>159/242 1.08 (0.80, 1.45)</td>
</tr>
<tr>
<td></td>
<td>Var/Var 27/46 0.95 (0.55, 1.65)</td>
<td>74/117 0.94 (0.63, 1.40)</td>
<td>48/72 1.13 (0.73, 1.74)</td>
</tr>
<tr>
<td>Current smokers</td>
<td>Wt/Wt 517/277 1 Reference</td>
<td>464/218 1 Reference</td>
<td>632/306 1 Reference</td>
</tr>
<tr>
<td></td>
<td>Wt/Var 475/250 0.99 (0.80, 1.23)</td>
<td>695/386 0.86 (0.69, 1.06)</td>
<td>656/346 0.92 (0.76, 1.12)</td>
</tr>
<tr>
<td></td>
<td>Var/Var 116/68 0.89 (0.63, 1.25)</td>
<td>305/137 1.01 (0.78, 1.33)</td>
<td>157/74 1.01 (0.73, 1.39)</td>
</tr>
</tbody>
</table>

Abbreviations: OR, odds ratio adjusted for country, age, sex, and smoking pack-years; 95% CI, 95% confidence interval; Wt, wild type; Var, gene variant.

<sup>a</sup>Data from Czech Republic and Slovakia were excluded owing to lack of HWE in the control group.

<sup>b</sup>Odds ratio adjusted for country, age, and sex.

**Statistical Analysis.** The frequency distribution of demographic variables and putative risk factors of lung cancer, including country of residence, age, sex, education, and smoking was examined for cases and controls. Former smokers were defined as smokers who quit smoking at least 2 years before interview or diagnosis. Tobacco pack-years were calculated as the product of smoking duration (years) and smoking intensity (packs per day). Hardy-Weinberg equilibrium (HWE) was separately tested in cases and in controls. We used logistic regression for multivariate analyses to assess the main effects of genetic polymorphisms on lung cancer risk. The primary endpoints of the analysis were odds ratios and associated confidence intervals. All the analyses were conducted with STATA software (StataCorp, College Station, TX).

**Results**

The allele frequencies at all loci among control group were in HWE (data not shown), except IL1B in controls of two of the countries (Slovakia and Czech Republic). The frequencies and distribution of the genotypes and the odds ratios for the associations of the polymorphisms are shown in Table 1.

We found that, in the three polymorphisms, there was no statistical difference in the distribution of genotypes among cases and distribution of the genotypes and the odds ratios were calculated as the product of smoking duration (years) and smoking intensity (packs per day). Hardy-Weinberg equilibrium (HWE) was separately tested in cases and in controls. We used logistic regression for multivariate analyses to assess the main effects of genetic polymorphisms on lung cancer risk. The primary endpoints of the analysis were odds ratios and associated confidence intervals. All the analyses were conducted with STATA software (StataCorp, College Station, TX).

**Statistical Power**

Our study has 80% power to detect a minimum odds ratio of 1.20 for these three SNPs, assuming $\alpha = 0.05$, two-sided test, and a codominant model.

**Study Limitations**

We found that controls were out of HWE for the IL1B -31C > T polymorphism. This was driven by subjects from only two of the countries (Czech Republic and Slovakia), whereas controls from the other countries were in HWE. Samples were ordered in the genotyping plates irrespectively of country of origin; therefore, the departure from HWE is unlikely to be due to genotyping misclassification. Recruitment bias is also unlikely because it has been done according to identical criteria across centers. We hypothesize that the departure from HWE is due to chance.

The present study failed to reproduce any of the associations observed in our previous studies (4, 5). The most likely explanation is that the previous observations were chance findings.

**Conclusion**

In conclusion, our study does not support major roles of polymorphisms of IL1B, COX2, and IL6 in lung carcinogenesis within this population.

**References**

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