Null Results in Brief

Lack of Association between –251 T>A Polymorphism of IL8 and Lung Cancer Risk

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Introduction

Inflammation is contributing factor in pathogenesis of many cancers (1). Cigarette smoke increases expression of inflammatory mediators in airway epithelial cells as well as immune cells (2). Chronic inflammation, arising as a result of continuous exposure to tobacco components, may result in oxidative stress and contribute to tumor promotion and progression in the lung (3).

Interleukin-8 is a member of the family of chemokines. It is mainly involved in the initiation and amplification of acute inflammatory reactions and in chronic inflammatory processes. Therefore, it plays an important role in diseases in which inflammation is a substantial pathophysiologic feature, namely in diseases with a chronic inflammatory component such as bronchial asthma and cancer.

Studies have shown that protein levels of interleukin-8 were significantly higher in small-airway epithelial cells from smokers. In current smokers, this was positively correlated with smoking history (2). Interleukin-8, originally discovered as a chemotactic factor for leukocytes, has recently been shown to contribute to human cancer progression through its potential functions as a mitogenic, angiogenic, and motogenic factor. Whereas it is constitutively detected in human cancer tissues and established cell lines, interleukin-8 expression is regulated by various tumor microenvironment factors, such as hypoxia, acidosis, nitric oxide, and cell density (4).

In this context, it is interesting to study whether polymorphic variation of the IL8 gene may have an impact of lung cancer susceptibility. A common single nucleotide polymorphism is known in the promoter of IL8, at position –251 from transcription start. The A-allele of this single nucleotide polymorphism was found to be related to higher in vitro levels of interleukin-8 after stimulation with lipopolysaccharide and associated with respiratory syncytial virus bronchiolitis in children (5).

We previously investigated the association between this polymorphism and lung cancer in a case-control study based on a Norwegian population, and we found a protective effect, although only in women (6).

Hypothesis. In the present study, we have investigated the role of a functional polymorphism in IL8, a key inflammation-related gene, as risk factor for lung cancer. The single nucleotide polymorphism was selected on the bases of reported functional and biological relevance, and of our previous results in a smaller case-control study.

Materials and Methods

Study Subjects. The study includes 2,144 cases and 2,116 controls recruited in 15 centers of six countries in Central and Eastern Europe including Czech Republic (Prague, Olomouc, Brno), Hungary (Borsod, Heves, Szabolcs, Szolnok, Budapest), Poland (Warsaw, Lodz), Romania (Bucharest), Russia (Moscow), and Slovakia (Banska Bystrica, Bratislava, Nitra). Details on the study setup and on subject recruitment have been previously reported (7). Cases and controls were recruited between 1998 and 2002. The study population consisted of 324 individuals from Romania (143 cases and 181 controls), 627 from Hungary (340 cases and 287 controls), 1,425 from Poland (699 cases and 726 controls), 723 from Russia (402 cases and 321 controls), 518 from Slovakia (301 cases and 217 controls), and 643 from Czech Republic (259 cases and 384 controls). Most centers recruited hospital controls, while 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 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 only the 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).
Table 1. Distribution of genotypes at IL8 –251 T>A in healthy controls and lung cancer patients

<table>
<thead>
<tr>
<th>IL8 –251 T&gt;A genotypes</th>
<th>Overall</th>
<th>Gender</th>
<th>Smoking status</th>
<th>Histology</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
<td>Odds ratio* (95% confidence interval)</td>
<td>Cases</td>
<td>Controls</td>
</tr>
<tr>
<td>T/T</td>
<td>578</td>
<td>574</td>
<td>1 (Reference)</td>
<td>T/T</td>
<td>125</td>
</tr>
<tr>
<td>A/A</td>
<td>1,081</td>
<td>1,084</td>
<td>0.97 (0.83-1.14)</td>
<td>T/A</td>
<td>243</td>
</tr>
<tr>
<td></td>
<td>485</td>
<td>488</td>
<td>1.05 (0.87-1.27)</td>
<td>A/A</td>
<td>105</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>453</td>
<td>421</td>
<td>1 (Reference)</td>
<td>T/T</td>
<td>838</td>
</tr>
<tr>
<td>A/A</td>
<td>485</td>
<td>488</td>
<td>1.05 (0.87-1.27)</td>
<td>A/A</td>
<td>819</td>
</tr>
<tr>
<td>Smoking status: Never smokers</td>
<td></td>
<td></td>
<td></td>
<td>Smoking status: Former*</td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>53</td>
<td>199</td>
<td>1 (Reference)</td>
<td>T/T</td>
<td>120</td>
</tr>
<tr>
<td>A/A</td>
<td>34</td>
<td>161</td>
<td>0.84 (0.51-1.41)</td>
<td>A/A</td>
<td>184</td>
</tr>
<tr>
<td>Small cell</td>
<td></td>
<td></td>
<td></td>
<td>Adenocarcinoma</td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>94</td>
<td>157</td>
<td>1 (Reference)</td>
<td>T/T</td>
<td>245</td>
</tr>
<tr>
<td>A/A</td>
<td>199</td>
<td>458</td>
<td>0.98 (0.77-1.26)</td>
<td>A/A</td>
<td>131</td>
</tr>
<tr>
<td>Squamous cell</td>
<td></td>
<td></td>
<td></td>
<td>Age &lt;50</td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>254</td>
<td>574</td>
<td>1 (Reference)</td>
<td>T/T</td>
<td>125</td>
</tr>
<tr>
<td>A/A</td>
<td>454</td>
<td>1,084</td>
<td>0.91 (0.74-1.12)</td>
<td>A/A</td>
<td>173</td>
</tr>
<tr>
<td>Small cell</td>
<td></td>
<td></td>
<td></td>
<td>≥50</td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>94</td>
<td>157</td>
<td>1 (Reference)</td>
<td>T/T</td>
<td>492</td>
</tr>
<tr>
<td>A/A</td>
<td>199</td>
<td>458</td>
<td>0.98 (0.77-1.26)</td>
<td>A/A</td>
<td>409</td>
</tr>
</tbody>
</table>

*Odds ratios, adjusted for age, sex, country, and tobacco pack-years.
†For gender-stratified analyses, odds ratios were adjusted for age, country, and tobacco pack-years.
‡For analyses of never smokers, odds ratios were adjusted for age, sex, and country.

DNA from cases and controls were randomized and mixed on PCR plates to assure that an equal number of cases and controls could be analyzed simultaneously. Genotyping was done 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 (1).

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 was tested in cases and in controls separately. We used logistic regression for multivariate analyses to assess the main effects of the genetic polymorphism on lung cancer risk. The primary end point of the analysis was odds ratios and associated confidence intervals. All the analyses were done with STATA software (StataCorp, College Station, TX).

Results
The genotype frequencies among the control group were in Hardy-Weinberg equilibrium (P = 0.20). The frequencies and distribution of the genotypes and the odds ratios for the associations of the polymorphism are shown in Table 1. We did not find an association between IL8 –251T>A polymorphism and lung cancer risk, either overall or when subjects were stratified on the basis of smoking status, gender, histology, and age (Table 1).

Statistical Power. Our study has 80% power to detect a minimum odds ratio of 1.20 for this single nucleotide polymorphism, assuming α = 0.05, two-sided test, and a codominant model.

Study Limitations. The present work failed to reproduce the association observed in our previous study (6). The previous association was found only in a subset of subjects of a study based on 250 cases and 214 controls. It is, therefore, probable that the previously reported association may be a false-positive finding.

Conclusion
Our study does not support a major role of polymorphism –251T>A of interleukin-8 in lung carcinogenesis within this population.

References
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