Apoptosis gene polymorphisms, age, smoking and the risk of non-small cell lung cancer

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Apoptosis is important for targeting cancer cells for destruction. Various single-nucleotide polymorphisms (SNPs) in apoptotic genes have been associated with increased risks in lung cancer, particularly FAS – 1377 G>A (rs2234767), FASLG – 844 C>T (rs763110), IL1B +3954 C>T Phe105Phe (rs1143634) and BAT3 Ser625Pro (rs1052486). We studied the association of these SNPs with non-small cell lung cancer (NSCLC) in a large case–control study (N = 4263: 2644 cases and 1619 controls). No associations with NSCLC were observed in the main effects analysis for all four SNPs, adjusting for age, gender, smoking status, pack-years and years since smoking cessation. In subjects under age 60, for FASLG – 844 C>T polymorphism, CT compared with the CC genotype, was significantly associated with increased risk of NSCLC, adjusted odds ratio (aOR) = 1.58 (1.22, 2.05), P = 0.0006 and TT aOR = 1.45 (1.01, 2.04), P = 0.04. In contrast, for those over age 60, the CT aOR = 0.91 (0.73, 1.13), P = 0.37 and TT aOR = 0.86 (0.64, 1.16), P = 0.32. The P-value for the age-genotype interaction was 0.004. For the IL1B +3954 C>T polymorphism, compared with the CC genotype, TT showed significant associations in former smokers and in men but tests of interaction were not significant (Psmoking = 0.24, Pgender = 0.17). No interactions were observed for FAS – 1377 G>A and BAT3 Ser625Pro polymorphisms. Our findings indicate that age and smoking may modify the association of the FASLG – 844 and IL1B +3954 SNPs with the risk of NSCLC.

Introduction

Apoptosis is the physiological mechanism of programmed cell death that is important in normal tissue development and homeostasis and plays a role in a number of human disorders including cancer. Aging, damaged and potentially malignant cells are eliminated through the activation of an intracellular cascade leading to controlled cell shrinkage, fragmentation and eventual phagocytosis (1). Defects in this process can lead to unchecked cell growth and proliferation in cancer development. One of the hallmarks of malignancies is the acquired ability to resist apoptosis (2), often achieved through somatic gene development. One of the hallmarks of malignancies is the acquired ability to resist apoptosis (2), often achieved through somatic gene development. Defects in this process can lead to unchecked cell growth and proliferation in cancer development.

No interactions were observed for FAS – 1377 G>A and BAT3 Ser625Pro polymorphisms. Our findings indicate that age and smoking may modify the association of the FASLG – 844 and IL1B +3954 SNPs with the risk of NSCLC.

FAS and FASLG

FAS ligand binds FAS (CD95, APO-1), a member of the transmembrane tumor necrosis factor superfamily of death receptors, initiating the extrinsic pathway of apoptosis. Both FAS and FASLG genes, located on chromosomes 10q24.1 and 1q23, respectively, have functional promoter SNPs. The FAS – 1377 G>A transition alters a SP-1 transcription factor GC-rich-binding site (4). This SNP is in tight linkage disequilibrium with another SNP – 670 A>G (rs1800682) that abolishes a STAT1-binding site (4). Both FAS SNPs have been shown to reduce transcription factor binding (8,9) and protein expression (10). The FASLG – 844 C>T transition lies within a binding motif for CAAT/enhancer-binding protein beta (11). The two alleles of this SNP show significantly different affinities for the CAAT/enhancer-binding protein beta transcription factor and the C allele has been shown to have increased expression with a luciferase reporter assay in Jurkat cells and increased expression of the FASL protein in fibroblasts by flow cytometry (5,11). While the T allele is the ancestral allele (National Center for Biotechnology Information dbSNP database) and the major allele in African and African American populations, the C allele is the major allele in other populations including Caucasian and Han Chinese. FAS AA versus GG and FASLG CC versus TT genotypes have been associated with increased risk of lung cancer in a Han Chinese population of 1000 patients and 1270 controls (FAS – 1377 AA conferred an adjusted odds ratio (aOR) = 1.59 (1.21, 2.10), P = 0.001 and FASLG – 844 CC conferred aOR = 1.70 (1.26, 2.52), P = 0.001) (5). Overall associations for both SNPs have also been reported in other common cancers such as esophageal cancer (12), cervical cancer (10), bladder (13) and breast cancer (14) in Han Chinese, but not in Caucasians for the FASLG – 844 SNP (15–20). There have been no reports published on the associations with non-small cell lung cancer (NSCLC) and FAS + 1377 or FASLG – 844 in a Caucasian population.

Interleukin 1 beta

Interleukin 1 beta, IL1B, is a cytokine that inhibits apoptosis and acts as a proinflammatory inducer of reactive oxygen species. Located on chromosome 2q14, IL1B +3954>C>T in exon 5 is a synonymous SNP, but an allele dosage effect has been observed for IL1B secretion from lipopoly saccharide-activated peripheral mononuclear cells from healthy controls (6,21). Engels et al. (21) reported a significantly increased odds ratio (OR) for lung cancer of 1.27 (1.10, 1.47), P = 0.001 for the combined heterozygous and homozygous variant compared with wildtype in 1553 Caucasian cases and 1730 controls. Promoter SNPs – 511 C>T (rs16944) and – 31 T>C (rs1143627) in IL1B have also been shown to be associated with lung cancer but with mixed results (22–24).

BAT3

BAT3 (HLA-B-associated transcript 3, Sycthe) is located on 6p21.3. BAT3 protein modulates p53 in p53-mediated responses to genotoxic stress, by affecting p53 stability and its ability to act as a transcription factor for specific genes such as NOX and PUMA (25). BAT3 also

Abbreviations: ADC, adenocarcinoma; aOR, adjusted odds ratio; HWE, Hardy–Weinberg equilibrium; LRT, likelihood ratio test; NSCLC, non-small cell lung cancer; OR, odds ratio; SCC, squamous cell carcinoma; SNP, single-nucleotide polymorphisms.

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interacts with HSP70 (25) and apoptosis-inducing factor induced by endoplasmic reticulum stress (26). Ruddle et al. (7) analyzed 1476 non-
synonymous SNPs in a Caucasian case–control study of 1529 cases and
2707 controls, reporting that the BAT3 SNP Ser625Pro had the most significant association with lung cancer OR = 0.69 (0.59–0.82),
P = 8.3 × 10^{-6} under a recessive model. This SNP is reported to have a ‘possibly damaging’ effect on protein structure or function as
predicted by the bioinformatic program PolyPhen (7).

These four putatively functional apoptosis SNPs have been shown to be individually associated with lung cancer in only a few studies. We hypothesized that these SNPs are associated with the risk of development of NSCLC, which we tested in a large hospital-based case–control study. Not all American Caucasians. Our secondary hypothesis was that these associations are modified by different measures of smoking and demographic factors such as age and gender.

Materials and methods

Study population

The study was approved by the Human Subjects Committees of Massachusetts General Hospital and the Harvard School of Public Health located in Boston, MA. Details of this case–control population have been described previously (27). Briefly, all eligible cases (patients with histologically confirmed lung
cancers) at Massachusetts General Hospital were recruited between December 1992 and April 2007. Before the year 1997, only early stage (stages I and II)
patients were recruited in this study; after 1997, all stages of lung cancer patients were recruited in this study. Controls were either case related or case
unrelated for those cases that did not have available controls. Case-related
controls were healthy friends and non-blood-related family members (usually
spouses). Case-unrelated controls were friends or spouses of other hospital pa-
tients from oncology or thoracic surgery units. These patients had similar age and
gender demographics as lung cancer patients. Importantly, none of the controls
were patients themselves. Potential controls who carried a previous diagnosis of
cancer (other than non-melanoma skin cancer) were excluded from participa-
tion. Over 85% of eligible cases and >90% of controls participated in this study
and provided blood samples. Interviewer-administered questionnaires
adapted from American Thoracic Society questionnaire (28) obtained informa-
tion on age, gender, smoking status and pack-years were elimi-
nated. Information on cases’ cancer cell type was available for 99.5%
and 2000 (32). Information on cases’ cancer cell type was available for 99.5%
of patients and 98% for cancer stage. ADC, SCC and large cell carci-
noma represented 57, 22 and 7% of cases. Fifteen percent were mixed,
not otherwise specified, and of uncertain classification. Bronchioloalveolar
carcinoma was 20% of the ADC cases. There were 4% more early
(stages I and II) than advanced (III and IV) stages of lung cancer.

SNP genotyping

DNA was extracted from peripheral blood samples using the Puregene DNA
Isolation Kit (Qiagen, Valencia, CA). Scant DNA from 956 subjects recruited
early in the study was processed with whole-genome amplification GenomiPhi
DNA Amplification Kit (GE Healthcare, Piscataway, NJ). The apoptotic gene
polymorphisms were genotyped by the 5V nuclease assay (Taqman) using the
ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster
City, CA). The primers, probes and reaction conditions are available upon
request. Genotyping was done by laboratory personnel blinded to case–control
status, and a random 5% of the samples were repeated to validate genotyping
procedures. Two authors reviewed independently all genotyping results.
Eighty-one percent of cases and 92% of controls were genotyped successfully.
BAT Ser625Pro had 1492 controls and 2135 cases [1220 adenocarcinoma
(ADC), 478 squamous cell carcinoma (SCC); 1014 early and 1083 late stage].
IL1B +3954 C>T had 1492 controls and 2150 cases (1228 ADC, 482 SCC;
1019 early and 1093 late stage). FAS –1377 G>A had 1497 controls and 2174
cases (1242 ADC, 484 SCC; 1023 early and 1112 late stage). FASLG –844
C>T had 1490 controls and 2147 cases (1224 ADC, 482 SCC; 1019 early and
1090 late stage). The concordance rate of 5% repeated samples was 100%.

Statistical analysis

Individuals of all races were recruited for this study. To reduce confounding by
allele frequency variation in different ethnic groups, otherwise known as pop-
ulation stratification, we restricted our analyses to Caucasians only (97%). We
also restricted the analysis to NSCLC. We retained subjects with complete
information on age, gender, smoking status, pack-years, years since smoking
cessation (for former smokers) and each SNP individually.

We compared the descriptive characteristics of the cases and controls using the
chi^2 test and the Wilcoxon rank sum test. We used SASS/Genetics software
(version 9.1.3; SAS Institute, Cary, NC) to perform the analyses. We deter-
mined allele frequencies in cases and controls separately and tested the differ-
cence with a x^2 test. To check for genotyping error, we examined departure from
Hardy–Weinberg equilibrium (HWE) in controls, using a x^2 test. All statistical
testing was done at the two-sided 0.05 level.

We used multiple logistic regression to determine the association between
each SNP and NSCLC estimated by ORs and their 95% confidence intervals.
Effect modification of the associations of single polymorphisms with NSCLC

IL1B +3954 C>T showed significant associations of the homozygous variant versus wild-type for NSCLC risk within strata of smoking status and within strata of gender (Table III). Compared with the CC genotype, TT conferred a significant deleterious effect for former smokers, aOR = 1.74 (1.06, 2.85), P = 0.03. The effect was significant neither for never smokers aOR = 0.77 (0.33, 1.79), P = 0.55 nor for current smokers aOR = 0.79 (0.39, 1.59), P = 0.5. The LRT of the overall smoking-genotype interaction was not significant (P = 0.24). The TT genotype also appeared to be significantly deleterious in men, aOR = 1.8 (1.04, 3.11), P = 0.034 and not women, aOR = 0.94 (0.58, 1.52), P = 0.79, but the LRT of the gender-genotype interaction was not significant (P = 0.17). The IL1B SNP associations were sensitive to modeling because combining strata as ever versus never smoking did not result in significant results and combining the variant groups with a dominant model also resulted in no associations for any smoking strata or strata of gender.

FASLG –844 C>T showed significant modification of genotype and risk by smoking status, tertile pack-years, age divided at 60 years old, cell type and stage (Tables III and IV). While there was no significant association between this SNP and NSCLC risk within each smoking group, the opposing directions of the ORs produced a significant LRT smoking-genotype interaction (P = 0.035). A subgroup of cases with squamous histology versus controls, however, shows significantly decreased risks for NSCLC in former smokers for this SNP: FASLG TT versus CC was protective, aOR = 0.37 (0.21, 0.67), P = 0.001. The P-value for the interaction with smoking status was significant at 0.019. Similarly, TT versus CC was marginally protective in all SCC cases versus controls, aOR = 0.66 (0.43, 1.00), P = 0.05. Evaluating associations with pack-year level as the smoking metric showed that for light smokers (those who smoked 1 to <15 pack-years) with CC as reference, CT was significant with an aOR of 1.74 (1.04, 2.85), P = 0.003. Evaluating the categories with smoking level as the smoking metric showed that for light smokers (those who smoked 1 to <15 pack-years) with CC as reference, CT was significant with an aOR of 1.74 (1.04, 2.85), P = 0.003. Upon further stratification by cell type and tertile pack-years, we found that similar risk relationships held true for ADC only: light smokers showed significant risks—CT versus CC aOR = 2.33 (1.45, 3.73), P = 0.0004 and TT versus CC aOR = 1.23 (0.63, 2.40), P = 0.54. The P-value for the interaction with tertile pack-years in the ADC subgroup was significant at 0.007. The stratum-specific associations were also significant in light smokers after combining variants for the total cases, CT + TT, aOR = 1.89 (1.26, 2.83), P = 0.02, with the overall interaction P_{tertile pack-year} = 0.033. Age was also a significant modifier: for those under age 60, both genotypes were associated with significant ORs: CT showed aOR = 1.58 (1.22, 2.05), P = 0.0006 and the TT showed aOR = 1.45 (1.01, 2.04), P = 0.04. In comparison, for those over age 60, both genotypes showed insignificant ORs: CT aOR = 0.91 (0.73, 1.13), P = 0.37 and TT aOR = 0.86 (0.64, 1.16), P = 0.32 (supplementary Table III shows count information, available at Carcinogenesis Online). The LRT P-value of the binary age-genotype interaction was P = 0.004 and with continuous age was 0.018. The significance of the age

### Table I. Descriptive characteristics of study population

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases (n = 2644)</th>
<th>Controls (n = 1619)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Males</td>
<td>1329 (50%)</td>
<td>713 (44%)</td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>1315 (50%)</td>
<td>906 (56%)</td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td>0.15</td>
</tr>
<tr>
<td>College degree</td>
<td>683 (30%)</td>
<td>515 (33%)</td>
<td></td>
</tr>
<tr>
<td>No college degree</td>
<td>1563 (70%)</td>
<td>1065 (67%)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>398</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Non-smoker</td>
<td>243 (9%)</td>
<td>567 (35%)</td>
<td></td>
</tr>
<tr>
<td>Former smoker</td>
<td>1416 (54%)</td>
<td>742 (46%)</td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>985 (37%)</td>
<td>310 (19%)</td>
<td></td>
</tr>
<tr>
<td>Among ever smokers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cigarettes per day</td>
<td>48 (0.03–231)</td>
<td>24 (0.03–218)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age started smoking</td>
<td>25 (0.14–120)</td>
<td>20 (0.14–100)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Former smokers’ years since quitting smoking</td>
<td>14 (1–59)</td>
<td>19 (1–65)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cell type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADC</td>
<td>1516 (57%)</td>
<td>1065 (67%)</td>
<td></td>
</tr>
<tr>
<td>SCC</td>
<td>583 (22%)</td>
<td>336 (20%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>533 (20%)</td>
<td>336 (20%)</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I and II</td>
<td>1356 (52%)</td>
<td>906 (56%)</td>
<td></td>
</tr>
<tr>
<td>III and IV</td>
<td>1241 (48%)</td>
<td>567 (35%)</td>
<td></td>
</tr>
</tbody>
</table>

*N = 3453: 2401 cases and 1052 controls.

**Table II. Apoptotic gene polymorphism associations with NSCLC**

<table>
<thead>
<tr>
<th>Gene/SNP</th>
<th>Cases, N (%)</th>
<th>Controls, N (%)</th>
<th>Crude OR (95% CI)</th>
<th>aOR* (95% CI)</th>
<th>P-value</th>
<th>P_trend*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAT3 T&gt;C Ser625Pro (rs1052486)</td>
<td>2135</td>
<td>1492</td>
<td>1.00</td>
<td>1.00</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>534 (0.25)</td>
<td>394 (0.27)</td>
<td>1.07 (0.92, 1.26)</td>
<td>1.06 (0.88, 1.28)</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>1089 (0.51)</td>
<td>749 (0.50)</td>
<td>1.08 (0.90, 1.31)</td>
<td>1.07 (0.86, 1.36)</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>512 (0.24)</td>
<td>349 (0.23)</td>
<td>0.97 (0.85, 1.12)</td>
<td>0.96 (0.82, 1.13)</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>C allele frequency</td>
<td>0.49</td>
<td>0.48</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL1B +3954 C&gt;T (rs1143634)</td>
<td>2150</td>
<td>1492</td>
<td>1.00</td>
<td>1.00</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>1262 (0.59)</td>
<td>872 (0.58)</td>
<td>1.13 (0.83, 1.54)</td>
<td>1.23 (0.86, 1.75)</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>775 (0.36)</td>
<td>551 (0.37)</td>
<td>1.11 (0.66, 1.88)</td>
<td>1.06 (0.58, 1.95)</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>T allele frequency</td>
<td>0.23</td>
<td>0.23</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FAS –1377 G&gt;A (rs2234767)</td>
<td>2174</td>
<td>1492</td>
<td>1.00</td>
<td>1.00</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>1645 (0.76)</td>
<td>1138 (0.76)</td>
<td>1.11 (0.86, 1.49)</td>
<td>1.01 (0.84, 1.21)</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>GA</td>
<td>492 (0.22)</td>
<td>336 (0.22)</td>
<td>1.11 (0.66, 1.88)</td>
<td>1.06 (0.58, 1.95)</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>37 (0.02)</td>
<td>23 (0.02)</td>
<td>1.00</td>
<td>1.00</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>A allele frequency</td>
<td>0.13</td>
<td>0.13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FASLG –844 T&gt;C (rs763110)</td>
<td>2147</td>
<td>1490</td>
<td>1.00</td>
<td>1.00</td>
<td>0.047</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>789 (0.37)</td>
<td>576 (0.39)</td>
<td>1.11 (0.96, 1.28)</td>
<td>1.13 (0.96, 1.34)</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>1036 (0.48)</td>
<td>684 (0.46)</td>
<td>1.03 (0.84, 1.25)</td>
<td>1.04 (0.82, 1.31)</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>T allele frequency</td>
<td>0.39</td>
<td>0.38</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Adapting for age, gender, smoking status, square root of pack-years and years since smoking cessation for former smokers.
interaction was robust to additional methods of modeling age. Age cut in quartiles (based on distribution in controls) also showed a significant interaction, \( P \text{-value} = 0.05 \); there were significant results in the second quartile (50–60 years old) for both homozygote and heterozygote variants versus wild-type, 1.65 (1.17, 2.33) and 1.59 (1.01, 2.52), respectively (also see supplementary Table II by decade, available at Carcinogenesis Online). Changing the median cut-point by ±5 years also did not change the strong significance of the interaction. Combining variants CT + TT showed similar results: <60 aOR = 1.57 (1.22, 2.02), \( P = 0.0004 \) and >60 aOR = 0.91 (0.74, 1.11), \( P_{\text{binary age}} = 0.0008 \). FASLG – 844 showed marginally increased risk when late stage patients were compared with controls, CT versus CC, aOR = 1.24 (1.04, 1.49), \( P = 0.04 \), but TT versus CC was not significant, aOR = 1.18 (0.90, 1.53). Comparing early versus late stage cases only, however, showed TT versus CC was deleterious for late stage, aOR = 1.34 (1.02, 1.75), \( P = 0.03 \).

**BAT3 Ser625Pro** showed no interactions by any variable tested (Table III). FAS – 1377 G>A showed a borderline protective effect for early stage versus late, aOR GG versus AA was 0.81 (0.65, 0.99), \( P = 0.04 \). None of the SNPs showed significant interactions with pack-years, transformed by the square root: **BAT3 Ser625Pro** \( P = 0.49 \), FAS – 1377 **P = 0.93**, FASLG – 844 **P = 0.49** and **IL1B + 3954** \( P = 0.39 \).

Testing the joint effects of the FASLG – 844 and FAS – 1377 SNPs (compared with the reference group of 0 variants in either SNP) did not show significant associations with NSCLC risk. The interaction between these SNPs was not significant, \( P = 0.16 \).

**Discussion**

We evaluated four SNPs in genes related to apoptosis that had previously been found individually to have strong associations in different case–control studies of lung cancer. FAS, FAS ligand, BAT3, and IL1B proteins are important to the competing processes of apoptosis and survival of the cancer cell. Overall, none of the SNPs were significantly associated with the risk of NSCLC in our study population; there were, however, significant associations within subgroups. The **IL1B + 3954** homozygote variant CT conferred a significant risk among former smokers and among men. Compared with **FASLG – 844** CC, CT carriers showed a significantly increased risk of NSCLC for people <60, for light smokers and for those in late stage. TT conferred a marginally protective effect in SCC cases, which appeared to be strongest in former smokers. In addition, the LRT of interaction showed significant differences in aORs within strata of age above and below 60, smoking status, tertile pack-years for the **FASLG SNP**. The association of the **FASLG T allele** with NSCLC in individuals <60 years old had the strongest biological (33) and statistical evidence, robust to different models of age.

To date, there are only two published reports of **FASLG – 844 C>T** associations with lung cancer. Park et al. (34) reported null results in a Korean population of 582 lung cancer patients and 582 age and sex-matched controls. They did not find significant differences in aORs within strata of age above and below 60, smoking status, tertile pack-years for the FASLG SNP. The association of the **FASLG T allele** with NSCLC in individuals <60 years old had the strongest biological (33) and statistical evidence, robust to different models of age.
induced by chronic smoking in human blood lymphocytes (35) and rat lung tissue with a dose response (36). However, the observation that this increased risk was significant for light-smoking cases with ADC histology and not SCC is consistent with the greater importance of stronger smoking intensity for SCC development compared with ADC, relative to non-smokers (37,38). The difference between ADC and SCC risk becomes less important at higher levels of smoking that may explain the lack of a dose response. The protective effect of the TT genotype in SCC cases, particularly in former smokers, is not as easy to explain since there is not as well-observed relationship between smoking status and histology as there is for smoking intensity. The significant interactions of FASLG with measures of smoking in ADC and SCC, however, may point to a differentiating genetic susceptibility factor in the development of these two cell types for light and former smokers.

Only the effect modification by age was robust to different measures of age, significant with both binary and continuous age measures, and the associations of both heterozygote and homozygote variant genotypes individually and combined were significantly deleterious in the <60 years age group. The T allele has been shown to be associated with lower FAS ligand expression compared with the C allele. In older ages, the FAS ligand messenger RNA expression has been shown to decrease in lymphocytes (33) so it is possible that at younger ages, when the FAS ligand has a greater role in apoptosis, the lower expression due to the T allele would have a greater effect. IL1B + 3954 TT had significant associations within strata of former smokers and for men but the P-values for smoking–genotype and gender–genotype interactions were not significant. The lack of an association seen in the current smoker stratum may be due to insufficient homozgyous genotype numbers. The counts of homozygous cases and homozygous controls in each smoking strata were 14 and 33 (current), 72 and 28 (former) and 8 and 27 (never). The associations did not remain significant after combining the variant genotypes or after combining strata of smoking to ever versus never. Associations within strata, without significant interactions, may also be due to significant linkage disequilibrium of this synonymous SNP with other SNPs in the promoter of IL1B and with SNPs in the nearby gene IL1A. Engels et al. (21) reported significant OR of the single IL1B + 3945 polymorphism for heavy smokers in a Caucasian population, 1.59 (1.28–1.97) for CT + TT versus CC but the smoking–SNP interaction was not significant, P = 0.10. The haplotype, however, containing IL1B + 3954 T allele with four other SNPs with wild-type alleles from IL1A −899 C>T, IL1A Ala141Ser, IL1B −511 T>C and IL1B −31 C>T showed an aOR of 3.53 (2.05, 6.10) in heavy smokers with a significant P-value for the smoking–haplotype interaction of 0.03. This OR was greater than that of the IL1A or IL1B SNPs alone suggesting that the haplotype of these SNPs may be a more effective measure of the genetic association in lung cancer. Also, −31 T>C and −31 C>T are in strong linkage disequilibrium with each other but are also in linkage disequilibrium with +3954 C>T in Caucasians (39) [D’ = 0.57, P = 0.03 and D’ = 0.59, P = 0.02, respectively (40)]. Positive associations have been shown for IL1B −511 T>C in a small Norwegian case–control study of 251 NSCLC cases and 271 controls (22), a Chinese case–control study of 122 cases and 122 controls (23) and a large European multicenter study (34). Hall et al. reported increased IL1B secretion from lipopolysaccharide-stimulated monocytes with the −511 C−31 T+C+3954 C haplotype in two independent populations, but did not observe differences in IL1B secretion due to the +3945T allele alone. Studying haplotypes of SNPs in IL1A, IL1B and IL1RN in this region of high linkage disequilibrium on chromosome 2q13–q21 may also give us greater power to detect associations for smoking intensity as well as smoking status.

The strengths of this study include the large sample size in a population limited to non-Hispanic Caucasians with information of the primary potential confounders of lung cancer. This provided sufficient power to detect gene–environment interactions and control for confounding including population stratification. Although this is a hospital-based study, selection bias should not be important since it is unlikely that SNPs in genes of apoptosis are related to case or control participation. Further study will include other genes in the apoptosis pathway and other pathways such as inflammation and DNA repair with additional coverage of the genes with other functional and tagging SNPs. This additional information may help us determine which aspect of the multiple roles these genes play is important in the development of lung cancer and how particular SNPs contribute to associations with respect to age, gender and smoking.

In summary, we report significant associations with NSCLC of IL1B + 3954 in subgroups of men and former smokers, but formal tests do not reveal interactions among these variables. Also, we report a novel association of the FASLG −844 SNP in North American Caucasians that appears to be significant in individuals <60 years old and shows statistically significant significant effect modification with different measures of age.

Supplementary material

Supplementary Tables I–III can be found at http://carcin.oxfordjournals.org/

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References


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