Carcinogenesis vol.29 no.11 pp.2147–2152, 2008
doi:10.1093/carcin/bgn205
Advance Access publication August 29, 2008

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

Monica Ter-Minassian[1,*], Rihong Zhai[1], Kofi Asomaning[1], Li Su[1], Wei Zhou[1], Geoffrey Liu[4,7], Rebecca Suk Heist[1,4], Thomas J.Lynch[5], John C.Wain[6], Xihong Lin[3], Immaculata DeVivo[2] and David C.Christiani[1,2,5]

[1]Department of Environmental Health, [2]Department of Epidemiology and [3]Department of Biostatistics, Harvard School of Public Health, Boston 02115, MA, USA, [4]Massachusetts General Hospital Cancer Center, [5]Pulmonary and Critical Care Unit and [6]Thoracic Surgery Unit, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston 02114, MA, USA and [7]Applied Molecular Oncology and Department of Medical Oncology, Princess Margaret Hospital, Ontario Cancer Institute, Toronto, Ontario M5G 2M9, Canada

[*]To whom correspondence should be addressed. Tel: +1 617 432 6458; Fax: +1 617 432 3441;
Email: mtermina@hsph.harvard.edu

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 ($P_{smoking} = 0.24$, $P_{gender} = 0.17$). 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 mutations. Various single-nucleotide polymorphisms (SNPs) in apoptotic genes, such as *TNF*, *TP53*, *DR4*, *FAS* and *FASL*, have also been consistently associated with susceptibility to cancer development at individual and multiple organ sites (reviewed in ref. 3). In lung cancer, associations with increased risk have been shown with *FAS* −1377 G>A (rs2234767), *FASLG* −844 C>T (rs763110), *IL1B* +3954 C>T Phe105Phe (rs1143634) and *BAT3* Ser625Pro (rs1052486) (4–7). FAS and FAS ligand are important in mediating the immune response to the presence of cancer cells by initiating the extrinsic pathway of apoptosis where cell surface membrane receptor binding triggers the caspase cascade through caspase 8. BAT3's role appears to be initiated by internal stresses that affect the caspase cascade primarily through the intrinsic mitochondrial pathway. Cytokine IL1B has both pro- and antiapoptotic effects. The SNPs in these genes have been shown to be functional either experimentally (*FAS* and *FASLG* and *IL1B*) (4–6) or through bioinformatic prediction (*BAT3*) (7). All the chosen SNPs had a main effect of $P \leq 0.001$ from at least one study (4–7).

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.

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 lipopolysaccharide-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 wild-type 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, Scythe) 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

interacts with HSP70 (25) and apoptosis-inducing factor induced by endoplasmic reticulum stress (26). Rudd *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), $P = 8.3 \times 10^{-6}$ 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 of North 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 patients 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 any cancer (other than non-melanoma skin cancer) were excluded from participation. 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 information on demographic and detailed smoking histories from each subject (29).

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 population 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 χ^2 test and the Wilcoxon rank sum test. We used SAS/Genetics software (version 9.1.3; SAS Institute, Cary, NC) to perform the analyses. We determined allele frequencies in cases and controls separately and tested the difference with a χ^2 test. To check for genotyping error, we examined departure from Hardy–Weinberg equilibrium (HWE) in controls, using a χ^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. We used the codominant genetic model for individual SNPs, controlling for age, gender, pack-years, years since smoking cessation and smoking status. The codominant model is defined as heterozygotes (1 variant genotype) versus wild-type (0 variant genotype) or homozygotes (2 variant genotype) versus wild-type. Smoking status is classified as current smokers, former smokers (those who quit smoking for >1 year prior to diagnosis or enrollment) and non-smokers (<100 cigarettes in their lifetime). Age, pack-years and years since smoking cessation were modeled as continuous covariates. The years since smoking cessation variable was set equal to zero for current and non-smokers. Previous analyses using generalized additive models (30,31) had shown that pack-years should be transformed by the square root to adjust for the non-linear relationship of pack-years with the logit probability of cancer risk. For stratified analyses, we created indicator variable of age greater or less than 60 years old (the median in controls), gender and smoking status, pack-years (in tertiles, cut-points 15 and 36 based on the distribution in smoking controls), cell type and cancer stage. We tested for effect modification using the likelihood ratio test (LRT) incorporating cross product interaction terms of each SNP allele and indicator variables for binary age, gender, smoking status and tertile pack-years. We also tested interactions with cross product interaction terms of each SNP allele and continuous square root of pack-years. Stratum-specific ORs were obtained using the interaction model with the stratum of interest as the reference group for smoking status and tertile pack-years. We compared early stage (I and II) cases only versus controls, late stage (III and IV) cases only versus controls, ADC cases only versus controls and SCC cases only versus controls. We also performed a case-only analysis of ADC and SCC, the two predominant histological cell types. Considering the biological interaction of FAS with its ligand FASLG, we also tested the joint effects of *FASLG* −844 and *FAS* −1377 SNPs on NSCLC risk and tested the statistical interaction with the LRT.

Results

Population characteristics

After restricting to Caucasians and NSCLC only, subjects with missing data on age, gender, smoking status and pack-years were eliminated, leaving 4263 subjects (2644 cases and 1619 controls). Demographics of the study population are summarized in Table I. Cases were older than controls and had a greater proportion of males. Cases had a significantly greater proportion of ever smokers. Among ever smokers, cases had consumed more cigarettes per day and over a lifetime (measured in pack-years). Both cases and controls had the same median age of starting to smoke (16 years old) with similar ranges. Cases who were former smokers averaged 5 fewer years since quitting smoking than former smokers who were controls. Overall, our controls were 19% smokers, 46% former smokers and 35% non-smokers. The distribution of smoking characteristics in the controls was comparable with the general Massachusetts population >45 in 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 carcinoma represented 57, 22 and 7% of cases. Fifteen percent were mixed, not otherwise specified, and of uncertain classification. Bronchioalveolar 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.

Associations of NSCLC with single apoptotic gene polymorphisms

Minor allele frequencies in controls were 48% for *BAT3* S625P (HWE *P*-value = 0.84), 13% for *FAS* −1377 G>A (HWE $P = 0.75$), 38% for *FASLG* −844 C>T (HWE $P = 0.25$) and 23% for *IL1B* +3954 C>T (HWE $P = 0.12$). In contrast, case minor allele frequencies were 49% for *BAT3* S625P, 13% for *FAS* −1377 G>A, 39% for *FASLG* −844 C>T and 23% for *IL1B* +3954 C>T. The differences between case and control allele frequencies were not significant for all four SNPs by the χ^2 test. Shown in Table II are the distributions of the homozygote wild-type, heterozygotes and homozygote variants in cases and controls for each SNP. There was no overall association between each SNP and lung cancer risk. After adjusting for age, gender, smoking status, pack-years and years since quitting, the associations continued to be non-significant. As also shown in Table II, the main effects aORs for all four SNPs were not associated with NSCLC. Tests for trend were also not significant. The age distribution of the SNPs is indicated in supplementary Table I (available at *Carcinogenesis* Online), which shows the total counts of cases and controls for each SNP by decade.

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 2.15 (1.41, 3.28), $P = 0.0003$ but TT was not significant. The overall tertile pack-year–genotype interaction was also significant at $P = 0.003$. 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 $P_{\text{tertile pack-year}} = 0.033$. 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 aORs: 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

Characteristics	Cases ($n = 2644$)	Controls ($n = 1619$)	*P*-value
Age	67 (30–94)	60 (19–96)	<0.001
Gender			<0.001
Males	1329 (50%)	713 (44%)	
Females	1315 (50%)	906 (56%)	
Education			0.15
College degree	683 (30%)	515 (33%)	
No college degree	1563 (70%)	1065 (67%)	
Missing	398	39	
Smoking status			<0.001
Non-smoker	243 (9%)	567 (35%)	
Former smoker	1416 (54%)	742 (46%)	
Current smoker	985 (37%)	310 (19%)	
Among ever smokers[a]			
Pack-years[b]	48 (0.03–231)	24 (0.03–218)	<0.001
Cigarettes per day[b]	25 (0.14–120)	20 (0.14–100)	<0.001
Age started smoking[b]	16 (5–59)	16 (6–54)	0.24
Former smokers' years since quitting smoking[b]	14 (1–59)	19 (1–65)	<0.001
Cell type			
ADC	1516 (57%)		
SCC	583 (22%)		
Other	533 (20%)		
Stage			
I and II	1356 (52%)		
III and IV	1241 (48%)		

[a]$N = 3453$: 2401 cases and 1052 controls.
[b]Median (range), significance calculated with Wilcoxon rank sum test.

Table II. Apoptotic gene polymorphism associations with NSCLC

Gene/SNP	Cases, *N* (%)	Controls, *N* (%)	Crude OR (95% CI)	aOR[a] (95% CI)	*P*-value[a]	P_{trend}[a]
BAT3 T>C Ser625Pro (rs1052486)	2135	1492				0.52
TT	534 (0.25)	394 (0.27)	1.00	1.00		
TC	1089 (0.51)	749 (0.50)	1.07 (0.92, 1.26)	1.06 (0.88, 1.28)	0.51	
CC	512 (0.24)	349 (0.23)	1.08 (0.90, 1.31)	1.07 (0.86, 1.36)	0.52	
C allele frequency	0.49	0.48				
IL1B +3954 C>T (rs1143634)	2150	1492				0.71
CC	1262 (0.59)	872 (0.58)	1.00	1.00		
CT	775 (0.36)	551 (0.37)	0.97 (0.85, 1.12)	0.96 (0.82, 1.13)	0.65	
TT	113 (0.05)	69 (0.05)	1.13 (0.83, 1.54)	1.23 (0.86, 1.75)	0.26	
T allele frequency	0.23	0.23				
FAS −1377 G>A (rs2234767)	2174	1497				0.87
GG	1645 (0.76)	1138 (0.76)	1.00	1.00		
GA	492 (0.22)	336 (0.22)	1.01 (0.86, 1.19)	1.01 (0.84, 1.21)	0.95	
AA	37 (0.02)	23 (0.02)	1.11 (0.66, 1.88)	1.06 (0.58, 1.95)	0.84	
A allele frequency	0.13	0.13				
FASLG −844 T>C (rs763110)	2147	1490				0.47
CC	789 (0.37)	576 (0.39)	1.00	1.00		
CT	1036 (0.48)	684 (0.46)	1.11 (0.96, 1.28)	1.13 (0.96, 1.34)	0.15	
TT	322 (0.15)	230 (0.15)	1.03 (0.84, 1.25)	1.04 (0.82, 1.31)	0.77	
T allele frequency	0.39	0.38				

[a]Adjusting for age, gender, smoking status, square root of pack-years and years since smoking cessation for former smokers.

Table III. Apoptotic gene polymorphism associations with NSCLC in subgroups, OR (95% CI)[a]

	BAT3 T>C Ser625Pro		*IL1B* +3954 C>T		*FAS* −1377 G>A		*FASLG* −844 C>T	
	CT versus TT	CC versus TT	TC versus CC	TT versus CC	GA versus GG	AA versus GG	CT versus CC	TT versus CC
Never smoker	1.27 (0.83, 1.94)	1.19 (0.71, 1.99)	0.83 (0.57, 1.20)	0.77 (0.33, 1.80)	1.07 (0.71, 1.62)	1.45 (0.34, 6.20)	0.81 (0.55, 1.19)	1.23 (0.74, 2.05)
Former smoker	1.15 (0.89, 1.48)	1.14 (0.85, 1.53)	0.99 (0.79, 1.23)	**1.74 (1.07, 2.85)**[c]	0.93 (0.73, 1.20)	1.11 (0.49, 2.50)	1.21 (0.96, 1.53)	0.84 (0.61, 1.14)
Current smoker	0.81 (0.56, 1.16)	0.89 (0.58, 1.35)	1.02 (0.75, 1.39)	0.79 (0.39, 1.59)	1.11 (0.79, 1.57)	0.83 (0.28, 2.44)	1.23 (0.90, 1.69)	1.44 (0.90, 2.30)
$P_{\text{smoking interaction}}$	0.52		0.24		0.88		**0.035**	
None	1.28 (0.83, 1.95)	1.20 (0.71, 2.01)	0.82 (0.56, 1.19)	0.77 (0.33, 1.80)	1.06 (0.70, 1.61)	1.45 (0.33, 6.28)	0.80 (0.54, 1.18)	1.22 (0.73, 2.05)
Mild[b]	1.29 (0.82, 2.02)	1.19 (0.70, 2.02)	0.71 (0.47, 1.08)	1.90 (0.84, 4.29)	1.16 (0.75, 1.80)	1.77 (0.37, 8.43)	**2.15 (1.41, 3.28)**[e]	1.19 (0.65, 2.16)
Moderate	0.84 (0.57, 1.23)	1.06 (0.69, 1.64)	1.17 (0.86, 1.62)	1.47 (0.75, 2.90)	0.76 (0.53, 1.10)	0.58 (0.20, 1.67)	1.16 (0.83, 1.62)	0.74 (0.47, 1.16)
Heavy	0.99 (0.73, 1.33)	0.92 (0.65, 1.31)	1.01 (0.78, 1.30)	1.04 (0.57, 1.88)	1.09 (0.81, 1.47)	1.25 (0.46, 3.36)	0.98 (0.75, 1.28)	1.12 (0.76, 1.64)
$P_{\text{pack-year interaction}}$	0.63		0.28		0.60		**0.003**	
Male	1.05 (0.80, 1.37)	1.02 (0.75, 1.40)	0.93 (0.73, 1.17)	**1.80 (1.04, 3.11)**[d]	0.91 (0.70, 1.18)	1.17 (0.43, 3.19)	1.20 (0.94, 1.53)	0.97 (0.70, 1.35)
Female	1.08 (0.83, 1.39)	1.11 (0.82, 1.51)	1.02 (0.81, 1.27)	0.94 (0.58, 1.52)	1.09 (0.84, 1.40)	0.99 (0.46, 2.14)	1.09 (0.86, 1.37)	1.13 (0.82, 1.57)
$P_{\text{gender interaction}}$	0.94		0.17		0.59		0.53	
Age <60	1.08 (0.81, 1.44)	1.24 (0.88, 1.73)	0.88 (0.68, 1.13)	1.00 (0.57, 1.76)	1.14 (0.86, 1.51)	1.74 (0.68, 4.44)	**1.58 (1.22, 2.05)**[f]	**1.45 (1.01, 2.07)**[g]
Age ≥60	1.06 (0.83, 1.34)	0.98 (0.74, 1.29)	1.01 (0.82, 1.24)	1.43 (0.91, 2.24)	0.94 (0.74, 1.18)	0.76 (0.35, 1.62)	0.91 (0.73, 1.13)	0.86 (0.64, 1.16)
$P_{\text{age interaction}}$	0.48		0.50		0.26		**0.004**	

[a]Adjusting for age, gender, smoking status, square root of pack-years and years since smoking cessation for former smokers.
[b]Based on tertiles of pack-years in controls: mild smoker = 1 to <15 pack-years; moderate smoker = 15 to <36 and heavy smoker = ≥36.
[c]$P = 0.026$.
[d]$P = 0.034$.
[e]$P = 0.0003$.
[f]$P = 0.0006$.
[g]$P = 0.04$.

interaction was robust to additional methods of modeling age. Age cut in quartiles (based on distribution in controls) also showed a significant interaction, *P*-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 TT compared with wild-type 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 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 gender frequency-matched controls. Zhang *et al.* (5) found in a Han Chinese population of 1000 patients and 1270 controls that the CC genotype compared with TT had an aOR of 1.79 (1.26, 2.52). They also reported effect modification by pack-years of smoking and showed an interaction between the *FASLG* and *FAS* SNPs. There are a few notable differences in the results of the Zhang study and our own. The gene frequencies in their Han Chinese population were significantly different from our Caucasian population. While we found *FASLG* minor allele (T) frequencies of 39 and 38% in cases and controls, respectively [similar to other Caucasian frequencies (17)], Zhang *et al.* reported minor allele (T) frequencies of 30.6 and 22.6%, a larger difference between cases and controls. The associations of the T allele were associated with increased risk of NSCLC in subgroups of our study, whereas the C allele associations were associated with increased risk in the Zhang study. Also, our heterozygote genotype associations (CT versus CC) were stronger than the homozygote associations (TT versus CC), whereas the Zhang study did not find significant associations with CT versus TT. This is may be due to their choice of the minor allele as referent allele. One possible explanation for the discrepancy is that *FASLG* −844 may be in linkage disequilibrium with the true causal SNP that is in a higher frequency in the Chinese population. To the best of our knowledge, we are the first to report associations with NSCLC of the *FASLG* −844 SNP in a Caucasian population.

We observed a number of significant associations within strata and significant interactions for the *FASLG* −844 SNP. The observed associations of *FASLG* −844 SNP heterozygotes and all variants combined with light smokers are not consistent with experimental evidence that has shown that the expression of the FAS ligand is

Table IV. Apoptotic gene polymorphism associations with NSCLC in subgroups of cases, OR (95% CI)[a]

	BAT3 T>C Ser625Pro		*IL1B* +3954 C>T		*FAS* −1377 G>A		*FASLG* −844 C>T	
	CT versus TT	CC versus TT	TC versus CC	TT versus CC	GA versus GG	AA versus GG	CT versus CC	TT versus CC
ADC	1.05 (0.86, 1.30)	1.00 (0.78, 1.29)	0.99 (0.82, 1.18)	1.22 (0.83, 1.80)	1.09 (0.89, 1.33)	0.86 (0.43, 1.70)	1.08 (0.90, 1.29)	1.02 (0.79, 1.31)
SCC	1.01 (0.73, 1.40)	0.94 (0.65, 1.37)	1.06 (0.80, 1.40)	1.51 (0.81, 2.81)	0.84 (0.61, 1.16)	1.16 (0.43, 3.10)	1.06 (0.79, 1.40)	**0.66 (0.43, 1.00)**[b]
Case-only ADC versus SCC	0.90 (0.69, 1.18)	0.85 (0.62, 1.18)	1.01 (0.79, 1.27)	1.06 (0.63, 1.78)	1.17 (0.89, 1.53)	0.74 (0.31, 1.77)	0.92 (0.73, 1.18)	1.27 (0.89, 1.82)
Early stage	0.92 (0.73, 1.16)	0.92 (0.70, 1.21)	0.98 (0.80, 1.20)	1.54 (1.00, 2.36)	0.90 (0.72, 1.14)	1.17 (0.57, 2.43)	0.96 (0.78, 1.18)	0.85 (0.63, 1.14)
Late stage	1.17 (0.94, 1.45)	1.15 (0.89, 1.48)	1.01 (0.84, 1.21)	1.09 (0.72, 1.66)	1.09 (0.88, 1.34)	0.93 (0.46, 1.88)	**1.23 (1.01, 1.49)**[c]	1.18 (0.90, 1.53)
Case-only early versus late	0.84 (0.68, 1.04)	0.90 (0.70, 1.16)	0.91 (0.76, 1.10)	1.17 (0.79, 1.74)	**0.81 (0.65, 0.99)**[d]	1.33 (0.68, 2.61)	0.86 (0.71, 1.04)	**0.75 (0.57, 0.98)**[e]

[a]Adjusting for age, gender, smoking status, square root of pack-years and years since smoking cessation for former smokers.
[b]$P = 0.05$.
[c]$P = 0.04$.
[d]$P = 0.04$.
[e]$P = 0.03$.

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 homozygous 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 aOR 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* Ala114Ser, *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, −511 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 (24). Hall *et al.* reported increased IL1B secretion from lipopolysaccharide-stimulated monocytes with the −511 C/−31 T/+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 effect modification with different measures of age.

Supplementary material

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

Funding

National Institutes of Health (CA074386); Centers for Disease Control and Prevention/The National Institute for Occupational Safety and Health (T42-OH008416); Flight Attendant Medical Research Institute (062459_YCSA); John F. and Virginia B. Taplin Scholarship Fund.

Acknowledgements

We thank Dr David P.Miller, Salvatore Mucci and Richard Rivera-Massa for valuable contributions in data collection and management.

Conflict of Interest Statement: None declared.

References

1. Danial,N.N. *et al*. (2004) Cell death: critical control points. *Cell*, **116**, 205–219.
2. Shivapurkar,N. *et al*. (2003) Apoptosis and lung cancer: a review. *J. Cell. Biochem.*, **88**, 885–898.
3. Zhivotovsky,B. *et al*. (2006) Carcinogenesis and apoptosis: paradigms and paradoxes. *Carcinogenesis*, **27**, 1939–1945.
4. Aguilar-Reina,J. *et al*. (2005) The -670A > G polymorphism in the promoter region of the FAS gene is associated with necrosis in periportal areas in patients with chronic hepatitis C. *J. Viral Hepat.*, **12**, 568–573.
5. Zhang,X. *et al*. (2005) Functional polymorphisms in cell death pathway genes FAS and FASL contribute to risk of lung cancer. *J. Med. Genet.*, **42**, 479–484.
6. Pociot,F. *et al*. (1992) A TaqI polymorphism in the human interleukin-1 beta (IL-1 beta) gene correlates with IL-1 beta secretion *in vitro*. *Eur. J. Clin. Invest.*, **22**, 396–402.
7. Rudd,M.F. *et al*. (2006) Variants in the GH-IGF axis confer susceptibility to lung cancer. *Genome Res.*, **16**, 693–701.
8. Sibley,K. *et al*. (2003) Functional FAS promoter polymorphisms are associated with increased risk of acute myeloid leukemia. *Cancer Res.*, **63**, 4327–4330.
9. Kanemitsu,S. *et al*. (2002) A functional polymorphism in fas (CD95/APO-1) gene promoter associated with systemic lupus erythematosus. *J. Rheumatol.*, **29**, 1183–1188.
10. Sun,T. *et al*. (2005) FASL -844C polymorphism is associated with increased activation-induced T cell death and risk of cervical cancer. *J. Exp. Med.*, **202**, 967–974.
11. Wu,J. *et al*. (2003) A novel polymorphic CAAT/enhancer-binding protein beta element in the FasL gene promoter alters FAS ligand expression: a candidate background gene in African American systemic lupus erythematosus patients. *J. Immunol.*, **170**, 132–138.
12. Sun,T. *et al*. (2004) Polymorphisms of death pathway genes FAS and FASL in esophageal squamous-cell carcinoma. *J. Natl Cancer Inst.*, **96**, 1030–1036.
13. Li,C. *et al*. (2006) Functional polymorphisms in the promoter regions of the FAS and FAS ligand genes and risk of bladder cancer in south China: a case-control analysis. *Pharmacogenet. Genomics*, **16**, 245–251.
14. Zhang,B. *et al*. (2007) Functional polymorphisms in FAS and FASL contribute to increased apoptosis of tumor infiltration lymphocytes and risk of breast cancer. *Carcinogenesis*, **28**, 1067–1073.
15. Ivansson,E.L. *et al*. (2007) Variants of chemokine receptor 2 and interleukin 4 receptor, but not interleukin 10 or Fas ligand, increase risk of cervical cancer. *Int. J. Cancer*, **121**, 2451–2457.
16. Zhang,H. *et al*. (2007) Importance of FAS-1377, FAS-670, and FASL-844 polymorphisms in tumor onset, progression, and pigment phenotypes of Swedish patients with melanoma: a case-control analysis. *Cancer J.*, **13**, 233–237.
17. Zhang,Z. *et al*. (2006) Polymorphisms of FAS and FAS ligand genes involved in the death pathway and risk and progression of squamous cell carcinoma of the head and neck. *Clin. Cancer Res.*, **12**, 5596–5602.
18. Crew,K.D. *et al*. (2007) Genetic polymorphisms in the apoptosis-associated genes FAS and FASL and breast cancer risk. *Carcinogenesis*, **28**, 2548–2551.
19. Krippl,P. *et al*. (2004) Re: polymorphisms of death pathway genes FAS and FASL in esophageal squamous-cell carcinoma. *J. Natl Cancer Inst.*, **96**, 1478–1479; author reply 1479.
20. Li,C. *et al*. (2006) Polymorphisms of the FAS and FAS ligand genes associated with risk of cutaneous malignant melanoma. *Pharmacogenet. Genomics*, **16**, 253–263.
21. Engels,E.A. *et al*. (2007) Systematic evaluation of genetic variants in the inflammation pathway and risk of lung cancer. *Cancer Res.*, **67**, 6520–6527.
22. Zienolddiny,S. *et al*. (2004) Polymorphisms of the interleukin-1 beta gene are associated with increased risk of non-small cell lung cancer. *Int. J. Cancer*, **109**, 353–356.
23. Lee,K.M. *et al*. (2007) Polymorphisms in immunoregulatory genes, smoky coal exposure and lung cancer risk in Xuan Wei, China. *Carcinogenesis*, **28**, 1437–1441.
24. Campa,D. *et al*. (2005) Lack of association between polymorphisms in inflammatory genes and lung cancer risk. *Cancer Epidemiol. Biomarkers Prev.*, **14**, 538–539.
25. Sasaki,T. *et al*. (2007) HLA-B-associated transcript 3 (Bat3)/Scythe is essential for p300-mediated acetylation of p53. *Genes Dev.*, **21**, 848–861.
26. Desmots,F. *et al*. (2007) Scythe regulates apoptosis-inducing factor stability during endoplasmic reticulum stress-induced apoptosis. *J. Biol. Chem.*, **283**, 3264–3271.
27. Miller,D.P. *et al*. (2002) Combinations of the variant genotypes of GSTP1, GSTM1, and p53 are associated with an increased lung cancer risk. *Cancer Res.*, **62**, 2819–2823.
28. Ferris,B.G. (1978) Epidemiology Standardization Project (American Thoracic Society). *Am. Rev. Respir. Dis.*, **118**, 1–120.
29. Zhou,W. *et al*. (2005) Gene-smoking interaction associations for the ERCC1 polymorphisms in the risk of lung cancer. *Cancer Epidemiol. Biomarkers Prev.*, **14**, 491–496.
30. Zhou,W. *et al*. (2001) The interaction between microsomal epoxide hydrolase polymorphisms and cumulative cigarette smoking in different histological subtypes of lung cancer. *Cancer Epidemiol. Biomarkers Prev.*, **10**, 461–466.
31. Thurston,S.W. *et al*. (2005) Modeling lung cancer risk in case-control studies using a new dose metric of smoking. *Cancer Epidemiol. Biomarkers Prev.*, **14**, 2296–2302.
32. Beiner,L. *et al*. (2001) *2000 Massachusetts Adult Tobacco Survey: Technical Report & Tables 1993–2000*. Center for Survey Research, University of Massachusetts, Boston, MA.
33. Pinti,M. *et al*. (2004) Balanced regulation of mRNA production for Fas and Fas ligand in lymphocytes from centenarians: how the immune system starts its second century. *Circulation*, **110**, 3108–3114.
34. Park,S.H. *et al*. (2006) Polymorphisms in the FAS and FASL genes and risk of lung cancer in a Korean population. *Lung Cancer*, **54**, 303–308.
35. Suzuki,N. *et al*. (1999) Effects of cigarette smoking on Fas/Fas ligand expression of human lymphocytes. *Cell. Immunol.*, **192**, 48–53.
36. Wu,C.H. *et al*. (2006) Immunohistochemical detection of apoptotic proteins, p53/Bax and JNK/FasL cascade, in the lung of rats exposed to cigarette smoke. *Arch. Toxicol.*, **80**, 328–336.
37. Khuder,S.A. (2001) Effect of cigarette smoking on major histological types of lung cancer: a meta-analysis. *Lung Cancer*, **31**, 139–148.
38. Yang,P. *et al*. (2002) Adenocarcinoma of the lung is strongly associated with cigarette smoking: further evidence from a prospective study of women. *Am. J. Epidemiol.*, **156**, 1114–1122.
39. Zabaleta,J. *et al*. (2008) Ethnic differences in cytokine gene polymorphisms: potential implications for cancer development. *Cancer Immunol. Immunother.*, **57**, 107–114.
40. Hall,S.K. *et al*. (2004) Correlation of polymorphic variation in the promoter region of the interleukin-1 beta gene with secretion of interleukin-1 beta protein. *Arthritis Rheum.*, **50**, 1976–1983.

Received May 12, 2008; revised August 22, 2008; accepted August 23, 2008