

# Cytokine and Cytokine Receptor Single-Nucleotide Polymorphisms Predict Risk for Non-Small Cell Lung Cancer among Women

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## Abstract

Studies on the relationships between inflammatory pathway genes and lung cancer risk have not included African-Americans and have only included a handful of genes. In a population-based case-control study on 198 African-American and 744 Caucasian women, we examined the association between 70 cytokine and cytokine receptor single-nucleotide polymorphisms (SNPs) and risk of non-small cell lung cancer (NSCLC). Unconditional logistic regression was used to estimate odds ratios and 95% confidence intervals in a dominant model adjusting for major risk factors for lung cancer. Separate analyses were conducted by race and by smoking history and history of chronic obstructive pulmonary disease among Caucasians. Random forest analysis was conducted by race. On logistic regression analysis, *IL6* (interleukin 6), *IL7R*, *IL15*, *TNF* (tumor necrosis factor), and *IL10* SNP were associated with risk of non-small cell

lung cancer among African-Americans; *IL7R* and *IL10* SNPs were also associated with risk of lung cancer among Caucasians. Although random forest analysis showed *IL7R* and *IL10* SNPs as being associated with risk for lung cancer among African-Americans, it also identified *TNFRSF10A* SNP as an important predictor. On random forest analysis, an *IL1A* SNP was identified as an important predictor of lung cancer among Caucasian women. Inflammatory SNPs differentially predicted risk for NSCLC according to race, as well as based on smoking history and history of chronic obstructive pulmonary disease among Caucasian women. Pathway analysis results are presented. Inflammatory pathway genotypes may serve to define a high risk group; further exploration of these genes in minority populations is warranted. (Cancer Epidemiol Biomarkers Prev 2009;18(6):1829–40)

## Introduction

In the United States, lung cancer is the second leading form of cancer, is expected to affect 215,020 persons in 2008, and is the leading cause of cancer-related death (1). Whereas lung cancer incidence and mortality among men have been decreasing for several years, mortality rates among women steadily increased from 1995 to 2002 (2). Although the 5-year survival rate for lung cancer diagnosed at a distant stage is 3%, that rate increases to 49% when disease is diagnosed at a localized stage, further underscoring the need for earlier diagnosis (3). Because only 15% of smokers develop lung cancer, identification of individuals for screening efforts needs to move beyond enumeration of pack-years of smoking. Screening for single-nucleotide polymorphisms (SNPs) in candidate pathway genes is one approach to defining a high-risk subgroup.

Inflammatory pathways have been implicated in lung cancer development. Research indicating a role for in-

flammation in lung cancer includes a relationship between *COX2* polymorphisms, *COX-2* expression, and lung tumorigenesis (4–6). Aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) may be associated with a decreased risk for lung cancer (7, 8). Moreover, chronic inflammatory conditions such as chronic obstructive pulmonary disease, chronic diffuse infiltrative lung diseases, and chronic lung infections have been associated with an increased risk for developing lung cancer (9).

Studies indicate that there are differences between lung cancer cases and controls in circulating cytokine expression profiles (10–15), and polymorphisms also have been investigated to determine whether variation in cytokine genes predicts risk for non-small cell lung cancer (NSCLC). Cytokine SNPs primarily examined thus far include those in *IL1B*, *IL1RN*, *TNF*, *IL10*, *IL6*, and *IL8*. These studies suggest an association between *IL1*, *TNF*, and *IL10*, and risk for NSCLC (16–19). Of the 11 previously published studies, none have focused on these relationships in African-Americans and only one was conducted in the United States (20). Differences in cytokine polymorphism allele frequencies by race have been seen, suggesting that the relationship between cytokine SNPs and risk of lung cancer may vary by race (21–25).

In a large population-based case-control study, we examined the association between cytokine and cytokine

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receptor polymorphisms and risk of NSCLC in African-American and Caucasian women.

## Materials and Methods

**Study Participants.** Population-based cases were identified via the Metropolitan Detroit Cancer Surveillance System, a member in the Surveillance, Epidemiology, and End Results program of the National Cancer Institute. Eligible participants included women ages 18 to 74 y, diagnosed with primary NSCLC in the metropolitan Detroit area between November 1, 2001, and October 31, 2005. Initially, participant eligibility was limited to adenocarcinoma cases. However, because many histologic diagnoses at the time of rapid case ascertainment were not more specific, after November 1, 2004, eligibility was expanded to include all NSCLC histologies. Women were targeted for this investigation because these data were collected as part of a study examining estrogen and other hormone exposure, and risk of NSCLC. Subsequently, men were not included in the study.

Because the questionnaire included detailed questions related to extensive family history and history of comorbidities, no proxy interviews were conducted; therefore, women deceased at ascertainment or first contact were ineligible. Five hundred seventy-seven (54.5%) women completed an interview. Because lung cancer is a rapidly fatal disease, many women were too ill ( $n = 129$ ) at the time of first contact to complete the interview. Two hundred sixty-three women refused to participate. We excluded women self-reporting race other than African-American or Caucasian ( $n = 15$ ) because there were too few cases of other racial backgrounds for analyses stratified by race. In total, 459 women with NSCLC who provided a blood specimen were available for analysis.

Population-based controls, identified by random-digit dialing, were frequency matched to cases on race and 5-y age group. Approximately 70% ( $n = 575$ ) of the households that completed the eligibility screening questionnaire participated in the interview; 209 women refused to participate. Eleven controls who reported race other than African-American or Caucasian were excluded. In total, 483 controls who provided a blood sample were included in the analysis.

**Data Collection.** This study was approved by all local institutional review boards; informed consent was obtained from each subject. In-person interviews were conducted to collect demographic information, smoking history, health history, and environmental tobacco smoke exposure. Exsmokers were defined as women who quit smoking  $>2$  y before diagnosis/interview. Never smokers included those who smoked  $<100$  cigarettes in their lifetime. Demographic information included age and residence at diagnosis/interview, date of diagnosis, date and place of birth, marital status, race, and number of years of education. Medical history included self-report of physician diagnoses of asthma, emphysema, allergies, pneumonia, bronchitis, chronic obstructive pulmonary disease (COPD), and tuberculosis. Reports of diagnoses of lung diseases within 1 y of lung cancer diagnosis (for cases) or interview (for the controls) were excluded. To limit misclassification, any patient who reported a physician diagnosis of COPD, chronic bronchitis, or emphysema was coded as having COPD.

Smoking history included age began and quit smoking, years of smoking, average number of cigarettes per day, type of cigarette, and total years of smoking interruption. Smoking pack-year history was calculated by multiplying the number of packs smoked per day by the number of years smoked where a pack was defined as 20 cigarettes. Never smokers are included in this analysis as having a zero smoking pack-year history. Environmental tobacco smoke exposure at home and within the workplace, as well as hours and years of exposure for each situation, were collected. Family history of lung cancer was coded as yes or no based on the detailed first-degree family history information. Lung cancer diagnosis dates and histology information were obtained through the Metropolitan Detroit Cancer Surveillance System.

Medication history included regular use of aspirin, defined as taking at least one pill thrice per week or more for at least a month during an individual's lifetime, ages at which participants started and stopped pill use, and number of pills per week. Aspirin use was distinguished between baby/senior citizen aspirin (81 mg) and adult-strength aspirin (325 mg). We excluded from analysis pill use 1 y before diagnosis/interview.

**Sample Collection and Genotyping.** Blood was collected in Vacutainer Plus tubes containing EDTA. DNA was isolated from whole blood with a Qiagen AutoPure LS Genomic DNA Purification System (Genra Systems) following the manufacturers' protocols.

Genomic DNA (250 ng) was submitted to the Wayne State University Applied Genomics Technology Center for genotyping. The Illumina GoldenGate assay using the Cancer SNP Panel was utilized. The candidate SNPs on the Cancer SNP Panel were identified from the Cancer Genome Anatomy Project SNP500 Database of the National Cancer Institute, were selected to be within 10 kb of each gene, and represent pathways involved in tumorigenesis. SNPs in cytokine genes, including those for ILs, TNF, TNF receptor superfamily members, IFNs, and transforming growth factor (TGF)  $\beta$ , were included in our analyses. This panel includes 83 cytokine and cytokine receptor SNPs. The GoldenGate assay was run according to the manufacturer's directions. The data were analyzed using Bead Studio software (Illumina).

**Statistical Analysis.** Cases were compared with controls on demographic factors using  $\chi^2$  tests for categorical variables and  $t$  tests for continuous variables. Hardy-Weinberg equilibrium was assessed by a  $\chi^2$  goodness-of-fit test for each SNP for African-American and Caucasian controls separately. Participating cases were compared with nonparticipating cases on race, age at diagnosis, and stage at diagnosis using  $t$  tests for continuous variables and  $\chi^2$  tests for categorical variables. The Kaplan-Meier method and log-rank test were used to compare median survival between the two groups. SNPs with minor allele frequencies  $<5\%$  or that were in Hardy-Weinberg disequilibrium among the controls were excluded from subsequent analyses. Multivariable unconditional logistic regression models were constructed by race using SAS V9.1.3 (SAS Institute) with adjustment for age at diagnosis, smoking pack-year history, smoking history (ever/never), family history of lung cancer, history of COPD, years since COPD diagnosis, adult aspirin use, education, and body

mass index (BMI). Model fit was assessed by race by calculating  $1-\hat{\gamma}$ , a measure of overfitting (26). This model was validated internally using a bootstrapping method to obtain a bias-corrected Somers'  $D_{xy}$  rank correlation. Heterozygotes were combined with the less frequent homozygotes in a dominant model testing for the relationship between presence of the minor allele and risk for NSCLC separately by race. To assess the associations between genes and lung cancer risk, a genetic risk score was calculated for each gene as a weighted summation of the SNPs for each gene where the weight equals the  $t$  statistical (coefficient divided by its SE) of that marker in the logistic regression model. The relationship between the minor allele and risk for lung cancer was also assessed separately by smoking history (never versus ever) and history of COPD among Caucasians. The number of African-Americans included in this study was too few for this stratified analysis.  $P$  values were adjusted for multiple comparisons according to the Benjamini and Hochberg (27) false discovery rate method. Analyses were repeated predicting risk for adenocarcinoma of the lung. Random forest analysis was also conducted by race using the randomForest package in *R* (version 2.7.2) as an alternative approach to model the data.

PLINK V1.01 and the haplo.ccs package in *R* were used for haplotype construction and testing, respectively (28, 29). Haplotypes were constructed separately by race among controls. To determine if a SNP was associated with risk for lung cancer independently of the other SNPs in the haplotype, tests of the association between a particular SNP and risk of NSCLC in the context of the haplotype were conducted. Associations between inflammatory pathway gene haplotypes and risk for NSCLC were estimated via unconditional logistic regression by race adjusting for other factors associated with lung cancer using *R*.

To predict the relationship between nonsynonymous SNPs in coding regions analyzed in this study and protein function, the Sorting Intolerant from Tolerant (SIFT) program was used (30). Using homologues in the protein alignment, SIFT scores  $<0.10$  were considered to be deleterious. Median sequence information content values  $>3.0$  indicate lower sequence diversity and higher chance for false positive error. To analyze the relationships among selected genes in a biologically informative manner

through pathway analysis, we used the Ingenuity Pathway Analysis V7 program (Ingenuity Systems, Inc.).

## Results

**Participant Characteristics.** Participant characteristics are displayed in Table 1. Approximately 20% of cases and controls were African-American. Cases did not differ from controls in age or race. Cases were significantly more likely than controls to report being current smokers and to have a history of COPD. We previously reported that cases more frequently reported having a family history of lung cancer in a first-degree relative were less likely to report having more than a high school education or general education development (GED) and had a longer smoking pack-year history and a lower BMI at the time of interview (7). Participating cases did not differ from nonparticipating cases in terms of race. Nonparticipating cases were slightly older (mean  $\pm$  SD,  $63.5 \pm 8.1$  versus  $60.1 \pm 9.3$  years;  $P < 0.0001$ ), were more likely to be diagnosed with distant disease (41% versus 32%;  $P = 0.03$ ), and had a significantly shorter median survival time [33 (29-37) versus 47 (41-56) months;  $P < 0.0001$ ].

**Cytokine SNPs.** SNPs not in Hardy-Weinberg equilibrium included rs9610-*IL10RA*, rs730690-*IL12B*, rs3093661-*TNF*, rs40401-*IL3*, and rs1800471-*TGFB1* for African-Americans and rs1126579-*IL8RB* and rs11914-*IFNGR1* for Caucasians. An additional six SNPs had minor allele frequencies  $<5\%$ , resulting in 70 of 83 SNPs meeting criteria for analyses.

**Cytokine SNPs and Risk for NSCLC by Race.** For 52 of the 70 SNPs, allele frequencies differed significantly between African-American and Caucasian controls (data not shown). Consequently, all analyses were stratified on race. The minor allele for rs1800629-*TNF* was associated with a decreased risk for lung cancer only among African-Americans (Table 2). For two *IL10* SNPs that were in linkage disequilibrium, rs3024496 and rs3024491, presence of the minor allele was associated with an increased risk of NSCLC among African-Americans but a decreased risk of lung cancer among Caucasians. rs2857261-*IL15*, rs1057972-*IL15*, rs1800797-*IL6*, and rs1800795-*IL6* were associated with an increased risk

**Table 1. Participant characteristics**

Variable	Caucasians			African-Americans		
	Cases	Controls	<i>P</i>	Cases	Controls	<i>P</i>
<i>n</i>	364	380		95	103	
Age (y), mean (SD)	61 (9)	60 (9)	0.08	58 (9)	58 (9)	0.83
Smoking status, <i>n</i> (%)						
Never	36 (10)	194 (52)		8 (8)	48 (47)	
Former	132 (36)	119 (32)		24 (25)	31 (30)	
Current	195 (54)	64 (17)	$<0.0001$	63 (66)	23 (23)	$<0.0001$
Pack-years among smokers, mean (SD)	51 (30)	26 (23)	$<0.0001$	30 (22)	23 (23)	0.07
History of COPD, <i>n</i> (%)						
No	242 (67)	326 (86)		72 (76)	90 (87)	
Yes	122 (34)	54 (14)	$<0.0001$	23 (24)	13 (13)	0.03
Years from COPD diagnosis to NSCLC diagnosis/interview among women with COPD, mean (SD)	20 (17)	22 (18)	0.42	14 (14)	18 (11)	0.41

Abbreviations: NSCLC, non-small cell lung cancer; COPD, chronic obstructive pulmonary disease.

Table 2. Cytokine and cytokine receptor SNPs and risk of NSCLC by race

Cytokine	SNP identifier		Caucasians			African-Americans		
			OR* (95% CI)	P <sup>†</sup>	P <sup>‡</sup>	OR* (95% CI)	P <sup>†</sup>	P <sup>‡</sup>
Proinflammatory cytokines and cytokine receptors								
IL1A	rs17561	Ala <sup>114</sup> Ser	0.79 (0.54-1.14)	0.63	0.27	1.54 (0.69-3.45)	0.87	0.37
IL1A	rs2071374	IVS4-109 A/C	1.05 (0.72-1.51)	0.89		0.82 (0.40-1.67)	0.94	
IL1B	rs1071676	Ex7-97 G/C	0.88 (0.60-1.28)	0.74	0.27	0.79 (0.34-1.83)	0.94	0.37
IL1B	rs1143634	Phe <sup>105</sup> Phe	0.87 (0.59-1.26)	0.72		0.72 (0.30-1.76)	0.94	
IL1B	rs3136558	IVS3-123 A/G	0.93 (0.64-1.36)	0.87		0.99 (0.43-2.25)	0.99	
IL1B	rs1143627	A-31G	1.23 (0.85-1.78)	0.63		1.29 (0.48-3.52)	0.94	
IL1B	rs16944	G-511A	1.23 (0.84-1.78)	0.63		0.77 (0.31-1.91)	0.94	
IL2	rs2069763	Leu <sup>38</sup> Leu	0.95 (0.66-1.38)	0.89	0.29	0.88 (0.39-2.00)	0.98	0.76
IL2	rs2069762	A-330C	1.38 (0.74-2.56)	0.64		1.23 (0.07-22.11)	0.98	
IL6	rs1800797	G-597A	1.25 (0.85-1.83)	0.63	0.27	3.06 (1.04-9.02)	0.43	0.13
IL6	rs1800795	C-174G	1.32 (0.90-1.94)	0.61		3.74 (1.26-11.10)	0.24	
IL6R	rs8192284	Asp <sup>358</sup> Ala	1.17 (0.80-1.71)	0.72		0.88 (0.40-1.96)	0.98	
IL7R	rs1494555	Ile <sup>138</sup> Val	1.65 (1.14-2.39)	0.54	0.002	0.56 (0.25-1.23)	0.74	0.04
IL7R	rs7737000	His <sup>165</sup> His	1.60 (1.05-2.45)	0.56		4.24 (1.61-11.17)	0.24	
IL8	rs4073	A-251T	1.24 (0.82-1.88)	0.63	0.27	0.24 (0.03-1.74)	0.74	0.30
IL8	rs2227306	IVS1+298 G/A	1.31 (0.88-1.94)	0.61		0.83 (0.37-1.86)	0.94	
IL8RA	rs2854386	1379bp 3' STP G/C	0.79 (0.44-1.42)	0.72		1.10 (0.53-2.29)	0.98	
IL12A	rs582537	IVS2-701 C/A	1.08 (0.72-1.62)	0.86		0.86 (0.42-1.79)	0.98	
IL12B	rs3212227	Ex8+159 A/C	0.74 (0.51-1.08)	0.61		1.86 (0.86-4.00)	0.73	
IL15	rs1493013	Ex3+163 A/G	1.11 (0.74-1.67)	0.83	0.27	1.06 (0.50-2.22)	0.98	0.04
IL15	rs2254514	Ex3-92 G/A	1.09 (0.76-1.57)	0.86		0.97 (0.44-2.13)	0.98	
IL15	rs2857261	IVS3+8 A/G	1.22 (0.82-1.83)	0.66		2.91 (1.22-6.96)	0.24	
IL15	rs1057972	Ex9-181 T/A	1.18 (0.79-1.76)	0.72		3.99 (1.32-12.08)	0.24	
IL15	rs10833	Ex9-66 G/A	1.09 (0.74-1.58)	0.86		1.16 (0.52-2.60)	0.98	
IL15RA	rs2296135	Ex8-361 C/A	1.37 (0.90-2.10)	0.61	0.27	1.21 (0.58-2.55)	0.94	0.30
IL15RA	rs2296141	IVS6-242 G/A	0.81 (0.49-1.34)	0.72		1.70 (0.73-3.96)	0.83	
IL15RA	rs2228059	Asn <sup>146</sup> Thr	1.18 (0.77-1.83)	0.72		1.57 (0.76-3.27)	0.83	
IL15RA	rs3136614	IVS4+32 A/G	1.05 (0.72-1.52)	0.89		1.27 (0.54-3.01)	0.94	
IFNAR2	rs3153	IVS1-4640 G/A	1.23 (0.85-1.78)	0.63	0.27	1.65 (0.73-3.71)	0.83	0.33
IFNAR2	rs7279064	Phe <sup>10</sup> Val	1.36 (0.94-1.99)	0.61		1.32 (0.62-2.82)	0.94	
IFNAR2	rs2236757	IVS6-50 G/A	1.23 (0.85-1.78)	0.63		1.54 (0.74-3.21)	0.86	
IFNG	rs1861494	IVS2+284 A/G	0.94 (0.65-1.35)	0.88		1.62 (0.69-3.80)	0.87	
IFNGR2	rs1059293	Ex7-134 G/A	0.88 (0.58-1.33)	0.79		1.11 (0.18-6.70)	0.98	
TNF	rs1799964	A-1031G	1.23 (0.84-1.80)	0.63	0.26	1.28 (0.58-2.86)	0.94	0.16
TNF	rs1800630	C-863A	1.39 (0.93-2.09)	0.61		1.27 (0.52-3.06)	0.94	
TNF	rs1800629	G-308A	0.75 (0.50-1.12)	0.61		0.38 (0.16-0.91)	0.34	
LTA	rs909253	IVS1+90 A/G	0.76 (0.53-1.10)	0.61		1.06 (0.48-2.34)	0.98	
TNFRSF10A	rs2235126	IVS7+218 G/A	0.98 (0.68-1.41)	0.94	0.85	0.78 (0.37-1.66)	0.94	0.61
TNFRSF10A	rs4871857	Arg <sup>209</sup> Thr	1.01 (0.66-1.53)	0.96		0.98 (0.48-2.00)	0.98	
TNFRSF1A	rs1800692	IVS4-33 G/A	0.76 (0.52-1.12)	0.61	0.27	0.94 (0.36-2.40)	0.98	0.30
TNFRSF1A	rs887477	IVS1-2572 C/A	0.73 (0.49-1.11)	0.61		0.56 (0.26-1.19)	0.74	
Anti-inflammatory cytokines and cytokine receptors								
IL1RN	rs419598	Ala <sup>57</sup> Ala	0.95 (0.66-1.37)	0.89	0.27	1.19 (0.40-3.48)	0.98	0.76
IL1RN	rs454078	IVS6+59 T/A	0.95 (0.66-1.37)	0.89		1.16 (0.40-3.40)	0.98	
IL1RN	rs380092	IVS6+166 T/A	0.80 (0.55-1.15)	0.63		1.05 (0.31-3.51)	0.98	
IL4	rs2243248	A-1098C	1.25 (0.75-2.09)	0.72	0.27	1.29 (0.58-2.83)	0.94	0.34
IL4	rs2243250	G-590A	1.34 (0.88-2.02)	0.61		1.41 (0.44-4.56)	0.94	
IL4	rs2070874	Ex1-168 G/A	1.21 (0.80-1.83)	0.71		0.84 (0.39-1.80)	0.94	
IL4	rs2243268	IVS2-1443 A/C	1.15 (0.76-1.74)	0.75		1.14 (0.55-2.37)	0.98	
IL4R	rs2243290	IVS3-9 C/A	1.12 (0.74-1.69)	0.83	0.29	1.23 (0.59-2.56)	0.94	0.16
IL4R	rs2057768	G-29429A	1.02 (0.70-1.47)	0.94		0.94 (0.46-1.91)	0.98	
IL4R	rs3024544	IVS3-85 G/A	1.16 (0.77-1.75)	0.72		0.44 (0.17-1.14)	0.64	
IL4R	rs1805011	Glu <sup>400</sup> Ala	0.79 (0.50-1.23)	0.63		1.37 (0.59-3.17)	0.94	
IL4R	rs1805012	Cys <sup>431</sup> Arg	0.84 (0.53-1.34)	0.72		0.79 (0.32-1.92)	0.94	
IL4R	rs1805015	Ser <sup>503</sup> Pro	0.91 (0.61-1.38)	0.86		0.97 (0.47-2.00)	0.98	
IL4R	rs1801275	Gln <sup>576</sup> Arg	0.76 (0.52-1.13)	0.61		1.58 (0.49-5.12)	0.94	
IL4R	rs1805016	Ser <sup>752</sup> Ala	0.97 (0.50-1.89)	0.94		1.49 (0.73-3.05)	0.87	
IL4R	rs8832	Ex12-313 G/A	1.02 (0.69-1.51)	0.94		1.19 (0.28-5.11)	0.98	
IL10	rs3024496	Ex5+210 A/G	0.64 (0.42-0.96)	0.56	0.26	1.76 (0.84-3.69)	0.74	0.13
IL10	rs3024491	IVS1-286 C/A	0.64 (0.42-0.96)	0.56		2.50 (1.19-5.25)	0.24	
IL10	rs1800871	G-819A	1.39 (0.96-2.02)	0.61		1.32 (0.60-2.88)	0.94	
IL10	rs1800896	A-1082G	0.70 (0.46-1.05)	0.61		1.38 (0.67-2.83)	0.94	
IL10	rs1800890	T-3575A	0.81 (0.55-1.18)	0.63		2.02 (0.96-4.25)	0.48	
IL13	rs1881457	A-1469C	0.93 (0.64-1.35)	0.86	0.35	1.06 (0.49-2.28)	0.98	0.14

(Continued on the following page)

**Table 2. Cytokine and cytokine receptor SNPs and risk of NSCLC by race (Cont'd)**

Cytokine	SNP identifier		Caucasians			African-Americans		
			OR* (95% CI)	P <sup>†</sup>	P <sup>‡</sup>	OR* (95% CI)	P <sup>†</sup>	P <sup>‡</sup>
<i>IL13</i>	rs1800925	G-1112A	0.95 (0.66-1.39)	0.89		0.67 (0.32-1.44)	0.87	
<i>IL13</i>	rs1295686	IVS3-24 G/A	0.87 (0.59-1.27)	0.72		1.44 (0.33-6.39)	0.94	
<i>IL13</i>	rs20541	Arg <sup>130</sup> Gln	0.80 (0.54-1.16)	0.63		0.46 (0.21-1.00)	0.44	
<i>TGFB1</i>	rs1800469	308 bp 3'STP G/A	0.98 (0.68-1.42)	0.94		1.00 (0.49-2.03)	0.99	
<i>TGFB1</i>	rs928180	IVS3-2409 A/G	0.67 (0.40-1.12)	0.61	0.26	0.40 (0.09-1.69)	0.83	0.30
<i>TGFB1</i>	rs334358	IVS8+547 C/A	0.77 (0.52-1.12)	0.61		1.47 (0.70-3.09)	0.87	
<i>TGFB1</i>	rs868	Ex9+195 A/G	0.75 (0.51-1.11)	0.61		1.46 (0.69-3.08)	0.87	

Abbreviation: OR, odds ratio.

\*Adjusted for age at diagnosis/interview, smoking history (pack-year), smoking (never/ever), years of education, family history of lung cancer, history of COPD, years since COPD diagnosis, adult aspirin use (never/ever), and BMI.

<sup>†</sup>P values for the association between individual SNPs and NSCLC risk adjusted for multiple comparisons through the Benjamini and Hochberg false discovery rate method.

<sup>‡</sup>P values for the association between each gene (for genes with more than one SNP represented in our analyses) and NSCLC risk adjusted for multiple comparisons through the Benjamini and Hochberg false discovery rate method.

among African-Americans, but no association was detected among Caucasians. rs20541-*IL13* was only marginally associated with a decreased risk of lung cancer among African-American women. Two SNPs in *IL7R*, rs1494555 and rs7737000, were associated with an increased risk among Caucasians, but only rs7737000 was associated with risk in African-Americans. This association between *IL7R* polymorphisms and lung cancer risk was supported by the significance of the genetic risk score for *IL7R*, which was statistically significant even after correction for multiple comparisons among Caucasians ( $P = 0.002$ ) and African-Americans ( $P = 0.04$ ). Also statistically significant after adjustment for multiple testing among African-Americans was the genetic risk score for *IL15* ( $P = 0.04$ ).

**Cytokine SNPs and Risk for NSCLC by Smoking History.** There are some relationships between SNPs

and risk for NSCLC by smoking history among Caucasians (Table 3). The *TNF* SNP, rs1800630, was associated with an increased risk of NSCLC among never smokers but not among smokers. Conversely, presence of the minor allele in SNPs associated with a decreased risk of NSCLC among never smokers but not among smokers included rs2857261-*IL15*, rs4871857-*TNFRSF10A*, rs3024496-*IL10*, and rs3024491-*IL10*. The minor allele for rs1494555-*IL7R* was associated with an increased risk of lung cancer among smokers but not among never smokers ( $P$  value<sub>interaction</sub> = 0.002).

**Cytokine SNPs and Risk of NSCLC by History of COPD.** For eight SNPs (rs2071374-*IL1A*, rs1494555-*IL7R*, rs7737000-*IL7R*, rs1057972-*IL15*, rs2857261-*IL15*, rs1799964-*TNF*, rs1800630-*TNF*, rs4871857-*TNFRSF10A*), presence of the minor allele was associated with an increased risk of lung cancer among Caucasian women with

**Table 3. Associations between SNPs and risk of NSCLC by smoking history among Caucasians**

Single-nucleotide polymorphism	Never smokers				Ever Smokers			
	Cases, n	Controls, n	OR* (95% CI)	P <sup>†</sup>	Cases, n	Controls, n	OR <sup>‡</sup> (95% CI)	P <sup>†</sup>
rs1494555- <i>IL7R</i>								
AA	18	90			128	103		
AG/GG	17	103	0.95 (0.45-2.03)	0.97	198	80	1.97 (1.28-3.03)	0.15
rs2857261- <i>IL15</i>								
AA	15	49			85	63		
AG/GG	21	145	0.46 (0.21-1.00)	0.46	242	120	1.75 (1.10-2.78)	0.65
rs1800630- <i>TNF</i>								
CC	21	151			231	134		
AC/AA	14	43	2.32 (1.06-5.07)	0.46	97	49	1.19 (0.74-1.91)	0.84
rs4871857- <i>TNFRSF10A</i>								
GG	14	47			90	54		
CG/CC	22	146	0.41 (0.19-0.90)	0.46	236	128	1.38 (0.85-2.23)	0.84
rs3024496- <i>IL10</i>								
AA	16	56			89	50		
AG/GG	20	137	0.46 (0.21-0.98)	0.46	238	133	0.75 (0.46-1.21)	0.84
rs3024491- <i>IL10</i>								
CC	16	56			89	50		
AC/AA	20	137	0.46 (0.21-0.98)	0.46	238	133	0.75 (0.46-1.21)	0.84

\*Adjusted for age at diagnosis/interview, years of education, family history of lung cancer, history of COPD, years since COPD diagnosis, adult aspirin use (never/ever), and BMI.

<sup>†</sup>Adjusted for multiple comparisons via the Benjamini and Hochberg false discovery rate method.

<sup>‡</sup>Adjusted for age at diagnosis/interview, smoking history (pack-year), years of education, family history of lung cancer, history of COPD, years since COPD diagnosis, adult aspirin use (never/ever), and BMI.

COPD but not among those without a history of COPD (Table 4). Two *IL1RN* SNPs, rs454078 and rs419598, were associated with a decreased risk of lung cancer among Caucasian women with COPD but not among women with no history of COPD. The two polymorphisms that marginally increased risk of NSCLC among Caucasian women without COPD but not among women with COPD were rs2227306-*IL8* and rs2296135-*IL15RA*.

**Risk of Adenocarcinoma of the Lung.** When analyses were restricted to predicting risk of adenocarcinoma of the lung, results were similar (data not shown).

**Random Forest Analysis.** Mean decrease accuracy variable importance measures for Caucasian and African-American women indicate that the most important predictor of lung cancer is, not surprisingly, smoking pack-year history (Table 5). rs77371000-*IL7R* was identified as being an important predictor of lung cancer among African-American women by both random forest analysis and logistic regression, and rs1494555-*IL7R* was identified by both methods as being an important predictor among Caucasian women. The second most important polymorphisms identified on random forest but not logistic regression among Caucasian and African-American

women, respectively, were rs17561-*IL1A* and rs4871857-*TNFRSF10A*.

**Haplotype Results.** Haplotypes in *IL1B*, *IL7R*, *IFNAR2*, and *IL13* predicted lung cancer risk differentially by self-reported race. However, the significance of each of these haplotypes determined by PLINK was driven by a single SNP (data not shown). Subsequently, haplotype analysis provided no additional risk information beyond that obtained from the single SNP analysis.

**Nonsynonymous SNPs and Predicted Protein Function.** Of the 12 nonsynonymous polymorphisms included in this study, predictions of the effect of amino acid substitutions on protein function were obtained for eight SNPs (rs17561, rs1494555, rs1801275, rs1805011, rs1805012, rs1805015, rs1805016, rs4871857). No prediction was made for two of the polymorphisms, and two were not found in the database. All eight of the SNPs analyzed through the SIFT program had prediction scores >0.10 and median sequence information content values <3.00.

**Pathway Analysis.** Of the 26 cytokines and cytokine receptors analyzed, all were incorporated into an in-

**Table 4. Associations between SNPs and risk of NSCLC by COPD history among Caucasians**

Single-nucleotide polymorphism	COPD				No COPD			
	Cases, <i>n</i>	Controls, <i>n</i>	OR* (95% CI)	<i>P</i> <sup>†</sup>	Cases, <i>n</i>	Controls, <i>n</i>	OR <sup>‡</sup> (95% CI)	<i>P</i> <sup>†</sup>
rs2071374- <i>IL1A</i>								
AA	60	31			132	170		
AC/CC	62	22	2.51 (1.02-6.18)	0.33	107	155	0.92 (0.61-1.40)	0.81
rs1494555- <i>IL7R</i>								
AA	41	33			105	161		
AG/GG	81	21	3.53 (1.46-8.51)	0.21	135	164	1.42 (0.94-2.16)	0.50
rs7737000- <i>IL7R</i>								
GG	94	46			176	249		
AG/AA	28	8	3.45 (1.02-11.71)	0.33	66	77	1.42 (0.89-2.26)	0.50
rs2227306- <i>IL8</i>								
GG	40	18			65	116		
AG/AA	81	36	0.70 (0.29-1.72)	0.72	173	207	1.60 (1.01-2.52)	0.50
rs1057972- <i>IL15</i>								
TT	28	20			75	92		
AT/AA	94	34	3.13 (1.26-7.80)	0.21	165	233	0.96 (0.61-1.50)	0.94
rs2857261- <i>IL15</i>								
AA	26	20			74	93		
AG/GG	96	34	3.53 (1.39-8.97)	0.21	167	233	0.98 (0.63-1.55)	0.98
rs2296135- <i>IL15RA</i>								
CC	34	9			55	93		
AC/AA	88	45	0.59 (0.22-1.63)	0.69	186	233	1.74 (1.07-2.82)	0.50
rs1799964- <i>TNF</i>								
AA	80	42			147	210		
AG/GG	42	12	3.50 (1.27-9.64)	0.21	94	116	0.97 (0.63-1.50)	0.96
rs1800630- <i>TNF</i>								
CC	91	45			161	242		
AC/AA	31	9	3.53 (1.14-10.98)	0.25	80	84	1.17 (0.74-1.84)	0.70
rs4871857- <i>TNFRSF10A</i>								
GG	27	19			77	84		
CG/CC	95	34	3.43 (1.28-9.24)	0.21	163	241	0.74 (0.47-1.18)	0.60
rs454078- <i>IL1RN</i>								
TT	73	27			120	175		
AT/AA	49	27	0.35 (0.14-0.84)	0.21	122	151	1.15 (0.76-1.74)	0.70
rs419598- <i>IL1RN</i>								
AA	72	27			119	174		
AG/GG	50	27	0.36 (0.15-0.86)	0.21	122	152	1.14 (0.75-1.72)	0.72

\*Adjusted for age at diagnosis/interview, smoking history (pack-years), smoking (ever/never), years since COPD diagnosis, years of education, family history of lung cancer, adult aspirin use (never/ever), and BMI.

<sup>†</sup>Adjusted for multiple comparisons via the Benjamini and Hochberg false discovery rate method.

<sup>‡</sup>Adjusted for age at diagnosis/interview, smoking history (pack-years), smoking (ever/never), years of education, family history of lung cancer, adult aspirin use (never/ever), and BMI.

**Table 5. Top 10 variable importance measures predicting NSCLC by race on random forest analysis**

Variable	Mean decrease accuracy
<b>Caucasians</b>	
Smoking history (pack-year)	1.24
COPD history	0.53
Family history of lung cancer	0.38
rs1494555- <i>IL7R</i>	0.18
rs17561- <i>IL1A</i>	0.17
rs16944- <i>IL1B</i>	0.16
rs1801275- <i>IL4R</i>	0.16
rs868- <i>TGFBR1</i>	0.16
rs20541- <i>IL13</i>	0.15
rs1805015- <i>IL4R</i>	0.14
<b>African-Americans</b>	
Smoking history (pack-year)	1.81
rs7737000- <i>IL7R</i>	0.51
rs4871857- <i>TNFRSF10A</i>	0.48
COPD history	0.27
Family history of lung cancer	0.24
rs3024496- <i>IL10</i>	0.23
rs1805016- <i>IL4R</i>	0.18
rs1805012- <i>IL4R</i>	0.16
rs928180- <i>TGFBR1</i>	0.15
rs1805015- <i>IL4R</i>	0.10

flammation network in addition to two other cytokines not analyzed in this study, IFN- $\alpha$  and IFN- $\beta$ . When only interactions between cytokines expressed in human lung and known to play a role in cancer were displayed (Fig. 1), 18 cytokines and cytokine receptors remained, including *IL-7R* and *IL-10*. SNPs in these two genes were most consistently shown to be associated with NSCLC on logistic regression and random forest analyses. Reported interactions between *IL-7R* and TNF- $\alpha$  and IFN- $\gamma$  and between *IL-10* and *IL-1 $\alpha$* , *IL-6*, *IL-8*, IFN- $\gamma$ , TNF- $\alpha$ , and TGF- $\beta$ 1 are presented.

## Discussion

We examined the relationship between NSCLC and 70 cytokine and cytokine receptor polymorphisms in 26 genes. Variant allele frequencies differed significantly between African-Americans and Caucasians for 74% of the SNPs included. SNPs in *IL7R* and *IL10* were associated with increased risk of lung cancer in both African-Americans and Caucasians on logistic regression analysis. An *IL10* SNP, rs3024491, was associated with an increased risk of lung cancer among African-Americans and a decreased risk among Caucasians. *IL7R* and *IL7R* and *IL10* were important predictors of lung cancer on random forest among Caucasians and African-Americans, respectively. Other SNPs associated with risk of NSCLC on logistic regression among African-Americans included those in *IL6*, *IL15*, *TNF*, and *IL13*. Polymorphisms in *TNF*, *TNFRSF10A*, *IL10*, and *IL15* were associated with risk of lung cancer among Caucasian never smokers and in *IL7R* among Caucasian ever smokers. When these associations were analyzed separately by history of COPD, single-nucleotide polymorphisms in *IL1A*, *IL7R*, *IL15*, *TNF*, *TNFRSF10A*, and *IL1RN* were associated with NSCLC risk in women with COPD and *IL8* in women without a history of COPD. Our study is the first to analyze the

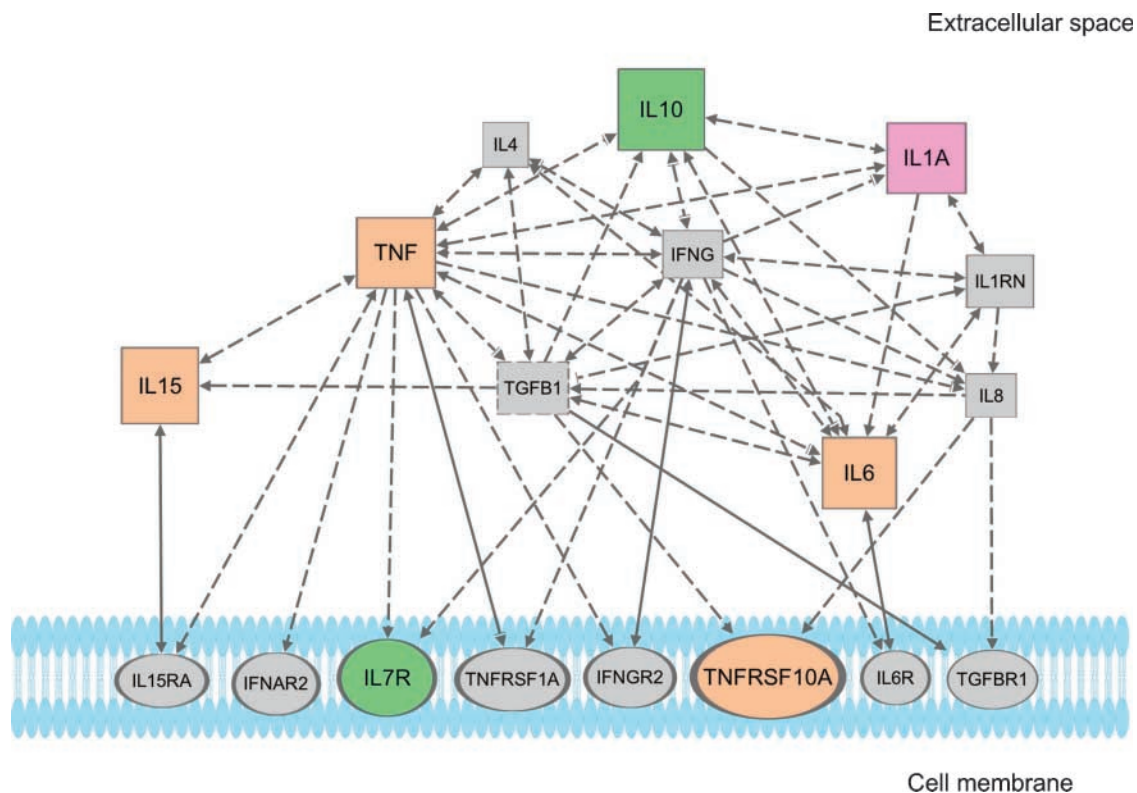
association between NSCLC risk and *IL7R*, *IL15*, *IL15RA*, *TNFRSF10A*, and *TNFRSF1A* polymorphisms.

**Cytokine SNPs, NSCLC Risk, and Race.** Distinct patterns of associations between NSCLC risk and cytokine polymorphisms were observed for Caucasians and African-Americans as evident in the relationships between *IL6*, *IL15*, *TNF*, and *IL13* SNPs and risk of NSCLC among African-Americans but not Caucasians on logistic regression analysis. The *IL10* polymorphism, rs3024491, was associated with an increased risk of NSCLC among African-Americans but a decreased risk of NSCLC among Caucasians on logistic regression analysis. These data suggest that the relationships between inflammatory pathway genes and lung cancer risk potentially differ between African-American and Caucasian women. Alternatively, particular SNPs included may not be causal but may be in linkage disequilibrium with other genetic changes that represent the observed association. This linkage disequilibrium may vary by race and account for the observed associations.

SNP associations conserved across both racial groups included those for *IL7R* and *IL10* polymorphisms. *IL-7* and *IL-10* play critical roles in immune modulation and have been implicated in tumorigenesis. *IL-7* is a proinflammatory cytokine involved in growth of pre-B lymphocytes and pre-T lymphocytes, is elevated in patients with T cell depletion, activates macrophages, up-regulates expression of the proangiogenic *IL-8*, and aids in T-lymphocyte survival. *IL-7R* expression has been shown in neoplastic lung cell lines (31), and *IL-7* systemic therapy has been shown to increase antitumor T-cell lytic activity in mice bearing NSCLC tumors (32). One potential mechanistic link among smoking, *IL-7*, and lung cancer may be the up-regulation of *IL-8* expression by *IL-7* (33).

A key anti-inflammatory cytokine, *IL-10*, is produced by monocytes, tumor associated macrophages, and T<sub>helper</sub> type 2 (T<sub>H</sub>2) cells; acts chiefly to down-regulate expression of proinflammatory cytokines, including TNF and TNF receptors; and inhibits the antitumor cytotoxic action of macrophages. However, it has also been shown to stimulate immune activity (34). Interestingly, murine models indicate that female mice, but not male mice, hemizygous for *IL10* were more susceptible to urethane induced lung adenocarcinomas (35). At least one review supports an antitumorigenic function of *IL-10* and disputes the dogma that *IL-10*, as an anti-inflammatory cytokine, serves to suppress immunosurveillance (34). However, a study involving 154 NSCLC cases and 205 controls indicated that alleles for three *IL10* promoter SNPs associated with increased *IL-10* production were found more frequently among lung cancer cases than controls (18). These contradictory findings between preclinical and clinical studies highlight the complexity of the role of cytokines in tumorigenesis.

**Cytokine SNPs, NSCLC Risk, and Smoking.** Of particular interest are the genes for which polymorphisms were associated with an increased risk of lung cancer differentially among never smokers (*TNF*) and among smokers (*IL7R* and *IL15*). TNF- $\alpha$  is produced by macrophages, CD4+ T cells, and natural killer cells in response to *IL-2* and IFNs, as well as by tumor cells. TNF- $\alpha$  binds to *TNFRSF1A* and *TNFRSF10A* receptors, resulting in programmed cell death. Alternatively, lung cancer cells have been shown to be resistant to TNF- $\alpha$ -induced death through activation of the transcription factor NF $\kappa$ B (36).



**Figure 1.** Pathway analysis depicting cytokine and cytokine receptor interactions relative to position in the cellular environment. Interactions between genes that are expressed in the human lung and that have been shown to play a role in cancer are presented. Solid lines, direct physical interactions; dotted lines, indirect interactions. Green, pink, orange, and gray figures, cytokines; associations between those cytokine and cytokine receptor polymorphisms and lung cancer risk were found among both racial groups, Caucasians only, African-Americans only, or neither group in our study, respectively.

Whereas some studies have shown no effect of cigarette smoke exposure on bronchoalveolar lavage TNF- $\alpha$  supernatant levels (37) or in exhaled breath condensate (38), others have observed lower levels of TNF- $\alpha$  in bronchoalveolar lavage fluid (39) and by peripheral blood mononuclear cells from smokers *in vitro* (40). Similar to the latter findings, the rs1800630-*TNFA* allele, which we observed to be associated with an increased risk of NSCLC among never smokers, has been shown to be associated with decreased TNF- $\alpha$  expression (41). If these findings are replicated, *TNF* may serve as a gene that increases a never smoker's risk of lung cancer by mimicking the cytokine profile in a smoker's lung. Thus, *TNF* death receptor and *IL7R* genes need to be further studied for roles in the development of lung cancer among never smokers and ever smokers, respectively.

Structurally similar to IL-2, IL-15 facilitates CD8+ memory T-lymphocyte survival and stimulates proliferation of natural killer cells, dependent on IL-15RA, in the tumor environment (42, 43). Adjuvant IL-15 therapy has been shown to result in tumor regression in a murine lung tumor model (44), underscoring the importance of examining polymorphisms in the *IL15* gene. Among the Caucasian women in our sample, rs2857261-*IL15* was associated with a decreased risk of NSCLC among never smokers and an increased risk among smokers, highlighting a potentially important interaction between *IL15* and the impact of cigarette smoke.

**Cytokine SNPs, NSCLC Risk, and COPD.** The relationships between cytokines and the development of lung cancer among people with and without COPD has yet to be elucidated. Our study revealed separate cytokine polymorphism signatures associated with risk of NSCLC among Caucasian women with COPD and those without COPD, even after taking into account smoking history. The increased risk of NSCLC among women without a history of COPD who carry the *A* allele for rs2227306-*IL8*, which is associated with increased IL-8 production, is expected because IL-8 serves as a chemotactic and powerful proangiogenic factor. Furthermore, the protective effect of polymorphisms in *IL1RN*, which codes for IL-1 receptor antagonist, among COPD patients may be related to suppression of IL-1-induced inflammation with cigarette smoke exposure; however, the effect of these two SNPs on expression remains to be determined. Although the association between TNF- $\alpha$  and COPD development has been well documented and anti-TNF therapies are being tested in COPD patients (45-48), we present here the association between *TNF* polymorphisms and risk of NSCLC among COPD patients but not among non-COPD patients. For both *TNF* promoter polymorphisms, the rs1799964 *G* allele and the rs1800630 *A* allele have been associated with increased expression, and both increased and decreased expression, respectively (49-51), illustrating the com-



plexities of regulation of genetic expression. Whether the association between these SNPs and genetic expression alters the risk of NSCLC among COPD patients remains to be determined. One potential mechanism involves increased TNF- $\alpha$  activating NF $\kappa$ B and subsequently contributing to a proinflammatory environment associated with tumorigenesis. In addition, the polymorphisms in *IL1A*, *IL7R*, *IL15*, and *TNFRSF10A* associated with increased risk of NSCLC among COPD patients suggest that the role of these cytokines and cytokine receptors needs to be studied in terms of lung tumorigenesis among COPD patients.

#### Logistic Regression versus Random Forest Results.

Comparisons between logistic regression and random forest have been studied extensively for classification purposes (52-54). In general, more sophisticated statistical methods yield better classification. In our study, we used these two methods to explore risk factors rather than to classify outcomes. Logistic regression is simple and straightforward, but SNPs are considered one at a time. Random forest is a suitable complement to the logistic regression method because the importance of a SNP was calculated, given other SNPs and risk factors. Given the different statistical approaches of these methods (one is linear and the other is nonlinear with a bagging algorithm), it is not unexpected that the results differ to some degree.

In our study, *IL7R* polymorphisms were identified in both racial groups as being predictive of lung cancer risk on logistic regression and random forest analysis. Furthermore, identification of *IL10* polymorphisms as predictive of NSCLC risk among African-American women was a consistent finding on logistic regression and random forest analysis. The remainder of the results differed between the two analytic methods. Although the findings were not statistically significant after adjusting for multiple comparisons on logistic regression analysis, *IL7R* and *IL10* polymorphisms showed the strongest relationship with lung cancer risk using both statistical approaches.

A few key cytokine genes with polymorphisms identified on random forest analysis but not on logistic regression analysis included rs17561-*IL1A* and rs16944-*IL1B* among Caucasian women and rs4871857-*TNFRSF10A* among African-American women. At least one other study involving non-Hispanic Caucasians found an 18% increased risk of lung cancer for rs17561-*IL1A* in a dominant model [odds ratio, 1.18; 95% confidence interval (95% CI), 1.03-1.36;  $P = 0.02$ ], especially among heavy smokers and participants with a history of emphysema (20). Although this particular SNP is not presumed to have an impact on IL-1 $\alpha$  function (SIFT score, 0.81; median sequence information content, 2.59), it may be in linkage disequilibrium with another polymorphism that affects IL-1 activity. We did not observe an association between rs16944-*IL1B* and lung cancer risk among either racial group on single SNP logistic regression analysis; however, relationships between *IL1B* polymorphisms and haplotypes and lung cancer have been previously reported among Caucasian and Japanese populations (16, 17, 20). Also known as lymphocyte activating factor, IL-1 is produced in two forms (IL-1 $\alpha$  and IL-1 $\beta$ ) primarily by monocytes and stimulates T helper cells, B-lymphocyte proliferation, and neutrophil chemotaxis. In addition, it is involved in the

production of prostaglandin E<sub>2</sub>, colony stimulating factor, IL-6, and IL-8, which plays a role in the pathogenesis of cancer and chronic inflammatory conditions such as COPD (46, 55).

Although rs4871857-*TNFRSF10A* was not associated with NSCLC risk among African-Americans on logistic regression analysis, it was identified by random forest as an important predictor. *TNFRSF10A* codes for the DR4 TNF receptor, also referred to as "the death receptor." The DR4 receptor is vital to induction of lung tumor cell death, but not normal cell death, by TNF-related apoptosis-inducing ligand (56) and was found to be expressed in almost 100% of NSCLC tumors in one study (57). These findings suggest that the TNF receptors may serve to reduce risk of clinically detectable lung cancer by keeping tumor cells in check. Although the rs4871857 polymorphism lies in a coding region for the ectodomain of DR4, it is not projected to have an impact on protein function (SIFT score, 0.73; median sequence information content, 1.84); however, further research into the role of *TNFRSF10A* genotypes in determining DR4 activity in the tumor microenvironment should be conducted.

Combined with the marginal results on logistic regression analysis, the low mean decrease accuracy for all of the SNPs, except for *IL7R* on random forest analysis, suggests that no single gene in the inflammatory pathway predominates in importance in predicting lung cancer risk. Rather, it is likely that the concert of cytokine signaling combined with other signaling pathways has an impact on lung cancer development.

**Pathway Analysis Results.** The inflammation network identified through pathway analysis emphasizes the proinflammatory role of TNF- $\alpha$  in up-regulation of *IL6*, *IL7*, *IL8*, *IL15*, *IL15RA*, and *IFNAR2* expression and/or activation and the anti-inflammatory role of IL-10 through its suppression of *IL1A*, *IL6*, *IL8*, and *IFNG* production. Mutual inhibition between proinflammatory and anti-inflammatory cytokines and cytokine receptors is evident through this immunosuppressive effect of IL-10. Further evidence lies in the inhibition of TGF- $\beta$ 1 by TNF- $\alpha$ , the relationship between IL-10 and decreased production of TNF- $\alpha$ , and the inhibition of IFN- $\gamma$  production by TGF- $\beta$ 1. The question then arises how a proinflammatory state induced by exposures to lung irritants such as cigarette smoke can lead to the development of a lung tumor microenvironment where immunosuppression predominates. The answer may lie in the stimulatory effects of TNF- $\alpha$ , the production of which is stimulated by chemicals in cigarette smoke, on NF $\kappa$ B expression by epithelial cells, IL-8 expression, and IL-10 activation. A chemokine that is elevated in smokers' lungs, IL-8, draws immune system cells to the site at which it is produced and facilitates angiogenesis through up-regulation of vascular endothelial growth factor expression (58, 59). Thus, a chronic proinflammatory state created by chronic cigarette smoke exposure, driven by TNF- $\alpha$ /NF $\kappa$ B positive feedback loop and monocyte chemotaxis to the lung tissue, may also promote tumorigenesis through immunosuppression induced by IL-10, in addition to the contribution of TGF- $\beta$ 1 production by transformed cells.

Of particular interest are the connections in the inflammation network involving IL-7R, which we identi-

fied as being associated with NSCLC risk, and IFN- $\gamma$ , which showed no relationship in our analyses. The interactions between IL-7R and TNF- $\alpha$ , as well as IFN- $\gamma$ , shown in the network support a proinflammatory role of IL-7R in tumorigenesis and in the human lung, emphasizing the importance of the association between *IL7R* polymorphisms and an increased risk of NSCLC. Although SNPs in *IFNG* and *IL8* were not identified as being predictors of NSCLC on either logistic regression or random forest analysis, they have been shown to interact with cytokine genes identified in our analyses as being associated with NSCLC risk. IFN- $\gamma$  is a key proinflammatory cytokine that activates macrophages, suppresses T<sub>H</sub>2 cytokine activity, including that of IL-10, and inhibits IL-8 secretion by human lung carcinoma cells (60). Simultaneously, IFN- $\gamma$  has been shown to decrease IL-7R protein production, suggesting another regulatory mechanism (61).

**Strengths and Limitations.** This study had a number of strengths. Only in-person interviews were conducted, increasing data validity and reliability. Extensive medical histories and environmental exposure histories were collected, permitting adjustment for potential confounders and analysis of SNP-smoking interactions and COPD strata-specific analyses. Because of the proportion of African-Americans included in this study, we were able to conduct race-specific analyses. Moreover, SNPs selected for these analyses were included in the Cancer 500 Panel from the National Cancer Institute's Cancer Genome Anatomy Project, allowing for comparison with future work involving this panel by other investigators. In addition, random forest and pathway analyses were used as alternative tools for data analysis.

This study is not without limitations. The functional consequences of a number of SNPs included in our analyses are as yet unknown. It is possible that the SNPs found to be associated with NSCLC risk in this study are in linkage disequilibrium with other SNPs, not included in our study, which have a functional consequence and are associated with risk of lung cancer. Because only in-person interviews were conducted, some women were too ill to participate, potentially creating a bias toward including cases with less aggressive forms of NSCLC as is evident in the greater proportion of nonparticipating cases diagnosed at a distant stage and with a shorter survival time. Although our study included ~20% African-Americans, the number of African-American participants was still too small to stratify analyses on race and smoking history, or history of COPD with adequate power. History of COPD was determined by patient report of a physician diagnosis of emphysema, chronic bronchitis, or COPD and not by objective measures. Subsequently, the possibility exists that some participants were misclassified according to history of COPD. This study included largely adenocarcinoma cases; therefore, the results may not be generalizable to all lung cancers. Whereas an estimate of overfitting for the model was 3% for Caucasians, it was 12% for African-Americans, suggesting that the number of African-Americans included in this study might have been too small for the number of parameters included in the logistic regression model. A bootstrap estimate of Somers'  $D_{xy}$ , a measure of model validity, was 0.68 for Caucasians and 0.63 for African-Americans, indi-

cating that model fit was fairly comparable for Caucasians and African-Americans. With respect to random forest analysis, the out of bag error rates for Caucasian and African-American women in this sample are 22% and 30%, respectively, further underscoring the need to conduct this study using a larger sample of African-American women. Finally, multiple comparisons were made, and some significant findings may be due to chance. However, the analysis was directed toward inflammatory pathway genes *a priori*, and adjusted *P* values are presented. Although these adjusted *P* values are not statistically significant, except for the association between *IL7R* and *IL15* genetic risk scores and NSCLC risk, the false discovery rate method is an overcorrection because it assumes independent tests of association; this assumption is violated, given linkage disequilibrium between some SNPs.

**Conclusions.** Although cigarette smoking has been shown to increase lung expression of IL-1 $\beta$ , IL-6, TNF- $\alpha$ , IL-4, and IL-8, we identified IL-7 and IL-15 as additional cytokines, which may confer an increased risk of lung cancer. Moreover, the increased risk associated with *IL7R* and *IL15* SNPs among Caucasian smokers, but not among never smokers, suggests that further research needs to be conducted about the interaction between cigarette smoke exposure and IL-7 and IL-15 expression in the prediction of lung cancer risk. Results also implicate *IL1A*, *IL7R*, *IL15*, *TNF*, and *TNFRSF10A* in the pathogenesis of lung cancer and *IL1RN* in the protection against developing lung cancer among COPD patients. The differential findings on logistic regression and random forest analyses by self-reported race indicate that further research about these cytokine SNPs in high-risk populations needs to be conducted by race. Finally, future integration of pathway analysis data with environmental risk factor data such as smoking behavior, demographic information, data on comorbidities, and disease status is necessary to understand how genetic networks in concert are modulated by other factors to affect disease risk.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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