ORIGINAL ARTICLE

Inflammatory Gene Polymorphisms in Lung Cancer Susceptibility

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ABSTRACT

Introduction: Chronic inflammation has been implicated in carcinogenesis, with increasing evidence of its role in lung cancer. We aimed to evaluate the role of genetic polymorphisms in inflammation-related genes in the risk for development of lung cancer.

Methods: A nested case-control study design was used, and 625 cases and 625 well-matched controls were selected from participants in the β-Carotene and Retinol Efficacy Trial, which is a large, prospective lung cancer chemoprevention trial. The association between lung cancer incidence and survival and 23 polymorphisms descriptive of 11 inflammation-related genes (interferon gamma gene [*IFNG*], interleukin 10 gene [*IL10*], interleukin 1 alpha gene [*IL1A*], interleukin 1 beta gene [*IL1B*], interleukin 2 gene [*IL2*], interleukin 4 receptor gene [*IL4R*], interleukin 4 gene [*IL4*], interleukin 6 gene [*IL6*], prostaglandin-endoperoxide synthase 2 gene [*PTGS2*] (also known as *COX2*), transforming growth factor beta 1 gene [*TGFB1*], and tumor necrosis factor alpha gene [*TNFA*]) was evaluated.

Results: Of the 23 polymorphisms, two were associated with risk for lung cancer. Compared with individuals with the wild-type (CC) variant, individuals carrying the minor allele variants of the *IL-1β-511*C>T promoter polymorphism (rs16944) (CT and TT) had decreased odds of lung cancer (OR = 0.74, [95% confidence interval (CI): 0.58–0.94] and OR = 0.71 [95% CI: 0.50–1.01], respectively, $p = 0.03$). Similar results were observed for the *IL-1β-1464* C>G promoter polymorphism (rs1143623), with presence of the minor variants CG and CC having decreased odds of lung cancer (OR = 0.75 [95% CI: 0.59–0.95] and OR = 0.69 [95% CI: 0.46–1.03], respectively, $p = 0.03$). Survival was not influenced by genotype.

Conclusions: This study provides further evidence that *IL1B* promoter polymorphisms may modulate the risk for development of lung cancer.

Keywords: Lung cancer; Polymorphism; Genetics; Cytokines; Inflammation

Introduction

Lung cancer is the leading cause of cancer mortality worldwide, with 155,870 deaths predicted in the United States in 2017.[1] Although the vast majority of lung cancer cases occur in the setting of tobacco use, lung cancer develops in less than 20% of smokers.[2] To explain these observations, a genetic basis for susceptibility to the carcinogenic effects of tobacco smoke has been postulated. Candidate genetic polymorphisms include genes involved in the metabolism and activation of the carcinogens in tobacco smoke, DNA repair, and cell cycle regulation.[3,4] Genome-wide association studies have demonstrated that single nucleotide polymorphisms (SNPs) located within the 15q25 region

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encoding for subunits of the nicotinic acetylcholine receptor are significantly associated with risk for lung cancer.[5,6]

Several lines of evidence support the hypothesis that persons with chronic inflammatory conditions, such as chronic obstructive pulmonary disease[7] or asthma,[8] have an increased risk for development of lung cancer, even when exposure to tobacco smoke is taken into account. Asbestos and cigarette smoke may exert some of their carcinogenic effects in the lung through chronic inflammation,[9] as tobacco use has been associated with higher levels of inflammatory cytokines and inflammatory cells.[10] Other evidence indicates that among persons with lung cancer, a proinflammatory state may lead to worse prognosis. This hypothesis is supported by the finding that patients with lung cancer with increased numbers of intratumoral macrophages had shorter relapse-free survival than do patients with low intratumoral macrophage density.[11] In addition, the total degree of systemic inflammation, as measured by C-reactive protein and albumin levels, has been found to be a powerful prognostic indicator in patients with lung cancer.[12]

The role of chronic infections in the etiology of hepatocellular carcinoma, gastric cancer, and cervical cancer is well established and is postulated to be mediated through the inflammatory response.[13] In humans, the role of inflammatory gene polymorphisms has been investigated in a variety of tumors, including gastric, liver, breast, ovarian, prostate, pancreas, skin, colon, and hematologic malignancies. Gene families studied have included interleukin 1 alpha (*IL1A*), interleukin 1 beta (*IL1B*), interleukin 1 receptor antagonist (*IL1RN*), interleukin 2 (*IL2*), interleukin 4 (*IL4*), interleukin 6 (*IL6*), interleukin 8 (*IL8*), interleukin 10 (*IL10*), tumor necrosis factor alpha (*TNFA*), lymphotoxin alpha (*LTA*), prostaglandin-endoperoxide synthase 2 (*PTGS2*) (also cyclooxygenase 2 [also known as *COX2*]), interferon gamma [*IFNG*], Toll like receptor 4 (*TLR4*), and others.[14,15] Inflammatory gene families have been studied most extensively in gastric cancer, where the results have been variable. Initial studies suggested that the proinflammatory alleles *IL-1β-31T* and *IL-1RN*2* increased effective interleukin-1 (IL-1) levels and inhibited gastric acid secretion, thereby increasing risk of noncardia gastric cancer.[16,17] These polymorphisms, in addition to polymorphisms in *TNFA* and *IL10*, were found to increase noncardia gastric cancer risk.[16] Further studies, however, have indicated that whereas *IL1B* and *IL-1RN* SNPs and haplotypes may be associated with atrophic gastritis, there is no association with gastric cancer.[18,19] Furthermore, results vary significantly depending on the population being studied, with variability most pronounced between white and Asian populations.[20]

Various studies have begun to understand the role of inflammatory genes in the pathogenesis of lung cancer. Measured free levels of circulating IL-17A, IL-17F, IL-22, IL-23, and tumor necrosis factor-α have been demonstrated to be significantly increased in patients with NSCLC compared with in healthy controls.[21] Research has focused on the role of SNPs within the *IL1B* gene and the *IL1RN* gene, similarly to prior studies in gastric cancer. Studies have demonstrated an increased risk of lung cancer as well as COPD among individuals carrying a -31T>C (rs1143627), -511C>T (rs16944), -3893A>G (rs12621220), or -1464 C>G (rs1143623) mutation.[22–25] Furthermore, studies have demonstrated haplotype linkage disequilibrium between the aforementioned *IL1B* SNPs and *IL1RN* variable number tandem repeats (VNTRs) with association between certain haplotypes and increased risk of lung cancer.[22–24,26] The success of checkpoint inhibitors in NSCLC has underscored the importance of immune regulations in the development and progression of lung cancer.[27]

Given these observations linking inflammatory gene SNPs and haplotypes with lung cancer risk, we tested the hypothesis that variation in the form of single-nucleotide polymorphisms (SNPs) in additional genes involved in the inflammatory response influences lung cancer risk and prognosis.

Materials and Methods

Subjects

Participants in this study were enrolled in the β-Carotene and Retinol Efficacy Trial (CARET), which is a large, randomized, multicenter trial that examined the effect of β-carotene and retinol versus placebo in a population at high risk for development of lung cancer.[28] Participants in CARET included heavy smokers who were age 50 to 69 years at the time of entry into the study, had a smoking history of 20 or more pack-years, and were either current smokers or had quit within the previous 6 years. As part of the study, genomic DNA was prospectively collected and banked from more than 12,000 subjects out of a total of 18,314 enrolled subjects. The Fred Hutchinson Cancer Research Center Institutional Review Board granted approval for the original intervention study, DNA collection, and use of the samples and data for the current study.

Criteria for the current study included (1) a diagnosis of lung cancer (both SCLC and NSCLC were included), (2) availability of a dried blood spot as source of DNA, and (3) availability of a matched control from the CARET data set. A matched, nested case-control design was used to minimize bias from population stratification. Demographic and exposure data on this population are well

characterized. The average length of follow-up for cases is more than 7 years. Cases and controls were matched by age, sex, race, occupational exposure to asbestos, year of enrollment, CARET intervention arm, smoking status (current versus former), and pack-years.

From participants entered into the original CARET trial with banked DNA, a total of 625 lung cancer cases and an equal number of matched controls were identified. The large study population allowed for near-perfect matching on all variables of interest, as shown in Table 1. Histological subtypes were determined from pathology reports when possible. For the purposes of analysis, NSCLC was divided into the following histological subtypes: adenocarcinoma (AC), squamous cell carcinoma (SCC), and other NSCLC. AC was the most frequent subtype with 169 cases, SCC accounted for 138 cases, other NSCLC accounted for 122 cases, and SCLC accounted for 98 cases. Because 98 cases did not have an accompanying pathology report, they were diagnosed by death certificate, clinically, or by self-report. The number of cases of each of the histological subtypes is shown in Table 1.

Table 1. Characteristics of the Study Population

Characteristic	Cases (n = 625)	Controls (n = 625)
Mean age ± SD, y	59.7 ± 5.3	59.6 ± 5.3
Sex, n (%)		
Female	217 (35%)	217 (35%)
Male	408 (65%)	408 (65%)
Race, n (%)		
White	614 (98%)	614 (98%)
Black	6 (1%)	6 (1%)
Other	5 (1%)	5 (1%)
Study center, n (%)		
Baltimore, MD	28 (4%)	22 (4%)
Irvine, CA	126 (20%)	139 (22%)
New Haven, CT	21 (3%)	33 (5%)
Portland, OR	190 (30%)	183 (29%)
San Francisco, CA	21 (3%)	16 (3%)
Seattle, WA	239 (38%)	232 (37%)
Smoking status at baseline, n (%)		
Current smoker	463 (74%)	463 (74%)
Former smoker	162 (26%)	162 (26%)
Mean pack-years of smoking ± SD	53 ± 18	53 ± 18
Asbestos exposure, % with occupational exposure	15%	15%
Mean body mass index ± SD, kg/m^2	26.9 ± 4.6	27.1 ± 4.7
CARET intervention assignment, n (%)		
Active vitamins	345 (55%)	345 (55%)
Placebo	280 (45%)	280 (45%)
Histological subtype, %		
Adenocarcinoma	169 (27%)	–
Squamous Cell	138 (22%)	–
Other NSCLC	122 (19%)	–
Small Cell	98 (16%)	–
Missing[a]	98 (16%)	–

[a]No pathology report available, diagnosed by death certificate only (n = 43), clinically (n = 32), or by self-report (n = 23).

DNA Extraction

DNA was extracted from archived dried blood spots stored on Schleicher and Schuell No. 907 filter paper stored at −70°C. Samples were collected from 1994 to 1997 and DNA extraction was performed in 2002. Prior work has demonstrated sufficient yield from dried blood samples when stored at −20°C for more than 20 years; samples in this study were stored for at most 8 years.[29] Samples were processed as follows. In brief, one 1/16-inch hole punch (MC Mieth Manufacturing, Port Orange, FL) from the dried blood spot was collected for each case and control. To prevent cross-contamination, the punch was flame-sterilized between samples. Filter paper punches without blood spots served as negative controls. To have sufficient DNA for multiple reactions, each sample was polymerase chain reaction–amplified by using a multistrand displacement reaction resulting in whole genome amplification (Qiagen, Valencia, CA) and yielding 20 μL of 1-μg/μL DNA. The amplified DNA was diluted 300-fold with reagent-grade water and used in all genotyping reactions. DNA amplified with use of the multistrand displacement reaction has been found to have better than 99% concordance with traditionally extracted DNA in subsequent Taqman genotyping reactions.[30]

Genotyping Protocol

In this study, a candidate gene selection strategy was used to choose genes implicated in cancer or disease.[14,15] Common polymorphisms in coding or promoter regions with an expected minor allele frequency greater than 5% were chosen.

All polymorphisms were genotyped by using Taqman-based allelic discrimination assays. We utilized previously validated assays described in the National Cancer Institute's SNP500Cancer project.[31] This resource validates the assays' performance against sequence-based typing on more than 100 ethnically diverse individuals. The assays used in this study are described by the internal SNP500Cancer identification and dbSNP reference SNP identification number and listed in Table 2.[32]

Statistical Analysis

Primary End Points. Lung cancer risk was assessed by comparing the distribution of SNP genotype frequencies between cases (of all histological subtypes) and controls

Table 2. Single-Nucleotide Polymorphisms Studied

Gene	SNP500Cancer ID	dbSNP ID	SNP Region[a]	Alternate Nomenclature	HWE *p* Value of Cases	HWE *p* Value of Controls	Minor Allele Frequency in Controls
Interferon gamma (*IFNG*)	IFNG-10	rs2069705	-1615C>T		0.11	0.86	0.34
Interleukin 10 (*IL10*)	IL10-01	rs1800871	-853C>T	-819	0.02	0.12	0.23
	IL10-02	rs1800872	-626A>C	-592	0.03	0.13	0.23
	IL10-03	rs1800896	-1116A>G	-1082	0.04	0.95	0.49
Interleukin 1 alpha (*IL1A*)	IL1A-01	rs17561	Ex5+21G>T	A114S	0.06	0.79	0.29
	IL1A-02	rs1800587	Ex1+12C>T	-889, 5′UTR	0.13	0.82	0.29
	IL1A-04	rs2071374	IVS4-109A>C		0.22	0.48	0.28
Interleukin 1 beta (*IL1B*)	IL1B-01	rs16944	-1060T>C	-511	0.01	0.23	0.36
	IL1B-02	rs1143634	Ex5+14C>T	F105F, +3954	0.26	0.68	0.21
	IL1B-09	rs1143623	-2022C>G	-1464	0.02	0.14	0.30
Interleukin 2 (*IL2*)	IL2-01	rs2069762	IVS1-100G>T		0.94	0.44	0.29
	IL2-03	rs2069763	Ex2-34G>T	L38L	0.05	0.08	0.35
Interleukin 4 receptor (*IL4R*)	IL4R-02	rs1805011	Ex11+300A>C	E400A	0.28	0.69	0.12
	IL4R-06	rs1801275	Ex11+828A>G	Q576R	0.45	0.32	0.21
	IL4R-23	rs2107356	-28120T>C		0.28	0.57	0.41
Interleukin 4 (*1L4*)	IL4-01	rs2243250	-588C>T	-524	0.19	0.31	0.17
Interleukin 6 (*IL6*)	IL6-01	rs1800795	-236C>G	-174	0.60	0.38	0.44
Prostaglandin-endoperoxide synthase 2 (*PTGS2* [also known as *COX2*])	PTGS2-33	rs5275	Ex10+837C>T	3′UTR	0.76	0.70	0.33
Transforming growth factor, beta 1 (*TGFB1*)	TGFB1-01	rs1982073	Ex1-327C>T	L10P	0.62	0.58	0.40
	TGFB1-05[b]	rs1800469	308 bp 3′ of STP C>T	-509C>T	0.29	0.70	0.32
Tumor necrosis factor (*TNF*)	TNF-02	rs1800629	-487A>G	-308	0.23	0.55	0.19
	TNF-04	rs361525	-417A>G	-238	0.55	0.70	0.05
	TNF-07	rs1799724	-1036C>T	-857	0.93	0.26	0.09

[a]The nomenclature is described in den Dunnen JT, Antonarakis SE.[32]
[b]TGFB1-05 has been reclassified as MGC4093-03.
HWE, Hardy-Weinberg equilibrium.

using a likelihood ratio test in a 2 × 3 cross-classification table with a *p* value threshold of 0.05 as a cutoff for statistical significance. In situations in which a genotype from either the case or control was not available on account of technical limitations such as amplification failure, both members of the case-control pair were dropped from the analysis. Haplotype analysis was performed by using PHASE version 2.1 with a permutation analysis to test for significant differences in haplotype frequencies between cases and controls.[33]

Stratified Analyses. To investigate the possibility that the effect of a genetic polymorphism may be biologically active only in a specific histological subgroup, exploratory analyses examining the effect of genotype within the histological subtypes based on pathology reports were conducted. The controls were each matched to a case in 1:1 fashion.

On the basis of prior multivariate analyses of the CARET data delineating the demographic variables predictive of lung cancer risk,[34] further exploratory subgroup analyses were performed within the following subpopulations: experimental arm (vitamin supplementation versus placebo), age older or younger than the median of 60 years, smoking status (current versus former), pack-years more or less than the median of 48, and sex. These analyses were restricted to case-control pairs as well. Within the analysis of pack-years and age, case-control pairs were dropped if one subject was below the median value and the other was above the median. Pack-years and age were the only variables for which this occurred; the matching was exact for the other variables (intervention arm, smoking status, and sex).

HWE. For all assays, genotype distributions in both cases and controls were assessed for Hardy-Weinberg equilibrium (HWE) by using the chi-square method.

Estimation of Power. Modeling of our data with assumptions of HWE with 80% power by using a 0.05-level test for heterogeneity in the 2 × 3 contingency allowed

detection of ORs for increased risk as low as 1.3 for a minor allele frequency of 0.5 and as low as 1.5 for a minor allele frequency of 0.1.

Survival Analysis. Patients in whom lung cancer had been diagnosed before the collection of the dried blood spots were censored to avoid bias, leaving 580 of the 625 original cases. The Kaplan-Meier method was used to construct survival curves, and Cox regression was performed to estimate hazard ratios (HRs). Differences in survival among subgroups of different genotypes were analyzed by using a log-rank test. All statistical tests were two sided. All analyses were implemented with SAS software (SAS Institute, Cary, NC).

Results

Genotyping

A genotype was assigned for 99.0% of all samples tested with a range of 97.4% to 99.7% among the SNPs tested. To estimate the rate of genotyping errors, we repeated genotyping of the *IL-1β-511* gene polymorphism for all samples and found 99.9% concordance with the original results.

Population Genetics

HWE was tested in the control group for each SNP genotyped and all p values were higher than 0.05. The minor allele frequencies observed in the control group in this predominantly (98%) white population are reported in Table 2.

The ORs for cancer risk by genotype are presented in Table 3. An association between the *IL1B* promoter polymorphisms rs16944 (also described as IL1B_01 or -511C>T) and rs1143623 (IL1B_09 or -1464 C>G) and lung cancer risk was found. The size of this effect was modest, with presence of the minor allele variants of *IL-1β-511*C>T (rs16944) (CT and TT) having decreased odds of lung cancer, compared with individuals carrying the wild-type (CC) variant (OR = 0.74 [95% confidence interval (CI): 0.58–0.94] and OR = 0.71 [95% CI: 0.50–1.01]; test of heterogeneity, $p = 0.03$). Similar results were observed for the *IL-1β-1464* C>G (rs1143623) promoter polymorphism, with presence of the minor variants CG and CC having decreased odds of lung cancer (OR = 0.75 [95% CI: 0.59–0.95] and OR = 0.69 [95% CI: 0.46–1.03]; test of heterogeneity, $p = 0.03$). Results were reanalyzed by intervention arm with no statistically significant difference noted between the intervention and the placebo groups (data not shown). Haplotypes inferred by the PHASE program are displayed for cases and controls in Table 4. A statistically significant ($p = 0.02$) difference between the haplotype distributions between cases and controls was observed for *IL-1β*. No other statistically significant association between genotype and lung cancer risk was found for other haplotypes or individual SNPs. The inflammatory gene polymorphisms studied showed no significant effect on survival.

Stratified Analyses

Exploratory subgroup analyses were conducted among case-control pairs. We analyzed subsets of the cases and compared them with their matched controls to determine whether there were any effects that were present only in a subgroup and whose effect might be diminished in the overall sample. We stratified by histological subtype (SCLC, NSCLC, SCC, or AC), experimental arm (active versus placebo), age (<60 versus >60), smoking status (current versus former), pack-years (<48 versus >48), and sex. However, given the large number of comparisons (23 polymorphisms × 14 subgroups) we chose $p = 0.01$ for a significance threshold and found no statistically significant associations within subgroups. Within the *IL1B* promoter polymorphisms, we found evidence for an effect across all subgroups (data not shown), with the strongest associations observed in the male current smokers with a smoking history of more than 48 pack-years in the SCC and SCLC subgroups. Differences between subgroups did not reach statistical significance in tests for interaction. To investigate the possibility that the effect was dependent on tobacco exposure, the subjects were divided into quartiles. A trend across pack-years was not observed. We also repeated all of the analyses with a data set that excluded the 98 cases without pathologic confirmation, and this did not alter our findings.

Discussion

The current study tested the hypothesis that variation in the genes that participate in the inflammatory response may increase or decrease risk of lung cancer and may predict survival among patients with lung cancer. With use of a nested case-control study design, this analysis further supports an association of *IL1B* promoter polymorphisms and lung cancer risk. These results suggest that susceptibility to lung cancer may in part be defined by the individual's genetic background of this proinflammatory cytokine. Additional proinflammatory genes tested in this study were not found to be associated with lung cancer risk.

Our cohort was defined prospectively from a large population at risk for lung cancer. We were able to achieve a very high degree of matching between cases and controls for all of the demographic variables that have previously been shown to be of importance in this cohort.[34] As such, our analysis is less prone to bias than

Table 3. Genotypes in Lung Cancer Cases and Controls

Gene Polymorphism	Genotype	Cases, n (%)	Controls, n (%)	OR (95% CI)
IFNG_10	TT	268 (43.3)	274 (44.3)	1
	TC	265 (42.8)	274 (44.3)	0.99 (0.78-1.26)
	CC	86 (13.9)	71 (11.5)	1.24 (0.87-1.77)
				$p = 0.44$
IL10_01	CC	382 (61.9)	371 (60.1)	1
	CT	195 (31.6)	206 (33.4)	0.92 (0.72-1.17)
	TT	40 (6.5)	40 (6.5)	0.97 (0.61-1.54)
				$p = 0.79$
IL10_02	CC	382 (61.6)	375 (60.5)	1
	CA	197 (31.8)	206 (33.2)	0.94 (0.74-1.20)
	AA	41 (6.6)	39 (6.3)	1.03 (0.65-1.64)
				$p = 0.85$
IL10_03	AA	160 (26.9)	158 (26.6)	1
	AG	273 (45.9)	297 (49.9)	0.91 (0.69-1.19)
	GG	162 (27.2)	140 (23.5)	1.14 (0.83-1.57)
				$p = 0.27$
IL1A_01	GG	303 (49.2)	316 (51.3)	1
	GT	272 (44.2)	249 (40.4)	1.14 (0.90-1.44)
	TT	41 (6.7)	51 (8.3)	0.84 (0.54-1.30)
				$p = 0.30$
IL1A_02	CC	299 (48.9)	312 (51.0)	1
	CT	269 (44.0)	249 (40.7)	1.13 (0.89-1.42)
	TT	44 (7.2)	51 (8.3)	0.90 (0.58-1.39)
				$p = 0.46$
IL1A_04	AA	319 (51.5)	322 (52.0)	1
	AC	242 (39.1)	253 (40.9)	0.97 (0.76-1.22)
	CC	58 (9.4)	44 (7.1)	1.33 (0.87-2.03)
				$p = 0.34$
IL1B_01	CC	303 (49.7)	256 (42.0)	1
	CT	234 (38.4)	267 (43.8)	0.74 (0.58-0.94)
	TT	73 (12.0)	87 (14.3)	0.71 (0.50-1.01)
				$p = 0.03$
IL1B_02	CC	361 (57.9)	383 (61.5)	1
	CT	233 (37.4)	213 (34.2)	1.16 (0.92-1.47)
	TT	29 (4.7)	27 (4.3)	1.14 (0.66-1.96)
				$p = 0.45$
IL1B_09	GG	360 (58.3)	313 (50.6)	1
	GC	209 (33.8)	243 (39.3)	0.75 (0.59-0.95)
	CC	49 (7.9)	62 (10.0)	0.69 (0.46-1.03)
				$p = 0.03$
IL2_01	TT	312 (50.2)	306 (49.3)	1
	TG	257 (41.4)	266 (42.8)	0.95 (0.75-1.20)
	GG	52 (8.4)	49 (7.9)	1.04 (0.68-1.59)
				$p = 0.86$
IL2_03	GG	256 (42.2)	246 (40.5)	1
	GT	292 (48.1)	296 (48.8)	0.95 (0.75-1.20)
	TT	59 (9.7)	65 (10.7)	0.87 (0.59-1.29)
				$p = 0.77$
IL4R_02	AA	450 (75.6)	459 (77.1)	1
	AC	138 (23.2)	126 (21.2)	1.12 (0.85-1.47)
	CC	7 (1.2)	10 (1.7)	0.71 (0.27-1.89)
				$p = 0.56$

(continued)

Table 3. Continued

Gene Polymorphism	Genotype	Cases, n (%)	Controls, n (%)	OR (95% CI)
IL4R_06	AA	376 (61.3)	387 (63.1)	1
	AG	212 (34.6)	195 (31.8)	1.12 (0.88-1.42)
	GG	25 (4.1)	31 (5.1)	0.83 (0.48-1.43)
				$p = 0.47$
IL4R_23	CC	211 (34.4)	219 (35.7)	1
	CT	307 (50.0)	290 (47.2)	1.10 (0.86-1.41)
	TT	96 (15.6)	105 (17.1)	0.95 (0.68-1.33)
				$p = 0.60$
IL4_01	CC	454 (73.7)	433 (70.3)	1
	CT	145 (23.5)	163 (26.5)	0.85 (0.65-1.10)
	TT	17 (2.8)	20 (3.2)	0.81 (0.42-1.57)
				$p = 0.41$
IL6_01	GG	190 (31.1)	194 (31.8)	1
	GC	295 (48.3)	292 (47.8)	1.03 (0.80-1.33)
	CC	126 (20.6)	125 (20.5)	1.03 (0.75-1.41)
				$p = 0.97$
PTGS2_33	TT	274 (44.7)	271 (44.2)	1
	CT	273 (44.5)	276 (45.0)	0.98 (0.77-1.24)
	CC	66 (10.8)	66 (10.8)	0.99 (0.68-1.45)
				$p = 0.98$
TGFB1_01	TT	238 (39.1)	224 (36.8)	1
	CT	281 (46.2)	286 (47.0)	0.92 (0.72-1.18)
	CC	89 (14.6)	98 (16.1)	0.85 (0.61-1.20)
				$p = 0.64$
TGFB1_05	CC	289 (47.8)	282 (46.6)	1
	CT	267 (44.1)	259 (42.8)	1.01 (0.79-1.27)
	TT	49 (8.1)	64 (10.6)	0.75 (0.50-1.12)
				$p = 0.33$
TNF_02	GG	409 (66.3)	404 (65.5)	1
	GA	181 (29.3)	193 (31.3)	0.93 (0.73-1.18)
	AA	27 (4.4)	20 (3.2)	1.33 (0.74-2.42)
				$p = 0.48$
TNF_04	GG	556 (89.5)	561 (90.3)	1
	GA	64 (10.3)	58 (9.3)	1.11 (0.77-1.62)
	AA	1 (0.2)	2 (0.3)	0.50 (0.05-5.58)
				$p = 0.72$
TNF_07	CC	501 (81.1)	516 (83.5)	1
	CT	111 (18.0)	95 (15.4)	1.20 (0.89-1.62)
	TT	6 (1.0)	7 (1.1)	0.88 (0.29-2.65)
				$p = 0.46$

CI, confidence interval.

traditional case-control studies. Many of the cohorts in the literature are hospital-based studies and are thus enriched for disease that is amenable to surgical treatment. This is less representative of the natural history of lung cancer than the present study, in which all incident cases were captured in a prospective cohort. Tobacco and asbestos exposures were well characterized in our study population before development of lung cancer in any of the subjects. Follow-up procedures were thorough. Although not as large as the multinational study of Campa et al.,[35] our study was adequately powered to detect effects that are likely to be of clinical significance, with ORs as low as 1.3 to 1.5. It should be noted that this study did not have numbers of cases adequate to perform an independent replication study, which inherently limits the generalizability of these results.

As with all gene association studies, multiple comparisons and subgroup analyses can lead to spurious associations. Our study population was predominantly of white origin. Different genotype frequencies between

Table 4. Haplotype Analysis

Haplotype	f (Controls)	f (Cases)	*p* Value
IL1A (-01/-04/-02)			$p = 0.43$
TAT	0.284	0.284	
GAC	0.436	0.419	
GCC	0.276	0.290	
IL1B (-02/-01/-09)			$p = 0.02$
CCG	0.455	0.484	
CTG	0.065	0.064	
CTC	0.266	0.216	
TCG	0.185	0.203	
TTC	0.029	0.030	
IL2 (-03/-01)			$p = 0.94$
GT	0.358	0.372	
GG	0.293	0.289	
TT	0.348	0.338	
IL4R (-02/-06)			$p = 1$
AA	0.792	0.785	
AG	0.087	0.089	
CG	0.120	0.126	
IL10 (-02/-01/-03)			$p = 0.67$
CCG	0.485	0.503	
CCA	0.283	0.272	
ATA	0.230	0.223	
TGFB1-01/MGC4093-03			$p = 0.55$
CT	0.321	0.300	
CC	0.077	0.080	
TC	0.600	0.619	
TNF (-07/-02/-04)			$p = 0.67$
CGG	0.675	0.658	
CGA	0.048	0.052	
CAG	0.189	0.189	
TGG	0.086	0.099	

f, frequency.

ethnic groups may partially explain the lack of association of inflammatory gene polymorphisms with lung cancer that have previously been described in Asian populations.[36–39] This is similar to the variable results demonstrated in the gastric cancer literature.[20] It should also be noted that population stratification within our predominantly white cohort may also influence our results and that further validation studies are needed.

The *IL1B* gene has been of interest because of its role as a proinflammatory cytokine produced by macrophages, monocytes, and lung epithelia. IL-1 has been shown to be up-regulated in lung cancer. Higher IL-1 concentrations within tumors have been associated with more virulent tumors and poor prognosis.[40] Furthermore, VNTR polymorphisms in the *IL1BRN* gene, which acts as a competitive ligand with IL-1, have been associated with lung cancer risk.[26] *IL1B* promoter polymorphisms have been shown to have an important role in the pathogenesis of gastric and other cancers. The *IL-1β*-511 and -31 polymorphisms lie in the promoter region and have been found to be in strong linkage disequilibrium.[17,25] The -31 polymorphic position lies within a TATA-box element, with the variant C allele causing disruption of binding to the TATA-box.[17] The variant -31C allele has been shown to be associated with a decreased promotion of *IL-1β* in in vitro studies of human lung epithelial cells.[41] This is thought to be secondary to the specific binding of a transcription factor (Yin Yang 1) to the -31C allele, decreasing transcription of *IL1B*.[42] This study did not test for the -31C>T SNP; however, in several studies *IL-1β-31* and *IL-1β-511* are perfectly concordant.[17] As has been previously demonstrated, this study found decreased odds of lung cancer in patients with either the homozygous or heterozygous -511T allele or -1464C allele.[22,23,25] In the report by Zienolddiny et al., the less frequent *IL-1β-31*CC/*IL-1β-511TT* genotypes were associated with a decreased risk of lung cancer (OR = 0.40 for *IL-1β-511* minor allele homozygotes and OR = 0.52 for heterozygotes) and a lower incidence of mutated p53 compared with the common allele.[25]

Interestingly, the study by Zienolddiny et al. of the *IL1* promoter polymorphism in NSCLC was expanded to a larger European cohort with 2135 cases and 2115 controls and no evidence of association between *IL1B* -31T>C and lung cancer risk was found.[34] Similarly, a study by Lind et al. demonstrated an increased risk of NSCLC for individuals with the haplotype homozygous *IL-1RN**1(an allele of the *IL1* VNTR with four repeats) and *IL-1β-31*T, but it did not show an increased risk in individuals with -31T who were not homozygous for *IL-1RN*1*.[26] It is possible that these discrepant results are secondary to varied genetic backgrounds of the populations being studied. Our study, which was conducted in a predominantly white population, did not show an association between alleles of *IL1B* rs1143634 and lung cancer, although prior studies in a Japanese population demonstrated an increased risk in individuals who were either homozygous or heterozygous for the T allele.[39]

This study provides further evidence for an *IL1B* risk haplotype. Although it examined three specific SNPs, rs1143634A>C, rs16944 (-511T>C), and rs1143623 (-1646C>G), this study demonstrates a significantly increased rate of the haplotype CTC among controls, and conversely an increased rate of the haplotype TCG among lung cancer cases ($p = 0.02$). This further supports findings in studies by Landvik et al. and Li et al. which demonstrated similar trends in -511 and -1464 containing haplotypes.[22,23] Rogus et al. demonstrate in vivo that subjects with haplotypes including homozygous -511C and -1464G alleles have 28% to 52% higher IL-1β levels.[43] It is possible, therefore, that one explanation for the decreased rate of lung cancer in individuals containing -511T and -1464C haplotypes is a decreased level of IL-1β production.

The fact that the same *IL-1* promoter polymorphism may be protective in lung cancer and cancer promoting

in gastric cancer may be explained by interactions with *Helicobacter pylori* and the effect of IL-1 on pH in gastric cancer. The direction of the effect noted in our study is consistent with that in the original report of Zienolddiny et al., although the magnitude is less.

The current study found a neutral effect of the T>C polymorphism in the 3′-UTR of cyclooxygenase 2 (*COX2*) (rs5275). Also known as prostaglandin-endoperoxide synthase 2, *COX2* is a key enzyme in the inflammatory response. Inhibitors of this enzyme have received significant attention as chemopreventive and anticancer agents.[44] *COX2* is overexpressed in NSCLC and is thought to be an adverse prognostic factor.[45] There are several reports in the literature regarding the role of the T>C polymorphism in the 3′-UTR of *COX2* (rs5275) that ascribe the allele as either a neutral,[35] protective,[38] or predisposing[46] risk factor for lung cancer. Our data are consistent with this allele having no effect on lung cancer risk.

This study did not find any correlation between genotype and lung cancer risk for the *TNFA*, *TGFB1*, *IL10*, and *IL6*, genes previously associated with risk in the literature.[36,47] There are many possible reasons for the failure to replicate these prior findings. The initial reports may arise from chance, as many of the findings were present only in retrospectively defined subsets of the subjects studied and multiple hypotheses were investigated. Another possibility is that varied ethnic background of study populations may result in a different genetic background that confers a different biologic significance to a given functional polymorphism. Furthermore, the specific SNPs genotyped vary between studies. For example, recent work has shown a possible association between the -634 C>G (rs1800796) SNP of the *IL6* gene, which was not tested in our study.[48,49] In our study of predominantly white individuals, the size of our study population and the minor allele frequencies for these four genes should have been sufficiently large to detect ORs as low as 1.3 to 1.5; thus, the absence of findings makes it highly unlikely that a gene with a large OR is present among the inflammatory gene polymorphisms studied.

This study provides further evidence of a relationship between the *IL1B* promoter polymorphisms and lung cancer risk. The central role of the IL-1 pathway in the initiation of the inflammatory response and the association of cancer risk with polymorphisms in this pathway justify further research into this area. Recombinant IL-1 receptor antagonists are already in clinical use for rheumatoid arthritis. Recently, the CANTOS trial demonstrated decreased incidence of and mortality due to lung cancer in patients treated with canakinumab, a humanized monoclonal antibody that inhibits IL-1β.[50] As further studies examine the role of this pathway in cancer promotion, translational research clarifying the role of this pathway in lung cancer could proceed rapidly.

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