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Genetic variation in innate immunity and inflammation pathways associated with lung cancer risk

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Abstract

Background—Pulmonary inflammation may contribute to lung cancer etiology. We conducted a broad evaluation of the association of single nucleotide polymorphisms (SNPs) in innate immunity and inflammation pathways with lung cancer risk, and conducted comparisons with a lung cancer genome wide association study (GWAS).

Methods—We included 378 lung cancer cases and 450 controls from the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial. An Illumina GoldenGate oligonucleotide pool assay was used to genotype 1,429 SNPs. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated for each SNP, and p-values for trend were calculated. For statistically significant SNPs (p-trend<0.05), we replicated our results with genotyped or imputed SNPs in the GWAS, and adjusted p-values for multiple testing.

Results—In our PLCO analysis, we observed a significant association between 81 SNPs located in 44 genes and lung cancer (p-trend<0.05). Of these 81 SNPS, there was evidence for confirmation in the GWAS for 10 SNPs. However, after adjusting for multiple comparisons, the only SNP that remained significantly associated with lung cancer in the replication phase was rs4648127 (NFKB1; multiple testing adjusted p-trend=0.02). The CT/TT genotype of NFKB1 was associated with reduced odds of lung cancer in the PLCO study (OR=0.56; 95% CI 0.37–0.86) and the GWAS (OR=0.79; 95% CI 0.69–0.90).

Conclusions—We found a significant association between a variant in the *NFKB1* gene and lung cancer risk. Our findings add to evidence implicating inflammation and immunity in lung cancer etiology.

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Keywords

lung cancer; genetics; inflammation; immunity; epidemiology

Introduction

With 1.38 million deaths annually, lung cancer causes the largest number of cancer-related deaths worldwide (1). Cigarette smoking is the primary cause of lung cancer, increasing risk by 15 to 30-fold (2). In addition, pulmonary inflammation may contribute to lung cancer etiology through the production of reactive oxygen and nitrogen species, the proliferation of cells and increase in angiogenesis during tissue repair, and the up-regulation of anti-apoptotic genes through the nuclear factor kappa B (NF-κB) pathway (3). Understanding the role of inflammation in lung cancer etiology may inform chemoprevention efforts and help identify high-risk individuals for screening.

Different strategies have been used to study the association between inflammation and lung cancer. Previous studies have shown associations of lung cancer risk with inflammatory lung conditions, such as chronic obstructive pulmonary disease, pulmonary tuberculosis and *Chlamydia* pneumonia (4–6). Further, circulating levels of C-reactive protein (CRP), interleukin (IL)-6 and IL-8, and polymorphisms in immunity and inflammation-related genes (e.g., *IL1B, IL1A, FCER2, IL-10, TNF-α, IL8RA, ICAM1* and *IL12A)* have also been associated with lung cancer risk (7–12). However, these genetic studies have been small, associations have not been replicated, and few studies have comprehensively evaluated polymorphisms in innate immunity and inflammation genes.

Information on genome-wide variation and lung cancer is available from genome-wide association studies (GWAS) (13–16). None of the SNPs found to be significantly associated with lung cancer were those previously identified in smaller genetic studies of inflammation-related genes. However, due to the stringent p-values required for statistical significance in GWAS to prevent false positive results, it is possible that moderate associations between polymorphisms in inflammation-related genes and lung cancer were missed.

We conducted a comprehensive evaluation of the association of 1,429 SNPs across 211 genes in innate immunity and inflammation pathways with lung cancer risk in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial. We independently confirmed our observations by conducting comparisons with the National Cancer Institute’s (NCI’s) lung cancer GWAS (13).

Methods

Study Population

Study participants were enrolled in the PLCO Cancer Screening Trial, which included approximately 155,000 people, aged 50–74 years (17). During 1992–2001 participants were randomized to receive cancer screening or routine health care. Our case-control study was nested within the screening arm of PLCO (N=77,464). Lung cancer screening included a baseline chest x-ray and two (never smokers) or three (current/former smokers) annual chest x-rays. Additionally, at baseline and annually for six years, blood samples were obtained and questionnaires were administered to collect demographic, behavioral and dietary information.

Lung cancers were ascertained through self-report and confirmed by medical chart and death certificate review. Of the 898 lung cancers that occurred between baseline and December 31, 2004, 378 were included in our analysis. Cases were excluded if they were missing the baseline questionnaire (n=17) or information on smoking behaviors (n=11), had a history of cancer (n=56), had multiple cancers during follow-up (n=88), did not consent to the use of their biological specimens for genetic analyses (n=217), or did not have available DNA (n=131). Controls were matched to cases based on the following criteria: age at randomization (55–59, 60–64, 65–69, 70–74 years), sex, year of randomization (1993–1995, 1996–1997, 1998–1999, 2000–2001), length of study follow-up (1-year intervals), and smoking behavior (status [current, former, never], pack-years smoked at baseline [0–29, 30–39, 40–49, 50+], and time since quitting [<15 and 15+ years]). Controls were matched to cases 1:1 for current and former smokers and 3:1 for never smokers.

Laboratory Methods

DNA was extracted from buffy coats collected at baseline using a Qiagen kit (Qiagen Inc., Valencia, CA). Genotyping for 1,536 SNPs across 148 gene regions was performed using an Illumina GoldenGate oligonucleotide pool assay (OPA, Illumina, San Diego, CA). Genes were selected onto the OPA platform based on their function in innate immunity and inflammation pathways (oxidative response, pattern recognition molecules and antimicrobials, integrins and adhesion molecules, complement, chemokines with their receptors and signaling molecules, and response genes and tissue factors). SNPs in these gene regions (defined as 20 kb upsteam to 10 kb past the polyA tail signal) from the International HapMap project were included using the Tagzilla algorithm (18). The following criteria were used for SNP inclusion: minor allele frequencies of >5% in the HapMap Caucasian samples, r^2 values of >0.8, and a greater weight for SNPs with a design score of 1.1. Additionally, <5% of SNPs were included based on prior evidence from association studies. High-sensitivity CRP was measured using a chemiluminescent immunoassay (Diagnostic Products Corporation, Los Angeles, CA).

Of the 1,536 SNPs genotyped, 99 were excluded due to assay failure and 8 were excluded due to deviations from Hardy-Weinberg equilibrium among controls (p-value<0.001). The assay completion rate for the included SNPs ranged from 91.2–94.9%, and the concordance among 23 quality control samples was 87.0–95.7% for 29 SNPs and 100% for the remaining 1,400 SNPs.

Statistical Analysis

Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated for lung cancer, comparing heterozygous and homozygous variant genotypes to homozygous wild type genotypes for each of 1,429 SNPs. P-values for trend were calculated, treating the number of alleles as continuous variables. We carried out stratified analyses by lung cancer histology, smoking status and family history, and evaluated interactions between SNPs and smoking status, family history and CRP levels. We conducted analyses adjusted for age, race, sex and smoking behavior (status, pack-years and time since quit) utilizing unconditional logistic regression to retain the maximum number of cases and controls. As adjustment did not alter the associations between SNPs and lung cancer, all results presented are unadjusted. In a sensitivity analysis restricted to white participants, the results also did not change. Therefore, we have presented results for all participants.

We identified SNPs that were significantly (p-trend<0.05) associated with lung cancer in our case-control study and then replicated our results in an independent sample, the NCI's lung cancer GWAS (13). The NCI GWAS included data on 5,739 lung cancer cases and 5,848 controls from the PLCO, the Environment and Genetics in Lung Cancer Etiology Study, the

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Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study, and the Cancer Prevention Study II Nutrition Cohort (13). We estimated ORs with logistic regression in the NCI GWAS for 1) the same SNP estimated in the case-control study, if genotyped in the GWAS; or 2) an imputed value for the SNP estimated in the case-control study, if not genotyped in the GWAS. Imputed values were estimated from genotyped SNPs in the same gene region (20 kb upsteam to 10 kb past the polyA tail signal) as the SNP of interest using the IMPUTE statistical program (19) and HapMap release #24 as the reference panel. We excluded PLCO participants from the GWAS analyses who were included in our case-control study (n=355).

We performed permutations to adjust for multiple testing of 81 SNPs in the replication stage. Briefly, we permuted the case-control status in the PLCO data 1000 times, as genotype data were not available from the GWAS. For each permutation, we computed the one-sided p-value for each SNP with direction specified by the PLCO results, and then computed the minimum p-value. Multiple testing adjusted p-values were calculated as the proportion of permutations (n=1000) where the minimum p-value was below the p-value in the GWAS data. When the direction of the association differed in the PLCO and GWAS datasets, the multiple testing adjusted p-value was 1.0. The multiple testing adjusted p-values calculated through permutation were very similar to those estimated with a Bonferroni correction.

Results

Baseline characteristics of cases (n=378) and controls (n=450) from the PLCO study are presented in Table 1. No significant differences were observed for the matching characteristics, with the exception of smoking status, as the study design oversampled never smoking controls. Cases were significantly more likely than controls to have a history of bronchitis/emphysema at baseline (p=0.007) and a family history of lung cancer (p=0.01). The most common lung cancer histologic types included adenocarcinoma (n=170), small cell carcinoma (n=88), squamous cell carcinoma (n=41) and large cell carcinoma (n=20).

Of the 1,429 SNPs included in our analysis, we observed significant associations between 81 SNPs located in 44 genes and lung cancer (p-trend<0.05). Of these 81 SNPS, there was evidence for confirmation in the GWAS data for 4 SNPs through direct replication, and 6 SNPs through imputation. Table 2 presents the ORs for the 10 confirmed SNPs in the PLCO and GWAS.

After adjusting for multiple comparisons, only NFKB1 (rs4648127) remained significantly associated with lung cancer in the replication phase (multiple testing adjusted p-value=0.02). The CT/TT genotype of NFKB1 (rs4648127) was associated with a 44% reduction in the odds (OR=0.56; 95% CI 0.37–0.86) of lung cancer in the PLCO case-control study and a 21% reduction in the odds (OR=0.79; 95% CI 0.69–0.90) of lung cancer when imputed in the GWAS study. None of the 13 additional *NFKB1* SNPs genotyped in the PLCO case-control study were significantly associated with lung cancer risk (all p-trend>0.05). Of note, rs4648127 was not correlated with the twelve other *NFKB1* SNPs genotyped in PLCO and available in HapMap (r^2 range 0–0.49).

We carried out additional analyses in the PLCO case-control study. Among controls, NFKB1 (rs4648127) was not associated with family history of lung cancer, bronchitis/emphysema or CRP (all p>0.3). Additionally, we found no significant interaction between NFKB1 (rs4648127) and smoking status (p-interaction=0.19), CRP level (p-interaction=0.97), or family history (p-interaction=0.78). Across lung cancer histologies, we found the association between NFKB1 (rs4648127) and lung cancer to be limited to

adenocarcinoma, though we had limited power to detect associations with other histology groups.

Discussion

A SNP in the *NFKB1* gene (rs4648127) was associated with lung cancer in the screening arm of the PLCO Cancer Screening Trial, with a 44% reduction in lung cancer risk with at least one T allele, compared to the CC genotype. Additionally, lung cancer risk was reduced by 21% when the same SNP was imputed with data from the NCI GWAS. Our observation of a significant association between the *NFKB1* gene and lung cancer risk underscores the etiologic role of inflammation and immunity in lung carcinogenesis

NF-κB, a transcription factor that is primarily activated by pro-inflammatory cytokines, plays an important role in development, immunity, tissue homeostasis, and inflammation, and regulates gene expression, cell apoptosis, and proliferation (20). Though variation in the *NFKB1* gene has not been previously associated with lung cancer risk, a polymorphism in the promoter region of *NFKB1* is associated with the severity of acute respiratory distress syndrome (21), suggesting biologic plausibility for *NFKB1*'s role in pulmonary diseases. SNPs in *NFKB1* have been associated with the risk of non-Hodgkin lymphoma, Hodgkin lymphoma, colon cancer, rectal cancer, meningioma and cervical cancer (18;22–25). Further, haplotypes including the same *NFKB1* SNP as our study (rs4648127) were significantly associated with rectal cancer risk (25). It is important to note that NF-κB can also be activated by inducers other than cytokines, including viral and bacterial products, DNA damage, and hypoxia (26). Thus, the association between *NFKB1*and lung cancer may also reflect associations between other factors and lung cancer risk.

It is important to place results from candidate gene studies in the context of GWAS results. The evaluation of 515,922 SNPs in the NCI GWAS necessitated the use of very stringent p-values to prevent false positive associations. Therefore, false negatives may have arisen for SNPs with more moderate associations and weaker p-values. Indeed, although 8 of 20 NFKB1 SNPs on the GWAS were significant at nominal levels ($p<0.05$, range=0.0004–0.7) (13), none of these met the GWAS p-value threshold. Additionally, there may have been poor coverage of gene regions associated with inflammation on the GWAS chip. Nonetheless, using a targeted approach focused on the immune and inflammation pathways, we found support for an association between *NFKB1* and lung cancer.

The main limitation of our study was the relatively small number of cases and controls, which limited our ability to detect weaker associations in a large number of SNPs or conduct pathway-based analyses in PLCO. However, we focused our analyses on genes with functions related to inflammation and innate immunity, which support emerging biologically plausible mechanisms in lung cancer development. Further, we confirmed our results with the NCI GWAS study. We believe that this method has a higher sensitivity than GWAS alone and can be used when there is a biologically-motivated hypothesis.

In conclusion, our study supports the role of genetic variation in innate immunity in the development of lung cancer. Future studies should further examine the *NFKB1* region to identify functional SNPs and corresponding protein levels that are associated with lung cancer risk. These findings add to the growing body of literature implicating inflammation and immunity in lung cancer etiology.

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Table 1

Characteristics of 378 lung cancer cases and 450 controls in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial

	Controls		Cases		p-value
	N	**%**	**N**	**%**	
Total	450		378		
Age at enrollment, years					
59	89	19.8	80	21.2	†
60–64	131	29.1	106	28.0	
65–69	147	32.7	128	33.9	
70–74	83	18.4	64	16.9	
Sex					
Female	180	40.0	131	34.7	†
Male	270	60.0	247	65.3	
Smoking status *					
Never smoker	99	22.0	30	7.9	†
Former smoker	217	48.2	217	57.4	
0–29 pack-years and quit for <15 years	21	4.7	23	6.1	
0–29 pack-years and quit for 15 years	49	10.7	44	11.6	
30–39 pack-years and quit for <15 years	32	7.1	30	7.9	
30–39 pack-years and quit for 15 years	16	3.6	17	4.5	
40–49 pack-years and quit for <15 years	15	3.3	14	3.7	
40–49 pack-years and quit for 15 years	8	1.8	11	2.9	
50+ pack-years and quit for <15 years	64	14.2	66	17.5	
50+ pack-years and quit for 15 years	12	2.7	12	3.2	
Current smoker	134	29.8	131	34.7	
0–29 pack-years	32	7.1	26	6.9	
30–39 pack-years	41	9.1	39	10.3	
40–49 pack-years	9	2.0	11	2.9	
50+ pack-years	52	11.6	55	14.6	
Race/ethnicity					
White	410	91.1	338	89.4	0.35

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	Controls		Cases		p-value
	N	%	N	%	
Black	20	4.4	27	7.1	
Hispanic	7	1.6	4	1.1	
Other	13	2.9	9	2.4	
Education					
High school graduate or less	157	34.9	148	39.2	0.21
More than high school graduate	293	65.1	230	60.8	
Body mass index at enrollment					
<18.5	8	1.8	7	1.9	0.59
18.5–24.9	142	31.6	121	32.0	
25.0–29.9	211	46.9	162	42.9	
30	89	19.8	88	23.3	
Regular use of aspirin or ibuprofen					
Yes	302	67.1	242	64.0	0.35
No	148	32.9	136	36.0	
History of bronchitis/emphysema					
Yes	47	10.4	62	16.4	0.007
No	394	87.6	299	79.1	
Missing	9	2.0	17	4.5	
History of heart disease					
Yes	55	12.2	62	16.4	0.06
No	386	85.8	300	79.4	
Missing	9	2.0	16	4.2	
Family history of lung cancer [††]					
Yes	48	10.7	66	17.5	0.01
No	387	86.0	288	76.2	
Missing	15	3.3	24	6.4	

[*] Proportions of cases and controls appear different due to differences in the number of controls per case for never smokers (3:1), former smokers (1:1) and current smokers (1:1).

[†] Matching variable.

[††] Family history of lung cancer in any first degree relative was self-reported.

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Table 2

Association of selected innate immunity and inflammation SNPs with lung cancer in the PLCO case-control study and NCI GWAS

	PLCO Study			Typed or imputed in GWAS	P-trend corrected for multiple comparisons**
	Controls	Cases	OR (95% CI)	OR (95% CI)	
NFKB1 (rs4648127)					
CC	379	342	1.0	1.0†	
CT	69	35	0.56 (0.36–0.87)	0.78 (0.68–0.90)	
TT	2	1	0.55 (0.05–6.14)	0.83 (0.55–1.25)	*0.023*‡
CT+TT			0.56 (0.37–0.86)	0.79 (0.69–0.90)	
*p-trend***			*0.0092*	*0.0006*	
C2 (rs497309)					
AA	374	293	1.0	1.0†	
AC	71	76	1.37 (0.96–1.95)	1.26 (1.09–1.46)	
CC	5	8	2.04 (0.66–6.31)	1.10 (0.86–1.41)	*0.18*
AC+CC			1.41 (1.00–1.99)	1.21 (1.08–1.37)	
*p-trend***			*0.040*	*0.0058*	
TLR3 (rs3775291)					
CC	229	172	1.0	1.0*	
CT	192	165	1.14 (0.86–1.52)	1.05 (0.97–1.13)	
TT	28	41	1.95 (1.16–3.28)	1.21 (1.06–1.38)	*0.32*
CT+TT			1.25 (0.95–1.64)	1.07 (1.00–1.16)	
*p-trend***			*0.024*	*0.010*	
ALOX5 (rs1487562)					
CC	321	237	1.0	1.0*	
CT	119	122	1.39 (1.03–1.88)	1.11 (1.03–1.21)	
TT	10	19	2.57 (1.18–5.64)	1.09 (0.91–1.30)	*0.44*
CT+TT			1.48 (1.11–1.98)	1.11 (1.03–1.20)	

	PLCO Study			Typed or imputed in GWAS	P-trend corrected for multiple comparisons**
	Controls	Cases	OR (95% CI)	OR (95% CI)	
*p-trend***			*0.0030*	*0.02*	
C2 (rs2734335)					
AA	149	96	1.0	1.0*	
AG	192	180	1.46 (1.05–2.02)	1.02 (0.93–1.11)	
GG	108	102	1.47 (1.01–2.13)	1.14 (1.03–1.27)	*0.46*
AG+GG			1.46 (1.08–1.98)	1.06 (0.97–1.14)	
*p-trend***			*0.038*	*0.017*	
FCER1G (rs4489574)					
CC	229	169	1.0	1.0*	
CT	169	152	1.22 (0.91–1.64)	1.14 (1.05–1.23)	
TT	51	57	1.51 (0.99–2.32)	1.05 (0.92–1.19)	*0.70*
CT+TT			1.29 (0.98–1.69)	1.12 (1.04–1.21)	
*p-trend***			*0.041*	*0.032*	
TLR4 (rs10759932)					
TT	341	265	1.0	1.0†	
CT	98	100	1.31 (0.95–1.81)	1.10 (1.01–1.21)	
CC	9	12	1.72 (0.71–4.13)	1.08 (0.88–1.34)	*0.75*
CT+CC			1.35 (0.99–1.84)	1.10 (1.01–1.20)	
*p-trend***			*0.049*	*0.039*	
TLR10 (rs7660429)					
CC	329	299	1.0	1.0†	
CG	110	73	0.73 (0.52–1.02)	1.09 (0.99–1.19)	
GG	11	6	0.60 (0.22–1.64)	1.14 (0.92–1.42)	*1.0*
CG+GG			0.72 (0.52–0.99)	1.10 (1.00–1.20)	
*p-trend***			*0.044*	*0.036*	

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	PLCO Study			Typed or imputed in GWAS	P-trend corrected for multiple comparisons**
	Controls	Cases	OR (95% CI)	OR (95% CI)	
ACAD11 (rs11927882)					
GG	273	201	1.0	1.0†	
AG	142	136	1.30 (0.97–1.75)	0.85 (0.72–0.99)	
AA	35	40	1.55 (0.95–2.53)	0.71 (0.42–1.16)	*1.0*
AG+AA			1.35 (1.02–1.78)	0.83 (0.72–0.97)	
*p-trend***			*0.027*	*0.013*	
CCR6 (rs9459883)					
GG	378	293	1.0	1.0†	
CG	70	73	1.35 (0.94–1.93)	0.90 (0.80–1.02)	
CC	2	12	7.74 (1.72–34.8)	0.72 (0.42–1.22)	*1.0*
CG+CC			1.52 (1.07–2.16)	0.89 (0.79–1.00)	
*p-trend***			*0.003*	*0.045*	

*Typed in GWAS;

†Imputed in GWAS;

‡Statistically significant after adjustment for multiple comparisons;

**P-trends in the PLCO study and the GWAS were two-sided and the p-trend corrected for multiple comparisons was one-sided.