POLYMORPHISMS IN INFLAMMATION-RELATED GENES AND RISK OF SMOKING-ASSOCIATED LUNG CANCER AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE

by

Yan Du

BS, Shanghai University of Traditional Chinese Medicine, China, 2005

MPH, Tulane University, 2006

MS, University of Pittsburgh, 2010

Submitted to the Graduate Faculty of

Graduate School of Public Health in partial fulfillment

of the requirements for the degree of

Doctor of Public Health

University of Pittsburgh

2011

UNIVERSITY OF PITTSBURGH

Graduate School of Public Health

This dissertation was presented

by

Yan Du

It was defended on

March 29, 2011

And approved by

Dissertation Advisor:
Joel L. Weissfeld, MD, MPH
Associate Professor of Epidemiology
Assistant Professor of Medicine
Graduate School of Public Health
School of Medicine
University of Pittsburgh

Brenda Diergaarde, PhD
Assistant Professor of Epidemiology
Graduate School of Public Health
University of Pittsburgh

Eleanor Feingold, PhD
Professor of Human Genetics and Biostatistics
Associate Dean for Education
Graduate School of Public Health
University of Pittsburgh

Marjorie Romkes, PhD
Associate Professor of Medicine
Department of Medicine
University of Pittsburgh

Frank C. Sciurba, MD, FCCP
Associate Professor of Medicine and Education
Department of Medicine
University of Pittsburgh

Joel L. Weissfeld, MD, MPH

POLYMORPHISMS IN INFLAMMATION-RELATED GENES AND RISK OF SMOKING-ASSOCIATED LUNG CANCER AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Yan Du, DrPH
University of Pittsburgh, 2011

Lung cancer and chronic obstructive pulmonary disease (COPD) are the leading causes of morbidity and mortality in the US. Despite the appreciation of the central role of smoking in the development of both diseases, only a relatively small number of smokers (15%-20%) develops lung cancer and/or COPD. This suggests that other factors including inherited genetic variation may play a role. Cigarette smoking induces inflammation; therefore, functionally relevant polymorphisms in inflammation-related genes may affect risk of smoking-associated lung cancer and/or COPD.

The primary goals of this research were to evaluate eicosanoid pathway (*IL1B, COX-2, PPARγ*) gene polymorphisms and cytokine (*TGFB1, IL6, IL10*) gene polymorphisms in relation to lung cancer risk (484 cases/866 controls); and cytokine (*TGFB1, IL6, IL10*) gene polymorphisms in relation to COPD (airflow obstruction and emphysema) risk (N=866). We utilized data and specimens from Project 4 of the University of Pittsburgh Cancer Institute Specialized Program of Research Excellence (SPORE) in Lung Cancer. In our study population, *IL1B* rs1143634 minor allele carriers had a decreased risk of lung cancer (OR=0.73, 95%CI=0.56-0.95) compared to major allele homozygote. There was a strong interaction between *PPARγ* rs1801282 and sex ($P_{interaction}$=0.003), female minor allele carriers were at a reduced risk of lung cancer (OR=0.58, 95%CI=0.37-0.91), while male minor allele carriers showed a non-significant increased risk (OR=1.45, 95%CI=0.96-2.19) compared to major allele

homozygotes. In the analyses of COPD, *TGFB1* rs2241712 was found associated with airflow obstruction severity as measured by Global Initiative for Obstructive Lung Disease (GOLD) (Cochran-Mantel-Haenszel 1degree freedom nonzero correlation P=0.02), minor allele carriers were at a decreased risk of developing the disease (any vs. no airflow obstruction, dominant model OR=0.73, 95%CI=0.55-0.98).

Enhancing our knowledge of lung cancer and COPD genetics is a significant contribution to public health as it may result in the development of new prevention and treatment strategies.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

I would like to take this opportunity to thank my mentors, family and colleagues who have helped me achieve my doctoral degree. The members of my committee have been invaluable, and I am fortunate to have had the opportunity to work with and learn from experts in the field of cancer epidemiology, genetics, and pulmonary disease research. I thank my committee members, Dr. Joel Weissfeld, Dr. Brenda Diergaarde, Dr. Eleanor Feingold, Dr. Marjorie Romkes, and Dr. Frank Sciurba for their mentorship, guidance, and encouragement throughout my doctoral training, all of which have helped me successfully complete this dissertation. This dissertation is part of the University of Pittsburgh Cancer Institute (UPCI) lung cancer SPORE development grant. I express my gratitude to Autumn Gaither Davis and to all PLuSS staff for their excellent work in operating and maintaining the database.

I thank Dr. Caterina Rosano for her mentorship during my years as a Graduate Student Researcher. I have enjoyed working with and learning from her and thankful for her encouragement and support.

I am grateful for the friendship of my friends and fellow students. They have supported my work by always being available to help me think through problems with my research or just to commiserate with the troubles that are typical of doctoral students.

I thank my parents and my other family members. I love you all very much! I thank my parents for instilling in me a strong work ethic, a love of learning, and the belief that I could achieve anything I put my mind to. Mom, thank you for being my confidant, and being there for me when I need it most. Dad, thank you for being my advisor and support system. Mom and Dad, I dedicate this work to you. I also would like to thank all my other family members for always supporting my educational pursuits. I hope I have made you all proud.

1.0 INTRODUCTION

Both lung cancer and chronic obstructive pulmonary disease (COPD) are associated with cigarette smoking, which induces a chronic inflammatory state in the lung. Interestingly, some smokers develop lung cancer, some develop COPD, some develop both diseases, and then there are some that stay free of disease all together. The reason for this is currently unclear but genetic factors may play an important role.

1.1 SPECIFIC AIMS

The goal of this project is to evaluate the role of genetic variability in inflammation-related genes in the development of lung cancer and COPD. We will use data and specimen from cases and controls from Project 4 of the University of Pittsburgh Cancer Institute (UPCI) Specialized Program of Research Excellence (SPORE) in Lung Cancer. We hypothesize that functionally relevant polymorphisms in inflammation-related genes may influence the risk of developing smoking-associated lung cancer and COPD. Specifically, we propose to:

Aim 1. Evaluate the association between *IL1B, COX-2,* and *PPARγ* gene polymorphisms and lung cancer risk.

Aim 2. Evaluate the association between *TGFB1, IL6 and IL10* gene polymorphisms and lung cancer risk.

Aim 3. Evaluate the association between *TGFB1, IL6 and IL10* gene polymorphisms and chronic obstructive pulmonary disease (COPD) risk.

2.0 LITERATURE REVIEW

2.1 LUNG CANCER

Lung cancer is the leading cause of cancer mortality in both men and women in the United States. The disease is responsible for more deaths than prostate, colon, pancreas, and breast cancer combined. In 2010, about 222,520 people will be diagnosed with lung cancer and about 157,300 people will die of lung cancer in the United State.[1]

Cigarette smoking is a well-established risk factor for lung cancer. Compared with never smokers, smokers have a 15-30 fold increased risk of developing lung cancer.[2-4] Other major risk factors for lung cancer include second-hand smoke[5], radon[6] and occupational exposures[5,7], high fat and cholesterol diet, alcohol consumption and family history.[8]

Current standard therapies for lung cancer include surgical resection, platinum-based doublet chemotherapy, and radiation therapy alone or in combination.[9] Unfortunately, these therapies rarely cure the disease. The overall 5-year survival rate hasn't improved much over the past three decades, and it is still only about 16%.[1]

2.2 CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

COPD is the fourth leading cause of death[10] and affects between 10 and 24 million adults in the United States.[10,11]

COPD is characterized by increased airway inflammation[12,13] and an abnormal inflammatory response of the lungs to noxious particles or gases, particularly cigarette smoke.[13] The term COPD describes patients with very different clinical phenotypes. The clinical presentations of COPD ranges from chronic bronchitis to hyperinflation and severe emphysema.[14]

Cigarette smoking is the main risk factor for COPD. Smoking accounts for 80%-90% of all COPD deaths: female smokers are nearly 13 times as likely to die from COPD as women who have never smoked; male smokers are nearly 12 times as likely to die from COPD as men who have never smoked.[15] Smoking causes two pathophysiologic processes in the lung. The first is inflammatory narrowing of peripheral airways, which is characterized by edema, mucus hypersecretion and fibrosis of peripheral airways. The second is proteolytic destruction of the lung parenchyma, which results in permanent enlargement of airspaces (emphysema) and, as a result, loss of lung elastic recoil. Usually, evidence of both pathophysiologic processes is observed in an individual COPD patient.[16]

Other risk factors for COPD include second-hand smoke, occupational hazards such as mineral dust, air pollution and history of childhood respiratory infections and heredity.[17]

2.3 CIGARETTE SMOKING AND INFLAMMATION

Cigarette smoking is the major cause of both lung cancer and COPD. Cigarette smoke contains a high concentration of oxidants in addition to a number of known carcinogens.[18] By generating reactive oxygen species (ROS), cigarette smoke induces an inflammatory state in the lung. Cigarette smoke elicits airway inflammation in all of those who smoke. The cigarette smoking-induced inflammatory response yields an array of deregulated cells, cytokines, and growth factors that are conducive to the development of both lung cancer and COPD.

A cigarette smoker's respiratory epithelium often contains multifocal premalignant lesions that can occur throughout the bronchial tree[19], which has been referred to as the field cancerization effect. This phenomenon implicates the capacity of tobacco carcinogens to extensively cause genetic mutations in the respiratory epithelium.[20] The smoking-induced pulmonary cellular network presents a distinctive environment in which carcinogenesis proceeds in complicity with surrounding lung inflammatory, structural, and stromal cells.[21]

Smoking-induced epithelial abnormalities can serve as targets for abnormal inflammatory responses. Alveolar macrophages, lymphocytes, neutrophils, endothelial cells, and fibroblasts release cytokines, chemokines, and growth factors, which may act to promote epithelial dysfunction and malignant progression.[21]

Cigarette smoke causes not only local airway and lung inflammation; but also systemic inflammatory responses including cellular and humoral inflammation, striking changes of vasomotor and endothelial function, systemic oxidative stress, and enhanced circulating concentrations of several procoagulant factors.[22,23]

2.4 ASSOCIATION BETWEEN COPD AND LUNG CANCER

Several lines of evidence indicate a connection between COPD and lung cancer. COPD and lung cancer are both smoking-related diseases that cluster in families and worsen with age. COPD is strongly associated with lung cancer risk and lung cancer mortality. Low lung function is an established risk factor for lung cancer; and among smokers, those with airflow obstruction have the greatest risk of developing lung cancer.[24] Studies have consistently reported that the prevalence of COPD in lung cancer cohorts is as much as two-fold greater than that reported in a cigarette-smoking control population.[17,25] Several epidemiological studies have suggested that chronic bronchitis and emphysema may increase the risk of lung cancer.[26-33] Other studies have suggested that the association may be subtype specific.[34-36]

Inflammation has been suggested as the potential link between COPD and lung cancer. Pulmonary inflammation could play a role in cancer initiation or promotion. For example, inflammatory cell-derived reactive nitrogen or oxygen species may bind to DNA and thus lead to genomic alterations.[37,38] The pulmonary environment of COPD, including ongoing tissue repair with enhanced cellular proliferation, could be conducive to both DNA mutation and angiogenesis. In addition, the pro-inflammatory cytokines released in this milieu elevate epithelial apoptosis resistance.[37]

2.5 INFLAMMATION IN CANCER AND COPD

Abnormal inflammation is related to both lung cancer and COPD.[22] Inflammation is an essential component of immune-mediated protection against pathogens and tissue damage. Inflammation

is a complex process involving a variety of interactions between immune and non-immune cells via a group of chemical mediators including eicosanoids, cytokines, nitric oxide (NO), and growth factors. [39]

2.5.1 Inflammation and cancer

There is a renaissance of research into the connection between inflammation and cancer.[40-42] Excessively and chronically produced pro-inflammatory mediators are hypothesized to contribute to tumor promotion and progression.[41-44] Chronic inflammation that results from inadequate pathogen eradication, prolonged inflammatory signaling, and defects in anti-inflammatory mechanisms can benefit tumor development.[45] There is a high rate of cell turnover in an inflammatory state, and the microenvironment is often highly oxidative and nitrosative, leading to increased opportunities for DNA damage and mutation. Chronic inflammation can promote an environment that is conducive to carcinogenesis, and it is involved in tumor initiation, promotion, and progression.[41,46-49]

The tumor microenvironment (TME) is created by the tumor and dominated by tumor-induced interactions.[50] In the TME, there is a subtle balance between antitumor immunity and pro-inflammatory activity, which originates from the tumor and weakens antitumor immunity.[43,51] The tumor can not only manage to escape from the host immune system (tumor escape), but it can also modify the functions of infiltrating cells to create the microenvironment favorable to tumor progression.[50] The net outcome of a persistent inflammatory microenvironment is enhanced tumor promotion, accelerated tumor progression, invasion of the surrounding tissues, angiogenesis, and often metastasis.[41]

Lung cancer is characterized by an aggressive clinical course and poor response to immunotherapy[52], probably because of the ability of lung cancer cells to produce a wide variety of immunosuppressive factors that may allow their escape from immune recognition.[53,54]

2.5.2 Inflammation and COPD

The pathology of COPD is that of a chronic inflammatory process with tissue damage and repair processes.[55] Tissue damage with airway wall remodeling and thickening, inflammation and fibrosis of the small airways appear to play an important role in patients with COPD. The accompanying emphysema leads to loss of lung elastic recoil, contributing to decreased expiratory flow. Many cytokines play a role in this condition. The cytokine profile in COPD patients includes lymphokines (e.g. IL6); chemotactic factors for neutrophils, eosinophils, monocytes/macrophages, and T cells; pro-inflammatory cytokines (e.g. IL1β); anti-inflammatory cytokines (e.g. IL10); and growth factors (e.g. TGFβ).[55]

The degree of inflammation increases with the severity of COPD, which is classified by the Global Initiative for Chronic Obstructive Lung Disease (GOLD).[56]

2.6 PATHWAYS TO LUNG CANCER AND COPD

Cigarette smoking, the main risk factor for both lung cancer and COPD, causes profound pulmonary inflammation in smokers' lungs. COPD is associated with abnormal inflammatory features. Pulmonary inflammation may also mechanistically related to lung cancer because

inflammatory cells secrete activated oxygen species, inflammatory mediators, and proteolytic enzymes that can both damage DNA and lead to increases in reparative cell proliferation rates.[57]

Even though cigarette smoking is the main risk factor for both lung cancer and COPD, only a fraction of smokers develop lung cancer[58] and / or COPD[59]. The majority of smokers develop neither lung cancer nor COPD. Why some smokers develop lung cancer, some develop COPD, some develop both diseases, and some stay disease free is currently unknown but genetic factors may play an important role.

Despite the shared exposure to cigarette smoking, which generates ROS and causes inflammation in both lung cancer and COPD, the resulting biological processes differ considerably. Cancer is an anti-apoptotic process, and cancer cells are prone to invade tissues and are characterized by unlimited cell proliferation and sustained angiogenesis; whereas COPD is characterized by increased apoptosis, extracellular matrix degradation, ineffective tissue repair, and limited angiogenesis.[24]

These different responses to the same exposure may be due to random somatic DNA mutations. However, it is also possible that inherited genetic factors determine the disease pathway taken. Single nucleotide polymorphisms (SNPs) are the most common form of human genetic variation and may contribute to individual susceptibility to lung cancer and COPD. Many studies have demonstrated that some variants affect either the expression or activities of particular proteins and therefore are associated with disease risk.

2.7 GENETIC VARIATION AND RISK OF LUNG CANCER AND COPD

Existing data support the notion that genetic factors play a role in the development of both lung cancer[60,61] and COPD.[62] Several studies in different populations have shown evidence for a large genetic contribution to the variability in pulmonary function[63] and for the familiar aggregation of lung cancer[64,65] and COPD patients[66]. Lung cancer, like most cancers, often involves the overexpression of oncogenes or the inhibition of tumor suppressor genes. Cigarette smoke can cause damage to DNA; this initiates a process of cell protection by specific repair mechanisms. It has been hypothesized that defective processes in DNA repair efficacy and quality may be a cause of lung cancer.[67] Recently, genowide-wide association studies (GWAS) have reported several specific susceptibility loci which are thought to impact on both smoking behavior and carcinogenesis.[68,69] Severe α1-Antitrypsin deficiency (α1ATD) is a proven genetic determinant of COPD in a small proportion of the population.[70] Genetic studies have provided evidence that polymorphisms in genes associated with inflammatory pathways can influence the risk of disease development.[71-74] Where genetic variants are found to be associated with both lung cancer and COPD, it is possible that these variants confer susceptibility to both through overlapping pathogenic pathways, such as those underlying smoking-induced inflammation.[75] Functionally relevant SNPs in inflammation-related genes may well modify the inflammatory response to cigarette smoking and, thus, risk of smoking-associated lung cancer or COPD.

Previous genetic epidemiological studies have investigated associations between several inflammatory pathway gene polymorphisms and lung cancer or COPD risk, some of them have identified genetic variants that are associated with risk of lung cancer (**Table 2-1**) or COPD (**Table 2-2**).

Table 2-1. Evidence from Genetic Association Studies: Selected Significant Results between Inflammatory Pathway Gene Polymorphisms and Lung Cancer Risk.

Studies	Setting	Cases/Controls	Variant	Level of Evidence
Vogel *et al.*, (2008)	Demark	Case-cohort: 403:744	*IL1B* -31T>C (rs1143627) *IL10* -592C>A (rs1800872)	IRR=1.51 (1.08-2.12), P=0.05[a] IRR=1.60 (1.13-2.27), P=0.001[a]
Engels *et al.*, (2007)	Texas, USA (non-Hispanic Caucasians)	1504/1684	*IL1B* 3954C>T (rs1143634)	OR=1.27 (1.10-1.47), P=0.001[a]
Hu *et al.*, (2005)	China	322/323	*COX-2* 8473T>C (rs5275)	OR=0.64 (0.45-0.92)[a]
Shih *et al.*, (2005)	Taiwan	154/205	*IL10* -1082G>A (rs1800896)	OR=5.98 (2.95-12.1)[a]
Campa *et al.*, (2004)	Norway	250/214	*COX-2* 8473T>C (rs5275)	TC vs. TT: OR=2.12 (1.25-3.59) CC vs. TT: OR=4.28 (2.44-7.49) P_{trend}<0.0001
Zienolddiny *et al.*, (2004)	Norway	251/271	*IL1B* -31T>C (rs1143627)	CT vs.CC: OR=1.89 (1.03-3.46) TT vs. CC: OR=2.39 (1.29-4.44)

a. Dominant model.

Table 2-2. Evidence from Genetic Association Studies: Selected Significant Results between Inflammatory Pathway Gene Polymorphisms and COPD Risk.

Studies	Setting	Cases/Controls	Variant	Level of Evidence
He *et al.*, (2009)	USA (Caucasians)	389/420	*IL6* -174C>G (rs1800795)	C allele is associated with COPD ($P_{additive}$=0.01)
Ito *et al.*, (2008)	Japan	70/99	*TGFB1* C-509T (rs1800469)	In emphysema, FEV_1 after bronchodilator was significantly associated with the T allele (P=0.007).
Su *et al.*, (2005)	China	84/97	*TGFB1* C-509T (rs1800469)	The frequency of T allele was significantly decreased in COPD compared with that in controls (P=0.008).
Celedon *et al.*, (2005)	USA (Caucasians)	304/441	*TGFB1* -10807G>A (rs2241712)	$P_{additive}$=0.01

2.8 SUMMARY

Lung cancer and COPD are both leading causes of morbidity and mortality in the U.S. and worldwide. Cigarette smoking is a well-established risk factor for both diseases. Cigarette smoke causes abnormal inflammatory response in those who smoke. Some changes persist long after smoking cessation. The risk of lung cancer remains elevated in former smokers and the progression of COPD continues in people who quit smoking. Identification of current or former smokers who are at the highest risk of developing lung cancer and/or COPD is a priority.

Despite the shared risk factor of cigarette smoking, only a small proportion of smokers develop lung cancer and/or COPD. There are also studies showing the familiar aggregation of both diseases. These properties of lung cancer and COPD suggest genetic predispositions to disease development. Single nucleotide polymorphisms (SNPs) are the most common form of human genetic variation, and studies have demonstrated that some variants can influence either expression or activities of inflammatory pathway genes. Thus, it is possible that inflammatory pathway gene polymorphisms may play a role in lung cancer and/or COPD development.

Previous genetic association studies of inflammatory pathway gene polymorphisms and lung cancer and/or COPD have yielded inconsistent results, partly due to small sample sizes and limited power, inadequate study design, genotyping with a limited number of informative SNPs, or population stratification. More studies are warranted to clarify the role of inflammatory pathway gene polymorphisms in lung cancer and/or COPD development.

Lung cancer and COPD are closely linked to cigarette smoking and inflammation. The investigation of these relationships will lead to a more comprehensive picture of the pulmonary environment at risk for the development of lung cancer and/or COPD, and eventually result in improved prevention strategies and treatments.

3.0 MANUSCRIPT 1: POLYMORPHISMS IN *IL1B*, *COX-2* AND *PPARG* GENES AND RISK OF LUNG CANCER

Manuscript in preparation

Yan Du, MS[1], Joel L. Weissfeld, MD, MPH[1], Marjorie Romkes, PhD[2], Frank Sciurba, MD[3], Eleanor Feingold, PhD[4], Brenda Diergaarde, PhD[1]

[1] Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA

[2] Department of Medicine, Center for Clinical Pharmacology, University of Pittsburgh, Pittsburgh, PA, USA

[3] Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, University of Pittsburgh, Pittsburgh, PA, USA

[4] Department of Human Genetics and Biostatistics, Graduate School of Public Health, University of Pittsburgh, PA, USA

3.1 ABSTRACT

Background Lung cancer is one of the leading causes of cancer death in the U.S. Cigarette smoking is the main risk factor for lung cancer; smoking induces inflammatory conditions in smokers' lungs. Interestingly, less than 20% of smokers develop lung cancer in their lifetime, suggesting that genetic factors may play a role in an individual's susceptibility to lung cancer. In this study, we evaluated associations between polymorphisms in three inflammation-related genes and lung cancer risk.

Methods We evaluated associations of nine polymorphisms in *IL1B, COX-2, and PPARγ* with lung cancer risk in 484 cases and 866 controls from the Pittsburgh metro area. Multiple logistic regression models adjusting for age, sex, and smoking pack-years were used to evaluate the associations. We further performed stratified analyses and tested gene-environment interactions.

Results Individuals carrying at least one T allele of *IL1B* rs1143634 had a lower risk of lung cancer (OR=0.73, 95%CI=0.56-0.95, P=0.02) than subjects with the CC genotype. In subgroup analyses, this protective effect remained significant in current smokers (OR=0.65, 95%CI=0.46-0.93, P=0.02), men (OR=0.58, 95%CI=0.40-0.85, P=0.004), and people of 58 years and older (OR=0.65, 95%CI=0.48-0.88, P=0.006). For *COX-2* rs689466, minor allele homozygotes (GG) had an increased risk of lung cancer (OR=2.34, 95%CI=1.15-5.77, P=0.02) compared to subjects with the AA genotype. *PPARγ* rs1801282 showed a significant interaction with sex (P for interaction=0.003). Minor allele carriers (CG/GG) had a decreased risk (OR=0.58, 95%CI=0.37-0.91, P=0.02) of lung cancer among women, but were associated with a non-significant increased risk (OR=1.45, 95%CI=0.96-2.19) among men.

Conclusions Our findings suggest that rs1143634 in *IL1B*, rs689466 in *COX-2* and rs1801282 in *PPARγ* may be related to the risk of smoking-associated lung cancer.

Implications for public health The better understanding of genetic factors linking inflammation and carcinogenesis will be beneficial to the development of efficacious prevention strategies and therapies for lung cancer.

3.2 INTRODUCTION

Lung cancer is the leading cause of cancer mortality in both men and women in the United States. The disease is responsible for more deaths than prostate, colon, pancreas, and breast cancer combined. In 2010, about 222,520 people will be diagnosed with lung cancer and about 157,300 people will die of lung cancer in the United States.[1] The most well established risk factor for lung cancer is cigarette smoking.[2] Compared with never smokers, smokers have 15-30 fold increased risk of developing lung cancer.[3,4] Cigarette smoke contains a high concentration of oxidants in addition to a number of known carcinogens.[18] By generating reactive oxygen species (ROS), cigarette smoke induces an inflammatory state in the lung. The cigarette smoking-induced inflammatory response produces a group of deregulated cells, cytokines, and growth factors that are favorable to the development of lung cancer.[21] There is a renaissance of research into the connection between inflammation and cancer.[40-42] Chronic inflammation is a cofactor in carcinogenesis.[41] The microenvironment of lung tumors and surrounding stromal tissue is characterized by an increased number of inflammatory cells and higher levels of pro-inflammatory cytokines. Lungs from smokers show an elevated number of macrophages in the alveoli and respiratory bronchiole and a higher level of pro-inflammatory cytokines.[40,76-78]

Recent data suggest that cigarette smoke stimulates airway epithelial cells and immune cells to release pro-inflammatory cytokines, such as interleukin-1β (*IL1B*), that lead to up-

regulation of various inflammation-related genes.[76,77,79] IL1B is a pro-inflammatory cytokine mainly produced by blood monocytes and tissue macrophages and has been implicated in mediating both acute and chronic inflammation. IL1B has both pro- and antiapoptotic effects.[80] Lung epithelial cells produce, as well as respond to IL1B. *IL1B* triggers a cascade of inflammation reactions through the induction of inflammation-related genes and ROS. This is known to result in activation of the nuclear factor-κB (*NF-κB*) signaling pathway, leading to up-regulation of various inflammation-related genes, including cyclooxygenase-2 (COX-2).[81]

COX-2 (PTGS2), also known as prostaglandin G/H synthase-2, is an inducible enzyme that catalyzes the rate-limiting step in the production of prostaglandins and plays a key role in inflammation.[82] Several studies reported that use of non-steroidal anti-inflammatory drugs (NSAIDs), of which COX-2 is a major target, was associated with a reduced risk of lung cancer[83-86], pointing to a chemopreventive effect of NSAIDs.

The *COX-2* gene is on chromosome 1q25.2-25.3, 8.3 kb in size, and has 10 exons.[87] COX-2 is rapidly induced in response to cytokines, growth factors and tumor promoters.[82,88] Expression and activity of COX-2 is thought to contribute to tumor promotion and carcinogenesis through stimulation of cell proliferation, inhibition of apoptosis, and promotion of angiogenesis and invasiveness.[89,90] The suppression of apoptosis associated with COX-2 over-expression may be an important factor in tumorigenesis. *In vitro* experiments indicate that modulation of COX-2 activity leads to altered apoptotic propensity.[91,92] Two products of the COX-2 pathway, prostaglandin-E1 (PGE1) and prostaglandin-E2 (PGE2), are reported to promote angiogenesis.[93] Moreover, COX-2 transfected cells induce expression of vascular endothelial growth factor (VEGF), one of the main regulators of angiogenesis.[94] COX-2 can mediate inhibition of anti-tumor immunity, thus indirectly affects the metastatic potential of

tumors.[95,96] In addition, several studies support the idea that COX-2 may be involved in matrix metalloproteinases (MMP) production and secretion.[97-100]

On the other hand, NSAID metabolites which lack COX-2 inhibitory activity still retain their chemopreventive potential, thus indicating that COX-2 is not the only target of anti-inflammatory drugs. Several NSAIDs, for example, can activate peroxisome proliferator-activated receptor gamma (*PPARγ*), and lead to inhibition of lung cancer cell growth.[101-103] *PPARγ* influences inflammatory gene expression, cell division, apoptosis, invasion, release of proangiogenic cytokines, and differentiation in many cancer types including lung cancer.[101,104-106] *PPARγ* is required for normal development of the lung.[106] *PPARγ* regulates cell growth by inducing differentiation and apoptosis.[101,102] These effects are mediated through inhibition of transcription factors, including NF-κB.[107] Forced over-expression of PPARγ in a non small cell lung cancer (NSCLC) cell line model inhibited the expression of COX-2 protein and promoter activity, resulting in decreased PGE2 production. One possible explanation of the inhibition of COX-2 expression is the increased activity of the phosphatase and tensin homologue (PTEN) causing a decrease in the level of phosphor-AKT and the resulting inhibition of NF-κB.[103] These properties have prompted extensive research on *PPARγ* in cancer treatment and prevention.

The relationship between *IL1B, COX-2* and *PPARγ* genes is shown in the schematic **figure 3.1**.

Even though cigarette smoking is the main risk factor for lung cancer, only a fraction of smokers develop lung cancer during their lifetime. This suggests that genetic variation may play a role in lung cancer development. Functionally relevant single nucleotide polymorphisms (SNPs) in inflammation-related pathway genes may modify the inflammatory response to cigarette smoking and, thus, an individual's susceptibility to lung cancer. In the present case-

control analysis, we evaluated associations between lung cancer and nine selected polymorphisms in three genes involved in the inflammatory response.

3.3 MATERIALS AND METHODS

3.3.1 Study Population

This study utilized data and specimens from the University of Pittsburgh Cancer Institute (UPCI) Specialized Program of Research Excellence (SPORE) in Lung Cancer.

Cases were newly diagnosed lung cancer patients treated by surgery between 1990 and 2008 at a University of Pittsburgh Medical Center hospital (Pittsburgh, PA). Eligibility criteria included: 1) current or ex-cigarette smoker, 2) cumulative cigarette dose exposure≥10 pack - years, 3) 45-85 years old at time of lung cancer diagnosis, 4) entry within 365 days of lung cancer diagnosis, and 5) pathologically verified lung cancer diagnosis (excluding carcinoid). These selection procedures identified 923 patients, including 588 (63.7% of 923) with DNA available and 567 (96.4% of 588) with genotype call rates >90%.

The control series are a random sample from a restricted set of participants in the Pittsburgh Lung Screening Study (PLuSS), a helical computed tomography (CT) lung cancer screening study.[34] Between 2002 and 2005, PLuSS enrolled 50-79 year-old current and ex-cigarette smokers of at least one-half pack/day for at least 25 years. Using self-report, PLuSS excluded individuals who: 1) quit smoking more than 10 years earlier, 2) had a history of lung cancer, or 3) had a chest CT scan within one year of enrollment. Selection as a control was restricted to the *N*=3,463 (92.2% of all PLuSS enrollees) white or black race CT-screened PLuSS

participants with buffy coat or whole blood available and no interval lung cancer diagnosis (as of 9/10/2008). A simple random sample of size n=1000 was selected, of which 929 (92.9%) samples had DNA available for the current study, and resulted in 919 (98.9% of 929) with genotype call rates >90%.

Information on smoking history was extracted from medical records for cases and from written questionnaires for controls.

The study was approved by the institutional review board (IRB) at the University of Pittsburgh. All participants provided written informed consent.

3.3.2 SNP Selection and Genotyping

Ten single nucleotide polymorphisms (SNPs) in three genes (*IL1B, COX-2,* and *PPARγ*) were genotyped. SNPs meeting the following criteria were given priority: (1) known and putative functional SNPs located in the promoter, untranslated region (UTR), or coding region of the gene; (2) previous report of an association with lung cancer or another cancer, or an inflammatory disorder. In addition, we selected tag SNPs for *COX-2* using data from the SeattleSNPs (http://pga.gs.washington.edu/), PGA-European-Panel. Using the online program LDSelect[108,109], we identified SNPs in the region including 5 kb up- and downstream of *COX-2* with minor allele frequencies (MAF) ≥5% and pairwise r^2 with untyped SNPs≥0.8. We forced the inclusion of three SNPs: rs5275, the most common *COX-2* polymorphism in Caucasians; rs20417 and rs689466, which were reported to be associated with inflammation.[110]

Six SNPs in *COX-2* (rs5275, rs5277, rs4648261, rs20417, rs689466, rs2745559), three SNPs in *IL1B* (rs1143634, rs1143633, rs1143627) and one SNP in *PPARγ* (rs1801282) were selected for genotyping. Information regarding each SNP was presented in **Table 3-1**.

Genomic DNA was extracted from whole blood samples using standard salt-based methods. Genotyping of 588 cases and 929 controls was performed at the University of Pittsburgh Genomics and Proteomics Core Laboratories (Pittsburgh, PA). All SNPs were genotyped using MassARRAY® iPLEX Gold (Sequenom, Inc., San Diego, CA); the SNP specific and mass extend oligonucleotides, and assays were designed using Sequenom RealSNP (www.realsnp.com) and MassARRAY Assay Design version 3.1 (Sequenom, Inc., San Diego, CA). Genotyping was performed for cases and controls together; laboratory technicians were blinded to case-control status. Fifty duplicate samples were included to assess laboratory reliability. The duplicates were 100% concordant.

More than 95% of all DNA samples were successfully genotyped for the 10 SNPs. Of the 1517 samples genotyped, 31 samples (21 cases and 10 controls) failed (call rate<90%), leaving 567 cases and 919 controls for analyses. The present study includes only Caucasians, the major racial/ethnic subgroup of subjects. 83 cases and 53 controls of black or unknown race were excluded. The study population consisted of 484 lung cancer cases and 866 controls.

3.3.3 Statistical Analysis

Participant characteristics in the cases and controls were compared by Wilcoxon rank sum test for continuous variables and Chi-square test for categorical variables.

Genotype and allele frequencies were calculated, observed genotype frequencies in the control population were tested for deviation from Hardy-Weinberg equilibrium (HWE) with the exact test.

Odds ratios (ORs) for lung cancer risk and the corresponding 95% confidence intervals (CIs) were calculated for each SNP using multiple logistic regression models, adjusting for age

(as a continuous variable), sex (male vs. female), and cumulative tobacco exposure (pack-years as a continuous variable). We used the genotype-based model for individual SNPs. Common allele homozygotes (0 minor alleles) were used as the reference group and the genotype variable had three levels (common allele homozygotes, heterozygotes [1 minor allele], rare allele homozygotes [2 minor alleles]).

Linear trend and per allele ORs were assessed using numerical scores assigned to the ordered categories (i.e., 0 to the category of major allele homozygotes, 1 to the category of heterozygotes, and 2 to the category of minor allele homozygotes) as continuous variable in the model.

In addition, we also tested the dominant model because the number of rare-allele homozygotes was relatively small. We combined heterozygotes and rare-allele homozygotes in the logistic regression analyses; common-allele homozygotes were used as the reference group.

We tested interactions with cross product interaction terms of each SNP and environmental factors such as sex (male vs. female), smoking status (current vs. former), and continuous age and pack-years under the dominant model. For stratified analyses, we created an indicator variable of age greater or less than 58 years old (the median in controls), sex (male vs. female) and smoking status (current vs. former smoker), pack-years (in tertiles, cut-points 37.5 and 57.5 based on the distribution in controls). Stratum-specific ORs were obtained under the dominant model.

We repeated the analysis restricting our cases to NSCLC only. We then performed subgroup analyses of adenocarcinoma (ADC) and squamous cell carcinoma (SCC), the two predominant histological types, comparing each subgroup of cases against the entire group of

controls. We also performed case-case comparison, comparing SCC against ADC, to see if different genes are involved in the etiology of developing lung cancer subtypes.

Haplotype frequencies and associations were calculated using PLINK (version 1.07) that uses the expectation maximization (EM) algorithm.

All significance tests were two sided; *P* values <0.05 were considered statistically significant. Data analyses were conducted using SAS software (version 9.1.3; SAS Institute, Cary, NC) and PLINK (version1.07).

3.4 RESULTS

3.4.1 Study Subjects

Baseline characteristics of the study population are presented in **Table 3-2**. Sex did not differ significantly between cases and controls (P=0.07). Cases were significantly older (median=67 years, inter quartile range [IQR] =61-74 years) than controls (median=58 years, IQR=54-63 years) (P<0.0001), and also reported greater cumulative tobacco exposure (median 50 vs. 47.5 pack-years, P=0.02). The proportion of current smokers among the controls was significantly higher than among the cases (59.1% vs. 48.9%, P=0.0004). The most common histological types of lung cancer were adenocarcinoma (n=230, 47.5%), followed by squamous cell carcinoma (n=173, 35.7%). Most NSCLC presented at the early stage (47.2% stage I, 16.1% stage II).

3.4.2 Association of Lung Cancer Risk with Genetic Polymorphisms

Two SNPs (rs20417, rs689466) were out of HWE in controls (P<0.0001 and P=0.01, respectively) (**Table 3-3**). One SNP, rs20417, deviated significantly from HWE (P<0.0001), with no heterozygotes at all. In addition, the minor allele frequency observed (2.7%) was much lower than expected (18.1%) in populations of European ancestry (CEU) based on information in the International HapMap Project database, suggesting potential genotyping issues. Thus, rs20417 was dropped from the analysis. Another SNP, rs689466, had a P value of 0.01; however, examination of the cluster plot for this SNP did not reveal any unusual patterns, therefore this SNP was included in the subsequent analyses.

The genotype distributions for each SNP among the cases and controls, and the genotype-based model and trend test results are shown in **Table 3-4**. There were no minor allele rs4648261 homozygote among the cases and only one in controls; therefore, for this SNP, the heterozygotes (GA) and minor allele homozygotes (AA) were combined. For SNP rs689466 in *COX-2*, we observed that the minor allele homozygotes (GG genotype) had a significantly increased risk of lung cancer (OR=2.34, 95%CI=1.15-5.77) compared to common allele homozygotes (AA genotype). Additionally, rs1143634 heterozygotes had a significantly decreased risk of lung cancer compared to rs1143634 common allele homozygotes (CT vs. CC: OR=0.76, 95%CI=0.59-0.96). This association continued to be significant after adjusting for age, sex, and pack-years. However, test for trend was not significant for rs1143634. All other SNPs showed no significant association with lung cancer risk. (**Table 3-4**)

The results under the dominant model are presented in **Table 3-5**. For *IL1B* rs1143634, a significant decreased risk of lung cancer was observed for carriers of at least one minor allele

compared to common allele homozygotes (CT+TT vs. CC: OR=0.73, 95%CI=0.56-0.95). No other significant association was observed.

3.4.3 Subgroup Analysis and Interaction Results

The results for NSCLC-only were similar to that of the main analysis (**Appendix A Table 6-1**). For histological subtypes, no significant associations between any of the SNPs and ADC (**Appendix A Table 6-2**) or SCC (**Appendix A Table 6-3**) risk were observed.

Because of the different features of lung cancer subtypes, we performed subgroup analysis of ADC and SCC. ADC is located mostly in the peripheral part of the lung, while SCC is located mostly in the central part of the lung. It is postulated that inflammation and irritation, particularly from smoke, might exercise different biological features in the central and peripheral part of the lung. It may thus be hypothesized that tobacco-induced inflammation is differentially associated with ADC and SCC, and that different inflammatory genes are involved in the process. However, no significant associations were observed with the case-case comparison (SCC vs. ADC). (**Appendix A Table 6-4**)

IL1B rs1143634 showed significant associations with lung cancer risk within strata of smoking status, sex and binary age. Compared to common allele homozygotes, subjects with at least one rare allele had a decreased risk of lung cancer among current smokers (OR=0.65, 95%CI=0.46-0.93), men (OR=0.58, 95%CI=0.40-0.85), and older (≥58 years) people (OR=0.65, 95%CI=0.48-0.88). However, there was no significant association between this SNP and lung cancer risk within each smoking (tertile pack-years) subgroup. No significant interaction between this SNP and any variable was observed (**Table 3-6**).

For SNP rs2745559 in *COX-2*, evaluating associations with pack-year levels as the smoking metric showed that among mild smokers (subjects who smoked <37.5 pack-years) subjects with at least one rare allele had a significantly decreased risk of lung cancer compared to common allele homozygotes (OR=0.57, 95%CI=0.33-0.99). The overall packyear-genotype interaction was not significant ($P_{interaction}$=0.98) (**Table 3-6**).

There was a significant interaction between *PPARγ* rs1801282 and sex ($P_{interaction}$=0.003). Female minor allele carriers had a reduced risk of lung cancer (OR=0.58, 95%CI=0.37-0.91), while male minor allele carriers were associated with a non-significant increased risk of the disease (OR=1.04, 95%CI=0.96-2.19). No other gene-environment interaction was observed. (**Table 3-6**)

3.4.4 Haplotype Analysis Results

In the haplotype analysis, we identified two haplotype block sets, rs5275-rs5277-rs689466-rs2745559 in *COX-2*, and rs1143634-rs1143633 in *IL1B*. For the *COX-2* block, TGAA haplotype showed a borderline significant reduced risk (OR=0.81, P=0.07) of lung cancer compared to all other haplotypes. No *IL1B* haplotypes showed significant association with lung cancer. Overall, the global association was not significant for either block (**Table 3-7**).

3.5 DISCUSSION

In the present case-control study, we evaluated the associations between nine polymorphisms in three inflammation-related genes (*IL1B*, *COX-2*, and *PPARγ*) and lung cancer risk. Our study

showed that a coding-synonymous polymorphism in *IL1B* (rs1143634) was significantly associated with risk of lung cancer. Although rs1143634 is a coding-synonymous SNP in exon 5 of *IL1B*, an allelic dosage effect on secretory capacity was observed after lipopolysaccharide stimulation.[111] In addition, *in vitro* studies have shown that the TT genotype of rs1143634 up-regulates production of IL1B levels.[111,112] In the current study, we found that the rare allele (T) of rs1143634 was associated with a decreased risk of lung cancer. Several other studies have previously investigated *IL1B* SNP rs1143634 and lung cancer risk in different populations. In a study conducted in Boston, Ter-minassian *et al.*[113] did not observe an association between rs1143634 and NSCLC risk in the overall Caucasian population (2150 NSCLC cases and 1492 controls). However, in the subgroup analysis, they reported TT compared with CC genotype conferred a significant risk among former smokers (OR=1.74, 95%CI=1.07-2.85) and among men (OR=1.80, 95%CI=1.04-3.11). In another study conducted by Engels *et al.*[71] in Houston, CT+TT genotypes were reported to be associated with an increased risk of lung cancer (OR=1.27, 95%CI=1.10-1.47) compared with CC genotype in Caucasians (1538 cases and 1705 controls). In a study conducted in a Japanese population of 462 cases and 379 controls, Kiyohara *et al.*[114] reported CT+TT genotypes conferred an increased risk of lung cancer in the unadjusted analysis (OR=1.53, 95%CI=1.03-2.27), the association was, however, attenuated after adjustment for smoking and other confounding variables (OR=1.45, 95%CI=0.93-2.26). They subsequently observed a modifying effect of ever smoking on genotype and lung cancer association (attributable proportion due to interaction: OR=0.45, 95%CI=0.08-0.83, P=0.02). The opposite finding in our study may be due to chance, or it may reflect true biological mechanisms and/ or population differences. Our population consists of smokers only, with a

relatively intense exposure to cigarette smoking. *IL1B* has both pro- and anti-apoptotic effects, thus it may play a different role in heavy smokers with prominent inflammation in their lungs.

Another extensively studied *IL1B* polymorphism is rs1143627 in the promoter region at position -31 of the gene. rs1143627 is a TATA-box polymorphism. It has been shown that this SNP can affect DNA-protein interactions *in vitro*, hence modulating the expression of *IL1B* gene.[115] Location of a C at this site will disrupt the TATA-box, which may lead to disruption of DNA-protein interactions or may change the affinity of regulatory proteins in binding to this sequence. As a result, it is likely that presence of a C allele at this site may reduce or abolish the TATA-box characteristic of the *IL1B* gene, thus lead to a reduced inflammatory reaction. However, we did not observe any association of this polymorphism with lung cancer risk in the current study. Similar to our findings, Engels *et al.*[71] did not find an association in their Caucasian population. Another study conducted by Campa *et al.*[81] in central and Eastern Europe also reported non-significant results. On the contrary, a nested case-cohort study[116] (403 cases, 744 sub-cohort) within the Danish "Diet, Cancer, and Health" cohort observed that minor allele carriers (TC+CC) were at 1.51-fold higher risk of lung cancer (95%CI=1.08-2.12) than major allele homozygotes (TT). They also observed an interaction between rs1143627 and NSAID use ($P_{interaction}$=0.02). A case-control study conducted in 251 NSCLC and 272 control subjects in Norway[72] reported that carriers of the T allele were associated with a higher risk of NSCLC, with OR of 1.89 (95%CI=1.03-3.46) for heterozygotes (CT) and OR of 2.39 (95%CI=1.29-4.44) for homozygotes (TT).

COXs catalyze the formation of prostaglandins from arachidonic acid. SNPs which influence the quantity of prostaglandins produced could be associated with inflammatory

diseases and tumor development.[117] SNPs in the gene may alter the COX-2 enzyme conformation, and therefore alter enzymatic function.[118]

COX-2 promoter region contains multiple regulatory elements, such as a *NF-κB* binding site. The regulation of *COX-2* gene expression could involve complex interaction among these factors.[119] It has been reported that the promoter polymorphism *COX-2* rs689466 (A1195G) can modify the *COX-2* transcription levels.[110] *In vivo* studies of esophageal tissues and in luciferase reporter assays performed in HeLa cells have shown that the A allele of *COX-2* rs689466 (A1195G) had a much higher transcriptional level than the G allele.[110] In this study, we observed an increased risk of lung cancer among the minor allele homozygotes (AA). However, because the number of minor allele homozygotes is relatively small in the current study, this finding needs to be confirmed in the future studies. There is one study in Taiwan[120] (358 cases and 716 controls) that also investigated this SNP, with non-significant results (P=0.82 for genotypic model and P=0.52 for allelic association).

It has been shown that the 3'UTR region of *COX-2* is an important determinant of the mRNA stability and therefore of the enzyme level.[121] The 3'UTR region of *COX-2* includes highly conserved adenosine- and uridine-rich elements that are constituted of the Shaw-Kamens sequence (AUUUA), which is also known as an AU-rich element. Elements in the 3'UTR region have an important role in polyadenylation, nuclear export, degradation, stabilization, and translation of the transcripts. Therefore, it is reasonable to hypothesize that the *COX-2* rs5275 (T8473C) polymorphism located within the functional region of 3'UTR could partly reduce mRNA stability and expression through changing the binding affinity of regulatory elements or by modifying the efficiency of polyadenylation signals. This could lead to decreased cellular *COX-2* activity and reduced inflammatory response, angiogenesis, and tumor growth.[122,123]

However, we didn't observe any significant association between rs5275 and lung cancer risk in our population. The Danish case-cohort study[116] discovered an interaction between rs5275 and smoking status, non-smoking minor allele carriers (TC+CC) were at 5.75-fold (95%CI=1.25-26.43) higher risk of lung cancer compared to major allele homozygotes (TT). In a case-control study conducted in 250 case and 214 control subjects of Caucasian origin from the Norwegian population, Campa *et al.*[124] found that the minor allele (C) of rs5275 was associated with a significantly increased risk of lung cancer, with OR of 2.12 (95%CI=1.25-3.59) for heterozygotes (TC) and 4.28 (95%CI=2.44-7.49) for homozygotes (CC) (P_{trend}<0.0001). On the contrary, studies in China[125] and South Korea[126] showed TC+CC genotypes were associated with significantly reduced risk of lung cancer. However, both Engels *et al.*[71] and Campa *et al.*[81] reported non-significant results from their studies of Caucasians and central-eastern European populations, respectively. The study conducted in Taiwan[120] didn't find any association between this polymorphism and lung cancer risk either.

The *PPARγ* rs1801282 (Pro12→Ala) is a coding region SNP. *PPARγ* has an anti-inflammatory action, and *in vitro* studies have reported the variant allele (G) of the rs1801282 polymorphism is related to less transcriptional activation of target genes.[127] Therefore, it is expected that the minor allele could be associated with an increased lung cancer risk. We observed that minor allele carriers had a reduced risk of lung cancer among females, but were at a non-significant increased risk among males, with a significant interaction with sex ($P_{interaction}$=0.003). This may be due to chance alone, or may indicate a true sex difference. Sex differences have not been found often for most polymorphism-associated lung cancer studies. However, one would expect to observe such differences if the mechanism underlying the function of the polymorphic site is affected by a sex-related phenomenon. PPARγ is a nuclear

hormone receptor, and a regulator of adipocyte differentiation. The observation that the effect was detectable only in women may be explained by hormonal factors. It is known that hormones can have a role in the carcinogenesis process in many tissues. Genetic polymorphisms may play an important role in mediating the hormonal effects. Furthermore, it has also been shown that NSAIDs could modify PPARγ activity.[101-103] In the Danish case-cohort study[116], Vogel *et al*. found a strong interaction between rs1801282 and NSAID use in relation to lung cancer risk ($P_{interaction}$=0.00009). Among non-users, minor allele carriers (CG+GG) were at lowered risk of lung cancer compared to homozygous major allele (C) carriers (incidence rate ratio [IRR] =0.62, 95%CI=0.39-0.99). Among NSAID-users, major allele homozygotes (CC) had a non-significant reduced risk of lung cancer (IRR=0.73, 95%CI=0.48-1.11), while minor allele carriers (CG+GG) had a non-significant higher risk of lung cancer (IRR=1.27, 95%CI=0.71-2.27), compared to non-users with the CC genotype.

Strengths of our study include that we had a fairly homogenous Caucasian population from western Pennsylvania, and population stratification is not likely to be a concern. Certain gene-environment interactions were tested. We characterized the *COX-2* variation across a large area of the gene. Our analysis encompassed more than 80% of the common genetic variants in the *COX-2* gene. As variation in the flanking region of *COX-2* is thought to contribute to gene function[110,123,128], investigations including these regions are important.

Like any other study, there are limitations to our study. The present study was not able to evaluate the effect of NSAIDs on polymorphism and lung cancer associations, since we didn't collect NSAIDs information. Previous studies[116,129] suggest that NSAIDs use may modify risk of cancer differently depending on the genotype. As NSAIDs bind with *COX-2*, it is possible that

polymorphisms in *COX-2* could alter an individual's response to NSAIDs and thus modify the chemopreventive effect of NSAIDs in lung cancer.

Second, as for most complex diseases, common genetic polymorphisms may only confer a modest effect on disease risk. Thus, the power to detect such associations was limited at the present study, especially in the subgroup analyses and tests for interaction. It will be important to follow-up on these findings in future studies with larger sample sizes. In our study population and with 80% power using log-additive model, for SNPs with MAF=0.05 we can detect ORs $\geq$1.6, for SNPs with MAF=0.10 we can detect ORs $\geq$1.4, for SNPs with MAF=0.25 we can detect ORs >1.3.

At this time, very little is known about the exact function of these variants, and we only speculate about their biologic roles. Our understanding of other factors influencing gene expression is also limited. It may be that these genetic variations and other factors such as epigenetic changes interact with each other in disease development, and that to understand this process, we need to know more about those dimensions as well.

Despite the many advances made in diagnostic and treatment strategies, lung cancer remains the leading cause of cancer-related mortality in the United States and its five year survival rate is still about 16%.[1] This emphasizes the need for novel strategies for early detection, prevention, and treatment of lung cancer. A large body of evidence indicates that increased PGE2 production contributes to tumorigenesis. COX-2 over-expression is frequently observed in lung cancers, and the accompanying increased proliferation, invasion, angiogenesis, and resistance to apoptosis have been attributed in part to elevated PGE2 production in the vicinity of the tumor. Thus, *COX-2* and its related signaling pathways represent potential targets for lung cancer chemoprevention and therapy.

Further study of the function of these polymorphisms is warranted to better understand what may be an important mechanism for lung carcinogenesis. Better understanding of the role of inflammation in lung carcinogenesis has potential to inform prevention strategies.

3.6 TABLES AND FIGURES

Table 3-1. SNP Information.

Gene	rs Number	Chr	Chr Position*	Allele	Location and Presumed Function
COX-2/PTGS2	rs5275	1q25.2-q25.3	186643058	T-->C	exon10, 3'UTR, C allele may decrease mRNA stability, thereby reducing inflammation response
COX-2/PTGS2	rs5277	1q25.2-q25.3	186648197	G-->C	exon3, coding-synonymous, V102V
COX-2/PTGS2	rs4648261	1q25.2-q25.3	186649004	G-->A	intron2
COX-2/PTGS2	rs20417	1q25.2-q25.3	186650321	G-->C	promoter region
COX-2/PTGS2	rs689466	1q25.2-q25.3	186650751	A-->G	promoter region
COX-2/PTGS2	rs2745559	1q25.2-q25.3	186652002	C-->A	promoter region
IL1B	rs1143634	2q14	113590390	C-->T	exon5, coding-synonymous, F105F
IL1B	rs1143633	2q14	113590467	G-->A	intron4
IL1B	rs1143627	2q14	113594387	T-->C	promoter region, T/C transversion is 31bp upstream of the transcription start site and the presence of the C allele causes disruption of a TATA box
PPARG	rs1801282	3p25	12393125	C-->G	coding-nonsynonymous, missense; Pro12Ala

*dbSNP Chromosome Report, GRCh37 Sequence
Database of Single Nucleotide Polymorphisms (dbSNP). Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine. (dbSNP Build ID: {build ID). Available from: http://www.ncbi.nlm.nih.gov/SNP/.

Table 3-2. Population Characteristics.

Characteristic	Cases (N=484)	Controls (N=866)	P*
Male, n(%)	266 (55.0)	432 (49.9)	0.07
Age (y), median (IQR)	67 (61, 74)	58 (54, 63)	<0.0001
Age (y), n (%)			<0.0001
<50	10 (2.1)	1 (0.1)	
50-59	83 (17.2)	516 (59.6)	
60-64	87 (18.0)	175 (20.2)	
65-69	112 (23.1)	103 (11.9)	
70+	192 (39.7)	71 (8.2)	
Smoke Status			
Current	224 (48.9)	511 (59.1)	0.0004
Former	234 (51.1)	354 (40.9)	
Smoking intensity duration, Pack-years, median (IQR)	50.00 (35.00, 75.00)	47.50 (33.00, 63.00)	0.02
Smoking intensity duration, Pack-years, n(%)			<0.0001
<30	77 (15.9)	161 (18.6)	
30-44	109 (22.5)	228 (26.3)	
45-59	112 (23.1)	228 (26.3)	
60-74	64 (13.2)	135 (15.6)	
75+	122 (25.2)	114 (13.2)	
Lung cancer histology, n(%)			
Adenocarcinoma	230 (47.5)	--	
Squamous cell carcinoma	173 (35.7)	--	
Large-cell carcinoma	11 (2.3)	--	
Other/unspecified non-small cell carcinoma	42 (8.7)	--	
Small-cell carcinoma	14 (2.9)	--	
Neuroendocrine	9 (1.9)	--	
Other/unspecified	5 (1.0)	--	
Lung cancer stage, n(%)†			
I	217 (47.2)	--	
II	74 (16.1)	--	
III	144 (31.3)	--	
IV	25 (5.4)	--	

Abbreviation: IQR, interquartile range.

* P values are derived by x^2 test, except for age and pack-years, where the Wilcoxon rank sum test was used.

† Stage is limited to non-small cell carcinoma

Table 3-3. Allele Frequencies and HWE in Controls and the International HapMap Project (CEU Population).

Gene	rs Number	N	Allele	Allele Counts	Allele Frequencies, %	HapMap CEU Frequencies, %	HWE P in controls (Exact Test)
COX-2	rs5275	860	T	1153	67.0	62.9	0.94
			C	567	33.0	37.1	
COX-2	rs5277	865	G	1458	84.3	79.3	0.92
			C	272	15.7	20.7	
COX-2	rs4648261	866	G	1691	97.6	94.2	0.45
			A	41	2.4	5.8	
COX-2	rs689466	866	A	1437	83.0	85.6	0.01
			G	295	17.0	14.4	
COX-2	rs2745559	862	C	1381	80.1	86.4	0.54
			A	343	19.9	13.6	
IL1B	rs1143634	866	C	1321	76.3	77.7	0.96
			T	411	23.6	22.3	
IL1B	rs1143633	863	G	1098	63.6	60.3	0.53
			A	628	36.4	39.7	
IL1B	rs1143627	866	T	1147	66.2	63.3	0.74
			C	585	33.8	36.7	
PPARG	rs1801282	866	C	1489	86.0	92.4	0.27
			G	243	14.0	7.6	

Table 3-4. Unadjusted and Adjusted Results of SNP and Lung Cancer Associations: Genotype-based Model and Trend Test.

			N (%)		Genotype-based Model		Genotype-based Model*	
Gene	**rs Number**	**Genotype**	**Cases**	**Controls**	**OR (95% CI)**	**P**	**OR (95% CI)***	**P***
COX-2	rs5275	T/T	213 (45.4)	386 (44.9)	1.00 (reference)		1.00 (reference)	
		T/C	199 (42.4)	381 (44.3)	0.95 (0.75, 1.20)	0.65	0.99 (0.75, 1.30)	0.93
		C/C	57 (12.2)	93 (10.8)	1.11 (0.77, 1.61)	0.58	0.94 (0.62, 1.44)	0.78
		Trend[a]			1.02 (0.86, 1.20)	0.85	0.98 (0.81, 1.18)	0.80
COX-2	rs5277	G/G	342 (71.0)	614 (71.0)	1.00 (reference)		1.00 (reference)	
		G/C	130 (26.8)	230 (26.6)	1.02 (0.79, 1.31)	0.91	1.17 (0.88, 1.56)	0.29
		C/C	10 (2.1)	21 (2.4)	0.86 (0.40, 1.84)	0.69	1.15 (0.49, 2.67)	0.75
		Trend[a]			0.99 (0.80, 1.23)	0.93	1.15 (0.90, 1.48)	0.26
COX-2	rs4648261	G/G	453 (93.6)	826 (95.4)	1.00 (reference)		1.00 (reference)	
		G/A+A/A†	31 (6.4)	40 (4.6)	1.45 (0.89, 2.36)	0.13	1.47 (0.84, 2.55)	0.18
		Trend[a]			1.32 (0.82, 2.13)	0.25	1.37 (0.80, 2.34)	0.25
COX-2	rs689466	A/A	321 (66.5)	585 (67.6)	1.00 (reference)		1.00 (reference)	
		A/G	144 (29.8)	267 (30.8)	0.98 (0.77, 1.26)	0.89	0.93 (0.70, 1.22)	0.60
		G/G	18 (3.7)	14 (1.6)	2.34 (1.15, 5.77)	0.02	2.15 (0.99, 4.67)	0.05
		Trend[a]			1.13 (0.92, 1.40)	0.24	1.08 (0.86, 1.37)	0.50
COX-2	rs2745559	C/C	330 (68.6)	556 (64.5)	1.00 (reference)		1.00 (reference)	
		C/A	136 (28.3)	269 (31.2)	0.85 (0.67, 1.09)	0.20	0.83 (0.63, 1.10)	0.19
		A/A	15 (3.1)	37 (4.3)	0.68 (0.37, 1.26)	0.22	0.55 (0.27, 1.10)	0.09
		Trend[a]			0.84 (0.70, 1.04)	0.10	0.80 (0.63, 1.00)	0.05
IL1B	rs1143634	C/C	305 (63.4)	504 (58.2)	1.00 (reference)		1.00 (reference)	
		C/T	143 (29.7)	313 (36.1)	0.76 (0.59, 0.96)	0.02	0.70 (0.53, 0.92)	0.01
		T/T	33 (6.9)	49 (5.7)	1.11 (0.70, 1.77)	0.65	0.96 (0.56, 1.64)	0.89
		Trend[a]			0.90 (0.75, 1.08)	0.26	0.83 (0.68, 1.03)	0.09
IL1B	rs1143633	G/G	194 (40.5)	345 (40.0)	1.00 (reference)		1.00 (reference)	
		G/A	210 (43.8)	408 (47.3)	0.92 (0.72, 1.17)	0.47	0.85 (0.64, 1.12)	0.24
		A/A	75 (15.7)	110 (12.8)	1.21 (0.86, 1.71)	0.27	1.36 (0.93, 2.01)	0.12
		Trend[a]			1.04 (0.89, 1.23)	0.61	1.06 (0.88, 1.28)	0.51

Table 3-4. (*Continued*)

Gene	rs Number	Genotype	N (%) Cases	N (%) Controls	Genotype-based Model OR (95%CI)	Genotype-based Model P	Genotype-based Model* OR (95%CI)*	Genotype-based Model* P*
IL1B	rs1143627	T/T	205 (42.5)	382 (44.1)	1.00 (reference)		1.00 (reference)	
		T/C	222 (46.1)	383 (44.2)	1.08 (0.85, 1.37)	0.52	0.98 (0.75, 1.28)	0.85
		C/C	55 (11.4)	101 (11.7)	1.02 (0.70, 1.47)	0.94	1.03 (0.67, 1.56)	0.91
		Trend[a]			1.03 (0.87, 1.21)	0.74	1.00 (0.82, 1.21)	0.97
PPARG	rs1801282	C/C	373 (77.7)	644 (74.4)	1.00 (reference)		1.00 (reference)	
		C/G	98 (20.4)	201 (23.2)	0.84 (0.64, 1.12)	0.22	0.97 (0.71, 1.32)	0.83
		G/G	9 (1.9)	21 (2.4)	0.74 (0.34, 1.63)	0.46	0.92 (0.71, 1.20)	0.43
		Trend[a]			0.85 (0.67, 1.07)	0.17	0.93 (0.72, 1.21)	0.59

* Adjusted for age, sex, and pack-years. a. Additive model, dose-response, 1df.

† GA and AA were combined because there was 0 A/A in cases and 1 A/A in controls.

Table 3-5. Adjusted Results of SNP and Lung Cancer Associations: Dominant Model.

Gene	rs Number	Subjects with data, N		Minor allele frequency, %		Dominant Model[b] Combined heterozygote and homozygote minor	
		Cases	Controls	Cases	Controls	OR (95% CI)	P
COX-2	rs5275	469	860	33.4	33.0	0.98 (0.76, 1.26)	0.86
COX-2	rs5277	482	865	15.6	15.7	1.17 (0.88, 1.54)	0.28
COX-2	rs4648261	484	866	3.2	2.4	1.43 (0.82, 2.47)	0.21
COX-2	rs689466	483	866	18.6	17.0	1.00 (0.76, 1.30)	0.97
COX-2	rs2745559	481	862	17.3	19.9	0.79 (0.60, 1.04)	0.09
IL1B	rs1143634	481	866	21.7	23.7	0.73 (0.56, 0.95)	0.02
IL1B	rs1143633	479	863	37.6	36.4	0.95 (0.73, 1.23)	0.70
IL1B	rs1143627	482	866	34.4	33.8	0.99 (0.76, 1.27)	0.91
PPARG	rs1801282	480	866	12.1	14.0	0.94 (0.70, 1.26)	0.68

Adjusted for age, sex, and pack-years.
b. Dominant Model: combine heterozygote and homozygote minor

Table 3-6. Associations within Subgroups and Interactions.

	***COX-2* rs5275** TC+CC vs. TT	***COX-2* rs5277** GC+CC vs. GG	***COX-2* rs4648261** GA+AA vs. GG	***COX-2* rs689466** AG+GG vs. AA	***COX-2* rs2745559** CA+AA vs. CC
Current Smoker	1.01 (0.71, 1.42)	1.10 (0.76, 1.60)	1.52 (0.75, 3.07)	1.16 (0.81, 1.66)	0.78 (0.54, 1.13)
Former Smoker	0.91 (0.61, 1.36)	1.29 (0.83, 2.00)	1.38 (0.53, 3.61)	0.86 (0.57, 1.31)	0.83 (0.54, 1.25)
P smoking interaction	0.66	0.58	0.69	0.43	0.65
Mild*	0.73 (0.43, 1.22)	1.34 (0.77, 2.33)	1.80 (0.68, 4.76)	1.06 (0.62, 1.83)	0.57 (0.33, 0.99)[a]
Moderate	1.36 (0.86, 2.14)	0.94 (0.58, 1.54)	1.26 (0.50, 3.19)	1.06 (0.66, 1.69)	0.94 (0.59, 1.52)
Heavy	0.91 (0.61, 1.38)	1.35 (0.86, 2.11)	1.17 (0.44, 3.15)	0.99 (0.65, 1.51)	0.83 (0.54, 1.28)
P pack-years interaction	0.58	0.56	0.41	0.34	0.98
Male	0.92 (0.64, 1.31)	1.43 (0.96, 2.12)	1.83 (0.88, 3.80)	0.95 (0.66, 1.37)	0.76 (0.52, 1.10)
Female	1.06 (0.73, 1.53)	0.95 (0.64, 1.41)	1.07 (0.46, 2.52)	1.05 (0.71, 1.55)	0.82 (0.55, 1.21)
P sex interaction	0.58	0.18	0.33	0.73	0.82
Age<58	1.04 (0.62, 1.74)	1.04 (0.60, 1.79)	2.34 (0.94, 5.82)	1.18 (0.69, 2.01)	0.68 (0.39, 1.19)
Age≥58	0.95 (0.70, 1.28)	1.25 (0.89, 1.74)	1.14 (0.59, 2.22)	0.95 (0.69, 1.29)	0.81 (0.59, 1.11)
P age interaction	0.38	0.33	0.20	0.98	0.91

OR (95%CI) is adjusted for age, sex, and pack-years.

* Based on tertiles of pack-years in controls: mild smoker < 37.5 pack-years; moderate smoker=37.5 to <57.5; heavy smoker=≥57.5.

a. P=0.046

Table 3-6. (*Continued*)

	***IL1B* rs1143634** CT+TT vs. CC	***IL1B* rs1143633** GA+AA vs. GG	***IL1B* rs1143627** TC+CC vs. TT	***PPARG* rs1801282** CG+GG vs. CC
Current Smoker	0.65 (0.46, 0.93)[b]	0.76 (0.54, 1.08)	1.17 (0.83, 1.65)	0.98 (0.66, 1.44)
Former Smoker	0.85 (0.57, 1.27)	1.24 (0.82, 1.85)	0.73 (0.49, 1.10)	0.81 (0.50, 1.32)
P smoking interaction	0.19	0.11	0.10	0.85
Mild*	0.81 (0.53, 1.24)	0.83 (0.50, 1.39)	1.17 (0.70, 1.96)	0.72 (0.40, 1.29)
Moderate	0.77 (0.52, 1.15)	0.89 (0.57, 1.41)	0.93 (0.59, 1.47)	1.00 (0.59, 1.67)
Heavy	0.75 (0.50, 1.12)	1.16 (0.77, 1.77)	0.90 (0.60, 1.35)	0.99 (0.61, 1.62)
P pack-years interaction	0.16	0.13	0.26	0.38
Male	0.58 (0.40, 0.85)[c]	1.15 (0.80, 1.64)	0.93 (0.66, 1.33)	1.45 (0.96, 2.19)
Female	0.92 (0.64, 1.34)	0.77 (0.53, 1.12)	1.05 (0.72, 1.51)	0.58 (0.37, 0.91)[d]
P sex interaction	0.10	0.13	0.66	0.003
Age<58	0.95 (0.56, 1.59)	1.00 (0.60, 1.66)	0.88 (0.53, 1.45)	0.58 (0.31, 1.10)
Age≥58	0.65 (0.48, 0.88)[e]	0.96 (0.71, 1.31)	1.01 (0.75, 1.37)	1.12 (0.79, 1.59)
P age interaction	0.94	0.89	0.81	0.63

OR (95%CI) is adjusted for age, sex, and pack-years.

* Based on tertiles of pack-years in controls: mild smoker < 37.5 pack-years; moderate smoker=37.5 to <57.5; heavy smoker=≥57.5.

b. P=0.019

c. P=0.004

d. P=0.016

e. P=0.006

Table 3-7. Haplotype Analysis Results.

Block	Haplotype	Case Freq (%)	Control Freq (%)	OR *	P*
Block 1	rs5275-rs5277-rs689466-rs2745559 (*COX-2*)				0.28**
	TGAC	14.6	14.8	1.04	0.77
	TCAC	15.7	15.6	1.15	0.27
	TGAA	17.4	19.8	0.81	0.07
	TGGC	18.8	16.9	1.11	0.40
	CGAC	33.5	32.8	1.00	0.98
Block 2	rs1143634-rs1143633 (*IL1B*)				0.24**
	TG	22.0	23.8	0.84	0.10
	CA	37.8	36.4	1.09	0.36
	CG	40.2	39.8	1.05	0.58

* Adjusted for age, sex, and pack-years.
** P for global association (H-1 df omnibus test)

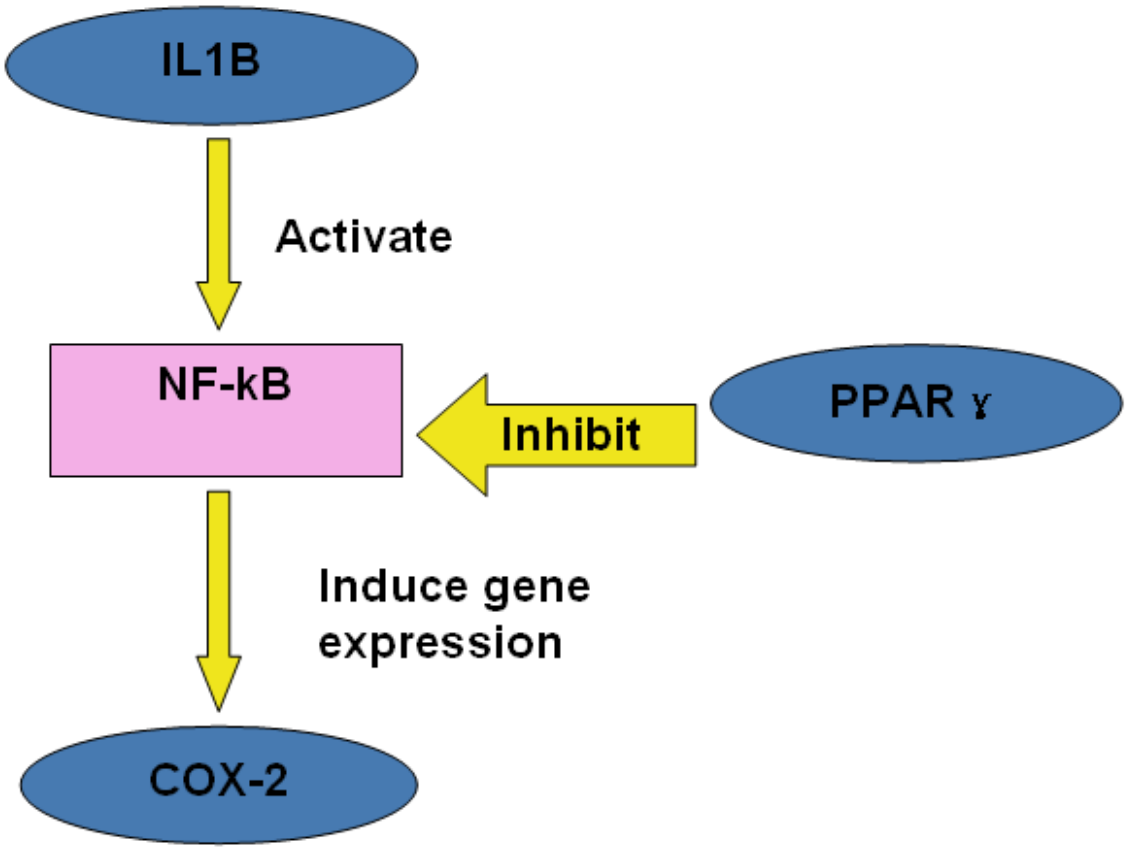

Figure 3.1. Schematic Figure of Relationship between *IL1B, COX-2* and *PPARG* Genes

Cigarette smoking stimulates airway epithelial cells and immune cells to release pro-inflammatory cytokines, such as IL1B. This is known to result in activation of the NF-kB signaling pathway, leading to up-regulation of various inflammation-related genes, including COX-2. PPARγ expression could lead to decrease in COX-2 expression through a negative feedback loop involving NF-kB.

4.0 MANUSCRIPT 2: POLYMORPHISMS IN TRANSFORMING GROWTH FACTOR BETA1 (*TGFB1*), INTERLEUKINE-6 (*IL6*) AND INTERLEUKINE-10 (*IL10*) AND LUNG CANCER RISK IN CAUCASIAN SMOKERS

Manuscript in preparation

Yan Du, MS[1], Joel L. Weissfeld, MD, MPH[1], Marjorie Romkes, PhD[2], Frank Sciurba, MD[3], Eleanor Feingold, PhD[4], Brenda Diergaarde, PhD[1]

[1] Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA

[2] Department of Medicine, Center for Clinical Pharmacology, University of Pittsburgh, Pittsburgh, PA, USA

[3] Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, University of Pittsburgh, Pittsburgh, PA, USA

[4] Department of Human Genetics and Biostatistics, Graduate School of Public Health, University of Pittsburgh, PA, USA

4.1 ABSTRACT

Background Inflammation plays a role in lung carcinogenesis. Cytokines are an important component of the inflammatory response. The aim of this study was to determine whether common single nucleotide polymorphisms (SNPs) in three cytokine genes were involved in predisposing an individual to lung cancer.

Methods We evaluated associations of seven polymorphisms in *TGFB1*, *IL6*, and *IL10* with lung cancer risk in 484 lung cancer cases and 866 controls from the Pittsburgh metro area. Multiple logistic regression models adjusting for age, sex, and pack-years were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs). Stratified analyses and gene-environment interaction tests were also conducted.

Results SNP rs1800872 in *IL10* was associated with lung cancer risk only in adjusted models. The A allele was associated with a decreased risk of lung cancer (per allele OR=0.81, 95%CI= 0.65-0.995, P_{trend}=0.04). In subgroup analysis, this protective effect remained, and was significant among mild (<37.5 pack-years) smokers (dominant model OR=0.57, 95%CI=0.33-0.96). Minor allele carriers (AG/GG) of another *IL10* SNP, rs1800896, also showed a protective effect in the younger age group (<58 years), with a dominant model OR of 0.56 (95%CI=0.33-0.95). No significant associations between lung cancer and other SNPs were identified.

Conclusions Our study suggests that common variation in *IL10* may influence susceptibility to lung cancer.

Implications for public health Valid markers of lung cancer susceptibility may help define higher risk groups that would benefit most from an individualized chemoprevention strategy and therapy.

4.2 INTRODUCTION

In the United States, lung cancer is the leading cause of cancer mortality in both men and women. It is the second leading form of cancer, and is expected to affect 222,520 people in 2010.[1] Smoking is the most well established risk factor for lung cancer.[2] However, only a fraction of smokers develop lung cancer[58], suggesting that other factors such as genetics may influence disease susceptibility. There is an increasing recognition of the role that inflammatory pathways play in malignant transformation including lung cancer development.[130,131] Activated inflammatory processes generate reactive oxygen and nitrogen species, secrete growth stimulatory cytokines, chemokines and pro-angiogenic factors, in consequence, favor lung cancer development.[132]

Cytokines are soluble proteins or glycoproteins that act as mediators of cell-to-cell communications, and they are an integral component of the immune response. Cytokines are pleiotropic, and the role of cytokines in tumorigenesis is complex. Studies indicate that there are differences between lung cancer cases and controls in circulating cytokine expression profiles.[79,133-137] The expression and functional effects of cytokine genes are influenced by genetic variants in these genes; therefore, polymorphisms in such genes might play a role in lung cancer development. However, results from genetic epidemiological studies are inconclusive.[71,81,116,138-141]

The present study includes seven putative functional single-nucleotide polymorphisms (SNPs) in three cytokine genes (*TGFB1, IL6, and IL10*) involved in the inflammatory response.

Transforming growth factor beta 1 (TGFB1) is a master regulator of the immune system.[142] TGFB1 inhibits the proliferation and induces the differentiation of normal bronchial epithelial cells.[143] Lung cancer patients were found to have higher levels of TGFB1 in the bronchoalveolar lavage (BAL) in comparison to BAL of healthy subjects.[144] TGFB1 expression is often up-regulated in non-small cell lung cancer (NSCLC) tumors, and elevated plasma levels of TGFB1 are related to a poorer prognosis for patients.[145]

Interleukin-6 (IL6) is a pro-inflammatory cytokine released in response to infection, trauma, and neoplasia. It has a key role in immune and acute-phase response, and hematopoiesis.[146-148] IL6 is a major cytokine that is expressed in tumor-infiltrating cells. Higher levels of serum IL6 were detected in lung cancer patients compared with healthy controls.[134] Several studies have demonstrated that anti-inflammatory drugs may inhibit angiogenesis and IL6 is one of the pivotal determinants of the angiogenic activity of NSCLC.[149,150]

Interleukin-10 (IL10) is mainly an anti-inflammatory cytokine produced by a number of cells including normal and neoplastic cells. It has been indicated in autoimmunity, transplantation tolerance and tumorigenesis. Elevated serum levels of IL10 were found in NSCLC patients when compared to healthy controls.[151] Furthermore, amplified production of immunosuppressive IL10 by NSCLC and higher serum concentrations of IL10 in NSCLC patients have both been shown to correlate with reduced survival.[151-153] The promoter region of *IL10* spans at least 5kb upstream of the transcription start point, and it is known to contain at least 27 polymorphic sites. Alterations in IL10 expression have been linked to polymorphisms in the promoter region of *IL10* gene, such as rs1800896 and rs1800872.[154]

The aim of the current study was to investigate whether common polymorphisms in the cytokine genes *TGFB1, IL6* and *IL10* are associated with lung cancer susceptibility in a Caucasian population.

4.3 MATERIALS AND METHODS

4.3.1 Study Population

This study utilized data and specimens from the University of Pittsburgh Cancer Institute (UPCI) Specialized Program of Research Excellence (SPORE) in Lung Cancer. The participants were recruited at the University of Pittsburgh Medical Center (Pittsburgh, PA). The study was approved by the institutional review board (IRB) at the University of Pittsburgh. All participants provided written informed consent.

Briefly, cases were newly diagnosed lung cancer patients treated by surgery between 1990 and 2008. Eligibility criteria included: 1) current or ex-cigarette smoker, 2) cumulative cigarette dose exposure $\geq$10 pack-years, 3) 45-85 years old at time of lung cancer diagnosis, 4) entry within one year of lung cancer diagnosis, and 5) pathologically verified lung cancer diagnosis (excluding carcinoid). These selection procedures identified 923 patients, including 588 (63.7% of 923) with DNA available.

The control series is a simple random sample from a restricted set of participants in the Pittsburgh Lung Screening Study (PLuSS), a helical computed tomography (CT) lung cancer screening study. The selection of the study participants has been previously described.[34] Briefly, between 2002 and 2005, PLuSS enrolled 50-79 year-old current and ex-cigarette smokers of at

least one-half pack/day for at least 25 years. Using self-report, PLuSS excluded individuals who: 1) quit smoking more than 10 years earlier, 2) had a history of lung cancer, or 3) had a chest CT scan within one year of enrollment. Selection as a control was restricted to the *N*=3,463 (92.2% of all PLuSS enrollees) white or black race CT-screened PLuSS participants with buffy coat or whole blood available and no interval lung cancer diagnosis (as of 9/10/2008). A simple random sample of size n=1000 was selected, of which 929 (92.9%) samples had DNA available for the current study.

For controls, a written questionnaire was used to obtain information about demographics and smoking history. For cases, information was extracted from medical records.

4.3.2 SNP Selection and Genotyping

Seven SNPs in three genes (*TGFB1, IL6, and IL10*) were genotyped. All SNPs were selected on the basis of reported functional or biological relevance; or previous report of an association with lung cancer or another cancer, or an inflammatory disorder. Two SNPs in *TGFB1* (rs2241712, rs1800469), two SNPs in *IL6* (rs2069860, rs1800795) and three SNP in *IL10* (rs1800896, rs1800872, rs3024509) were selected for genotyping. A summary of SNP location and presumed function was presented in **Table 4-1**.

Genomic DNA was extracted from whole blood samples using standard salt-based methods. Genotyping of 588 cases and 929 controls was performed at the University of Pittsburgh Genomics and Proteomics Core Laboratories (Pittsburgh, PA). All SNPs were genotyped using MassARRAY® iPLEX Gold (Sequenom, Inc., San Diego, CA); the SNP specific and mass extend oligonucleotides, and assays were designed using Sequenom RealSNP (www.realsnp.com) and MassARRAY Assay Design version 3.1 (Sequenom, Inc., San Diego,

CA). Genotyping was performed for cases and controls together; laboratory technicians were blinded to case-control status. Fifty duplicate samples were included to assess laboratory reliability. The concordance rate was 100%.

More than 95% of all DNA samples were successfully genotyped for all 7 SNPs. Of the 1517 samples genotyped, 31 samples (21 cases and 10 controls) failed (call rate<90%), leaving 567 cases and 919 controls for analysis. The present study includes only self-reported Caucasians, the major racial/ethnic subgroup. 83 cases and 53 controls of black or unknown race were excluded, leaving 484 cases and 866 controls for subsequent analyses.

4.3.3 Statistical Analysis

Participant characteristics in the cases and controls were compared by Wilcoxon rank sum test for continuous variables and Chi-square test for categorical variables.

Genotype and allele frequencies were calculated, observed genotype frequencies in the control population were tested for deviation from Hardy-Weinberg equilibrium (HWE) using the exact test.

For each SNP, odds ratios (ORs) for lung cancer risk and the corresponding 95% confidence intervals (CIs) were calculated using multiple logistic regression models, adjusting for known risk factors for lung cancer, i.e., age, sex, and cumulative tobacco exposure (pack-years). We used the genotype-based genetic model for individual SNPs. The genotype-based model is defined as heterozygotes (1 minor allele) versus common allele homozygotes (0 rare alleles) or rare allele homozygotes (2 minor alleles) versus common allele homozygotes.

Linear trend and allelic ORs were assessed using numerical scores assigned to the ordered categories (i.e., 0 to the category of major allele homozygotes, 1 to the category of

heterozygotes, and 2 to the category of minor allele homozygotes) as continuous variable in the model.

Additionally, because the number of rare-allele homozygotes was relatively small, we then combined heterozygotes and rare-allele homozygotes in the logistic regression analyses; common-allele homozygotes were used as the reference group (indicating dominant model).

We tested interactions with cross product interaction terms of each SNP and environmental factors such as sex (males vs. females), smoking status (current vs. former), and continuous age and pack-years. For stratified analyses, we created an indicator variable of age greater or less than 58 years old (the median in controls), sex (male vs. female) and smoking status (current vs. former smoker), pack-years (in tertiles, cut-points 37.5 and 57.5 based on the distribution in controls). Stratum-specific ORs were obtained under the dominant model.

For each SNP, we further evaluated whether sex, age, or smoking pack-years were distributed differentially among different genotypes within lung cancer patients and within controls.

We repeated the analysis restricting our cases to NSCLC only. We then performed subgroup analyses of adenocarcinoma (ADC) and squamous cell carcinoma (SCC), the two predominant histological types, comparing each subgroup of cases against the entire group of controls. We also performed case-case comparison, comparing SCC against ADC, to assess whether any of these SNPs may play a role in a specific disease etiology.

PLINK (version 1.07) was used to estimate haplotype frequencies and risk estimates.

All significance tests were two sided; *P* values <0.05 were considered statistically significant. Data analyses were conducted using SAS/Genetics software (version9.1.3; SAS Institute, Cary, NC) and PLINK (version 1.07).

4.4 RESULTS

4.4.1 Study Subjects

Table 4-2 presents characteristics of the lung cancer cases (N=484) and controls (N=866). The proportions of males and females were similar in cases and controls (P=0.07). Cases were significantly older (median=67 years, inter quartile range [IQR] = 61-74 years) than controls (median=58 years, IQR=54-63 years) (P<0.0001). Cases also reported greater cumulative tobacco exposure than controls (median 50 vs. 47.5 pack-years, P=0.02). There was a greater proportion of current smokers among the controls than among the cases (59.1% vs. 48.9%, P=0.0004). The most common histological types of lung cancer were adenocarcinoma (n=230, 47.5%) and squamous cell carcinoma (n=173, 35.7%). Most NSCLC presented at the early stage (47.2% stage I, 16.1% stage II).

4.4.2 Associations of Lung Cancer with Genetic Polymorphisms

All SNPs were in HWE in controls (P>0.05) (**Table 4-3**). The genotype distributions for each SNP among the cases and controls, and the genotype-based model and trend test results are shown in **Table 4-4**. There were no rs2069860 minor allele homozygotes among the cases and only one among the controls, therefore, AT and TT genotypes were combined. In unadjusted results, there was no statistically significant association between any SNP and lung cancer risk

from the genotype-based test or trend test. However, after adjusting for age, sex, and pack-years, the A allele of *IL10* rs1800872 showed a protective effect against lung cancer, with a per allele OR of 0.81 (95%CI=0.65-0.995, P_{trend}=0.04). All other SNP and lung cancer associations remained non-significant (**Table 4-4**).

The results under the dominant model are presented in **Table 4-5**. No significant associations were observed.

4.4.3 Stratified Analysis and Interaction Results

Table 4-6 shows results for SNP and lung cancer associations within subgroups and SNP-environment interaction P-values. The protective effect of *IL10* rs1800872 A allele remained significant only in mild (smoking exposure<37.5 pack-years) smokers, with an OR of 0.57 (95%CI=0.33-0.96). Another *IL10* SNP, rs1800896, showed a borderline significant interaction ($P_{interaction}$=0.05) with continuous age. CT+TT genotypes showed a significant protective effect among people younger than 58 (OR=0.56, 95%CI=0.33-0.95, P=0.03), but a non-significant increased risk among people of 58 or older (OR=1.23, 95%CI=0.92-1.65). No other statistically significant gene-environment interaction was observed.

4.4.4 Risk of NSCLS, ADC, and SCC

When analyses were restricted to NSCLC cases only, results were similar to the main analyses. The A allele of *IL10* rs1800872 showed a borderline protective effect with an adjusted per allele OR=0.80 (95%CI=0.65-0.998, P_{trend}=0.048). No significant association between any SNP and ADC was found. However, for SCC, the A allele of *IL10* rs1800872 again showed a protective

effect both in unadjusted (P_{trend}=0.03) and adjusted results (P_{trend}=0.02). The case-case comparison (SCC vs. ADC) also showed that the same SNP, *IL10* rs1800872, was associated with the differences between SCC and ADC (ADC as the reference group, dominant model OR=0.72, 95%CI=0.40-0.94, P=0.03).

4.4.5 Haplotype Analysis Results

Two haplotype block sets were identified, rs1800469-rs2241712 in *TGFB1*, and rs3024509-rs1800872-rs1800896 in *IL10*. TAA haplotype of *IL10* showed a borderline significant reduced risk of lung cancer (OR=0.82, P=0.06) compared to all other haplotypes. No significant association was observed for *TGFB1* haplotypes. The global association was not significant for either block (**Table 4-7**).

4.5 DISCUSSION

In the present case-control study, we evaluated the relationship of seven polymorphisms in three cytokine genes with lung cancer susceptibility. We observed that the minor allele (A) of rs1800872 in *IL10* was associated with a decreased risk of lung cancer (per allele OR=0.81, 95%CI=0.65-0.995, P_{trend}=0.04), after adjustment for age, sex, and pack-years. We also detected a borderline significant interaction between *IL10* rs1800896 and age, minor allele showed a protective effect in the younger age group, but was associated with a non-significant increased risk in the older age group.

IL10 is a key anti-inflammatory cytokine and is produced by a number of different cells including lymphocytes, monocytes/macrophages and various tumor cell lines.[155,156] IL10 was originally designated as a cytokine synthesis inhibitory factor because of its ability to inhibit synthesis of pro-inflammatory cytokines such as TNF-α, IL1, IL6 and IL12.[157,158] Studies have reported raised levels of serum and peri-tumoral IL10 production in many malignancies[159-161], including lung cancer,[162] which have been inferred in support of a role for IL10 in tumor escape from the immune response. *IL10* rs1800896 (-1082) AG/GG genotypes and *IL10* rs1800872 (-592) CA/CC genotypes are generally considered to be associated with a higher IL10 production.[154]

Several studies have investigated the role of rs1800872 in lung cancer development. Similar to our finding, in a study conducted by Engels *et al.*[71] in a Caucasian population (1538 cases and 1705 controls), a non-significant reduced risk of lung cancer was observed among minor allele carriers of rs1800872 (CA+AA vs. CC: OR=0.93, 95%CI: 0.80-1.07). In a study conducted in Taiwan, Shih *et al.*[140] reported an increased risk of NSCLC among their minor allele carriers (CA+CC vs. AA, OR=1.70, 95%CI: 1.12-2.60). Contrary to our results, a nested case-cohort study (403 cases, 744 sub-cohort)[116] within the Danish "Diet, Cancer, and Health" cohort observed that minor allele carriers (CA/AA) were at higher risk (IRR=1.60, 95%CI: 1.13-2.27) of lung cancer than major allele homozygotes (CC), but only in the fully adjusted model. The reasons for the discrepancies are unclear. It may be caused by random error, or it may be due to different population characteristics. The Shih[140] study was conducted in an Asian population with a different minor allele (C). The Danish cohort[116] included never smokers, while our population was consisted of current or former smokers only, with a relatively intense pack-year exposure. *IL10* has both immunosuppressive and anti-angiogenic functions and consequently has

both tumor-promoting and tumor-inhibiting properties. The various overlapping functions of cytokines are determined by their local concentration, the type and the maturational stage of the responding cell, and the presence of other cytokines and their mediators. *IL10* may act differently in heavy smokers with more prominent respiratory and systematic inflammation. Thus, the conflicting results may reflect the complicated mechanism of *IL10* in disease etiology.

We also looked at confounding. The association observed for the *IL10* rs1800872 polymorphism and lung cancer risk became statistically significant only after adjustment for age, sex and smoking pack-years. In further analysis, we observed minor allele of this SNP was significantly associated with increased age in the control group (P=0.03). In post-hoc analysis, we did forward selection of covariates. We observed that age was the factor that confounded the association. Age itself may not be the confounder, since the evaluated genetic polymorphisms are unlikely to be associated with age. But age is an index of many other factors in the aging process, the adjustment of age may have adjusted for other factors. For example, this cytokine SNP may be associated with aging- and inflammation-related proteins.

The AA genotype of rs1800896 (-1082), causing a lower IL10 expression, has been associated with several cancers.[154,163,164] We observed a borderline significant interaction between this SNP and age: minor allele carriers (AG/GG) had a reduced risk of lung cancer in the younger age group, but were at higher risk for people aged 58 or older. The study by Engels *et al.*[71] reported a non-significant association (AG+GG vs. AA: OR=0.94, 95%CI=0.80-1.10). Another study conducted in a Chinese population[141] also reported no association (AG+GG vs. AA: OR=1.02, 95%CI=0.49-2.13). However, Shih *et al.*[140] reported AG+GG genotypes were at increased risk of NSCLC compared to AA genotype (OR=5.98, 95%CI=2.95-12.1). We can't draw definitive conclusions from our exploratory subgroup analysis due to the small sample size.

Nevertheless, the effect observed was statistically significant and follow-up studies are needed to clarify the association.

Lung cancer is characterized by an aggressive clinical course and poor response to immunotherapy[52], probably because lung cancer cells are capable to produce a wide variety of immunosuppressive factors that may allow their escape from immune recognition. [53,54,165] TGFB1 exerts inhibitory effects on cells of all aspects of the immune system.[142] Over-expression of TGFB1 has been indicated in immune suppression.[143] TGFB1 is involved in the process to depress the immune response including T-helper cells, cytotoxic T lymphocytes, dendritic cells, macrophages, natural killer cells and B cells.[166] Cancer cells acquire the ability to escape the immune-surveillance during tumorigenesis. One of the multiple mechanisms by which tumors can evade to immune system is the secretion of TGFB1 by cancer and stromal cells.[142] The therapeutic potential of TGFB1 antagonists and their receptor antagonists in cancer therapy have been investigated.[167] Several drugs are currently under evaluation to inhibit various stages of TGFB1 signaling, and some promising data have been accumulated.[167,168] Since functional polymorphisms can alter the expression of *TGFB1*, it is important to know patients' individual genetic profile in order to evaluate which individuals will benefit most from the therapeutic use of these molecules. However, we did not observe any significant association between two promoter region polymorphisms (rs2241712 and rs1800469) of *TGFB1* and lung cancer risk. To our knowledge, there is only one study[138] that has investigated the association between *TGFB1* rs1800469 and NSCLC risk, and they did not find any association in Caucasian women (CT+TT vs. CC: OR=0.98, 95%CI=0.68-1.42), nor in African American women (CT+TT vs. CC: OR=1.00, 95%CI=0.49-2.03).

IL6 is a major cytokine that is expressed in tumor-infiltrating cells. Significantly higher serum levels of IL6 have been observed in lung cancer patients compared with healthy controls.[134] We studied one coding non-synonymous SNP (rs2069860) and one promoter SNP (rs1800795) in *IL6*, and no significant association was found. Several other studies[71,81,116,138,139] also looked at SNP rs1800795 in Caucasian populations. Similar to our results, they did not observe any significant association. *IL6* cytokine gene transcription and expression is tightly regulated at the level of its promoter, which acts as a sophisticated biosensor for environmental stress, thus controlling immunological homeostasis.[169] Studies provide evidence that genetic polymorphisms in the promoter region influence *IL6* transcription not by a simple additive mechanism but rather through complex interactions determined by the haplotype.[170] Thus, study a single polymorphism (rs1800795) in isolation will not reveal the overall functional effect of this polymorphism in combination with other functional polymorphisms.

Because of the different features of lung cancer subtypes, we performed subgroup analysis of ADC and SCC. ADC is located mostly in the peripheral part of the lung, while SCC is located mostly in the central part of the lung. It is postulated that inflammation and irritation, particularly from smoke, might exercise different biological features in the central and peripheral part of the lung. It may thus be hypothesized that tobacco-induced inflammation is differentially associated with ADC and SCC, and that different inflammatory genes are involved in the process. Our result of *IL10* rs1800982 is associated with SCC but not ADC has provided some evidence to support this theory. However, due to the small sample size in the subgroup analysis, no definite conclusion could be drawn and the result needs to be investigated in future studies.

We have limited power to detect gene-environment interactions and subgroup associations. To assess a two-way gene environment interaction in a case-control analysis with

80% power, given a 10% frequency of both the genetic polymorphism and the environmental factor, a main effect relative risk (RR) of 1.3 for both the genetic and environmental exposure, an expected RR associated with interaction=2.0, and an alpha level of 0.05, a minimum of one thousand cases is required.[171-173]

It is also important to note that the tested SNPs themselves may not necessarily be functional, but may be in linkage disequilibrium (LD) with the true functional SNPs. We included a few SNPs per gene. However, the SNPs we included are in LD with many other SNPs. For example, *IL6* rs1800795 is in strong LD with at least eight other SNPs across the gene (Seattle SNP database, http://pga.gs.washington.edu/). If any of these SNPs is the functional one, we would be able to detect an effect.

We only included three cytokine genes in the current study. Ideally, many genes with functional significance should be assessed, especially because it is known that different cytokines interact with each other, forming networks that initiate gene activation and suppression.[174] Future studies should integrate pathway analysis data in larger sample sizes, and include environmental risk factor data such as demographic information, smoking behavior, comorbidities, and disease status. Understanding how genetic networks are modulated by other factors to affect disease risk may help clarify etiologic relationships that are presently confusing or inconsistent.

4.6 TABLES

Table 4-1. SNP Information.

Gene	rs Number	Chr	Chr Position*	Nucleotide	Allele	Locations and Presumed Function
TGFB1	rs2241712	19q13.1	41869756	-10807G>A	A→G	In the promoter region; alter gene expression.
TGFB1	rs1800469	19q13.1	41860296	-509T>C	C→T	In the promoter region; T allele is associated with increased circulating concentrations of TGF-B1 in plasma.[I]
IL6	rs2069860	7p21	22771038	Ex5+14A	A→T	Coding non-synonymous; Val[V]→ Asp[D].
IL6	rs1800795	7p21	22766645	-236C>G (-174C>G)	G→C	In the promoter region; C allele is associated with higher levels of IL-6 protein and the C-reactive protein.[II]
IL10	rs1800896	1q31-q32	206946897	-1082G>A (-1116A>G)	A→G	5' near gene.
IL10	rs1800872	1q31-q32	206946407	592C>A (-627A>C, -6653A>C)	C→A	5' near gene.
IL10	rs3024509	1q31-q32	206943297	IVS3-58T>C	T→C	Intron_3.

*dbSNP Chromosome Report, GRCh37 Sequence

Database of Single Nucleotide Polymorphisms (dbSNP). Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine. (dbSNP Build ID: {build ID}). Available from: http://www.ncbi.nlm.nih.gov/SNP/.

I. Grainger DJ, Heathcote K, Chiano M, et al. Genetic control of the circulating concentration of transforming growth factor type beta1. Hum Mol Genet 1999;8:93-7.

II. Vickers MA, Green FR, Terry C, et al. Genotype at a promoter polymorphism of the interleukin-6 gene is associated with baseline levels of plasma C-reactive protein. Cardiovasc Res 2002;53:1029-34.

Table 4-2. Population Characteristics.

Characteristic	Cases (N=484)	Controls (N=866)	P*
Male, n(%)	266 (55.0)	432 (49.9)	0.07
Age (y), median (IQR)	67 (61, 74)	58 (54, 63)	<0.0001
Age (y), n (%)			<0.0001
<50	10 (2.1)	1 (0.1)	
50-59	83 (17.2)	516 (59.6)	
60-64	87 (18.0)	175 (20.2)	
65-69	112 (23.1)	103 (11.9)	
70+	192 (39.7)	71 (27.0)	
Smoke Status			0.0004
Current	224 (48.9)	511 (59.1)	
Former	234 (51.1)	354 (40.9)	
Smoking intensity duration, Pack-years, median (IQR)	50 (35, 75)	47.5 (33, 63)	0.02
Smoking intensity duration, Pack-years, n(%)			<0.0001
<30	77 (15.9)	161 (18.6)	
30-44	109 (22.5)	228 (26.3)	
45-59	112 (23.1)	228 (26.3)	
60-74	64 (23.1)	135 (15.6)	
75+	122 (25.2)	114 (13.2)	
Lung cancer histology, n(%)			
Adenocarcinoma	230 (47.5)	--	
Squamous cell carcinoma	173 (35.7)	--	
Large-cell carcinoma	11 (2.3)	--	
Other/unspecified non-small cell carcinoma	42 (8.7)	--	
Small-cell carcinoma	14 (2.9)	--	
Neuroendocrine	9 (1.9)	--	
Other/unspecified	5 (1.0)	--	
Lung cancer stage, n(%)†			
I	217 (47.2)	--	
II	74 (16.1)	--	
III	144 (31.3)	--	
IV	25 (5.4)	--	

Abbreviation: IQR, interquartile range.

* P values are derived by x^2 test, except for age and pack-years, where the Wilcoxon rank sum test was used.

† Stage is limited to non-small cell carcinoma

Table 4-3. Allele Frequencies and HWE in Controls.

Gene	rs Number	N	Allele	Allele Counts	Allele Frequencies, %	HWE P in controls (Exact Test)
TGFB1	rs2241712	865	A	1128	65.2	0.45
			G	602	34.8	
TGFB1	rs1800469	865	C	1142	66.0	0.36
			T	588	34.0	
IL6	rs2069860	866	A	1718	99.2	0.06
			T	14	0.8	
IL6	rs1800795	866	G	1027	59.3	0.29
			C	705	40.7	
IL10	rs1800896	866	A	924	53.3	0.38
			G	808	46.7	
IL10	rs1800872	866	C	1307	75.5	0.71
			A	425	24.5	
IL10	rs3024509	866	T	1642	94.8	0.50
			C	90	5.2	

Table 4-4. Unadjusted and Adjusted Results of SNP and Lung Cancer Associations: Genotype-based Model and Trend Test.

Gene	rs Number	Genotype	N (%) Lung Cancer	N (%) Control	Genotype-based Model OR (95% CI)	Genotype-based Model P	Genotype-based Model* OR (95% CI)*	Genotype-based Model* P*
TGFB1	rs2241712	AA	210 (44.1)	373 (43.1)	1.00 (reference)		1.00 (reference)	
		AG	209 (43.9)	382 (44.2)	0.97 (0.77, 1.23)	0.81	1.04 (0.80, 1.37)	0.76
		GG	57 (12.0)	110 (12.7)	0.92 (0.64, 1.32)	0.65	0.93 (0.62, 1.40)	0.73
		Trend[a]			0.96 (0.82, 1.14)	0.66	0.99 (0.82, 1.19)	0.90
TGFB1	rs1800469	CC	216 (45.2)	383 (44.3)	1.00 (reference)		1.00 (reference)	
		CT	210 (43.9)	376 (43.5)	0.99 (0.78, 1.26)	0.94	1.02 (0.78, 1.34)	0.86
		TT	52 (10.9)	106 (12.2)	0.87 (0.60, 1.26)	0.46	0.91 (0.60, 1.39)	0.66
		Trend[a]			0.95 (0.81, 1.12)	0.55	0.98 (0.81, 1.18)	0.80
IL6	rs2069860	AA	475 (98.1)	853 (98.5)	1.00 (reference)		1.00 (reference)	
		AT+TT†	9 (1.9)	13 (1.5)	1.35 (0.56, 3.22)	0.50	1.58 (0.59, 4.21)	0.36
		Trend[a]			1.14 (0.51, 2.56)	0.75	1.35 (0.55, 3.31)	0.52
IL6	rs1800795	GG	184 (38.3)	312 (36.0)	1.00 (reference)		1.00 (reference)	
		GC	216 (44.9)	403 (46.5)	0.91 (0.71, 1.16)	0.45	0.96 (0.72, 1.26)	0.75
		CC	81 (16.9)	151 (17.5)	0.91 (0.66, 1.26)	0.57	1.12 (0.78, 1.62)	0.54
		Trend[a]			0.95 (0.81, 1.11)	0.48	1.04 (0.87, 1.24)	0.67
IL10	rs1800896	AA	145 (30.0)	253 (29.2)	1.00 (reference)		1.00 (reference)	
		AG	228 (47.2)	418 (48.3)	0.95 (0.73, 1.24)	0.71	1.06 (0.79, 1.43)	0.68
		GG	110 (22.8)	195 (22.5)	0.98 (0.72, 1.34)	0.92	1.11 (0.78, 1.57)	0.57
		Trend[a]			0.99 (0.85, 1.16)	0.89	1.05 (0.88, 1.25)	0.57
IL10	rs1800872	CC	288 (60.0)	495 (57.2)	1.00 (reference)		1.00 (reference)	
		CA	170 (35.4)	317 (36.6)	0.92 (0.73, 1.17)	0.50	0.84 (0.64, 1.09)	0.19
		AA	22 (4.6)	54 (6.2)	0.70 (0.42, 1.17)	0.18	0.59 (0.34, 1.05)	0.07
		Trend[a]			0.88 (0.73, 1.06)	0.19	0.81 (0.65, 0.995)	0.04
IL10	rs3024509	TT	433 (89.7)	779 (90.0)	1.00 (reference)		1.00 (reference)	
		TC	47 (9.7)	84 (9.7)	1.01 (0.69, 1.47)	0.97	1.15 (0.75, 1.76)	0.51
		CC	3 (0.6)	3 (0.3)	1.80 (0.36, 8.95)	0.47	1.60 (0.26, 10.03)	0.61
		Trend[a]			1.06 (0.75, 1.49)	0.75	1.17 (0.80, 1.73)	0.42

* Adjusted for age, sex, and pack-years. a. Additive Model, dose-response, 1df.

† AT and TT were combined because there was 0 TT in cases and 1 TT in controls.

Table 4-5. Adjusted Results of SNP and Lung Cancer Associations: Dominant Model.

Gene	rs Number	Subjects with data, N		Minor allele frequency, %		Dominant Model[b] Combined heterozygote and homozygote minor	
		Cases	Controls	Cases	Controls	OR (95% CI)	P
TGFB1	rs2241712	476	865	33.9	34.8	1.02 (0.79, 1.31)	0.89
TGFB1	rs1800469	478	865	32.9	34.0	1.00 (0.78, 1.29)	1.00
IL6	rs2069860	484	866	0.9	0.8	1.48 (0.56, 3.87)	0.43
IL6	rs1800795	481	866	39.3	40.7	1.00 (0.77, 1.30)	0.99
IL10	rs1800896	483	866	46.4	46.7	1.08 (0.82, 1.42)	0.60
IL10	rs1800872	483	866	22.3	24.5	0.80 (0.62, 1.03)	0.09
IL10	rs3024509	480	866	5.5	5.2	1.17 (0.77, 1.77)	0.46

Adjusted for age, sex and pack-years.

b. Dominant Model: combine heterozygote and homozygote minor.

Table 4-6. Associations within Subgroups and Interactions.

	N		*TGFB1* rs2241712	*TGFB1*rs1800469	*IL6* rs2069860	*IL6* rs1800795
	Cases	Controls	AG+GG vs. AA	CT+TT vs. CC	AT+TT vs. AA	GC+CC vs. GG
Current Smoker	224	511	1.05 (0.74, 1.48)	0.97 (0.69, 1.37)	1.96 (0.58, 6.62)	0.90 (0.63, 1.29)
Former Smoker	234	354	1.00 (0.67, 1.50)	1.07 (0.72, 1.60)	1.01 (0.20, 4.99)	1.23 (0.82, 1.85)
P smoking interaction			0.79	0.78	0.76	0.36
Mild*	133	266	0.98 (0.59, 1.63)	0.92 (0.55, 1.53)	0.55 (0.07, 4.32)	0.98 (0.58, 1.66)
Moderate	161	307	1.16 (0.74, 1.83)	1.20 (0.77, 1.88)	0.90 (0.21, 3.81)	1.00 (0.62, 1.60)
Heavy	190	293	0.96 (0.65, 1.43)	0.94 (0.63, 1.40)	8.57 (0.86, 85.53)	0.99 (0.67, 1.48)
P pack-years interaction			0.61	0.52	0.07	0.98
Male	266	432	1.09 (0.76, 1.55)	1.10 (0.77, 1.56)	1.73 (0.46, 6.51)	0.89 (0.62, 1.28)
Female	218	434	0.95 (0.65, 1.37)	0.90 (0.62, 1.30)	1.25 (0.31, 5.05)	1.12 (0.77, 1.65)
P sex interaction			0.60	0.45	0.74	0.38
Age<58	73	427	1.33 (0.79, 2.24)	1.24 (0.74, 2.07)	1.48 (0.30, 7.19)	1.58 (0.90, 2.78)
Age≥58	411	439	0.95 (0.73, 1.25)	0.97 (0.74, 1.27)	1.56 (0.49, 4.97)	0.82 (0.62, 1.08)
P age interaction			0.20	0.27	0.91	0.23

OR (95%CI) is adjusted for age, sex, and pack-years.

* Based on tertiles of pack-years in controls: mild smoker < 37.5 pack-years; moderate smoker=37.5 to <57.5; heavy smoker=≥57.5.

Table 4-6. (*Continued*)

	N		***IL10* rs1800896**	***IL10* rs1800872**	***IL10* rs3024509**
	Cases	Controls	AG+GG vs. AA	CA+AA vs. CC	TC+CC vs. TT
Current Smoker	224	511	1.20 (0.83, 1.76)	0.89 (0.63, 1.25)	1.82 (1.07, 3.10)
Former Smoker	234	354	0.97 (0.63, 1.49)	0.68 (0.45, 1.03)	0.73 (0.37, 1.44)
P smoking interaction			0.27	0.59	0.35
Mild*	133	266	1.00 (0.58, 1.72)	0.57 (0.33, 0.96)[a]	0.95 (0.44, 2.05)
Moderate	161	307	1.11 (0.68, 1.81)	0.94 (0.60, 1.47)	0.80 (0.37, 1.75)
Heavy	190	293	1.14 (0.74, 1.75)	0.84 (0.56, 1.26)	1.96 (1.00, 3.83)[b]
P pack-years interaction			0.33	0.90	0.09
Male	266	432	1.23 (0.84, 1.80)	0.76 (0.53, 1.09)	1.37 (0.78, 2.42)
Female	218	434	0.93 (0.62, 1.39)	0.85 (0.59, 1.23)	0.99 (0.53, 1.83)
P sex interaction			0.35	0.71	0.43
Age<58	73	427	0.56 (0.33, 0.95)[c]	0.76 (0.45, 1.29)	1.62 (0.76, 3.42)
Age≥58	411	439	1.23 (0.92, 1.65)	0.82 (0.63, 1.08)	0.95 (0.60, 1.49)
P age interaction			0.05	0.55	0.22

OR (95%CI) is adjusted for age, sex, and pack-years.

* Based on tertiles of pack-years in controls: mild smoker < 37.5 pack-years; moderate smoker=37.5 to <57.5; heavy smoker=≥57.5.

f. P=0.03

g. P=0.05

h. P=0.03

Table 4-7. Haplotype Analysis Results.

Block	Haplotype	Case Freq (%)	Control Freq (%)	OR *	P*
Block 1	rs1800469-rs2241712 (*TGFB1*)				0.96**
	CG	1.1	1.0	1.00	0.99
	TG	32.7	33.8	0.98	0.84
	CA	66.2	65.2	1.03	0.79
Block 2	rs3024509-rs1800872-rs1800896 (*IL10*)				0.28**
	CCG	5.5	5.1	1.19	0.39
	TAA	22.3	24.5	0.82	0.06
	TCA	31.3	28.9	1.12	0.27
	TCG	40.9	41.5	1.03	0.75

* Adjusted for age, sex, and pack-years.
** P for global association (H-1 df omnibus test)

5.0 MANUSCRIPT 3: VARIATION IN TRANSFORMING GROWTH FACTOR BETA1 (TGFB1) IS ASSOCIATED WITH SMOKING-RELATED CHRONIC OBSTRUCTIVE PULMONARY DISEASE IN A CAUCASIAN POPULATION

Manuscript in preparation

Yan Du, MS[1], Joel L. Weissfeld, MD, MPH[1], Marjorie Romkes, PhD[2], Frank Sciurba, MD[3], Eleanor Feingold, PhD[4], Brenda Diergaarde, PhD[1]

[1] Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA

[2] Department of Medicine, Center for Clinical Pharmacology, University of Pittsburgh, Pittsburgh, PA, USA

[3] Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, University of Pittsburgh, Pittsburgh, PA, USA

[4] Department of Human Genetics and Biostatistics, Graduate School of Public Health, University of Pittsburgh, PA, USA

5.1 ABSTRACT

Background Chronic obstructive pulmonary disease (COPD) is defined as a disease state characterized by the presence of airflow obstruction due to emphysema, chronic bronchitis, and/or small airway diseases. Cigarette smoking is the main risk factor of COPD and it induces abnormal inflammatory reactions in smokers' lungs. Therefore, inflammatory mediators such as cytokines are postulated to be of distinct importance in COPD development. The expression and functional effects of cytokine genes are influenced by genetic variants in these genes.

Methods In the present study, we evaluated seven single nucleotide polymorphisms (SNPs) in *TGFB1, IL6,* and *IL10* in relation to airflow obstruction and emphysema risk in a Caucasian population from the Pittsburgh metro area. Cochran-Mantel-Haenszel statistics were used to evaluate the relationships between genotypes and airflow obstruction/emphysema severity. Logistic regression adjusting for covariates was used to obtain odds ratios (ORs) and 95% confidence intervals (CIs) with airflow obstruction or emphysema treated as a two-category variable.

Results SNP rs2241712 in *TGFB1* was associated with airflow obstruction severity (P for nonzero correlation=0.02), the minor allele was associated with a decreased risk of the disease (any vs. no airflow obstruction: per allele OR=0.80, 95%CI=0.65-0.99, P_{trend}=0.04). No other significant association was observed.

Conclusions Our study suggests that common variation in *TGFB1* may be associated with the development of COPD.

Implications for public health It is anticipated that increased understanding of the genetics of COPD will improve identification of individuals susceptible to developing this disease, as well as result in more effective treatment.

5.2 INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death in the United States.[175] COPD is defined as a disease state characterized by the presence of airflow obstruction due to emphysema, chronic bronchitis, and/or small airway diseases.[176] The Global Initiative for Chronic Obstructive Lung Disease (GOLD) severity criteria based on spirometry are used to classify COPD into no, mild (GOLD I), moderate (GOLD II), and severe (GOLD III-IV) airflow obstruction categories.[177] Emphysema is defined anatomically as abnormal permanent enlargement of the airspaces distal to the terminal bronchioles, accompanied by destruction of their walls without obvious fibrosis.[178] Computed tomography (CT) technology has provided a non-invasive method of emphysema identification and grading.[179]

Smoking is the major environmental risk factor for COPD.[180,181] However, the fact that only a minority (15%-20%) of smokers develop COPD[182], and COPD clusters in families[62] suggest the presence of genetic predisposing factors in its pathogenesis. Constant exposure to cigarette smoke is associated with immune inflammatory cells infiltration and tissue remodeling to produce bronchial mucus glands enlargement, thickening of the walls and narrowing of the lumen of the smaller conducting airways, as well as emphysematous destruction of the alveolar surface.[56,183] Many cytokines play an important role in this chronic inflammatory process with tissue damage and repair. The pattern of cytokines involved depends on the inflammatory stage. Cytokine gene polymorphisms have been postulated as one of the possible genetic risk factors for COPD because they can affect gene expression and functions, thus alter inflammatory response.

The ***transforming growth factor beta 1*** (*TGFB1*) is located within the region of suggestive linkage to pulmonary function on chromosome 19q.[184] It is a multifunctional

cytokine, and regulates immune responses, cellular proliferation and differentiation, tissue repair, and extracellular matrix (ECM) production.[185,186] Some of these functions could protect against the development of emphysema. For example, TGFB1 can inhibit matrix metalloproteinase, consequently may contribute to the development of emphysema through the digestion of elastic fibers.[187,188] It is furthermore involved in repair of the ECM after inflammation and tissue injury by promoting synthesis of elastin and collagen[189,190], and this could help repair damage to the lungs of smokers who are at risk of developing emphysema. Although initially TGFB1 may be involved in repair as in wound healing, studies also suggest that large amounts of this cytokine may ultimately lead to destruction.[191] These results suggest that compromised TGFB1 signaling may be correlated with the development of emphysema.

Interleukin-6 (*IL6*) is a pleiotropic pro-inflammatory and immunomodulatory cytokine[192,193], and it has been proposed as a marker of systemic inflammation.[194] IL6 is a potential mediator of inflammation in COPD.[195] It has been shown to be increased in serum, exhaled breath condensate and sputum during stable conditions and exacerbation[195], to predict a faster decline in FEV_1[192], and to be associated with low exercise capacity.[196] In addition, it has been found to decline after oral and inhaled corticosteroid therapy.[197] Furthermore, IL6 is an important mediator of the acute phase response and can up-regulate C-reactive protein (CRP) at the transcriptional level.[198] CRP has been associated with lung function levels in healthy individuals and/or lung function decline in smoking-induced airflow obstruction.[199,200]

Interleukin-10 (*IL10*) is an anti-inflammatory cytokine, and is produced by T cells and macrophages.[201,202] Conflicting results concerning the modulation of IL10 secretion have been reported. It has been observed that there is an increased release of IL10 from the alveolar macrophages of cigarette smokers and emphysema patients.[203] Other studies have reported

significantly lower IL10 levels in induced sputum of airflow obstruction patients compared to healthy non-smokers.[204] These findings may be ascertained in established disease and may not be responsible for the induction of the disease. Nonetheless, differences in the level of *IL10* expression may have effects on COPD pathogenesis: IL10 can induce a non-allergic type of airway inflammation through suppressing the Th2 response, and increased IL10 production may lead to decreased immunity against bacteria and viruses and in turn support a risk factor in COPD—recurrent airway infections.

In the current study, we investigated single-nucleotide polymorphisms (SNPs) in three cytokine genes (*TGFB1*, *IL6*, and *IL10*) in relation to airflow obstruction as measured by GOLD stages and radiographic emphysema assessed semi-quantitatively with CT scan in a Caucasian population.

5.3 MATERIALS AND METHODS

5.3.1 Participants

The Pittsburgh Lung Screening Study (PLuSS), is a community-based study of lung cancer screening with low-dose multidetector helical CT. The recruitment of study participants has been described previously.[34] Briefly, between 2002 and 2005, PLuSS enrolled 50-79 year-old current and ex-cigarette smokers of at least one-half pack/day for at least 25 years. Using self-report, PLuSS excluded individuals who: 1) quit smoking more than 10 years earlier, 2) had a history of lung cancer, or 3) had a chest CT scan within one year of enrollment. A simple random sample of size n=1000 was selected from the *N*=3,463 (92.2% of all PLuSS enrollees) white or black

race CT-screened PLuSS participants with buffy coat or whole blood available and no interval lung cancer diagnosis (as of 9/10/2008). There were 929 (92.9%) samples with DNA available for the current study. Ten individuals (1.1% of 929) with genotype call rates <90% were excluded.

The present study included only Caucasians, the major racial/ethnic subgroup of subjects. All subsequent data analyses used the 866 self-reported white subjects.

Spirometry Protocol. Enrolled participants underwent a low-dose multidetector helical CT, and spirometry for pulmonary function testing (PFT), and also completed a written questionnaire. The questionnaire obtained information about medical history, current health problems, signs and symptoms of pulmonary disease, and smoking history. [205] A certified technician, using an office-based OMI-3000 spirometer (OMI Spirometry System, Houston, TX), performed PFT without a bronchodilator in accordance with the American Thoracic Society (ATS) criteria.[206] FEV_1 and forced vital capacity (FVC) were measured, and the FEV_1/FVC ratio was calculated for each participant. The highest value of at least 3 measurements was used. FEV_1 predicted was calculated using Hankinson's equations from sex, race, age, and height.[207] Severity of airflow obstruction was determined according to spirometric classification of GOLD[177] as follows: Stage I: FEV_1/FVC<0.7 and $FEV_1 \geq 80\%$ predicted; Stage II: FEV_1/FVC<0.7 and $50\% \leq FEV_1 < 80\%$ predicted; Stage III: FEV_1/FVC<0.7 and $30\% \leq FEV_1 < 50\%$ predicted; Stage IV: FEV_1/FVC<0.7 and $FEV_1 < 30\%$ predicted or $FEV_1 < 50\%$ predicted plus chronic respiratory failure.

Protocol for Primary Interpretation of the Baseline Screening CT. Three readers visually scored the baseline CT scan for emphysema presence and severity. Based on National Emphysema Treatment Trial criteria, scoring procedures used a five-level semi-quantitative scale

to represent no, trace, mild, moderate, and severe emphysema, the latter four categories roughly corresponding to emphysema affecting less than 10, 10-25, 25-50%, and greater than 50% of the lung, respectively. The inter-reader reliability was high.[205]

5.3.2 Genetic Polymorphisms and Genotyping

Seven SNPs in three genes (*TGFB1*, *IL6,* and *IL10*) were genotyped. Two SNPs in the promoter region of *TGFB1* (rs2241712, rs1800469), one coding non-synonymous SNP (rs2069860) and one promoter region SNP of *IL6* (rs1800795), and two SNPs in 5' near *IL10* (rs1800896, rs1800872) and one SNP (rs3024509) in intron 3 of *IL10* were selected. (**Table 5-1**)

Genotyping of 929 samples was performed at the University of Pittsburgh Genomics and Proteomics Core Laboratories (Pittsburgh, PA). All SNPs were genotyped using MassARRAY® iPLEX Gold (Sequenom, Inc., San Diego, CA); the SNP specific and mass extend oligonucleotides, and assays were designed using Sequenom RealSNP (www.realsnp.com) and MassARRAY Assay Design version 3.1 (Sequenom, Inc., San Diego, CA). Fifty duplicate samples were included to assess laboratory reliability. Call rates for these 7 SNPs were ≥95% and concordance of duplicates was 100%.

5.3.3 Statistical Analysis

We used chi-square tests to evaluate baseline factors and radiographic emphysema severity. Analysis of variance (ANOVA) was used to test associations between continuous variables of lung function (FEV_1, FEV_1% predicted, and FEV_1/FVC) and emphysema severity. Due to the

small numbers of the moderate (n=68) and severe (n=8) emphysema subgroups, we combined these two categories.

Hardy-Weinberg Equilibrium (HWE) was assessed in the whole population (n=866), the no-airflow-obstruction subgroup (n=521), and the no-emphysema subgroup (n=507) using an exact test.

We used a Cochran-Mantel-Haenszel (CMH) test to evaluate genotype (modeled as a 0, 1, 2 ordinal variable representing major allele homozygotes, heterozygotes, and minor allele homozygotes, respectively) relationships with airway obstruction (modeled as either a 0, 1, 2, 3 categorical or ordinal variable representing no, mild, moderate, and severe obstruction, respectively) and with emphysema (modeled as 0, 1, 2, 3 categorical or ordinal variable representing no, trace, mild, and moderate-severe emphysema, respectively).

Logistic regression adjusting for covariates (age, sex, and smoking pack-years) was used to obtain ORs and 95%CIs when airflow obstruction or radiographic emphysema was treated as a two category variable (any vs. no airflow obstruction, any vs. no emphysema, respectively). Genotype-based, additive (test for linear trend), and dominant models were examined. The genotype-based model is defined as heterozygotes (1 minor allele) versus common allele homozygotes (0 rare alleles), or rare allele homozygotes (2 minor alleles) versus common allele homozygotes. Linear trend was assessed using numerical scores assigned to the ordered categories (i.e., 0 to the category of major allele homozygotes, 1 to the category of heterozygotes, and 2 to the category of minor allele homozygotes) as continuous variable in the model. Because the number of rare-allele homozygotes was relatively small, we also combined heterozygotes and rare-allele homozygotes in the logistic regression analyses; common-allele homozygotes were used as the reference group (indicating dominant model).

Interaction between genotypes and any other airflow obstruction/emphysema risk factor (age in years, sex, smoking pack-years, smoking status, years of smoking, and number of cigarettes per day) was tested using the appropriate cross-product term in logistic regression under the dominant model.

All significance tests were two sided; *P* values <0.05 were considered statistically significant. Data analyses were conducted using SAS/Genetics software (version9.1.3; SAS Institute, Cary, NC).

5.4 RESULTS

5.4.1 Characteristics of Study Subjects

Table 5-2 shows the baseline characteristics of the study subjects. Our study population included 866 self-reported white subjects that fulfilled quality control criteria. Half (50.1%) of the population were women, and the majority of subjects (61.1%) were less than 60 years of age. The majority (59.1%) were current smokers, with a relatively intense cigarette smoking exposure (duration of cigarette use=39.1±7.4 years; dose intensity=25.7±9.8 cigarettes/day; smoking intensity duration=50.2±21.6 pack-years). More than one fifth (22.9%) reported a history of emphysema, bronchitis, or asthma; and about two-thirds (65.5%) reported symptoms of cough, phlegm, or wheezing. There is a high prevalence of airflow obstruction (39.8%) defined as GOLD stage I-IV and emphysema (41.5%) based on the CT scan.

Airflow obstruction (as measured by diminishing FEV_1% predicted and FEV_1/FVC) increased with emphysema severity (both P<0.0001). (**Table 5-2**)

5.4.2 Radiographic Emphysema Risk Factors

Factors associated with emphysema include age (P=0.0002), smoking status (P=0.03), years of cigarette use (P<0.0001), cigarettes per day (P=0.04), pack-years of smoking (P<0.0001), history of emphysema, bronchitis, or asthma (P<0.0001), and symptoms of cough, phlegm, or wheezing (P=0.03). Emphysema severity did not differ according to sex (P=0.77). (**Table 5-2**)

Airflow obstruction and emphysema correlated strongly; moderate or severe emphysema was 2.1-, 3.5-, and 2.9-fold more common in persons with mild, moderate, and severe airflow obstruction, respectively, than in persons without airflow obstruction. (**Table 5-2**)

5.4.3 Association between SNPs and COPD Phenotypes

The allele frequencies and HWE P values of the examined SNPs are shown in **Appendix C Table 6-9**. All SNPs were in HWE in the whole study population (P>0.05). While in both no-airflow obstruction and no-emphysema subjects, *IL6* rs2069860 was slightly deviated from HWE (P=0.04). However, examination of the cluster plot for rs2069860 did not reveal any unusual patterns; therefore this SNP was included in the subsequent analyses. We calculated the pairwise linkage disequilibrium (LD) values for the SNPs using PLINK (version 1.07). There was a tight LD between the two *TGFB1* SNPs (rs2241712, rs1800469) in the promoter region (r^2=0.95).

We observed a statistically significant association between *TGFB1* rs2241712 and airflow obstruction (P=0.02 for both airflow obstruction and SNP genotype as ordinal variables). We also observed a borderline significant association between *TGFB1* rs1800469 genotype and airflow obstruction (P=0.05 for both as ordinal variables).

No significant association of any SNP genotypes and emphysema was observed (**Table 5-3B**).

When airflow obstruction and emphysema were treated as a two category variable, we obtained similar results. For the airflow obstruction phenotype, we observed that the minor allele (G) of *TGFB1* rs2241712 was associated with a decreased risk of the disease (per allele OR=0.80, 95%CI=0.65-0.98, P_{trend}=0.03), and the result remained significant (per allele OR=0.80, 95%CI=0.65-0.99, P_{trend}=0.04) after adjusting for age, sex and smoking pack-years (**Table 5-4**). Under the dominant model, minor allele carriers of *TGFB1* rs2241712 again showed a reduced risk of airflow obstruction (OR=0.73, 95%CI=0.55-0.98).

When comparing subjects with and without emphysema, no significant association of any SNP with emphysema was observed. (**Appendix C Table 6-12**) None of the (seven) SNPs were associated with emphysema phenotype under the genotype-based, additive, (**Appendix C Table 6-12**) or dominant models (**Appendix C Table 6-13**).

We then explored the association of the significant *TGFB1* SNP (rs2241712) and COPD severity defined by combining both GOLD stages and CT scan emphysema score (**Figure 5-1**. Definition of the new COPD severity classification based on both GOLD stages and emphysema score.). Relative to healthy persons without airflow obstruction and CT emphysema no worse than trace, *TGFB1* rs2241712 G decreased the per allele odds of non-obstructive mild to severe emphysema or non-emphysematous (CT emphysema<mild) obstruction by 8% (adjusted OR=0.92, 95%CI=0.73-1.15, p=N.S.). Relative to healthy persons without airflow obstruction and CT emphysema no worse than trace, *TGFB1* rs2241712 G decreased the per allele odds of obstructive mild to severe emphysema by 27% (adjusted OR=0.73, 95%CI=0.53-0.996, p<0.05).

5.4.4 Interaction between Genotypes and Other COPD Risk Factors

We observed a statistically significant interaction between *IL10* rs1800896 and sex when emphysema was treated as a two-category variable (P for interaction=0.004). In men, minor allele carriers showed a non-significant protective effect against emphysema (dominant model OR=0.66, 95%CI=0.43-1.03); but in women, minor allele carriers were at a higher risk of emphysema (dominant model OR=1.51, 95%CI=0.97-2.35).

5.5 DISCUSSION

We observed that SNP rs2241712 in *TGFB1* was associated with airflow obstruction in our study population. Together with previous findings, this study adds evidence to the *TGFB1* gene as a risk factor for the development of COPD. Associations of COPD with other variants were not evident.

COPD is a pulmonary disease with prominent inflammatory features, and appears to be associated with the presence of increased systemic inflammatory markers.[208,209] Only some people exposed to cigarette smoke develop COPD, suggesting that genetic factors are important determinants of susceptibility. Inherited differences in the inflammatory response may contribute to COPD by initiating or maintaining airway inflammation. Different cytokines may be part of the airway inflammatory response in COPD. It is likely that variations in the phenotypic expression of these evaluated cytokines are influenced by polymorphisms in these genes. Thus, these polymorphisms may be of particular interest.

TGFB1 is produced by epithelial cells and macrophages and can cause fibrotic changes in lung tissue. Increased expression of TGFB1 and its mRNA was found in the bronchiolar and alveolar epithelium of COPD patients, and correlated with the number of intraepithelial macrophages.[210] COPD is characterized by inflammation with increased numbers of CD8+ lymphocytes and macrophages in the airways.[12,194] In emphysema there is disruption and fragmentation of elastic fibres in the alveolar walls. The resulting loss of elastic recoil leads to premature collapse of the small airways during expiration and this is one explanation of the airflow obstruction that occurs in emphysema.[211] TGFB1 has anti-inflammatory actions[185,212], it could plausibly act to prevent the degradation of elastin by inhibiting the expression of matrix metalloproteases. It is also possible that TGFB1 may be acting to promote the synthesis of elastin [188,189] and therefore, it could play a role in repairing the loss of elastic fibres that is due to smoking.

Studies have indicated that SNP rs1800469 in the promoter region of *TGFB1* is functional. Previous studies have reported that the T allele of rs1800469 (-509T>C) is associated with increased TGFB1 circulating concentrations in plasma,[213] and with an allele dose effect (TT>TC>CC).[214] This allele alters a Ying Yang 1 (YY1) transcription-factor consensus-binding site, enhances YY1 binding and promoter function, and is associated with higher TGFB1 circulating concentrations.[213,214] Since TGFB1 has anti-inflammatory and pro-repair activities; the T allele of this SNP is thought to be protective against the development of COPD.

In our study and other studies of Caucasians[74], rs2241712 and rs1800469 in the promoter region are in strong LD. Cross-sectional studies have investigated associations of SNPs in *TGFB1* with the presence of COPD, and with lower levels of FEV_1% predicted and FEV_1/FVC in several populations. In a family-based association analysis of severe, early-onset COPD

pedigrees, Celedon *et al.*[74] found modest evidence for association between rs2241712 G allele and decreased airflow obstruction. Furthermore, in a comparison of subjects with severe emphysema detected by high resolution CT (HRCT) from the National Emphysema Treatment Trial and smoking controls, rs2241712 and rs1800469 minor alleles were associated with reduced emphysema risk. Su *et al*[215]. also reported the T allele frequency of rs1800469 was significantly decreased in airflow obstruction compared with that in controls in a Chinese population. In contrast, van Diemen *et al.*[216] failed to find association of rs1800469 with airflow obstruction among Caucasians of Dutch descent. In a case-control study conducted in Korea, Yoon *et al.*[217] did not find significant association for the two SNPs with airflow obstruction either. Ito *et al.*[218] did not detect any association of the two SNPs with the emphysema phenotype detected by HRCT in a Japanese population; however, they reported that FEV_1 after bronchodilator was significantly associated with the T allele of rs1800469 (P=0.007), and that T was significantly more prevalent in GOLD stage III-IV vs. GOLD stage I-II (OR=2.86, 95%CI=1.33-6.14) in emphysema patients. A meta-analysis by Smolonska *et al.*[219] reported the minor allele of rs2241712 was protective for COPD (OR=0.73, 95%CI=0.57-0.94); and rs1800469 showed borderline significant protective effect for COPD (OR=0.76, 95%CI=0.54-1.08). One possible contributor to inconsistent results relates to genetic heterogeneity between study populations. Different genetic factors might influence the development of COPD in different ethnic groups. There is also the possibility that these polymorphisms are not the functional variants affecting COPD susceptibility. The effects that we detected may be due to LD with nearby functional variants. Even if the same functional genetic variant was involved in each population, the LD relationships of this functional variant with neighboring genetic polymorphisms could vary between ethnic groups.

The polymorphisms in the *IL6* gene promoter region appear to be related to cytokine levels in plasma.[220] There are five previous reports analyzing the *IL6* rs1800795 (-174G>C) polymorphism and COPD in Caucasian populations. In the study by He *et al.*[73], they reported that all SNPs genotyped and in high LD with the *IL6* rs1800795 (-174G>C) showed significant or borderline association with rapid decline of lung function and with airflow obstruction. However, in a German study conducted by Seifart *et al.*[221], 113 patients and 356 controls were analyzed, no significant association was found of *IL6* rs1800795 (-174G>C) with airflow obstruction. In a Dutch study, Broekhuizen *el al.*[222] studied rs1800795 in 99 patients with a cachexia phenotype and did not find any association between this SNP and airflow obstruction. Studies conducted in Spain[223] and the Republic of Bashkortostan[224] also reported no significant association between this SNP and airflow obstruction. The meta-analyses by Smolonska *et al.*[219] included the latter four publications[221-224] of white populations, and found no significant association of rs1800795 and airflow obstruction risk, with an OR of 1.15 (95%CI=0.92-1.43). Similarly, no significant association was observed in our study.

IL10 is hypothesized to inhibit inflammatory response by reducing the production of pro-inflammatory cytokine.[201,202] In addition, IL10 may prevent apoptosis in T[225] and B cells by up-regulating bcl-2 protein[226] that may result in the persistence of inflammatory cells, leading to ongoing airway inflammation. Furthermore, IL10 was demonstrated to influence airway macrophages releasing proteases and anti-proteases[203], a balanced system that play an important role in emphysema development and progression. However, genetic association studies[221,224] failed to demonstrate relationships between *IL10* SNPs and airflow obstruction risk. In accordance to previous studies, we did not observe any association either.

However, in the current study, we observed that sex modified the association between *IL10* rs1800896 and emphysema risk. Several studies have shown the existence of sex differences in clinical presentation and phenotypes of COPD.[227-230] To our knowledge, this is the first study reporting a modifying effect of sex on this SNP and emphysema risk. Even though the interaction is quite significant (P=0.004), we can't rule out the possibility of chance finding. Follow-up studies with adequate sample sizes are warranted to confirm this finding and both *in vivo* and *in vitro* studies are needed to investigate the impact of this polymorphism on disease pathogenesis.

Inconsistent results in the associations between genetic variations in candidate genes and COPD are quite frequent.[231] Besides heterogeneity between study populations, another possible contributor to inconsistent genetic association results relates to the phenotypic differences of COPD, and published association studies have used different phenotype definitions. For example, genetic association studies of COPD have defined cases on the basis of airflow obstruction[217], emphysema[218], decline in lung function[73], or chronic bronchitis.[232] It is possible that a given genetic variant may confer susceptibility to a specific COPD-related phenotype. However, it is difficult to define a distinct COPD subtype since different manifestations of COPD overlap with each other. Furthermore, patients with COPD may also have co-existent asthma even though their predominant problem was COPD. The dissimilarities could also be explained by patient characteristics. For instance, our COPD patients had milder airflow obstruction (FEV_1<80% predicted) than the COPD patients in the Celedon study[74] (FEV_1<45% predicted).

The strengths of our study include, first, we have a relatively homogeneous population. Our study participants are Caucasians from southwestern Pennsylvania, and population

stratification is not likely to have a large impact on our findings. Our data were collected for epidemiological study purposes, and the dataset is quite complete and has high quality compare to other study data that were retrospectively abstracted from medical records.

There are several limitations to our study. Our sample size is modest, and we did not have power to detect genetic determinants of minor effect. Using airflow obstruction (no vs. any) as an example, in our study population and with 80% power using log-additive model, for SNPs with MAF=0.05 we can detect ORs ≥ 1.8, for SNPs with MAF=0.10 we can detect ORs >1.5, for SNPs with MAF=0.30 we can detect ORs >1.3. We genotyped only two or three SNPs per gene, assuming that the variants tested have functional effects on COPD susceptibility. However, if another variant in or near the gene not in LD with the studied SNPs were the causal variant, then the true association could be easily missed.

Misclassification of airflow obstruction may have occurred since spirometry data were collected at one point of time, and without administration of any pulmonary function improving medications (e.g., bronchodilator). However, participants were asked to come in for the measurement during their usual health state, thus the day-to-day variation of the pulmonary function was assumed to be minimal.

We did not adjust for multiple comparisons in this study. False positive associations may arise from multiple testing in studies that assess many genes, markers, and phenotypes.[233] There is no consensus on the optimal method to adjust for multiple comparisons in case-control genetic association studies, though replication in an independent study may provide the strongest evidence for true association. Our results of *TGFB1* SNPs are similar to previous studies provided additional support that this gene may play an important role in COPD development.

Future candidate gene association studies need to employ strict genetic epidemiology criteria, including adequate sample sizes, adjustment for multiple testing, and control for population stratification. Genome-wide linkage analysis will be required to identify genomic regions that likely contain COPD susceptibility genes, and positional candidate gene association testing and/or SNP-based fine mapping will likely be required to identify novel COPD susceptibility genes within these regions. A more systematic approach to COPD genetics may lead to more consistent results in the search for genetic determinants of COPD. Functional studies are necessary to pinpoint the exact role of such SNPs in genes.

In summary, evaluating associations of seven cytokine polymorphisms with COPD, we found rs2241712 in the promoter region of *TGFB1* gene is associated with airflow obstruction. Our findings need to be validated in large, prospectively accrued populations and incorporating additional genetic markers in the cytokine pathway.

5.6 TABLES AND FIGURES

Table 5-1. SNP Information.

Gene	rs Number	Chr	Chr Position*	Nucleotide	Allele	Location and Presumed Function
TGFB1	rs2241712	19q13.1	41869756	-10807G>A	A→G	In the promoter region; alter gene expression.
TGFB1	rs1800469	19q13.1	41860296	-509T>C	C→T	In the promoter region; T allele is associated with increased circulating concentrations of TGF-B1 in plasma.[I]
IL6	rs2069860	7p21	22771038	Ex5+14A	A→T	Coding non-synonymous; Val[V]→ Asp[D].
IL6	rs1800795	7p21	22766645	-236C>G (-174C>G)	G→C	In the promoter region; associated with levels of IL-6 protein and the C-reactive protein.[II]
IL10	rs1800896	1q31-q32	206946897	-1082G>A (-1116A>G)	A→G	5' near gene.
IL10	rs1800872	1q31-q32	206946407	-592C>A (-627A>C, -6653A>C)	C→A	5' near gene.
IL10	rs3024509	1q31-q32	206943297	IVS3-58T>C	T→C	Intron_3.

*dbSNP Chromosome Report, GRCh37 Sequence

Database of Single Nucleotide Polymorphisms (dbSNP). Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine. (dbSNP Build ID: {build ID}). Available from: HUhttp://www.ncbi.nlm.nih.gov/SNP/U

I. Grainger DJ, Heathcote K, Chiano M, et al. Genetic control of the circulating concentration of transforming growth factor type beta1. Hum Mol Genet 1999;8:93-7.

II. Vickers MA, Green FR, Terry C, et al. Genotype at a promoter polymorphism of the interleukin-6 gene is associated with baseline levels of plasma C-reactive protein. Cardiovasc Res 2002;53:1029-34.

Table 5-2. Study Population Characteristics by Emphysema Severity Status.

Characteristic	n	Total (%)	Radiographic Emphysema Severity (Row %)†				P Value*
			None (n=507)	Trace (n=179)	Mild (n=104)	Moderate + Severe (n=76)	
Sex							0.77
Men	432	49.9	251 (49.5)	95 (53.1)	49 (47.1)	37 (48.7)	
Women	434	50.1	256 (50.5)	84 (46.9)	55 (52.9)	39 (51.3)	
Age, yr							0.0002
49-59	517	61.1	332 (67.3)	101 (57.1)	50 (49.5)	34 (45.3)	
60-69	258	30.5	132 (26.8)	59 (33.3)	38 (37.6)	29 (38.7)	
70+	71	16.9	29 (5.9)	17 (9.6)	13 (12.9)	12 (16.0)	
Smoking status							0.03
Current smoker	511	59.1	281 (55.4)	116 (64.8)	71 (68.3)	43 (57.3)	
Former smoker	354	40.9	226 (44.6)	63 (35.2)	33 (31.7)	32 (42.7)	
Duration of cigarette use, yr							<0.0001
<40	482	55.7	317 (62.5)	98 (54.8)	38 (36.5)	29 (38.7)	
40+	383	44.3	190 (37.5)	81 (45.3)	66 (63.5)	46 (61.3)	
Dose intensity, cigarettes/d							0.04
<20	264	30.5	175 (34.5)	53 (29.6)	25 (24.0)	11 (14.7)	
20-29	354	40.9	201 (39.6)	74 (41.4)	45 (43.3)	34 (45.3)	
30-39	156	18.0	79 (15.6)	33 (18.4)	24 (23.1)	20 (26.7)	
40+	91	10.5	52 (10.3)	19 (10.6)	10 (9.6)	10 (13.3)	
Smoking intensity duration, pack-years							<0.0001
<30	161	18.6	120 (23.7)	28 (15.6)	10 (9.6)	3 (4.00)	
30-44	228	26.3	138 (27.2)	52 (29.1)	20 (19.2)	18 (23.6)	
45-59	228	26.3	131 (25.8)	41 (22.9)	35 (33.7)	21 (27.6)	
60-74	135	15.4	63 (12.4)	35 (19.6)	20 (19.2)	17 (22.4)	
75+	114	13.2	55 (10.9)	23 (12.8)	19 (18.3)	17 (22.4)	

Table 5-2. (*Continued*)

History of emphysema, bronchitis, or asthma							<0.0001
No	668	77.1	412 (81.3)	141 (78.8)	77 (74.0)	38 (50.0)	
Yes	198	22.9	95 (18.7)	38 (21.2)	27 (26.0)	38 (50.0)	
Cough, phlegm, or wheeze							0.03
No	299	34.5	192 (37.9)	55 (30.7)	35 (33.6)	17 (22.4)	
Yes	567	65.5	315 (62.1)	124 (69.3)	69 (66.4)	59 (77.6)	
Airflow obstruction							<0.0001
None	521	60.2	365 (72.0)	107 (60.1)	41 (39.4)	8 (10.5)	
GOLD I	127	14.7	56 (11.0)	27 (15.2)	27 (26.0)	17 (22.4)	
GOLD II	171	19.8	74 (14.6)	39 (21.9)	30 (28.8)	28 (36.9)	
GOLD III-IV	46	5.3	12 (2.4)	5 (2.8)	6 (5.8)	23 (30.2)	

Characteristic	**Mean ± SD of Total (N=866)**	**Radiographic Emphysema Severity**				**P Value‡**
		None (n=507)	**Trace (n=179)**	**Mild (n=104)**	**Moderate + Severe (n=76)**	
FEV_1obs (liters)	2.56 ± 0.81	2.70 (0.03)	2.59 (0.06)	2.35 (0.08)	1.93 (0.09)	<0.0001
FEV_1% Predicted	83.52 ±18.75	86.60 (0.80)	83.72 (1.34)	80.62 (1.76)	66.48 (2.05)	<0.0001
FEV_1/FVC	70.32 ± 10.44	73.49 (0.39)	70.73 (0.66)	66.33 (0.86)	53.64 (1.01)	<0.0001

*Independence between characteristic and radiographic emphysema severity, level of statistical significance (chi-square test).

†Row % is the percentage of the study subgroup total (n) with the indicated characteristic.

‡GLM (3df) to compare the mean of 4 radiographic emphysema severity categories. Characteristics within each category are presented as mean (SE).

FEV_1obs: Forced expiratory volume in first second, liters.

FEV_1% Predicted: Forced expiratory volume in first second as percent of expected.

FEV_1/FVC: 100×FEV_1/FVC.

Table 5-3A. Genotype Distribution by GOLD Stage (Airflow Obstruction Severity).

Gene	rs Number	Genotype	GOLD Stage†				II-IV (n=217)	P *
			0 (n=521)	I (n=127)	II (n=171)	III + IV (n=46)		
TGFB1	rs2241712	AA	208 (55.8)	58 (15.6)	83 (22.3)	24 (6.4)	107 (28.7)	0.02 (1df)
		AG	241 (63.3)	51 (13.4)	73 (19.2)	16 (4.2)	89 (23.4)	0.049 (2df)
		GG	71 (64.6)	18 (13.4)	15 (13.6)	6 (5.5)	21 (19.1)	0.23 (6df)
TGFB1	rs1800469	CC	217 (56.7)	58 (15.1)	84 (21.9)	24 (6.3)	108 (28.2)	0.05 (1df)
		CT	235 (62.7)	53 (14.1)	71 (18.9)	16 (4.3)	87 (23.2)	0.12 (2df)
		TT	68 (64.2)	16 (15.1)	16 (15.1)	6 (5.7)	22 (20.8)	0.51 (6df)
IL6	rs2069860‡	AA	513 (60.2)	124 (14.6)	169 (19.8)	46 (5.4)	215 (25.2)	0.54 (1df)
		AT	7 (58.3)	3 (25.0)	2 (16.7)	0 (0.0)	2 (16.7)	0.54 (1df)
		TT	1 (100)	0 (0.0)	0 (0.00)	0 (0.0)	0 (0.0)	0.69 (3df)
IL6	rs1800795	GG	188 (60.5)	43 (13.8)	64 (20.6)	16 (5.1)	80 (25.7)	0.93 (1df)
		CG	245 (60.8)	58 (14.4)	75 (18.6)	25 (6.2)	100 (24.8)	1.00 (2df)
		CC	88 (58.3)	26 (17.2)	32 (21.2)	5 (3.3)	37 (24.5)	0.78 (6df)
IL10	rs1800896	AA	152 (60.1)	39 (15.4)	49 (19.4)	13 (5.1)	62 (24.5)	0.98 (1df)
		AG	248 (59.3)	62 (14.8)	90 (21.5)	18 (4.3)	108 (25.8)	0.98 (2df)
		GG	121 (62.4)	26 (13.4)	32 (16.5)	15 (7.7)	47 (24.2)	0.52 (6df)
IL10	rs1800872	CC	297 (60.1)	74 (15.0)	91 (18.4)	32 (6.5)	123 (24.9)	0.90 (1df)
		CA	193 (60.9)	47 (14.8)	64 (20.2)	13 (4.1)	77 (24.3)	0.78 (2df)
		AA	31 (57.4)	6 (11.1)	16 (29.6)	1 (1.9)	17 (31.5)	0.31 (6df)
IL10	rs3024509‡	TT	467 (60.0)	113 (14.5)	157 (20.2)	42 (5.4)	199 (25.6)	0.45 (1df)
		TC	54 (64.3)	12 (14.3)	14 (16.7)	4 (4.8)	18 (21.5)	0.45 (1df)
		CC	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0.82 (3df)

*P values are for 1df nonzero correlation statistic, 2df row mean score differ statistic, 6df general association statistic.

†Row percentages

‡When calculated P-values from CMH, *IL6* rs2069860 AT/ TT genotypes were combined, and *IL10* rs3024509 TC/CC genotypes were combined, due to small cell counts.

Table 5-3B. Genotype Distributions by Radiographic Emphysema Severity.

Gene	rs Number	Genotype	Radiographic Emphysema Severity (Row %)†					P *
			None (n=507)	Trace (n=179)	Mild (n=104)	Moderate + Severe (n=76)	Mild+ (n=180)	
TGFB1	rs2241712	AA	214 (57.4)	74 (19.8)	44 (11.8)	41 (11.0)	85 (22.8)	0.13 (1df)
		AG	227 (59.4)	79 (20.7)	45 (11.8)	31 (8.1)	76 (19.9)	0.32 (2df)
		GG	65 (59.1)	26 (23.6)	15 (13.6)	4 (3.6)	19 (17.2)	0.36 (6df)
TGFB1	rs1800469	CC	218 (56.9)	78 (20.4)	44 (11.5)	43 (11.2)	87 (22.7)	0.14 (1df)
		CT	226 (60.1)	77 (20.5)	45 (12.0)	28 (7.5)	73 (19.5)	0.29 (2df)
		TT	62 (58.5)	24 (22.6)	15 (14.2)	5 (4.7)	20 (18.9)	0.38 (6df)
IL6	rs2069860‡	AA	499 (58.5)	178 (20.9)	103 (12.1)	73 (8.6)	176 (20.7)	0.43 (1df)
		AT	7 (58.3)	1 (8.3)	1 (8.3)	3 (25.0)	4 (33.3)	0.43 (1df)
		TT	1 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.22 (3df)
IL6	rs1800795	GG	183 (58.7)	69 (22.1)	35 (11.2)	25 (8.0)	60 (19.2)	0.76 (1df)
		CG	234 (58.1)	83 (20.6)	46 (11.4)	40 (9.9)	86 (21.3)	0.82 (2df)
		CC	90 (59.6)	27 (17.9)	23 (15.2)	11 (7.3)	34 (22.5)	0.72 (6df)
IL10	rs1800896	AA	145 (57.3)	63 (24.9)	28 (11.1)	17 (6.7)	45 (17.8)	0.24 (1df)
		AG	251 (60.1)	78 (18.7)	53 (12.7)	36 (8.6)	89 (21.3)	0.46 (2df)
		GG	111 (56.9)	38 (19.5)	23 (11.8)	23 (11.8)	46 (23.6)	0.32 (6df)
IL10	rs1800872	CC	288 (58.2)	107 (21.6)	53 (10.7)	47 (9.5)	100 (20.2)	0.87 (1df)
		CA	188 (59.3)	59 (18.6)	46 (14.5)	24 (7.6)	70 (22.1)	0.99 (2df)
		AA	31 (57.4)	13 (24.1)	5 (9.3)	5 (9.3)	10 (18.6)	0.58 (6df)
IL10	rs3024509‡	TT	455 (58.4)	165 (21.2)	91 (11.7)	68 (8.7)	159 (20.4)	0.80 (1df)
		TC	52 (61.9)	12 (14.3)	12 (14.3)	8 (9.5)	20 (23.8)	0.80 (1df)
		CC	0 (0.0)	2 (66.7)	1 (33.3)	0 (0.0)	1 (33.3)	0.63 (3df)

* P values are for 1df nonzero correlation statistic, 2df row mean score differ statistic, 6df general association statistic.

†Row percentages

‡ When calculated P-values from CMH, *IL6* rs2069860 AT/ TT genotypes were combined, and *IL10* rs3024509 TC/CC genotypes were combined, due to small cell counts.

Table 5-4. Unadjusted and Adjusted Results of SNP and Airflow Obstruction Associations: Genotype-based Model and Trend Test

Gene	rs Number	Genotype	N (%) GOLDI-IV	N (%) Control	Genotype-based Model OR (95% CI)	Genotype-based Model P	Genotype-based Model* OR (95% CI)*	Genotype-based Model* P*
TGFB1	rs2241712	AA	165 (48.0)	208 (40.0)	1.00 (reference)		1.00 (reference)	
		AG	140 (40.7)	241 (46.3)	0.73 (0.55, 0.98)	0.04	0.75 (0.55, 1.01)	0.06
		GG	39 (11.3)	71 (13.7)	0.69 (0.45, 1.08)	0.10	0.69 (0.43, 1.09)	0.11
		Trend[a]			0.80 (0.65, 0.98)	0.03	0.80 (0.65, 0.99)	0.04
TGFB1	rs1800469	CC	166 (48.3)	217 (41.7)	1.00 (reference)		1.00 (reference)	
		CT	140 (40.7)	235 (45.2)	0.78 (0.58, 1.04)	0.09	0.79 (0.58, 1.06)	0.12
		TT	38 (11.0)	68 (13.1)	0.73 (0.47, 1.14)	0.17	0.74 (0.47, 1.18)	0.21
		Trend[a]			0.83 (0.68, 1.02)	0.07	0.84 (0.68, 1.03)	0.10
IL6	rs2069860	AA	339 (98.5)	513 (98.5)	1.00 (reference)		1.00 (reference)	
		AT	5 (1.5)	7 (1.3)	1.08 (0.34, 3.43)	0.90	1.32 (0.40, 4.28)	0.65
		TT	0 (0.0)	1 (0.2)	---	--	---	--
		Trend[a]			0.86 (0.31, 2.41)	0.77	0.99 (0.35, 2.82)	0.99
IL6	rs1800795	GG	123 (35.8)	188 (36.1)	1.00 (reference)		1.00 (reference)	
		GC	158 (45.9)	245 (47.0)	0.99 (0.73, 1.33)	0.93	1.01 (0.73, 1.38)	0.98
		CC	63 (18.3)	88 (16.9)	1.09 (0.74, 1.63)	0.66	1.16 (0.77, 1.75)	0.48
		Trend[a]			1.04 (0.85, 1.26)	0.72	1.07 (0.87, 1.30)	0.54
IL10	rs1800896	AA	101 (29.4)	152 (29.2)	1.00 (reference)		1.00 (reference)	
		AG	170 (49.4)	248 (47.6)	1.03 (0.75, 1.42)	0.85	1.14 (0.82, 1.60)	0.43
		GG	73 (21.2)	121 (23.2)	0.91 (0.62, 1.33)	0.62	1.00 (0.67, 1.49)	0.99
		Trend[a]			0.96 ().79, 1.16)	0.66	1.01 (0.83, 1.23)	0.94
IL10	rs1800872	CC	197 (57.3)	297 (57.0)	1.00 (reference)		1.00 (reference)	
		CA	124 (36.0)	193 (37.0)	0.97 (0.73, 1.29)	0.83	0.96 (0.71, 1.30)	0.80
		AA	23 (6.7)	31 (6.0)	1.12 (0.63, 1.98)	0.70	0.97 (0.54, 1.76)	0.92
		Trend[a]			1.01 (0.81, 1.27)	0.91	0.97 (0.77, 1.23)	0.82
IL10	rs3024509	TT	312 (90.7)	467 (89.6)	1.00 (reference)		1.00 (reference)	
		TC	30 (8.7)	54 (10.4)	0.83 (0.52, 1.33)	0.44	0.93 (0.57, 1.52)	0.78
		CC	2 (0.6)	0 (0.0)	---	--	---	--
		Trend[a]			0.95 (0.61, 1.48)	0.82	1.07 (0.67, 1.68)	0.79

* Adjusted for age, sex and pack-years. a. Additive Model, dose-response, 1df.

Table 5-5. Adjusted Results of SNP and Airflow Obstruction Associations: Dominant Model

Gene	rs Number	Subjects with data, N		Minor allele frequency, %		Dominant Model[b] Combined heterozygote and homozygote minor	
		Cases	Controls	Cases	Controls	OR (95% CI)	P
TGFB1	rs2241712	344	520	31.7	36.8	0.73 (0.55, 0.98)	0.03
TGFB1	rs1800469	344	520	31.4	35.7	0.78 (0.58, 1.03)	0.08
IL6	rs2069860	344	521	0.7	0.9	1.13 (0.36, 3.57)	0.83
IL6	rs1800795	344	521	41.3	40.4	1.05 (0.78, 1.41)	0.77
IL10	rs1800896	344	521	45.9	47.0	1.10 (0.80, 1.50)	0.57
IL10	rs1800872	344	521	24.7	24.5	0.96 (0.72, 1.28)	0.80
IL10	rs3024509	344	521	4.9	5.2	1.00 (0.62, 1.61)	0.99

Adjusted for age, sex, pack-years.
b. Dominant Model: combine heterozygote and homozygote minor.

	Emphysema Score				
GOLD Stages		**None**	**Trace**	**Mild**	**Moderate + Severe**
	0	1 (n=471)		2 (n=49)	
	I	2 (n=214)		3 (n=131)	
	II				
	III-IV				

Figure 5.1. Definition of the new COPD severity classification based on both GOLD stages and emphysema score.
1 [Normal]: GOLD(0)&Emphy(None), GOLD(0)&Emphy(Trace)
2 [Moderate]: GOLD(I)&Emphy(None), GOLD(I)&Emphy(Trace), GOLD(II-IV)&Emphy(None), GOLD(II-IV)&Emphy(Trace), GOLD(0)&Emphy(Mild), GOLD(0)&Emphy(Moderate+Severe)
3 [Severe]: GOLD(I)&Emphy(Mild), GOLD(I)&Emphy(Moderate-Severe), GOLD(II-IV)&Emphy(Mild), GOLD(II-IV)&Emphy(Moderate+Severe)

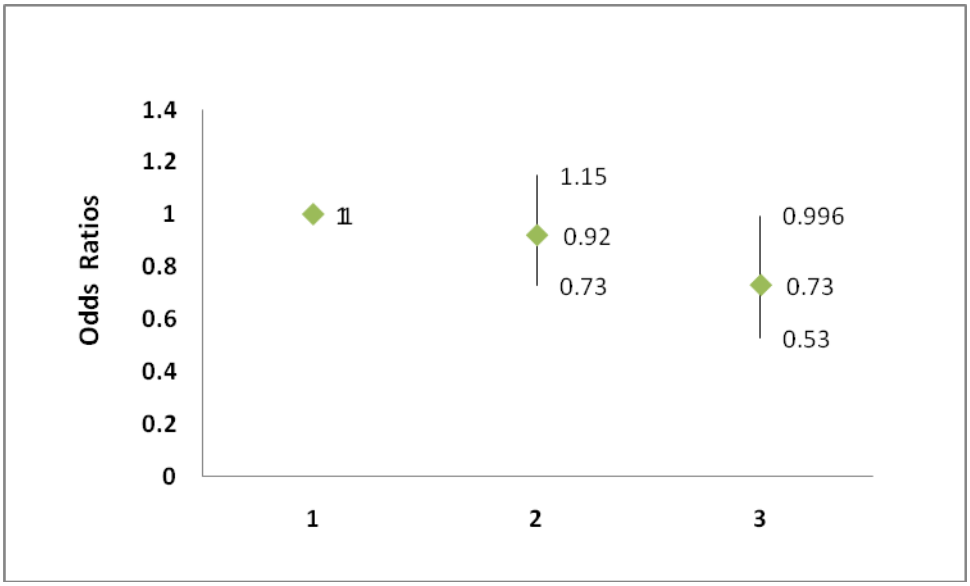

Figure 5.2. Association between *TGFB1* rs2241712 and COPD severity (per allele odds ratio and 95% confidence interval), adjusting for age, sex, and smoking pack-years.

6.0 DISCUSSION

6.1 SUMMARY OF FINDINGS

Lung cancer is the leading cause of cancer mortality in the U.S. The 5-year survival for the disease is approximately 16%, and despite therapeutic advances there has been little improvement over the past three decades.[1] COPD is the fourth leading cause of death in the US.[10] Despite the availability of effective treatments for COPD, no existing therapy halts or reverses the progressive and accelerated decline in lung function that is characteristic of this condition. Both diseases are associated with cigarette smoking, which causes abnormal inflammatory changes. However, only a fraction of smokers develop lung cancer[58] and/or COPD[59], suggesting genetic predispositions to both diseases. While the relationship between inflammation and lung cancer or COPD is becoming better understood, the effects of inflammatory pathway genes in disease development and prevention have not been extensively studied.

In this study we utilized data and specimens from Project 4 of the UPCI SPORE in Lung Cancer and were able to examine several inflammatory pathway genes and smoking-related lung cancer and/or lung disease risk, including: 1) polymorphisms in eicosanoid pathway genes (*IL1B, COX2, PPARG*) and lung cancer risk; 2) polymorphisms in cytokine genes (*TGFB1, IL6, IL10*)

and lung cancer risk; and 3) polymorphisms in cytokine genes (*TGFB1*, *IL6*, *IL10*) and COPD risk.

Our findings suggest that *IL1B* rs1143634 may be associated with lung cancer risk. However, our study showed that the minor allele had a protective effect against disease development, which is contradictory to previous findings. One possible explanation is that *IL1B* has both pro- and anti-apoptotic effects, thus it may play a different role in heavy smokers (such as our population) with chronic and persistent inflammation. We also observed an interaction between *PPARγ* rs1801282 and sex. This may be explained by hormonal effects. *PPARγ* is a nuclear hormone receptor, and a regulator of adipocyte differentiation. It is known that hormones can have a role in the progression of tumors in many tissues. Results from genetic association studies of lung cancer are inconclusive. One probable explanation is that most study sample sizes have limited power to detect SNPs with moderate effects, which is usual for complex disease. Nevertheless, our study results point out potential areas for further investigation.

The pathology of COPD is that of a chronic inflammatory process with tissue damage and repair, many cytokines play a role in this condition. We investigated associations between several cytokine gene polymorphisms and COPD risk in PLuSS participants. We found that *TGFB1* rs2241712 was associated with COPD, especially with the airflow obstruction phenotype. This result is consistent with previous studies. Together with other findings, our study suggests the important role of *TGFB1* in COPD, and *TGFB1* is a promising gene for future COPD research.

Our findings support the need for further research on inflammatory pathway genes and smoking-related lung cancer and lung disease.

6.2 FUTURE RESEARCH

Further functional studies for inflammatory pathway gene polymorphisms are needed to elucidate the mechanisms behind lung cancer and COPD development. Developing and using animal and other preclinical models are needed to investigate the pathogenetic mechanisms and to disentangle the complex associations of the two diseases.

Because associations of disease and genetic variations in inflammatory genes are often relatively modest, it is likely that polymorphisms in multiple inflammatory genes cooperate in an additive or synergistic manner to impact disease risk. Pathway analyses may help to reveal gene-gene interactions or risks imparted independently from other genes in the pathway.

Gene-gene and gene-environment interactions have a significant influence on susceptibility to lung cancer or COPD development. However, our current knowledge of these interactions is limited, and continued research efforts in this area will be important for a full understanding of predisposition to both diseases. Well-designed follow-up studies with adequate sample sizes are needed to investigate these associations.

New knowledge about the genome may discover variation that is more relevant to common disease, such as lung cancer and COPD, than the common polymorphisms. One type of variation that is frequent but has not been well evaluated with respect to both diseases is copy number variation (CNV). Another possibility is that the risk of common disease is caused by rare variants at lock with alleles of large effect. These rare alleles are not readily detected by common polymorphisms because they have arisen independently and have no common haplotype. This hypothesis remains to be generally tested because it required resequencing of genes in many people.

More specific for our project, there are several follow-up studies could be conducted. To better understand the role of *TGFB1* gene in COPD development, we can evaluate the association between *TGFB1* SNPs and measurements of bronchial thickening. The ongoing PLuSS also collects yearly follow-up PFT data and CT measurements, thus we can test whether inflammatory pathway genes are associated with progression of COPD and/or lung function decline. With more complete COPD information in cases, we can also evaluate whether the association between inflammatory pathway genes and lung cancer is mediated by COPD.

Future research will make it possible to create new tools involving genetics, genomics, proteomics, metabolomics, and molecular imaging. These will aid better risk determination, identification of specific therapeutic targets, and personalized treatments for lung cancer and COPD.

6.3 PUBLIC HEALTH SIGNIFICANCE

Lung cancer and COPD are significant causes of morbidity and mortality in the U.S. and worldwide. One shared risk factor is cigarette smoking, an avoidable behavior. Despite the smoking rate is declining in western world, the incidence is rising in developing countries, such as China and India. Moreover, elevated lung cancer risk remains a long term in heavily exposed former smokers. In people who develop COPD, the inflammatory process is amplified and persists long after smoking cessation. Lung cancer and COPD will continue to pose huge public health burdens in the foreseeable future. Great efforts are still needed: 1) to understand the common processes/molecules that are central to chronic inflammation and lung cancer/COPD; 2)

to identify current and former smokers at the highest risk of developing lung cancer/COPD; 3) to discover novel therapeutic strategies to prevent and treat lung cancer/COPD.

Only a small proportion of smokers develop lung cancer and/or COPD, and the familiar clustering of both diseases suggest the genetic predisposition to lung cancer and COPD. Improved understanding of lung cancer and COPD genetics may aid in the early identification of higher risk groups. Screening high-risk populations will permit timely intervention and education. Interventions based on an understanding of inflammatory processes are particularly promising because inflammatory processes are modifiable through behavioral changes and medical treatments.

Smoking-related lung cancer and COPD are problems with an enormous public health burden that will require a matching effort and commitment from all stakeholders to achieve tangible progress in reducing morbidity, mortality, and health care utilization. Further insight into the genetic factors involved may eventually result in: 1) effective genetic-based methods for selecting current or former cigarette smokers for more intensive screening, surveillance, or chemoprevention, and 2) effective genetic-based methods for selecting biologically targeted treatments of lung cancer and/or COPD.

APPENDIX A

SUPPLEMENTARY TABLES FOR MANUSCRIPT 1

Table 6-1. Unadjusted and Adjusted Results of SNP and NSCLC associations: Genotype-based Model and Trend Test.

			N (%)		Genotype-based Model		Genotype-based Model*	
Gene	**rs Number**	**Genotype**	**NSCLC**	**Controls**	**OR (95% CI)**	**P**	**OR (95% CI)***	**P***
COX-2	rs5275	T/T	199 (45.2)	386 (44.9)	1.00 (reference)		1.00 (reference)	
		T/C	187 (42.5)	381 (44.3)	0.95 (0.75, 1.22)	0.69	0.98 (0.74, 1.29)	0.88
		C/C	54 (12.3)	93 (10.8)	1.13 (0.77, 1.64)	0.54	0.98 (0.64, 1.52)	0.93
		Trend[a]			1.03 (0.86, 1.22)	0.78	0.99 (0.81, 1.20)	0.89
COX-2	rs5277	G/G	325 (71.7)	614 (71.08)	1.00 (reference)		1.00 (reference)	
		G/C	120 (26.5)	230 (26.6)	0.99 (0.76, 1.28)	0.91	1.13 (0.84, 1.52)	0.43
		C/C	8 (1.8)	21 (2.4)	0.72 (0.32, 1.64)	0.43	1.04 (0.42, 2.60)	0.93
		Trend[a]			0.95 (0.76, 1.19)	0.63	1.10 (0.85, 1.42)	0.48
COX-2	rs4648261	G/G	426 (93.6)	826 (95.4)	1.00 (reference)		1.00 (reference)	
		G/A+A/A†	29 (6.4)	40 (4.6)	1.44 (0.88, 2.36)	0.15	1.52 (0.87, 2.68)	0.14
		Trend[a]			1.36 (0.84, 2.20)	0.22	1.42 (0.82, 2.46)	0.21
COX-2	rs689466	A/A	302 (66.5)	585 (67.6)	1.00 (reference)		1.00 (reference)	
		A/G	136 (30.0)	267 (30.8)	0.99 (0.77, 1.27)	0.92	0.93 (0.70, 1.23)	0.60
		G/G	16 (3.5)	14 (1.6)	2.21 (1.07, 4.60)	0.03	2.04 (0.91, 4.56)	0.08
		Trend[a]			1.11 (0.90, 1.38)	0.33	1.06 (0.83, 1.35)	0.64
COX-2	rs2745559	C/C	305 (67.5)	556 (64.5)	1.00 (reference)		1.00 (reference)	
		C/A	133 (29.4)	269 (31.2)	0.90 (0.70, 1.16)	0.42	0.88 (0.66, 1.17)	0.39
		A/A	14 (3.1)	37 (4.3)	0.69 (0.37, 1.30)	0.25	0.54 (0.26, 1.13)	0.10
		Trend[a]			0.87 (0.71, 1.07)	0.20	0.83 (0.65, 1.05)	0.11
IL1B	rs1143634	C/C	285 (63.0)	504 (58.2)	1.00 (reference)		1.00 (reference)	
		C/T	136 (30.1)	313 (36.1)	0.77 (0.60, 0.99)	0.04	0.71 (0.53, 0.95)	0.02
		T/T	31 (6.9)	49 (5.7)	1.12 (0.70, 1.80)	0.64	1.01 (0.58, 1.74)	0.98
		Trend[a]			0.91 (0.75, 1.09)	0.30	0.85 (0.68, 1.05)	0.14
IL1B	rs1143633	G/G	186 (41.1)	345 (40.0)	1.00 (reference)		1.00 (reference)	
		G/A	200 (44.2)	408 (47.3)	0.91 (0.71, 1.16)	0.45	0.83 (0.63, 1.10)	0.20
		A/A	67 (15.0)	110 (12.7)	1.13 (0.79, 1.61)	0.50	1.20 (0.80, 1.79)	0.38
		Trend[a]			1.02 (0.86, 1.21)	0.81	1.02 (0.84, 1.23)	0.84

Table 6-1. (*Continued*)

Gene	rs Number	Genotype	N (%) NSCLC	N (%) Controls	Genotype-based Model OR (95%CI)	Genotype-based Model P	Genotype-based Model* OR (95%CI)*	Genotype-based Model* P*
IL1B	rs1143627	T/T	188 (41.5)	382 (44.1)	1.00 (reference)		1.00 (reference)	
		T/C	211 (46.6)	383 (44.2)	1.12 (0.88, 1.43)	0.36	1.01 (0.77, 1.34)	0.93
		C/C	54 (11.9)	101 (11.7)	1.09 (0.75, 1.58)	0.66	1.12 (0.73, 1.72)	0.59
		Trend[a]			1.07 (0.90, 1.26)	0.46	1.05 (0.86, 1.27)	0.66
PPARG	rs1801282	C/C	348 (77.2)	644 (74.4)	1.00 (reference)		1.00 (reference)	
		C/G	94 (20.8)	201 (23.2)	0.87 (0.66, 1.14)	0.31	1.02 (0.74, 1.40)	0.92
		G/G	9 (2.0)	21 (2.4)	0.79 (0.36, 1.75)	0.57	0.76 (0.31, 1.85)	0.55
		Trend[a]			0.87 (0.69, 1.11)	0.26	0.97 (0.74, 1.26)	0.80

* Adjusted for age, sex, and pack-years. a. Additive model, dose-response, 1df.

† GA and AA were combined because there was 0 A/A in NSCLC cases and 1 A/A in controls.

Table 6-2. Unadjusted and Adjusted Results of SNP and ADC Associations: Genotype-based Model and Trend Test.

			N (%)		Genotype-based Model		Genotype-based Model*	
Gene	**rs Number**	**Genotype**	**ADC**	**Controls**	**OR (95% CI)**	**P**	**OR (95% CI)***	**P***
COX-2	rs5275	T/T	107 (47.6)	386 (44.9)	1.00 (reference)		1.00 (reference)	
		T/C	95 (42.2)	381 (44.3)	0.90 (0.66, 1.23)	0.50	0.90 (0.63, 1.27)	0.54
		C/C	23 (10.2)	93 (10.8)	0.89 (0.54, 1.48)	0.66	0.75 (0.43, 1.33)	0.33
		Trend[a]			0.93 (0.74, 1.16)	0.51	0.88 (0.69, 1.13)	0.30
COX-2	rs5277	G/G	165 (71.7)	614 (71.0)	1.00 (reference)		1.00 (reference)	
		G/C	59 (25.7)	230 (26.6)	0.96 (0.68, 1.33)	0.78	1.14 (0.79, 1.66)	0.48
		C/C	6 (2.6)	21 (2.4)	1.06 (0.42, 2.68)	0.90	1.45 (0.52, 4.08)	0.48
		Trend[a]			0.98 (0.74, 1.30)	0.88	1.16 (0.85, 1.60)	0.35
COX-2	rs4648261	G/G	215 (93.5)	826 (95.4)	1.00 (reference)		1.00 (reference)	
		G/A+A/A†	15 (6.5)	40 (4.6)	1.48 (0.80, 2.73)	0.21	1.46 (0.73, 2.92)	0.28
		Trend[a]			1.39 (0.76, 2.52)	0.28	1.35 (0.69, 2.62)	0.38
COX-2	rs689466	A/A	147 (63.9)	585 (67.6)	1.00 (reference)		1.00 (reference)	
		A/G	74 (32.2)	267 (30.8)	1.10 (0.81, 1.51)	0.54	1.07 (0.75, 1.52)	0.70
		G/G	9 (3.9)	14 (1.6)	2.56 (1.09, 6.03)	0.03	2.01 (0.79, 5.11)	0.14
		Trend[a]			1.24 (0.94, 1.62)	0.12	1.18 (0.87, 1.58)	0.29
COX-2	rs2745559	C/C	154 (67.3)	556 (64.5)	1.00 (reference)		1.00 (reference)	
		C/A	66 (28.8)	269 (31.2)	0.89 (0.64, 1.22)	0.46	0.87 (0.61, 1.25)	0.45
		A/A	9 (3.9)	37 (4.3)	0.88 (0.42, 1.86)	0.73	0.62 (0.26, 1.48)	0.28
		Trend[a]			0.91 (0.70, 1.18)	0.46	0.84 (0.62, 1.12)	0.23
IL1B	rs1143634	C/C	142 (62.0)	504 (58.2)	1.00 (reference)		1.00 (reference)	
		C/T	67 (29.3)	313 (36.1)	0.76 (0.55, 1.05)	0.10	0.70 (0.49, 1.00)	0.05
		T/T	20 (8.7)	49 (5.7)	1.45 (0.83, 2.52)	0.19	1.30 (0.70, 2.43)	0.41
		Trend[a]			0.98 (0.77, 1.25)	0.87	0.92 (0.70, 1.20)	0.52
IL1B	rs1143633	G/G	98 (42.8)	345 (40.0)	1.00 (reference)		1.00 (reference)	
		G/A	98 (42.8)	408 (47.3)	0.85 (0.62, 1.16)	0.30	0.73 (0.51, 1.03)	0.08
		A/A	33 (14.4)	110 (12.7)	1.06 (0.67, 1.66)	0.81	1.13 (0.68, 1.86)	0.64
		Trend[a]			0.98 (0.79, 1.21)	0.82	0.96 (0.75, 1.22)	0.73

Table 6-2. (*Continued*)

Gene	rs Number	Genotype	N (%) ADC	N (%) Controls	Genotype-based Model OR (95%CI)	Genotype-based Model P	Genotype-based Model* OR (95%CI)*	Genotype-based Model* P*
IL1B	rs1143627	T/T	98 (42.8)	382 (44.1)	1.00 (reference)		1.00 (reference)	
		T/C	109 (47.6)	383 (44.2)	1.11 (0.82, 1.51)	0.51	1.01 (0.72, 1.43)	0.95
		C/C	22 (9.6)	101 (11.7)	0.85 (0.51, 1.42)	0.53	1.01 (0.58, 1.77)	0.98
		Trend[a]			0.98 (0.79, 1.22)	0.88	1.01 (0.79, 1.29)	0.96
PPARG	rs1801282	C/C	180 (78.3)	644 (74.4)	1.00 (reference)		1.00 (reference)	
		C/G	48 (20.9)	201 (23.2)	0.85 (0.60, 1.22)	0.39	0.99 (0.67, 1.47)	0.96
		G/G	2 (0.9)	21 (2.4)	0.34, 0.08 (1.47)	0.15	0.30 (0.07, 1.41)	0.13
		Trend[a]			0.79 (0.57, 1.08)	0.13	0.86 (0.61, 1.21)	0.37

* Adjusted for age, sex, and pack-years. a. Additive model, dose-response, 1df.

† GA and AA were combined because there was 0 A/A in ADC cases and 1 A/A in controls.

Table 6-3. Unadjusted and Adjusted Results of SNP and SCC Associations: Genotype-based Model and Trend Test.

			N (%)		Genotype-based Model		Genotype-based Model*	
Gene	**rs Number**	**Genotype**	**SCC**	**Controls**	**OR (95% CI)**	**P**	**OR (95% CI)***	**P***
COX-2	rs5275	T/T	67 (41.1)	386 (44.9)	1.00 (reference)		1.00 (reference)	
		T/C	72 (44.2)	381 (44.3)	1.09 (0.76, 1.56)	0.64	1.13 (0.75, 1.68)	0.56
		C/C	24 (14.7)	93 (10.8)	1.49 (0.89, 2.50)	0.13	1.19 (0.66, 2.15)	0.57
		Trend[a]			1.18 (0.93, 1.51)	0.18	1.10 (0.84, 1.45)	0.50
COX-2	rs5277	G/G	119 (70.0)	614 (71.0)	1.00 (reference)		1.00 (reference)	
		G/C	49 (28.8)	230 (26.6)	1.10 (0.76, 1.58)	0.61	1.29 (0.85, 1.95)	0.23
		C/C	2 (1.2)	21 (2.4)	0.49 (0.11, 2.12)	0.34	0.98 (0.22, 4.47)	0.98
		Trend[a]			0.99 (0.72, 1.37)	0.95	1.21 (0.84, 1.75)	0.31
COX-2	rs4648261	G/G	162 (94.2)	826 (95.4)	1.00 (reference)		1.00 (reference)	
		G/A+A/A†	10 (5.8)	40 (4.6)	1.31 (0.64, 2.67)	0.46	1.19 (0.53, 2.70)	0.99
		Trend[a]			1.23 (0.61, 2.47)	0.56	1.15 (0.52, 2.55)	0.73
COX-2	rs689466	A/A	121 (70.8)	585 (67.6)	1.00 (reference)		1.00 (reference)	
		A/G	46 (26.9)	267 (30.8)	0.83 (0.58, 1.21)	0.33	0.82 (0.54, 1.24)	0.34
		G/G	4 (2.3)	14 (1.6)	1.38 (0.45, 4.27)	0.57	1.28 (0.38, 4.25)	0.69
		Trend[a]			0.91 (0.65, 1.26)	0.56	0.89 (0.62, 1.28)	0.54
COX-2	rs2745559	C/C	117 (68.8)	556 (64.5)	1.00 (reference)		1.00 (reference)	
		C/A	51 (30.0)	269 (31.2)	0.90 (0.63, 1.29)	0.57	0.92 (0.61, 1.37)	0.67
		A/A	2 (1.2)	37 (4.3)	0.26 (0.06, 1.08)	0.06	0.17 (0.04, 0.80)	0.02
		Trend[a]			0.78 (0.57, 1.06)	0.11	0.74 (0.52, 1.05)	0.09
IL1B	rs1143634	C/C	111 (65.3)	504 (58.2)	1.00 (reference)		1.00 (reference)	
		C/T	51 (30.0)	313 (36.1)	0.74 (0.52, 1.06)	0.10	0.70 (0.47, 1.05)	0.08
		T/T	8 (4.7)	49 (5.7)	0.74 (0.34, 1.61)	0.45	0.68 (0.29, 1.60)	0.37
		Trend[a]			0.79 (0.59, 1.06)	0.11	0.75 (0.54, 1.04)	0.08
IL1B	rs1143633	G/G	65 (38.0)	345 (40.0)	1.00 (reference)		1.00 (reference)	
		G/A	77 (45.0)	408 (47.3)	1.00 (0.70, 1.44)	0.99	1.01 (0.68, 1.51)	0.96
		A/A	29 (17.0)	110 (12.7)	1.40 (0.86, 2.28)	0.18	1.49 (0.86, 2.59)	0.16
		Trend[a]			1.14 (0.90, 1.45)	0.28	1.17 (0.90, 1.53)	0.25

Table 6-3. (*Continued*)

Gene	rs Number	Genotype	N (%) SCC	N (%) Controls	Genotype-based Model OR (95%CI)	Genotype-based Model P	Genotype-based Model* OR (95%CI)*	Genotype-based Model* P*
IL1B	rs1143627	T/T	70 (40.9)	382 (44.1)	1.00 (reference)		1.00 (reference)	
		T/C	78 (45.6)	383 (44.2)	1.11 (0.78, 1.58)	0.56	0.93 (0.63, 1.38)	0.71
		C/C	23 (13.5)	101 (11.7)	1.24 (0.74, 2.09)	0.41	1.19 (0.66, 2.15)	0.57
		Trend[a]			1.11 (0.88, 1.42)	0.38	1.04 (0.79, 1.37)	0.79
PPARG	rs1801282	C/C	126 (75.0)	644 (74.4)	1.00 (reference)		1.00 (reference)	
		C/G	38 (22.6)	201 (23.2)	0.97 (0.65, 1.44)	0.87	1.20 (0.77, 1.87)	0.43
		G/G	4 (2.4)	21 (2.4)	0.97 (0.33, 2.89)	0.96	0.85 (0.26, 2.79)	0.79
		Trend[a]			0.97 (0.70, 1.36)	0.87	1.09 (0.76, 1.57)	0.65

* Adjusted for age, sex, and pack-years. a. Additive model, dose-response, 1df.

† GA and AA were combined because there was 0 A/A in SCC cases and 1 A/A in controls.

Table 6-4. Unadjusted and Adjusted Results of Case-Case Comparison: Genotype-based Model and Trend Test.

			N (%)		Genotype-based Model		Genotype-based Model*	
Gene	**rs Number**	**Genotype**	**SCC**	**ADC**	**OR (95% CI)**	**P**	**OR (95% CI)** ^{R} *	**P***
COX-2	rs5275	T/T	67 (41.1)	107 (47.6)	1.00 (reference)		1.00 (reference)	
		T/C	72 (44.2)	95 (42.2)	1.21 (0.79, 1.87)	0.39	1.23 (0.79, 1.91)	0.37
		C/C	24 (14.7)	23 (10.2)	1.67 (0.87, 3.19)	0.12	1.50 (0.76, 2.93)	0.24
		Trend[a]			1.27 (0.94, 1.70)	0.12	1.22 (0.90, 1.66)	0.19
COX-2	rs5277	G/G	119 (70.0)	165 (71.7)	1.00 (reference)		1.00 (reference)	
		G/C	49 (28.8)	59 (25.7)	1.15 (0.74, 1.80)	0.54	1.16 (0.73, 1.84)	0.52
		C/C	2 (1.2)	6 (2.6)	0.46 (0.09, 2.33)	0.35	0.50 (0.10, 2.61)	0.41
		Trend[a]			1.01 (0.68, 1.50)	0.95	1.03 (0.69, 1.54)	0.89
COX-2	rs4648261	G/G	162 (94.2)	215 (93.5)	1.00 (reference)		1.00 (reference)	
		G/A+A/A†	10 (5.8)	15 (6.5)	0.89 (0.39, 2.02)	0.77	0.96 (0.41, 2.27)	0.93
		Trend[a]			0.89 (0.39, 2.02)	0.77	0.96 (0.41, 2.27)	0.93
COX-2	rs689466	A/A	121 (70.8)	147 (63.9)	1.00 (reference)		1.00 (reference)	
		A/G	46 (26.9)	74 (32.2)	0.76 (0.49, 1.17)	0.21	0.74 (0.47, 1.16)	0.19
		G/G	4 (2.3)	9 (3.9)	0.54 (0.16, 1.80)	0.32	0.56 (0.17, 1.91)	0.36
		Trend[a]			0.75 (0.52, 1.09)	0.13	0.74 (0.51, 1.09)	0.13
COX-2	rs2745559	C/C	117 (68.8)	154 (67.3)	1.00 (reference)		1.00 (reference)	
		C/A	51 (30.0)	66 (28.8)	1.02 (0.66, 1.58)	0.94	1.04 (0.66, 1.63)	0.87
		A/A	2 (1.2)	9 (3.9)	0.29 (0.06, 1.38)	0.12	0.32 (0.06, 1.54)	0.15
		Trend[a]			0.86 (0.59, 1.25)	0.42	0.88 (0.60, 1.30)	0.52
IL1B	rs1143634	C/C	111 (65.3)	142 (62.0)	1.00 (reference)		1.00 (reference)	
		C/T	51 (30.0)	67 (29.3)	0.97 (0.63, 1.51)	0.91	1.07 (0.68, 1.68)	0.79
		T/T	8 (4.7)	20 (8.7)	0.51 (0.22, 1.21)	0.13	0.48 (0.20, 1.16)	0.10
		Trend[a]			0.83 (0.60, 1.14)	0.25	0.84 (0.60, 1.18)	0.32
IL1B	rs1143633	G/G	65 (38.0)	98 (42.8)	1.00 (reference)		1.00 (reference)	
		G/A	77 (45.0)	98 (42.8)	1.19 (0.77, 1.83)	0.44	1.12 (0.72, 1.75)	0.63
		A/A	29 (17.0)	33 (14.4)	1.33 (0.74, 2.39)	0.35	1.21 (0.66, 2.22)	0.55
		Trend[a]			1.16 (0.88, 1.53)	0.30	1.10 (0.83, 1.47)	0.51

Table 6-4. (*Continued*)

Gene	rs Number	Genotype	N (%) SCC	N (%) ADC	Genotype-based Model OR (95%CI)	Genotype-based Model P	Genotype-based Model* OR (95%CI)[R]*	Genotype-based Model* P*
IL1B	rs1143627	T/T	70 (40.9)	98 (42.8)	1.00 (reference)		1.00 (reference)	
		T/C	78 (45.6)	109 (47.6)	1.00 (0.66, 1.53)	0.99	1.01 (0.65, 1.56)	0.97
		C/C	23 (13.5)	22 (9.6)	1.46 (0.76, 2.83)	0.26	1.48 (0.75, 2.92)	0.27
		Trend[a]			1.14 (0.84, 1.54)	0.39	1.14 (0.84, 1.56)	0.40
PPARG	rs1801282	C/C	126 (75.0)	180 (78.2)	1.00 (reference)		1.00 (reference)	
		C/G	38 (22.6)	48 (20.9)	1.13 (0.70, 1.83)	0.62	1.18 (0.72, 1.94)	0.52
		G/G	4 (2.4)	2 (0.9)	2.86 (0.52, 15.84)	0.23	2.86 (0.50, 16.26)	0.24
		Trend[a]			1.25 (0.81, 1.91)	0.31	1.29 (0.83, 2.00)	0.26

* Adjusted for age, sex, and pack-years. a. Additive model, dose-response, 1df. R. Reference group: Adenocarcinoma.

† GA and AA were combined because there was 0 A/A in ADC and 0 A/A in SCC.

APPENDIX B

SUPPLEMENTARY TABLES FOR MANUSCRIPT 2

Table 6-5. Unadjusted and Adjusted Results of SNP and NSCLC Associations: Genotype-based Model and Trend Test.

			N (%)		Genotype-based Model		Genotype-based Model*	
Gene	**rs Number**	**Genotype**	**NSCLC**	**Control**	**OR (95% CI)**	**P**	**OR (95%CI)***	**P ***
TGFB1	rs2241712	AA	197 (44.0)	373 (43.1)	1.00 (reference)		1.00 (reference)	
		AG	197 (44.0)	382 (44.2)	0.98 (0.77, 1.15)	0.85	1.06 (0.80, 1.40)	0.68
		GG	54 (12.0)	110 (12.7)	0.93 (0.64, 1.34)	0.70	0.93 (0.61, 1.42)	0.75
		Trend[a]			0.97 (0.82, 1.15)	0.70	0.99 (0.82, 1.20)	0.95
TGFB1	rs1800469	CC	203 (45.1)	383 (44.3)	1.00 (reference)		1.00 (reference)	
		CT	198 (44.0)	376 (43.5)	0.99 (0.78, 1.27)	0.96	1.04 (0.79, 1.36)	0.80
		TT	49 (10.9)	106 (12.2)	0.87 (0.60, 1.27)	0.48	0.91 (0.59, 1.40)	0.66
		Trend[a]			0.95 (0.81, 1.13)	0.58	0.98 (0.81, 1.19)	0.83
IL6	rs2069860	AA	447 (98.0)	853 (98.5)	1.00 (reference)		1.00 (reference)	
		AT	9 (2.0)	12 (1.4)	1.43 (0.60, 3.42)	0.42	1.69 (0.63, 4.55)	0.30
		TT	0 (0.0)	1 (0.1)	---	--	---	--
		Trend[a]			1.21 (0.54, 2.71)	0.65	1.44 (0.58, 3.55)	0.43
IL6	rs1800795	GG	172 (38.0)	312 (36.1)	1.00 (reference)		1.00 (reference)	
		GC	203 (44.8)	403 (46.5)	0.91 (0.71, 1.18)	0.48	0.97 (0.73, 1.29)	0.84
		CC	78 (17.2)	151 (17.4)	0.94 (0.67, 1.30)	0.70	1.16 (0.80, 1.69)	0.44
		Trend[a]			0.96 (0.82, 1.13)	0.60	1.06 (0.88, 1.27)	0.54
IL10	rs1800896	AA	136 (29.9)	253 (29.2)	1.00 (reference)		1.00 (reference)	
		AG	217 (47.7)	418 (48.3)	0.97 (0.74, 1.26)	0.80	1.09 (0.80, 1.47)	0.59
		GG	102 (22.4)	195 (22.5)	0.97 (0.71, 1.34)	0.87	1.12 (0.78, 1.60)	0.55
		Trend[a]			0.99 (0.84, 1.15)	0.85	1.06 (0.88, 1.27)	0.54
IL10	rs1800872	CC	272 (60.2)	495 (57.2)	1.00 (reference)		1.00 (reference)	
		CA	159 (35.2)	317 (36.6)	0.91 (0.72, 1.16)	0.46	0.82 (0.62, 1.08)	0.17
		AA	21 (4.6)	54 (6.2)	0.71 (0.42, 1.20)	0.20	0.61 (0.34, 1.09)	0.10
		Trend[a]			0.88 (0.73, 1.07)	0.19	0.80 (0.65, 0.998)	0.048
IL10	rs3024509	TT	411 (90.3)	779 (90.0)	1.00 (reference)		1.00 (reference)	
		TC	41 (9.0)	84 (9.7)	0.93 (0.63, 1.37)	0.70	1.06 (0.68, 1.65)	0.80
		CC	3 (0.7)	3 (0.3)	1.90 (0.38, 9.43)	0.43	1.70 (0.27, 10.77)	0.58
		Trend[a]			0.99 (0.70, 1.42)	0.97	1.10 (0.74, 1.64)	0.65

* Adjusted for age, sex and pack-years. a. Additive Model, dose-response, 1df.

Table 6-6. Unadjusted and Adjusted Results of SNP and ADC Associations: Genotype-based Model and Trend Test.

			N (%)		Genotype-based Model		Genotype-based Model*	
Gene	**rs Number**	**Genotype**	**ADC**	**Control**	**OR (95% CI)**	**P**	**OR (95% CI)***	**P***
TGFB1	rs2241712	AA	98 (43.8)	373 (43.1)	1.00 (reference)		1.00 (reference)	
		AG	102 (45.5)	382 (44.2)	1.02 (0.74, 1.39)	0.92	1.11 (0.78, 1.56)	0.57
		GG	24 (10.7)	110 (12.7)	0.83 (0.51, 1.36)	0.46	0.91 (0.53, 1.56)	0.72
		Trend[a]			0.94 (0.76, 1.17)	0.60	1.00 (0.78, 1.27)	0.99
TGFB1	rs1800469	CC	102 (45.1)	383 (44.3)	1.00 (reference)		1.00 (reference)	
		CT	105 (46.5)	376 (43.5)	1.05 (0.77, 1.43)	0.76	1.11 (0.79, 1.56)	0.57
		TT	19 (8.4)	106 (12.2)	0.67 (0.39, 1.15)	0.15	0.78 (0.44, 1.40)	0.41
		Trend[a]			0.90 (0.72, 1.12)	0.35	0.96 (0.75, 1.23)	0.74
IL6	rs2069860	AA	227 (98.7)	853 (98.5)	1.00 (reference)		1.00 (reference)	
		AT	3 (1.3)	12 (1.4)	0.94 (0.26, 3.36)	0.92	0.93 (0.22, 3.83)	0.91
		TT	0 (0.0)	1 (0.1)	---	--	---	--
		Trend[a]			0.82 (0.25, 2.71)	0.75	0.85 (0.22, 3.23)	0.81
IL6	rs1800795	GG	87 (38.3)	312 (36.0)	1.00 (reference)		1.00 (reference)	
		GC	103 (45.4)	403 (46.5)	0.92 (0.67, 1.26)	0.60	0.96 (0.67, 1.38)	0.83
		CC	37 (16.3)	151 (17.5)	0.88 (0.57, 1.35)	0.56	1.09 (0.68, 1.76)	0.72
		Trend[a]			0.93 (0.76, 1.15)	0.51	1.03 (0.82, 1.30)	0.82
IL10	rs1800896	AA	74 (32.3)	253 (29.2)	1.00 (reference)		1.00 (reference)	
		AG	109 (47.6)	418 (48.3)	0.89 (0.65, 1.25)	0.50	1.09 (0.75, 1.58)	0.67
		GG	46 (20.1)	195 (22.5)	0.81 (0.53, 1.22)	0.31	0.93 (0.59, 1.48)	0.77
		Trend[a]			0.90 (0.73, 1.10)	0.30	0.98 (0.78, 1.22)	0.84
IL10	rs1800872	CC	128 (55.9)	495 (57.2)	1.00 (reference)		1.00 (reference)	
		CA	91 (39.7)	317 (36.6)	1.11 (0.82, 1.50)	0.50	1.01 (0.72, 1.42)	0.94
		AA	10 (4.4)	54 (6.2)	0.72 (0.36, 1.45)	0.35	0.58 (0.27, 1.24)	0.16
		Trend[a]			0.98 (0.77, 1.25)	0.89	0.89 (0.68, 1.16)	0.39
IL10	rs3024509	TT	205 (89.5)	779 (90.0)	1.00 (reference)		1.00 (reference)	
		TC	24 (10.5)	84 (9.7)	1.09 (0.67, 1.75)	0.74	1.15 (0.68, 1.96)	0.60
		CC	0 (0.0)	3 (0.3)	---	--	---	--
		Trend[a]			1.01 (0.64, 1.60)	0.97	1.03 (0.62, 1.72)	0.91

* Adjusted for age, sex and pack-years. a. Additive Model, dose-response, 1df.

Table 6-7. Unadjusted and Adjusted Results of SNP and SCC Associations: Genotype-based Model and Trend Test.

			N (%)		Genotype-based Model		Genotype-based Model*	
Gene	**rs Number**	**Genotype**	**SCC**	**Control**	**OR (95% CI)**	**P**	**OR (95% CI)***	**P***
TGFB1	rs2241712	AA	75 (43.9)	373 (43.1)	1.00 (reference)		1.00 (reference)	
		AG	72 (42.1)	382 (44.2)	0.94 (0.66, 1.34)	0.72	0.96 (0.65, 1.42)	0.83
		GG	24 (14.0)	110 (12.7)	1.09 (0.65, 1.80)	0.75	0.94 (0.53, 1.68)	0.83
		Trend[a]			1.01 (0.80, 1.29)	0.92	0.97 (0.74, 1.26)	0.80
TGFB1	rs1800469	CC	77 (45.0)	383 (44.3)	1.00 (reference)		1.00 (reference)	
		CT	70 (40.9)	376 (43.5)	0.93 (0.65, 1.32)	0.67	0.91 (0.61, 1.34)	0.63
		TT	24 (14.1)	106 (12.2)	1.13 (0.68, 1.87)	0.65	1.01 (0.56, 1.80)	0.98
		Trend[a]			1.02 (0.81, 1.30)	0.86	0.97 (0.75, 1.27)	0.85
IL6	rs2069860	AA	167 (96.5)	853 (98.5)	1.00 (reference)		1.00 (reference)	
		AT	6 (3.5)	12 (1.4)	2.55 (0.95, 6.90)	0.06	3.42 (1.09, 10.76)	0.04
		TT	0 (0.0)	1 (0.1)	---	--	---	--
		Trend[a]			2.02 (0.80, 5.07)	0.13	2.70 (0.96, 7.58)	0.06
IL6	rs1800795	GG	61 (35.3)	312 (36.0)	1.00 (reference)		1.00 (reference)	
		GC	79 (45.6)	403 (46.5)	1.00 (0.70, 1.45)	0.99	1.02 (0.68, 1.53)	0.94
		CC	33 (19.1)	151 (17.5)	1.12 (0.70, 1.78)	0.64	1.20 (0.71, 2.02)	0.50
		Trend[a]			1.05 (0.83, 1.32)	0.68	1.08 (0.84, 1.40)	0.55
IL10	rs1800896	AA	44 (25.4)	253 (29.2)	1.00 (reference)		1.00 (reference)	
		AG	84 (48.6)	418 (48.3)	1.16 (0.78, 1.72)	0.48	1.37 (0.88, 2.14)	0.16
		GG	45 (26.0)	195 (22.5)	1.33 (0.84, 2.09)	0.22	1.63 (0.98, 2.71)	0.06
		Trend[a]			1.15 (0.92, 1.45)	0.22	1.28 (0.99, 1.64)	0.06
IL10	rs1800872	CC	115 (67.2)	495 (57.2)	1.00 (reference)		1.00 (reference)	
		CA	47 (27.5)	317 (36.6)	0.64 (0.44, 0.92)	0.02	0.59 (0.39, 0.89)	0.01
		AA	9 (5.3)	54 (6.2)	0.72 (0.34, 1.50)	0.38	0.62 (0.28, 1.37)	0.24
		Trend[a]			0.73 (0.55, 0.97)	0.03	0.68 (0.50, 0.93)	0.02
IL10	rs3024509	TT	161 (93.0)	779 (90.0)	1.00 (reference)		1.00 (reference)	
		TC	10 (5.8)	84 (9.7)	0.58 (0.29, 1.13)	0.11	0.72 (0.35, 1.50)	0.38
		CC	2 (1.2)	3 (0.3)	3.23 (0.54, 19.46)	0.20	4.19 (0.58, 30.35)	0.16
		Trend[a]			0.78 (0.44, 1.37)	0.38	0.98 (0.54, 1.78)	0.94

* Adjusted for age, sex and pack-years. a. Additive Model, dose-response, 1df.

Table 6-8. Unadjusted and Adjusted Results of Case-Case Comparison: Genotype-based Model and Trend Test.

Gene	rs Number	Genotype	N (%) SCC	N (%) ADC	Genotype-based Model OR (95% CI) [R]	Genotype-based Model P	Genotype-based Model* OR (95% CI) [R*]	Genotype-based Model* P*
TGFB1	rs2241712	AA	75 (43.9)	98 (43.8)	1.00 (reference)		1.00 (reference)	
		AG	72 (42.1)	102 (45.5)	0.92 (0.60, 1.41)	0.71	0.92 (0.59, 1.43)	0.71
		GG	24 (14.0)	24 (10.7)	1.31 (0.69, 2.48)	0.41	1.23 (0.63, 2.38)	0.54
		Trend[a]			1.07 (0.80, 1.44)	0.64	1.05 (0.78, 1.42)	0.76
TGFB1	rs1800469	CC	77 (45.0)	102 (45.1)	1.00 (reference)		1.00 (reference)	
		CT	70 (41.0)	105 (46.5)	0.88 (0.58, 1.35)	0.56	0.86 (0.56, 1.33)	0.49
		TT	24 (14.0)	19 (8.4)	1.67 (0.86, 3.27)	0.13	1.60 (0.80, 3.20)	0.18
		Trend[a]			1.14 (0.84, 1.53)	0.40	1.11 (0.82, 1.51)	0.50
IL6	rs2069860	AA	167 (96.5)	227 (98.7)	1.00 (reference)		1.00 (reference)	
		AT	6 (3.5)	3 (1.3)	2.72 (0.67, 11.03)	0.16	2.99 (0.72, 12.38)	0.13
		TT	0 (0.0)	0 (0.0)	---	--	---	--
		Trend[a]			2.72 (0.67, 11.03)	0.16	2.99 (0.72, 12.38)	0.13
IL6	rs1800795	GG	61 (35.3)	87 (38.3)	1.00 (reference)		1.00 (reference)	
		GC	79 (45.7)	103 (45.4)	1.09 (0.71, 1.70)	0.69	1.17 (0.74, 1.84)	0.50
		CC	33 (19.0)	37 (16.3)	1.27 (0.72, 2.25)	0.41	1.43 (0.79, 2.60)	0.24
		Trend[a]			1.12 (0.85, 1.48)	0.42	1.19 (0.89, 1.59)	0.24
IL10	rs1800896	AA	44 (25.4)	74 (32.3)	1.00 (reference)		1.00 (reference)	
		AG	84 (48.6)	109 (47.6)	1.30 (0.81, 2.07)	0.28	1.26 (0.78, 2.04)	0.35
		GG	45 (26.0)	46 (20.1)	1.65 (0.94, 2.87)	0.08	1.65 (0.93, 2.91)	0.09
		Trend[a]			1.28 (0.97, 1.69)	0.08	1.28 (0.97, 1.70)	0.09
IL10	rs1800872	CC	115 (67.2)	128 (55.9)	1.00 (reference)		1.00 (reference)	
		CA	47 (27.5)	91 (39.7)	0.58 (0.37, 0.89)	0.01	0.57 (0.37, 0.89)	0.01
		AA	9 (5.3)	10 (4.4)	1.00 (0.39, 2.55)	1.00	1.01 (0.39, 2.63)	0.98
		Trend[a]			0.73 (0.52, 1.04)	0.08	0.73 (0.52, 1.05)	0.09
IL10	rs3024509	TT	161 (93.0)	205 (89.5)	1.00 (reference)		1.00 (reference)	
		TC	10 (5.8)	24 (10.5)	0.53 (0.25, 1.14)	0.10	0.48 (0.22, 1.07)	0.07
		CC	2 (1.2)	0 (0.0)	---	--	---	--
		Trend[a]			0.77 (0.40, 1.49)	0.44	0.73 (0.37, 1.44)	0.36

* Adjusted for age, sex and pack-years. a. Additive Model, dose-response, 1df. R. Reference group: Adenocarcinoma.

APPENDIX C

SUPPLEMENTARY TABLES FOR MANUSCRIPT 3

Table 6-9. Allele Frequencies and HWE in the Whole Population (N=866).

Gene	rs Number	N	Allele	Allele Counts	Allele Frequencies, %	HWE P in controls (Exact Test)
TGFB1	rs2241712	865	A	1128	65.2	0.45
			G	602	34.8	
TGFB1	rs1800469	865	C	1142	66.0	0.36
			T	588	34.0	
IL6	rs2069860	866	A	1718	99.2	0.06
			T	14	0.8	
IL6	rs1800795	866	G	1027	59.3	0.29
			C	705	40.7	
IL10	rs1800896	866	A	924	53.4	0.38
			G	808	46.6	
IL10	rs1800872	866	C	1307	75.5	0.71
			A	425	24.5	
IL10	rs3024509	866	T	1642	94.8	0.50
			C	90	5.2	

Table 6-10. Allele Frequencies and HWE in No-Emphysema Subgroup (Emphysema Score=No).

Gene	rs Number	N	Allele	Allele Counts	Allele Frequencies, %	HWE P in controls (Exact Test)
TGFB1	rs2241712	506	A	655	64.7	0.70
			G	357	35.3	
TGFB1	rs1800469	506	C	662	65.4	0.76
			T	350	34.6	
IL6	rs2069860	507	A	1005	99.1	0.04
			T	9	0.9	
IL6	rs1800795	507	G	600	59.2	0.32
			C	414	40.8	
IL10	rs1800896	507	A	541	53.4	0.93
			G	473	46.6	
IL10	rs1800872	507	C	764	75.4	1.00
			A	250	24.6	
IL10	rs3024509	507	T	962	94.9	0.64
			C	52	5.1	

Table 6-11. Allele Frequencies and HWE in No-Airway Obstruction Subgroup (GOLD=0).

Gene	rs Number	N	Allele	Allele Counts	Allele Frequencies, %	HWE P in controls (Exact Test)
TGFB1	rs2241712	520	A	657	63.2	0.92
			G	383	36.8	
TGFB1	rs1800469	520	C	669	64.3	0.77
			T	371	35.7	
IL6	rs2069860	521	A	1033	99.1	0.04
			T	9	0.9	
IL6	rs1800795	521	G	621	59.6	0.60
			C	421	40.4	
IL10	rs1800896	521	A	552	53.0	0.34
			G	490	47.0	
IL10	rs1800872	521	C	787	75.5	1.00
			A	255	24.5	
IL10	rs3024509	521	T	988	94.8	0.39
			C	54	5.2	

Table 6-12. Unadjusted and Adjusted Results of SNP and Emphysema Associations: Genotype-based Model and Trend Test.

			N (%)		Genotype-based Model		Genotype-based Model*	
Gene	**rs Number**	**Genotype**	**Emphysema**	**Control**	**OR (95% CI)**	**P**	**OR (95% CI)***	**P***
TGFB1	rs2241712	AA	159 (44.3)	214 (42.3)	1.00 (reference)		1.00 (reference)	
		AG	155 (43.2)	227 (44.9)	0.92 (0.69, 1.23)	0.57	0.95 (0.71, 1.28)	0.74
		GG	45 (12.5)	65 (12.8)	0.93 (0.61, 1.44)	0.75	0.96 (0.61, 1.49)	0.84
		Trend[a]			0.95 (0.78, 1.16)	0.62	0.97 (0.79, 1.19)	0.76
TGFB1	rs1800469	CC	165 (46.0)	218 (43.1)	1.00 (reference)		1.00 (reference)	
		CT	150 (41.8)	226 (44.7)	0.88 (0.66, 1.17)	0.37	0.89 (0.66, 1.20)	0.46
		TT	44 (12.2)	62 (12.2)	0.94 (0.61, 1.45)	0.77	0.98 (0.62, 1.53)	0.91
		Trend[a]			0.94 (0.77, 1.15)	0.54	0.96 (0.78, 1.18)	0.68
IL6	rs2069860	AA	354 (98.6)	499 (98.4)	1.00 (reference)		1.00 (reference)	
		AT	5 (1.4)	7 (1.4)	1.01 (0.32, 3.20)	0.99	1.18 (0.37, 3.80)	0.78
		TT	0 (0.0)	1 (0.2)	---	--	---	--
		Trend[a]			0.81 (0.29, 2.27)	0.68	0.90 (0.32, 2.54)	0.84
IL6	rs1800795	GG	129 (35.9)	183 (36.1)	1.00 (reference)		1.00 (reference)	
		GC	169 (47.1)	234 (46.5)	1.03 (0.76, 1.38)	0.87	1.04 (0.77, 1.42)	0.80
		CC	61 (17.0)	90 (17.8)	0.96 (0.65, 1.43)	0.85	1.00 (0.67, 1.50)	1.00
		Trend[a]			0.99 (0.82, 1.20)	0.90	1.01 (0.83, 1.23)	0.95
IL10	rs1800896	AA	108 (30.1)	145 (28.6)	1.00 (reference)		1.00 (reference)	
		AG	167 (46.5)	251 (49.5)	0.89 (0.65, 1.23)	0.49	0.95 (0.69, 1.32)	0.76
		GG	84 (23.4)	111 (21.9)	1.02 (0.70, 1.48)	0.93	1.10 (0.75, 1.63)	0.62
		Trend[a]			1.00 (0.83, 1.21)	1.00	1.04 (0.86, 1.27)	0.66
IL10	rs1800872	CC	207 (57.7)	288 (56.8)	1.00 (reference)		1.00 (reference)	
		CA	129 (35.9)	188 (37.1)	0.96 (0.72, 1.27)	0.75	0.95 (0.71, 1.27)	0.72
		AA	23 (6.4)	31 (6.1)	1.03 (0.59, 1.82)	0.91	0.91 (0.51, 1.63)	0.74
		Trend[a]			0.99 (0.79, 1.23)	0.89	0.95 (0.76, 1.19)	0.66
IL10	rs3024509	TT	324 (90.3)	455 (89.7)	1.00 (reference)		1.00 (reference)	
		TC	32 (8.9)	52 (10.3)	0.86 (0.54, 1.37)	0.54	0.95 (0.59, 1.53)	0.83
		CC††	3 (0.8)	0 (0.0)	---	--	---	--
		Trend[a]			1.03 (0.68, 1.58)	0.88	1.13 (0.73, 1.75)	0.59

* Adjusted for age, sex and pack-years. a. Additive Model, dose-response, 1df.

Table 6-13. Adjusted Results of SNP and Emphysema Associations: Dominant Model.

Gene	rs Number	Subjects with data, N		Minor allele frequency, %		Dominant Model[b] Combined heterozygote and homozygote minor	
		Cases	Controls	Cases	Controls	OR (95% CI)	P
TGFB1	rs2241712	359	506	34.1	35.3	0.95 (0.72, 1.26)	0.73
TGFB1	rs1800469	359	506	33.2	34.6	0.91 (0.69, 1.20)	0.51
IL6	rs2069860	359	507	0.7	0.9	1.01 (0.32, 3.17)	0.98
IL6	rs1800795	359	507	40.5	40.8	1.03 (0.77, 1.37)	0.85
IL10	rs1800896	359	507	46.7	46.7	1.00 (0.74, 1.35)	0.98
IL10	rs1800872	359	507	24.4	24.7	0.94 (0.71, 1.25)	0.67
IL10	rs3024509	359	507	5.3	5.1	1.04 (0.65, 1.65)	0.87

Adjusted for age, sex, pack-years.

b. Dominant Model: combine heterozygote and homozygote minor.

Table 6-14. Associations within Subgroups and Interaction [Airflow Obstruction GOLD=I-IV vs. GOLD=0].

	***TGFB1* rs2241712** AG+GG vs. AA	***TGFB1*rs1800469** CT+TT vs. CC	***IL6* rs2069860** AT+TT vs. AA	***IL6* rs1800795** GC+CC vs. GG
Current Smoker	0.72 (0.50, 1.03)	0.73 (0.50, 1.05)	1.30 (0.28, 5.90)	1.35 (0.92, 2.00)
Former Smoker	0.77 (0.48, 1.23)	0.88 (0.55, 1.40)	0.97 (0.16, 5.83)	0.63 (0.39, 1.01)
P smoke status interaction	0.86	0.57	0.70	0.02
Mild*	1.08 (0.57, 2.05)	1.03 (0.55, 1.93)	---	1.06 (0.56, 2.02)
Moderate	0.63 (0.37, 1.06)	0.66 (0.39, 1.10)	1.79 (0.43, 7.51)	1.87 (1.04, 3.35)[b]
Heavy	0.65 (0.43, 0.99)[a]	0.74 (0.49, 1.13)	0.52 (0.05, 5.94)	0.74 (0.49, 1.13)
P pack-years interaction	0.46	0.65	0.86	0.48
P years of smoke interaction	0.97	0.92	0.54	0.65
P number of cig/d interaction	0.37	0.58	0.79	0.70
Male	0.73 (0.48, 1.09)	0.79 (0.53, 1.19)	0.29 (0.03, 2.55)	0.93 (0.61, 1.42)
Female	0.74 (0.49, 1.11)	0.76 (0.51, 1.14)	3.65 (0.65, 20.44)	1.16 (0.76, 1.76)
P sex interaction	0.98	0.87	0.07	0.47
Age<56	0.63 (0.38, 1.05)	0.70 (0.42, 1.15)	1.96 (0.32, 12.04)	1.58 (0.91, 2.73)
Age≥56	0.80 (0.57, 1.14)	0.84 (0.60, 1.19)	0.88 (0.21, 3.80)	0.89 (0.62, 1.28)
P age interaction	0.96	0.98	0.57	0.30

^ OR (95%CI) is adjusted for age, sex, and pack-years.

* Based on tertiles of pack-years in controls: mild smoker < 33 pack-years; moderate smoker=33 to <51.25; heavy smoker=≥51.25.

a. P=0.04

b. P=0.04

Table 6-14. (*Continued*)

	***IL10* rs1800896** AG+GG vs. AA	***IL10* rs1800872** CA+AA vs. CC	***IL10* rs3024509** TC+CC vs. TT
Current Smoker	0.95 (0.64, 1.42)	1.08 (0.75, 1.56)	1.27 (0.67, 2.41)
Former Smoker	1.36 (0.81, 2.28)	0.75 (0.46, 1.21)	0.87 (0.41, 1.83)
P smoke status interaction	0.32	0.25	0.30
Mild*	1.13 (0.56, 2.28)	1.14 (0.61, 2.13)	1.30 (0.53, 3.18)
Moderate	0.76 (0.44, 1.31)	1.32 (0.79, 2.19)	1.36 (0.61, 3.05)
Heavy	1.35 (0.86, 2.12)	0.74 (0.48, 1.12)	0.57 (0.27, 1.23)
P pack-years interaction	0.17	0.04	0.43
P years of smoke interaction	0.89	0.29	0.09
P number of cig/d interaction	0.31	0.23	0.71
Male	1.09 (0.70, 1.69)	0.95 (0.63, 1.43)	0.88 (0.44, 1.75)
Female	1.10 (0.70, 1.73)	0.98 (0.65, 1.46)	1.14 (0.59, 2.21)
P sex interaction	0.91	0.95	0.58
Age<56	1.68 (0.91, 3.09)	1.29 (0.78, 2.12)	1.83 (0.84, 3.96)
Age≥56	0.87 (0.60, 1.26)	0.85 (0.60, 1.20)	0.75 (0.41, 1.35)
P age interaction	0.60	0.17	0.06

^ OR (95%CI) is adjusted for age, sex, and pack-years.

* Based on tertiles of pack-years in controls: mild smoker < 33 pack-years; moderate smoker=33 to <51.25; heavy smoker=≥51.25.

Table 6-15. Associations within Subgroups and Interaction [any vs. no emphysema].

	***TGFB1* rs2241712** AG+GG vs. AA	***TGFB1*rs1800469** CT+TT vs. CC	***IL6* rs2069860** AT+TT vs. AA	***IL6* rs1800795** GC+CC vs. GG
Current Smoker	1.18 (0.83, 1.69)	1.13 (0.79, 1.61)	1.04 (0.23, 4.73)	1.01 (0.69, 1.46)
Former Smoker	0.69 (0.44, 1.09)	0.66 (0.42, 1.05)	1.05 (0.18, 6.25)	0.95 (0.59, 1.52)
P smoke status interaction	0.06	0.06	0.92	0.86
Mild*	1.42 (0.80, 2.52)	1.36 (0.77, 2.41)	2.20 (0.29, 16.45)	1.10 (0.62, 1.94)
Moderate	0.82 (0.50, 1.35)	0.78 (0.47, 1.27)	0.80 (0.14, 4.55)	1.40 (0.82, 2.39)
Heavy	0.87 (0.56, 1.34)	0.83 (0.54, 1.28)	0.57 (0.05, 6.41)	0.81 (0.52, 1.26)
P pack-years interaction	0.28	0.21	0.97	0.40
P years of smoke interaction	0.90	0.94	0.15	0.67
P number of cig/d interaction	0.08	0.05	0.21	0.80
Male	1.17 (0.77, 1.78)	1.11 (0.73, 1.68)	1.56 (0.32, 7.59)	0.90 (0.59, 1.38)
Female	0.82 (0.56, 1.21)	0.79 (0.5, 1.16)	0.73 (0.13, 4.04)	1.10 (0.74, 1.65)
P sex interaction	0.26	0.26	0.61	0.48
Age<57	1.02 (0.65, 1.60)	1.01 (0.65, 1.58)	2.88 (0.55, 14.98)	1.28 (0.81, 2.04)
Age≥57	0.98 (0.68, 1.41)	0.93 (0.65, 1.33)	0.55 (0.10, 2.88)	0.85 (0.58, 1.23)
P age interaction	0.50	0.41	0.15	0.51

^ OR (95%CI) is adjusted for age, sex, and pack-years.

* Based on tertiles of pack-years in controls: mild smoker < 34.5 pack-years; moderate smoker=34.5 to <53.75; heavy smoker=≥53.75.

Table 6-15. (*Continued*)

	***IL10* rs1800896** AG+GG vs. AA	***IL10* rs1800872** CA+AA vs. CC	***IL10* rs3024509** TC+CC vs. TT
Current Smoker	0.91 (0.62, 1.34)	1.03 (0.72, 1.48)	1.32 (0.70, 2.50)
Former Smoker	1.16 (0.70, 1.92)	0.74 (0.46, 1.18)	0.93 (0.45, 1.92)
P smoke status interaction	0.51	0.31	0.35
Mild*	1.67 (0.88, 3.20)	1.12 (0.64, 1.95)	0.83 (0.36, 1.90)
Moderate	0.65 (0.38, 1.11)	0.80 (0.49, 1.31)	1.77 (0.78, 4.02)
Heavy	1.02 (0.64, 1.64)	0.95 (0.61, 1.46)	0.71 (0.32, 1.57)
P pack-years interaction	0.66	0.40	0.61
P years of smoke interaction	0.25	0.68	0.72
P number of cig/d interaction	0.27	0.64	0.38
Male	0.66 (0.43, 1.03)	1.04 (0.69, 1.58)	0.69 (0.34, 1.43)
Female	1.51 (0.97, 2.35)	0.84 (0.57, 1.23)	1.49 (0.80, 2.79)
P sex interaction	0.004	0.37	0.09
Age<57	0.83 (0.51, 1.37)	1.20 (0.77, 1.88)	1.06 (0.51, 2.23)
Age≥57	1.06 (0.72, 1.56)	0.80 (0.56, 1.15)	1.12 (0.61, 2.05)
P age interaction	0.15	0.29	0.60

^ OR (95%CI) is adjusted for age, sex, and pack-years.

* Based on tertiles of pack-years in controls: mild smoker < 34.5 pack-years; moderate smoker=34.5 to <53.75; heavy smoker=≥53.75.

BIBLIOGRAPHY

1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin 2010;60:277-300.
2. Youlden DR, Cramb SM, Baade PD. The International Epidemiology of Lung Cancer: geographical distribution and secular trends. J Thorac Oncol 2008;3:819-31.
3. Sasco AJ, Secretan MB, Straif K. Tobacco smoking and cancer: a brief review of recent epidemiological evidence. Lung Cancer 2004;45 Suppl 2:S3-9.
4. Proctor RN. Tobacco and the global lung cancer epidemic. Nat Rev Cancer 2001;1:82-6.
5. Cigarette Smoking, Asbestos Exposure and Your Health. . Centers for Disease Control and Prevention. Agency for Toxic Substances and Disease Registry. , June 2006. (Accessed July 26, 2009, at http://www.atsdr.cdc.gov/asbestos/site-kit/docs/CigarettesAsbestos2.pdf)
6. Fact Sheet. Radon and Cancer: Questions and Answers. U.S. National Institutes of Health. National Cancer Institute. , 07/13/2004. (Accessed July 26, 2009, at http://www.cancer.gov/cancertopics/factsheet/Risk/radon)
7. 11th Report on Carcinogens (RoC). U.S. Department of Health and Human Services. National Toxicology Program. , 01/31/2005. (Accessed July 26 2009, at http://ntp.niehs.nih.gov/go/19914)
8. Lung Cancer Risk Factors. Centers for Disease Control and Prevention, 06/13/2009 (Accessed July 26, 2009, at http://www.cdc.gov/cancer/lung/basic_info/risk_factors.htm)
9. Berhoune M, Banu E, Scotte F, Prognon P, Oudard S, Bonan B. Therapeutic strategy for treatment of metastatic non-small cell lung cancer. Ann Pharmacother 2008;42:1640-52.
10. Mannino DM, Braman S. The epidemiology and economics of chronic obstructive pulmonary disease. Proc Am Thorac Soc 2007;4:502-6.
11. Mannino DM. COPD and lung cancer have come a long way ...baby. Am J Respir Crit Care Med 2007;176:108-9.
12. Saetta M, Di Stefano A, Turato G, et al. CD8+ T-lymphocytes in peripheral airways of smokers with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1998;157:822-6.
13. Di Stefano A, Capelli A, Lusuardi M, et al. Severity of airflow limitation is associated with severity of airway inflammation in smokers. Am J Respir Crit Care Med 1998;158:1277-85.
14. Nussbaumer-Ochsner Y, Rabe KF. Systemic manifestations of COPD. Chest 2011;139:165-73.
15. Rabe KF, Beghe B, Luppi F, Fabbri LM. Update in chronic obstructive pulmonary disease 2006. Am J Respir Crit Care Med 2007;175:1222-32.

16. Joos L, He JQ, Shepherdson MB, et al. The role of matrix metalloproteinase polymorphisms in the rate of decline in lung function. Hum Mol Genet 2002;11:569-76.
17. Skillrud DM, Offord KP, Miller RD. Higher risk of lung cancer in chronic obstructive pulmonary disease. A prospective, matched, controlled study. Ann Intern Med 1986;105:503-7.
18. Hecht SS. Tobacco carcinogens, their biomarkers and tobacco-induced cancer. Nat Rev Cancer 2003;3:733-44.
19. Auerbach O, Stout AP, Hammond EC, Garfinkel L. Changes in bronchial epithelium in relation to cigarette smoking and in relation to lung cancer. N Engl J Med 1961;265:253-67.
20. Wistuba, II. Genetics of preneoplasia: lessons from lung cancer. Curr Mol Med 2007;7:3-14.
21. Walser T, Cui X, Yanagawa J, et al. Smoking and lung cancer: the role of inflammation. Proc Am Thorac Soc 2008;5:811-5.
22. Yanbaeva DG, Dentener MA, Creutzberg EC, Wesseling G, Wouters EF. Systemic effects of smoking. Chest 2007;131:1557-66.
23. Agusti A. Thomas a. Neff lecture. Chronic obstructive pulmonary disease: a systemic disease. Proc Am Thorac Soc 2006;3:478-81.
24. Punturieri A, Szabo E, Croxton TL, Shapiro SD, Dubinett SM. Lung cancer and chronic obstructive pulmonary disease: needs and opportunities for integrated research. J Natl Cancer Inst 2009;101:554-9.
25. Tockman MS, Anthonisen NR, Wright EC, Donithan MG. Airways obstruction and the risk for lung cancer. Ann Intern Med 1987;106:512-8.
26. Turner MC, Chen Y, Krewski D, Calle EE, Thun MJ. Chronic obstructive pulmonary disease is associated with lung cancer mortality in a prospective study of never smokers. Am J Respir Crit Care Med 2007;176:285-90.
27. Schabath MB, Delclos GL, Martynowicz MM, et al. Opposing effects of emphysema, hay fever, and select genetic variants on lung cancer risk. Am J Epidemiol 2005;161:412-22.
28. Brownson RC, Alavanja MC. Previous lung disease and lung cancer risk among women (United States). Cancer Causes Control 2000;11:853-8.
29. Mayne ST, Buenconsejo J, Janerich DT. Previous lung disease and risk of lung cancer among men and women nonsmokers. Am J Epidemiol 1999;149:13-20.
30. Wu AH, Fontham ET, Reynolds P, et al. Previous lung disease and risk of lung cancer among lifetime nonsmoking women in the United States. Am J Epidemiol 1995;141:1023-32.
31. Osann KE. Lung cancer in women: the importance of smoking, family history of cancer, and medical history of respiratory disease. Cancer Res 1991;51:4893-7.
32. Wu AH, Yu MC, Thomas DC, Pike MC, Henderson BE. Personal and family history of lung disease as risk factors for adenocarcinoma of the lung. Cancer Res 1988;48:7279-84.
33. Samet JM, Humble CG, Pathak DR. Personal and family history of respiratory disease and lung cancer risk. Am Rev Respir Dis 1986;134:466-70.
34. Wilson DO, Weissfeld JL, Fuhrman CR, et al. The Pittsburgh Lung Screening Study (PLuSS): outcomes within 3 years of a first computed tomography scan. Am J Respir Crit Care Med 2008;178:956-61.

35. de Torres JP, Bastarrika G, Wisnivesky JP, et al. Assessing the relationship between lung cancer risk and emphysema detected on low-dose CT of the chest. Chest 2007;132:1932-8.
36. Papi A, Casoni G, Caramori G, et al. COPD increases the risk of squamous histological subtype in smokers who develop non-small cell lung carcinoma. Thorax 2004;59:679-81.
37. Engels EA. Inflammation in the development of lung cancer: epidemiological evidence. Expert Rev Anticancer Ther 2008;8:605-15.
38. Weitzman SA, Gordon LI. Inflammation and cancer: role of phagocyte-generated oxidants in carcinogenesis. Blood 1990;76:655-63.
39. Ballaz S, Mulshine JL. The potential contributions of chronic inflammation to lung carcinogenesis. Clin Lung Cancer 2003;5:46-62.
40. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet 2001;357:539-45.
41. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002;420:860-7.
42. Balkwill F, Charles KA, Mantovani A. Smoldering and polarized inflammation in the initiation and promotion of malignant disease. Cancer Cell 2005;7:211-7.
43. Ben-Baruch A. Inflammation-associated immune suppression in cancer: the roles played by cytokines, chemokines and additional mediators. Semin Cancer Biol 2006;16:38-52.
44. Smyth MJ, Cretney E, Kershaw MH, Hayakawa Y. Cytokines in cancer immunity and immunotherapy. Immunol Rev 2004;202:275-93.
45. Han J, Ulevitch RJ. Limiting inflammatory responses during activation of innate immunity. Nat Immunol 2005;6:1198-205.
46. Shacter E, Weitzman SA. Chronic inflammation and cancer. Oncology (Williston Park) 2002;16:217-26, 29; discussion 30-2.
47. Hussain SP, Hofseth LJ, Harris CC. Radical causes of cancer. Nat Rev Cancer 2003;3:276-85.
48. Dobrovolskaia MA, Kozlov SV. Inflammation and cancer: when NF-kappaB amalgamates the perilous partnership. Curr Cancer Drug Targets 2005;5:325-44.
49. de Visser KE, Eichten A, Coussens LM. Paradoxical roles of the immune system during cancer development. Nat Rev Cancer 2006;6:24-37.
50. Whiteside TL. The tumor microenvironment and its role in promoting tumor growth. Oncogene 2008;27:5904-12.
51. Kim R, Emi M, Tanabe K, Arihiro K. Tumor-driven evolution of immunosuppressive networks during malignant progression. Cancer Res 2006;66:5527-36.
52. Dubinett SM, Kradin RL. Cytokine immunotherapy of non-small cell lung cancer. Reg Immunol 1993;5:232-43.
53. Huang M, Wang J, Lee P, et al. Human non-small cell lung cancer cells express a type 2 cytokine pattern. Cancer Res 1995;55:3847-53.
54. Kradin RL, Boyle LA, Preffer FI, et al. Tumor-derived interleukin-2-dependent lymphocytes in adoptive immunotherapy of lung cancer. Cancer Immunol Immunother 1987;24:76-85.
55. Chung KF. Cytokines in chronic obstructive pulmonary disease. Eur Respir J Suppl 2001;34:50s-9s.
56. Hogg JC, Chu F, Utokaparch S, et al. The nature of small-airway obstruction in chronic obstructive pulmonary disease. N Engl J Med 2004;350:2645-53.

57. Smith CJ, Perfetti TA, King JA. Perspectives on pulmonary inflammation and lung cancer risk in cigarette smokers. Inhal Toxicol 2006;18:667-77.
58. Amos CI, Xu W, Spitz MR. Is there a genetic basis for lung cancer susceptibility? Recent Results Cancer Res 1999;151:3-12.
59. Fletcher C, Peto R. The natural history of chronic airflow obstruction. Br Med J 1977;1:1645-8.
60. Yang P, Schwartz AG, McAllister AE, Swanson GM, Aston CE. Lung cancer risk in families of nonsmoking probands: heterogeneity by age at diagnosis. Genet Epidemiol 1999;17:253-73.
61. Gorlova OY, Zhang Y, Schabath MB, et al. Never smokers and lung cancer risk: a case-control study of epidemiological factors. Int J Cancer 2006;118:1798-804.
62. Kueppers F, Miller RD, Gordon H, Hepper NG, Offord K. Familial prevalence of chronic obstructive pulmonary disease in a matched pair study. Am J Med 1977;63:336-42.
63. Hallberg J, Iliadou A, Anderson M, et al. Genetic and environmental influence on lung function impairment in Swedish twins. Respir Res 2010;11:92.
64. McDuffie HH. Clustering of cancer in families of patients with primary lung cancer. J Clin Epidemiol 1991;44:69-76.
65. Bailey-Wilson JE, Sellers TA, Elston RC, Evens CC, Rothschild H. Evidence for a major gene effect in early-onset lung cancer. J La State Med Soc 1993;145:157-62.
66. Hersh CP, Hokanson JE, Lynch DA, et al. Family history is a risk factor for chronic obstructive pulmonary disease. Chest 2011.
67. Popanda O, Edler L, Waas P, et al. Elevated risk of squamous-cell carcinoma of the lung in heavy smokers carrying the variant alleles of the TP53 Arg72Pro and p21 Ser31Arg polymorphisms. Lung Cancer 2007;55:25-34.
68. Hung RJ, McKay JD, Gaborieau V, et al. A susceptibility locus for lung cancer maps to nicotinic acetylcholine receptor subunit genes on 15q25. Nature 2008;452:633-7.
69. Genome-wide meta-analyses identify multiple loci associated with smoking behavior. Nat Genet 2010;42:441-7.
70. Falk GA, Briscoe WA. Alpha-1-antitrypsin deficiency in chronic obstructive pulmonary disease. Ann Intern Med 1970;72:427-9.
71. Engels EA, Wu X, Gu J, Dong Q, Liu J, Spitz MR. Systematic evaluation of genetic variants in the inflammation pathway and risk of lung cancer. Cancer Res 2007;67:6520-7.
72. Zienolddiny S, Ryberg D, Maggini V, Skaug V, Canzian F, Haugen A. Polymorphisms of the interleukin-1 beta gene are associated with increased risk of non-small cell lung cancer. Int J Cancer 2004;109:353-6.
73. He JQ, Foreman MG, Shumansky K, et al. Associations of IL6 polymorphisms with lung function decline and COPD. Thorax 2009;64:698-704.
74. Celedon JC, Lange C, Raby BA, et al. The transforming growth factor-beta1 (TGFB1) gene is associated with chronic obstructive pulmonary disease (COPD). Hum Mol Genet 2004;13:1649-56.
75. Brody JS, Spira A. State of the art. Chronic obstructive pulmonary disease, inflammation, and lung cancer. Proc Am Thorac Soc 2006;3:535-7.
76. Kuschner WG, D'Alessandro A, Wong H, Blanc PD. Dose-dependent cigarette smoking-related inflammatory responses in healthy adults. Eur Respir J 1996;9:1989-94.

77. Takizawa H, Tanaka M, Takami K, et al. Increased expression of inflammatory mediators in small-airway epithelium from tobacco smokers. Am J Physiol Lung Cell Mol Physiol 2000;278:L906-13.
78. van Eeden SF, Tan WC, Suwa T, et al. Cytokines involved in the systemic inflammatory response induced by exposure to particulate matter air pollutants (PM(10)). Am J Respir Crit Care Med 2001;164:826-30.
79. Matanic D, Beg-Zec Z, Stojanovic D, Matakoric N, Flego V, Milevoj-Ribic F. Cytokines in patients with lung cancer. Scand J Immunol 2003;57:173-8.
80. Bird S, Zou J, Wang T, Munday B, Cunningham C, Secombes CJ. Evolution of interleukin-1beta. Cytokine Growth Factor Rev 2002;13:483-502.
81. Campa D, Hung RJ, Mates D, et al. Lack of association between polymorphisms in inflammatory genes and lung cancer risk. Cancer Epidemiol Biomarkers Prev 2005;14:538-9.
82. Dubois RN, Abramson SB, Crofford L, et al. Cyclooxygenase in biology and disease. FASEB J 1998;12:1063-73.
83. Slatore CG, Au DH, Littman AJ, Satia JA, White E. Association of nonsteroidal anti-inflammatory drugs with lung cancer: results from a large cohort study. Cancer Epidemiol Biomarkers Prev 2009;18:1203-7.
84. Van Dyke AL, Cote ML, Prysak G, Claeys GB, Wenzlaff AS, Schwartz AG. Regular adult aspirin use decreases the risk of non-small cell lung cancer among women. Cancer Epidemiol Biomarkers Prev 2008;17:148-57.
85. Harris RE, Beebe-Donk J, Schuller HM. Chemoprevention of lung cancer by non-steroidal anti-inflammatory drugs among cigarette smokers. Oncol Rep 2002;9:693-5.
86. Akhmedkhanov A, Toniolo P, Zeleniuch-Jacquotte A, Koenig KL, Shore RE. Aspirin and lung cancer in women. Br J Cancer 2002;87:49-53.
87. Kosaka T, Miyata A, Ihara H, et al. Characterization of the human gene (PTGS2) encoding prostaglandin-endoperoxide synthase 2. Eur J Biochem 1994;221:889-97.
88. Smith WL, DeWitt DL, Garavito RM. Cyclooxygenases: structural, cellular, and molecular biology. Annu Rev Biochem 2000;69:145-82.
89. Cao Y, Prescott SM. Many actions of cyclooxygenase-2 in cellular dynamics and in cancer. J Cell Physiol 2002;190:279-86.
90. Kanaoka S, Takai T, Yoshida K. Cyclooxygenase-2 and tumor biology. Adv Clin Chem 2007;43:59-78.
91. Chan TA, Morin PJ, Vogelstein B, Kinzler KW. Mechanisms underlying nonsteroidal antiinflammatory drug-mediated apoptosis. Proc Natl Acad Sci U S A 1998;95:681-6.
92. Tsujii M, DuBois RN. Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. Cell 1995;83:493-501.
93. Nie D, Honn KV. Cyclooxygenase, lipoxygenase and tumor angiogenesis. Cell Mol Life Sci 2002;59:799-807.
94. Tsujii M, Kawano S, DuBois RN. Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. Proc Natl Acad Sci U S A 1997;94:3336-40.
95. Huang M, Sharma S, Mao JT, Dubinett SM. Non-small cell lung cancer-derived soluble mediators and prostaglandin E2 enhance peripheral blood lymphocyte IL-10 transcription and protein production. J Immunol 1996;157:5512-20.
96. Huang M, Stolina M, Sharma S, et al. Non-small cell lung cancer cyclooxygenase-2-dependent regulation of cytokine balance in lymphocytes and macrophages: up-

regulation of interleukin 10 and down-regulation of interleukin 12 production. Cancer Res 1998;58:1208-16.
97. Shattuck-Brandt RL, Lamps LW, Heppner Goss KJ, DuBois RN, Matrisian LM. Differential expression of matrilysin and cyclooxygenase-2 in intestinal and colorectal neoplasms. Mol Carcinog 1999;24:177-87.
98. Byers SW, Sommers CL, Hoxter B, Mercurio AM, Tozeren A. Role of E-cadherin in the response of tumor cell aggregates to lymphatic, venous and arterial flow: measurement of cell-cell adhesion strength. J Cell Sci 1995;108 (Pt 5):2053-64.
99. Dempke W, Rie C, Grothey A, Schmoll HJ. Cyclooxygenase-2: a novel target for cancer chemotherapy? J Cancer Res Clin Oncol 2001;127:411-7.
100. Attiga FA, Fernandez PM, Weeraratna AT, Manyak MJ, Patierno SR. Inhibitors of prostaglandin synthesis inhibit human prostate tumor cell invasiveness and reduce the release of matrix metalloproteinases. Cancer Res 2000;60:4629-37.
101. Chang TH, Szabo E. Induction of differentiation and apoptosis by ligands of peroxisome proliferator-activated receptor gamma in non-small cell lung cancer. Cancer Res 2000;60:1129-38.
102. Satoh T, Toyoda M, Hoshino H, et al. Activation of peroxisome proliferator-activated receptor-gamma stimulates the growth arrest and DNA-damage inducible 153 gene in non-small cell lung carcinoma cells. Oncogene 2002;21:2171-80.
103. Wick M, Hurteau G, Dessev C, et al. Peroxisome proliferator-activated receptor-gamma is a target of nonsteroidal anti-inflammatory drugs mediating cyclooxygenase-independent inhibition of lung cancer cell growth. Mol Pharmacol 2002;62:1207-14.
104. Su CG, Wen X, Bailey ST, et al. A novel therapy for colitis utilizing PPAR-gamma ligands to inhibit the epithelial inflammatory response. J Clin Invest 1999;104:383-9.
105. Han S, Roman J. Peroxisome proliferator-activated receptor gamma: a novel target for cancer therapeutics? Anticancer Drugs 2007;18:237-44.
106. Simon DM, Arikan MC, Srisuma S, et al. Epithelial cell PPAR[gamma] contributes to normal lung maturation. FASEB J 2006;20:1507-9.
107. Tegeder I, Pfeilschifter J, Geisslinger G. Cyclooxygenase-independent actions of cyclooxygenase inhibitors. FASEB J 2001;15:2057-72.
108. Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. Am J Hum Genet 2004;74:106-20.
109. ldSelect. (Accessed at http://droog.gs.washington.edu/ldSelect.html)
110. Zhang X, Miao X, Tan W, et al. Identification of functional genetic variants in cyclooxygenase-2 and their association with risk of esophageal cancer. Gastroenterology 2005;129:565-76.
111. Pociot F, Molvig J, Wogensen L, Worsaae H, Nerup J. A TaqI polymorphism in the human interleukin-1 beta (IL-1 beta) gene correlates with IL-1 beta secretion in vitro. Eur J Clin Invest 1992;22:396-402.
112. Hernandez-Guerrero C, Monzon-Bordonaba F, Jimenez-Zamudio L, et al. In-vitro secretion of proinflammatory cytokines by human amniochorion carrying hyper-responsive gene polymorphisms of tumour necrosis factor-alpha and interleukin-1beta. Mol Hum Reprod 2003;9:625-9.
113. Ter-Minassian M, Zhai R, Asomaning K, et al. Apoptosis gene polymorphisms, age, smoking and the risk of non-small cell lung cancer. Carcinogenesis 2008;29:2147-52.

114. Kiyohara C, Horiuchi T, Takayama K, Nakanishi Y. IL1B rs1143634 polymorphism, cigarette smoking, alcohol use, and lung cancer risk in a Japanese population. J Thorac Oncol;5:299-304.
115. El-Omar EM, Carrington M, Chow WH, et al. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature 2000;404:398-402.
116. Vogel U, Christensen J, Wallin H, et al. Polymorphisms in genes involved in the inflammatory response and interaction with NSAID use or smoking in relation to lung cancer risk in a prospective study. Mutat Res 2008;639:89-100.
117. Ermert L, Dierkes C, Ermert M. Immunohistochemical expression of cyclooxygenase isoenzymes and downstream enzymes in human lung tumors. Clin Cancer Res 2003;9:1604-10.
118. Fritsche E, Baek SJ, King LM, Zeldin DC, Eling TE, Bell DA. Functional characterization of cyclooxygenase-2 polymorphisms. J Pharmacol Exp Ther 2001;299:468-76.
119. Wang JM, Ko CY, Chen LC, Wang WL, Chang WC. Functional role of NF-IL6beta and its sumoylation and acetylation modifications in promoter activation of cyclooxygenase 2 gene. Nucleic Acids Res 2006;34:217-31.
120. Liu CJ, Hsia TC, Wang RF, et al. Interaction of cyclooxygenase 2 genotype and smoking habit in Taiwanese lung cancer patients. Anticancer Res;30:1195-9.
121. Gou Q, Liu CH, Ben-Av P, Hla T. Dissociation of basal turnover and cytokine-induced transcript stabilization of the human cyclooxygenase-2 mRNA by mutagenesis of the 3'-untranslated region. Biochem Biophys Res Commun 1998;242:508-12.
122. Shaw G, Kamen R. A conserved AU sequence from the 3' untranslated region of GM-CSF mRNA mediates selective mRNA degradation. Cell 1986;46:659-67.
123. Dixon DA. Dysregulated post-transcriptional control of COX-2 gene expression in cancer. Curr Pharm Des 2004;10:635-46.
124. Campa D, Zienolddiny S, Maggini V, Skaug V, Haugen A, Canzian F. Association of a common polymorphism in the cyclooxygenase 2 gene with risk of non-small cell lung cancer. Carcinogenesis 2004;25:229-35.
125. Hu Z, Miao X, Ma H, et al. A common polymorphism in the 3'UTR of cyclooxygenase 2/prostaglandin synthase 2 gene and risk of lung cancer in a Chinese population. Lung Cancer 2005;48:11-7.
126. Park JM, Choi JE, Chae MH, et al. Relationship between cyclooxygenase 8473T>C polymorphism and the risk of lung cancer: a case-control study. BMC Cancer 2006;6:70.
127. Masugi J, Tamori Y, Mori H, Koike T, Kasuga M. Inhibitory effect of a proline-to-alanine substitution at codon 12 of peroxisome proliferator-activated receptor-gamma 2 on thiazolidinedione-induced adipogenesis. Biochem Biophys Res Commun 2000;268:178-82.
128. Cok SJ, Morrison AR. The 3'-untranslated region of murine cyclooxygenase-2 contains multiple regulatory elements that alter message stability and translational efficiency. J Biol Chem 2001;276:23179-85.
129. Brasky TM, Bonner MR, Moysich KB, et al. Genetic variants in COX-2, non-steroidal anti-inflammatory drugs, and breast cancer risk: the Western New York Exposures and Breast Cancer (WEB) Study. Breast Cancer Res Treat 2011;126:157-65.
130. Fitzpatrick FA. Inflammation, carcinogenesis and cancer. Int Immunopharmacol 2001;1:1651-67.

131. O'Byrne KJ, Dalgleish AG. Chronic immune activation and inflammation as the cause of malignancy. Br J Cancer 2001;85:473-83.
132. Bauer AK, Malkinson AM, Kleeberger SR. Susceptibility to neoplastic and non-neoplastic pulmonary diseases in mice: genetic similarities. Am J Physiol Lung Cell Mol Physiol 2004;287:L685-703.
133. Martin F, Santolaria F, Batista N, et al. Cytokine levels (IL-6 and IFN-gamma), acute phase response and nutritional status as prognostic factors in lung cancer. Cytokine 1999;11:80-6.
134. Brichory FM, Misek DE, Yim AM, et al. An immune response manifested by the common occurrence of annexins I and II autoantibodies and high circulating levels of IL-6 in lung cancer. Proc Natl Acad Sci U S A 2001;98:9824-9.
135. Tas F, Duranyildiz D, Argon A, et al. Serum levels of leptin and proinflammatory cytokines in advanced-stage non-small cell lung cancer. Med Oncol 2005;22:353-8.
136. Kaminska J, Kowalska M, Kotowicz B, et al. Pretreatment serum levels of cytokines and cytokine receptors in patients with non-small cell lung cancer, and correlations with clinicopathological features and prognosis. M-CSF - an independent prognostic factor. Oncology 2006;70:115-25.
137. McKeown DJ, Brown DJ, Kelly A, Wallace AM, McMillan DC. The relationship between circulating concentrations of C-reactive protein, inflammatory cytokines and cytokine receptors in patients with non-small-cell lung cancer. Br J Cancer 2004;91:1993-5.
138. Van Dyke AL, Cote ML, Wenzlaff AS, et al. Cytokine and cytokine receptor single-nucleotide polymorphisms predict risk for non-small cell lung cancer among women. Cancer Epidemiol Biomarkers Prev 2009;18:1829-40.
139. Reyes-Gibby CC, Spitz M, Wu X, et al. Cytokine genes and pain severity in lung cancer: exploring the influence of TNF-alpha-308 G/A IL6-174G/C and IL8-251T/A. Cancer Epidemiol Biomarkers Prev 2007;16:2745-51.
140. Shih CM, Lee YL, Chiou HL, et al. The involvement of genetic polymorphism of IL-10 promoter in non-small cell lung cancer. Lung Cancer 2005;50:291-7.
141. Lee KM, Shen M, Chapman RS, et al. Polymorphisms in immunoregulatory genes, smoky coal exposure and lung cancer risk in Xuan Wei, China. Carcinogenesis 2007;28:1437-41.
142. Kirkbride KC, Blobe GC. Inhibiting the TGF-beta signalling pathway as a means of cancer immunotherapy. Expert Opin Biol Ther 2003;3:251-61.
143. Elliott RL, Blobe GC. Role of transforming growth factor Beta in human cancer. J Clin Oncol 2005;23:2078-93.
144. Domagala-Kulawik J, Hoser G, Safianowska A, Grubek-Jaworska H, Chazan R. Elevated TGF-beta1 concentration in bronchoalveolar lavage fluid from patients with primary lung cancer. Arch Immunol Ther Exp (Warsz) 2006;54:143-7.
145. Kong F, Jirtle RL, Huang DH, Clough RW, Anscher MS. Plasma transforming growth factor-beta1 level before radiotherapy correlates with long term outcome of patients with lung carcinoma. Cancer 1999;86:1712-9.
146. Papanicolaou DA, Wilder RL, Manolagas SC, Chrousos GP. The pathophysiologic roles of interleukin-6 in human disease. Ann Intern Med 1998;128:127-37.
147. Ishihara K, Hirano T. IL-6 in autoimmune disease and chronic inflammatory proliferative disease. Cytokine Growth Factor Rev 2002;13:357-68.

148. Ershler WB, Keller ET. Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. Annu Rev Med 2000;51:245-70.
149. Srisuma S, Biswal SS, Mitzner WA, Gallagher SJ, Mai KH, Wagner EM. Identification of genes promoting angiogenesis in mouse lung by transcriptional profiling. Am J Respir Cell Mol Biol 2003;29:172-9.
150. White ES, Strom SR, Wys NL, Arenberg DA. Non-small cell lung cancer cells induce monocytes to increase expression of angiogenic activity. J Immunol 2001;166:7549-55.
151. De Vita F, Orditura M, Galizia G, et al. Serum interleukin-10 levels as a prognostic factor in advanced non-small cell lung cancer patients. Chest 2000;117:365-73.
152. Hatanaka H, Abe Y, Naruke M, et al. Significant correlation between interleukin 10 expression and vascularization through angiopoietin/TIE2 networks in non-small cell lung cancer. Clin Cancer Res 2001;7:1287-92.
153. Hatanaka H, Abe Y, Kamiya T, et al. Clinical implications of interleukin (IL)-10 induced by non-small-cell lung cancer. Ann Oncol 2000;11:815-9.
154. Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV. An investigation of polymorphism in the interleukin-10 gene promoter. Eur J Immunogenet 1997;24:1-8.
155. Burdin N, Rousset F, Banchereau J. B-cell-derived IL-10: production and function. Methods 1997;11:98-111.
156. Yssel H, De Waal Malefyt R, Roncarolo MG, et al. IL-10 is produced by subsets of human CD4+ T cell clones and peripheral blood T cells. J Immunol 1992;149:2378-84.
157. Fiorentino DF, Zlotnik A, Mosmann TR, Howard M, O'Garra A. IL-10 inhibits cytokine production by activated macrophages. J Immunol 1991;147:3815-22.
158. de Waal Malefyt R, Yssel H, Roncarolo MG, Spits H, de Vries JE. Interleukin-10. Curr Opin Immunol 1992;4:314-20.
159. Dummer W, Becker JC, Schwaaf A, Leverkus M, Moll T, Brocker EB. Elevated serum levels of interleukin-10 in patients with metastatic malignant melanoma. Melanoma Res 1995;5:67-8.
160. Fortis C, Foppoli M, Gianotti L, et al. Increased interleukin-10 serum levels in patients with solid tumours. Cancer Lett 1996;104:1-5.
161. Gotlieb WH, Abrams JS, Watson JM, Velu TJ, Berek JS, Martinez-Maza O. Presence of interleukin 10 (IL-10) in the ascites of patients with ovarian and other intra-abdominal cancers. Cytokine 1992;4:385-90.
162. Wojciechowska-Lacka A, Matecka-Nowak M, Adamiak E, Lacki JK, Cerkaska-Gluszak B. Serum levels of interleukin-10 and interleukin-6 in patients with lung cancer. Neoplasma 1996;43:155-8.
163. Howell WM, Turner SJ, Bateman AC, Theaker JM. IL-10 promoter polymorphisms influence tumour development in cutaneous malignant melanoma. Genes Immun 2001;2:25-31.
164. McCarron SL, Edwards S, Evans PR, et al. Influence of cytokine gene polymorphisms on the development of prostate cancer. Cancer Res 2002;62:3369-72.
165. Kamiya T, Hatanaka H, Abe Y, et al. Interleukin-10 expression is closely correlated with the expression of granulocyte-macrophage colony-stimulating factor in non-small cell lung cancer. Anticancer Res 2003;23:2909-13.
166. Prud'homme D. Pathobiology of transforming growth factor beta in cancer, fibrosis and immunologic disease, and therapeutic considerations. Lab Invest 2008;87:1077-91.

167. Saunier EF, Akhurst RJ. TGF beta inhibition for cancer therapy. Curr Cancer Drug Targets 2006;6:565-78.
168. Colakogullari M, Ulukaya E, Yilmaztepe Oral A, et al. The involvement of IL-10, IL-6, IFN-gamma, TNF-alpha and TGF-beta gene polymorphisms among Turkish lung cancer patients. Cell Biochem Funct 2008;26:283-90.
169. Vanden Berghe W, Vermeulen L, De Wilde G, De Bosscher K, Boone E, Haegeman G. Signal transduction by tumor necrosis factor and gene regulation of the inflammatory cytokine interleukin-6. Biochem Pharmacol 2000;60:1185-95.
170. Terry CF, Loukaci V, Green FR. Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation. J Biol Chem 2000;275:18138-44.
171. Garcia-Closas M, Lubin JH. Power and sample size calculations in case-control studies of gene-environment interactions: comments on different approaches. Am J Epidemiol 1999;149:689-92.
172. Geisler SA, Olshan AF. GSTM1, GSTT1, and the risk of squamous cell carcinoma of the head and neck: a mini-HuGE review. Am J Epidemiol 2001;154:95-105.
173. Green J, Banks E, Berrington A, Darby S, Deo H, Newton R. N-acetyltransferase 2 and bladder cancer: an overview and consideration of the evidence for gene-environment interaction. Br J Cancer 2000;83:412-7.
174. Keen LJ. The extent and analysis of cytokine and cytokine receptor gene polymorphism. Transpl Immunol 2002;10:143-6.
175. Jemal A, Ward E, Hao Y, Thun M. Trends in the leading causes of death in the United States, 1970-2002. JAMA 2005;294:1255-9.
176. Rabe KF, Hurd S, Anzueto A, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med 2007;176:532-55.
177. Global Initiative for chronic obstructive lung disease (GOLD). Global strategy for the diagnosis, management, and prevention of chronic obstructive lung disease.: National Institutes of health: National heart, lung, and blood institute.; 2006.
178. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease. American Thoracic Society. Am J Respir Crit Care Med 1995;152:S77-121.
179. Stern EJ, Frank MS. CT of the lung in patients with pulmonary emphysema: diagnosis, quantification, and correlation with pathologic and physiologic findings. AJR Am J Roentgenol 1994;162:791-8.
180. Mannino DM. COPD: epidemiology, prevalence, morbidity and mortality, and disease heterogeneity. Chest 2002;121:121S-6S.
181. Doll R, Peto R, Boreham J, Sutherland I. Mortality in relation to smoking: 50 years' observations on male British doctors. BMJ 2004;328:1519.
182. Bascom R. Differential susceptibility to tobacco smoke: possible mechanisms. Pharmacogenetics 1991;1:102-6.
183. Wright JL, Lawson LM, Pare PD, Wiggs BJ, Kennedy S, Hogg JC. Morphology of peripheral airways in current smokers and ex-smokers. Am Rev Respir Dis 1983;127:474-7.
184. Silverman EK, Mosley JD, Palmer LJ, et al. Genome-wide linkage analysis of severe, early-onset chronic obstructive pulmonary disease: airflow obstruction and chronic bronchitis phenotypes. Hum Mol Genet 2002;11:623-32.

185. Sporn MB, Roberts AB. Transforming growth factor-beta. Multiple actions and potential clinical applications. JAMA 1989;262:938-41.
186. Letterio JJ, Roberts AB. Regulation of immune responses by TGF-beta. Annu Rev Immunol 1998;16:137-61.
187. Eickelberg O, Kohler E, Reichenberger F, et al. Extracellular matrix deposition by primary human lung fibroblasts in response to TGF-beta1 and TGF-beta3. Am J Physiol 1999;276:L814-24.
188. Fang KC, Wolters PJ, Steinhoff M, Bidgol A, Blount JL, Caughey GH. Mast cell expression of gelatinases A and B is regulated by kit ligand and TGF-beta. J Immunol 1999;162:5528-35.
189. McGowan SE, Jackson SK, Olson PJ, Parekh T, Gold LI. Exogenous and endogenous transforming growth factors-beta influence elastin gene expression in cultured lung fibroblasts. Am J Respir Cell Mol Biol 1997;17:25-35.
190. Kucich U, Rosenbloom JC, Abrams WR, Rosenbloom J. Transforming growth factor-beta stabilizes elastin mRNA by a pathway requiring active Smads, protein kinase C-delta, and p38. Am J Respir Cell Mol Biol 2002;26:183-8.
191. Rennard SI. Inflammation and repair processes in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1999;160:S12-6.
192. Donaldson GC, Seemungal TA, Patel IS, et al. Airway and systemic inflammation and decline in lung function in patients with COPD. Chest 2005;128:1995-2004.
193. Walston JD, Fallin MD, Cushman M, et al. IL-6 gene variation is associated with IL-6 and C-reactive protein levels but not cardiovascular outcomes in the Cardiovascular Health Study. Hum Genet 2007;122:485-94.
194. Keatings VM, Collins PD, Scott DM, Barnes PJ. Differences in interleukin-8 and tumor necrosis factor-alpha in induced sputum from patients with chronic obstructive pulmonary disease or asthma. Am J Respir Crit Care Med 1996;153:530-4.
195. Agusti AG, Noguera A, Sauleda J, Sala E, Pons J, Busquets X. Systemic effects of chronic obstructive pulmonary disease. Eur Respir J 2003;21:347-60.
196. Broekhuizen R, Wouters EF, Creutzberg EC, Schols AM. Raised CRP levels mark metabolic and functional impairment in advanced COPD. Thorax 2006;61:17-22.
197. Sin DD, Lacy P, York E, Man SF. Effects of fluticasone on systemic markers of inflammation in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2004;170:760-5.
198. Majello B, Arcone R, Toniatti C, Ciliberto G. Constitutive and IL-6-induced nuclear factors that interact with the human C-reactive protein promoter. EMBO J 1990;9:457-65.
199. Aronson D, Roterman I, Yigla M, et al. Inverse association between pulmonary function and C-reactive protein in apparently healthy subjects. Am J Respir Crit Care Med 2006;174:626-32.
200. Shaaban R, Kony S, Driss F, et al. Change in C-reactive protein levels and FEV1 decline: a longitudinal population-based study. Respir Med 2006;100:2112-20.
201. Howard M, O'Garra A, Ishida H, de Waal Malefyt R, de Vries J. Biological properties of interleukin 10. J Clin Immunol 1992;12:239-47.
202. de Waal Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE. Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. J Exp Med 1991;174:1209-20.

203. Lim S, Roche N, Oliver BG, Mattos W, Barnes PJ, Chung KF. Balance of matrix metalloprotease-9 and tissue inhibitor of metalloprotease-1 from alveolar macrophages in cigarette smokers. Regulation by interleukin-10. Am J Respir Crit Care Med 2000;162:1355-60.
204. Takanashi S, Hasegawa Y, Kanehira Y, et al. Interleukin-10 level in sputum is reduced in bronchial asthma, COPD and in smokers. Eur Respir J 1999;14:309-14.
205. Wilson DO, Weissfeld JL, Balkan A, et al. Association of radiographic emphysema and airflow obstruction with lung cancer. Am J Respir Crit Care Med 2008;178:738-44.
206. Ferguson GT, Enright PL, Buist AS, Higgins MW. Office spirometry for lung health assessment in adults: A consensus statement from the National Lung Health Education Program. Chest 2000;117:1146-61.
207. Lung function testing: selection of reference values and interpretative strategies. American Thoracic Society. Am Rev Respir Dis 1991;144:1202-18.
208. Eid AA, Ionescu AA, Nixon LS, et al. Inflammatory response and body composition in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2001;164:1414-8.
209. de Torres JP, Cordoba-Lanus E, Lopez-Aguilar C, et al. C-reactive protein levels and clinically important predictive outcomes in stable COPD patients. Eur Respir J 2006;27:902-7.
210. de Boer WI, van Schadewijk A, Sont JK, et al. Transforming growth factor beta1 and recruitment of macrophages and mast cells in airways in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1998;158:1951-7.
211. Finlay GA, O'Donnell MD, O'Connor CM, Hayes JP, FitzGerald MX. Elastin and collagen remodeling in emphysema. A scanning electron microscopy study. Am J Pathol 1996;149:1405-15.
212. Hoidal JR. Genetics of COPD: present and future. Eur Respir J 2001;18:741-3.
213. Grainger DJ, Heathcote K, Chiano M, et al. Genetic control of the circulating concentration of transforming growth factor type beta1. Hum Mol Genet 1999;8:93-7.
214. Silverman ES, Palmer LJ, Subramaniam V, et al. Transforming growth factor-beta1 promoter polymorphism C-509T is associated with asthma. Am J Respir Crit Care Med 2004;169:214-9.
215. Su ZG, Wen FQ, Feng YL, Xiao M, Wu XL. Transforming growth factor-beta1 gene polymorphisms associated with chronic obstructive pulmonary disease in Chinese population. Acta Pharmacol Sin 2005;26:714-20.
216. van Diemen CC, Postma DS, Vonk JM, Bruinenberg M, Nolte IM, Boezen HM. Decorin and TGF-beta1 polymorphisms and development of COPD in a general population. Respir Res 2006;7:89.
217. Yoon HI, Silverman EK, Lee HW, et al. Lack of association between COPD and transforming growth factor-beta1 (TGFB1) genetic polymorphisms in Koreans. Int J Tuberc Lung Dis 2006;10:504-9.
218. Ito M, Hanaoka M, Droma Y, et al. The association of transforming growth factor beta 1 gene polymorphisms with the emphysema phenotype of COPD in Japanese. Intern Med 2008;47:1387-94.
219. Smolonska J, Wijmenga C, Postma DS, Boezen HM. Meta-analyses on suspected chronic obstructive pulmonary disease genes: a summary of 20 years' research. Am J Respir Crit Care Med 2009;180:618-31.

220. Fishman D, Faulds G, Jeffery R, et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest 1998;102:1369-76.
221. Seifart C, Dempfle A, Plagens A, et al. TNF-alpha-, TNF-beta-, IL-6-, and IL-10-promoter polymorphisms in patients with chronic obstructive pulmonary disease. Tissue Antigens 2005;65:93-100.
222. Broekhuizen R, Grimble RF, Howell WM, et al. Pulmonary cachexia, systemic inflammatory profile, and the interleukin 1beta -511 single nucleotide polymorphism. Am J Clin Nutr 2005;82:1059-64.
223. Cordoba-Lanus E, de-Torres JP, Lopez-Aguilar C, et al. Association of IL-6 gene polymorphisms and COPD in a Spanish population. Respir Med 2008;102:1805-11.
224. Danilko KV, Korytina GF, Akhmidishina LZ, Ianbaeva DG, Zagidullin Sh Z, Victorova TV. [Association of cytokines genes (ILL, IL1RN, TNF, LTA, IL6, IL8, IL0) polymorphic markers with chronic obstructive pulmonary disease]. Mol Biol (Mosk) 2007;41:26-36.
225. Taga K, Chretien J, Cherney B, Diaz L, Brown M, Tosato G. Interleukin-10 inhibits apoptotic cell death in infectious mononucleosis T cells. J Clin Invest 1994;94:251-60.
226. Levy Y, Brouet JC. Interleukin-10 prevents spontaneous death of germinal center B cells by induction of the bcl-2 protein. J Clin Invest 1994;93:424-8.
227. Watson L, Vestbo J, Postma DS, et al. Gender differences in the management and experience of Chronic Obstructive Pulmonary Disease. Respir Med 2004;98:1207-13.
228. de Torres JP, Campo A, Casanova C, Aguirre-Jaime A, Zulueta J. Gender and chronic obstructive pulmonary disease in high-risk smokers. Respiration 2006;73:306-10.
229. de Torres JP, Casanova C, Hernandez C, et al. Gender associated differences in determinants of quality of life in patients with COPD: a case series study. Health Qual Life Outcomes 2006;4:72.
230. Langhammer A, Johnsen R, Holmen J, Gulsvik A, Bjermer L. Cigarette smoking gives more respiratory symptoms among women than among men. The Nord-Trondelag Health Study (HUNT). J Epidemiol Community Health 2000;54:917-22.
231. Hersh CP, Demeo DL, Lange C, et al. Attempted replication of reported chronic obstructive pulmonary disease candidate gene associations. Am J Respir Cell Mol Biol 2005;33:71-8.
232. Huang SL, Su CH, Chang SC. Tumor necrosis factor-alpha gene polymorphism in chronic bronchitis. Am J Respir Crit Care Med 1997;156:1436-9.
233. Silverman EK, Palmer LJ. Case-control association studies for the genetics of complex respiratory diseases. Am J Respir Cell Mol Biol 2000;22:645-8.