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PTEN IDENTIFIED AS IMPORTANT RISK FACTOR OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Abstract

Common genetic variation may play an important role in altering chronic obstructive pulmonary disease (COPD) risk. In Xuanwei, China, the COPD rate is more than twice the Chinese national average, and COPD is strongly associated with in-home coal use. To identify genetic variation that may be associated with COPD in a population with substantial in-home coal smoke exposures, we evaluated 1,261 single nucleotide polymorphisms (SNPs) in 380 candidate genes potentially relevant for cancer and other human diseases in a population-based case-control study in Xuanwei (53 cases; 107 controls). *PTEN* was the most significantly associated gene with COPD in a minP analysis using 20,000 permutations (P = 0.00005). SNP-based analyses found that homozygote variant carriers of *PTEN* rs701848 (OR_{TT} = 0.12, 95%CI = 0.03 - 0.47) had a significant decreased risk of COPD. PTEN, or phosphatase and tensin homolog, is an important regulator of cell cycle progression and cellular survival via the AKT signaling pathway. Our exploratory analysis suggests that genetic variation in *PTEN* may be an important risk factor of COPD in Xuanwei. However, due to the small sample size, additional studies are needed to evaluate these associations within Xuanwei and other populations with coal smoke exposures.

Keywords

COPD; cell cycle; apoptosis; *AKT*; *PTEN*

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by an inflammation of the lower airways and lung. By 2020, COPD is estimated to be the third most common cause of death and fifth most common cause of disability in the world (1). In-home solid fuel burning for heating and cooking, to which 3 billion people are exposed worldwide (2), is one environmental exposure associated with COPD (3). Since family history of COPD is also a

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None of the authors have a conflict of interest to declare in relation to this work.

risk factor for COPD, genetic variation and its interaction with environmental exposures may contribute to COPD etiology (4).

Xuanwei, China poses a unique opportunity to assess COPD susceptibility in a population with substantial in-home coal smoke exposure. The primary source of indoor air pollution in Xuanwei is smoke from burning coal for heating and cooking. Xuanwei's COPD rate is more than twice the national average in China, and is strongly associated with coal use (3;5). In Xuanwei, 90% of residents are farmers with minimal industrial and automotive air pollution exposure (6). Nearly all women and few men cook, and most men and nearly no women smoke tobacco (7).

We hypothesized that a large-scale candidate gene analysis would provide insight into the etiology of COPD in Xuanwei. Therefore, we analyzed 1261 single nucleotide polymorphisms (SNPs) in 380 candidate genes using an Oligo Pool (OPA) with an Illumina® GoldenGate assay. Candidate SNPs were genotyped if they were potentially relevant for cancer and other human diseases or had possible functional significance, based on previously identified candidate genes.

Methods

This study population has been previously described (8). Briefly, all residents of Xuanwei, China from March 1995 to March 1996 were eligible for inclusion in a lung cancer population-based case-control study, of which 122 enrolled. Controls were selected from the Xuanwei general population using a household registration list and were individually matched (n = 122) to lung cancer cases by sex, age (± 2 years), village, and type of fuel used for in-home cooking and heating at time of interview. Controls with previously diagnosed COPD were not eligible for this study. At the same time, from March 1995 to March 1996, all newly diagnosed chronic bronchitis and/or emphysema (COPD) patients by the Xuanwei County Hospital, who had lived in Xuanwei their entire lifetime, were also enrolled (n = 53). COPD patients were identified from those attending the Xuanwei hospital for respiratory problems. Chronic bronchitis patients were diagnosed by the hospital if their medical history included cough and sputum on most days for at least 3 months over 2 consecutive years. Further, emphysema patients were identified by clinical symptoms, X-ray, and/or other hospital records. The participation rate for controls and COPD cases was 100%. A standardized questionnaire evaluating demographic information, smoking history, family and personal medical history, fuel use history, and other covariates was administered by trained interviewers to cases in the hospital and to controls in their homes. Smoking histories were determined by questions on smoking status, age started smoking, amount of tobacco smoked per day from cigarettes, and age quit smoking, if applicable. Coal use histories were collected by questions on type of fuel use, frequency of fuel use, and amount of fuel used for all homes throughout the subject's lifetime.

Genotyping was performed on DNA extracted from sputum samples via phenol-chloroform extraction (9). High throughput genotyping was successful for 53 cases and 111 controls with an Oligo Pool (OPA) by the Illumina GoldenGate Assay (www.illumina.com) at the National Cancer Institute's Core Genotyping Facility (Gaithersburg, MD). There was insufficient DNA available for 10 controls. Four additional controls were excluded for missing demographic data. Duplicate samples (n = 18) of both cases and controls were randomly distributed throughout study plates to ensure quality control and determine the intra-subject concordance rate for all assays (>98%). Initially, 1,442 SNPs from the SNP500Cancer (http://snp500cancer.nci.nih.gov) database in over 400 genes were genotyped successfully. Candidate SNPs were selected based on their potentially relevance to cancer and other human diseases or they had possible functional significance, based on previously identified candidate

genes. Hardy-Weinberg equilibrium (HWE) for each SNP was tested in controls with a Pearson χ^2 test or a Fischer's exact test if any of the cell counts were less than five. Exclusion of 165 SNPs with low minor allele frequency (<0.01) and 16 SNPs with substantial deviation from HWE ($p < 0.001$) left 1,261 SNPs in 380 genes for analysis.

The association between each gene and COPD was assessed by the minP test in MatLab, which assesses the significance of the minimal p-value in each gene using a permutation-based resampling procedure (20,000 permutations) that takes into account the number of SNPs genotyped in each gene region and their underlying linkage disequilibrium structure (10). False Discovery Rates (FDRs) were calculated to evaluate the significance of the minP results (11). Unconditional logistic regression was used to estimate the odds ratio (OR) and 95% confidence interval (CI) for the association between COPD risk and each SNP independently, using the homozygote of the common allele as the reference group and adjusting for age (<55 years, ≥55 years), sex, smoking (0 pack-years, 0< pack-years <25, ≥25 pack-years), and lifetime coal use (<130 tons, ≥130 tons). Dose effects for each SNP were estimated by a linear trend test by coding the genotypes based on the number of variant alleles present (0, 1, 2). Finally, interactions between the dominant genetic models for the *PTEN* SNPs and lifetime coal use (<130 tons, ≥130 tons), as well as smoking (0 pack-years, 0< pack-years <25, ≥25 pack-years), were tested on the multiplicative scale, adjusting for age, sex, smoking, and coal exposure.

All statistical methods were performed using SAS (Cary, NC), unless stated otherwise.

Results

Cases and controls were similar in sex, smoking status, and tons of coal used over the course of their lifetime, but not in age (Table 1).

Gene-based minP analysis identified 22 genes significantly associated with COPD risk (minP < 0.05) (Table 2). After adjusting for multiple comparisons, *PTEN* was the most significantly associated gene with COPD risk (FDR = 0.019). *POLB* (FDR = 0.076) and *ARNT* (FDR = 0.22) were also highly associated with COPD risk.

SNP analyses found that both of the two *PTEN* SNPs that were genotyped were associated with COPD risk (Table 3). Homozygote variant carriers of *PTEN* rs701848 ($OR_{TT} = 0.12$, 95% CI = 0.03 - 0.47) and rs1903858 ($OR_{AA} = 0.13$, 95%CI = 0.04 - 0.44) were associated with about a 9-fold decreased COPD risk. Since *PTEN* rs701848 and rs1903858 are in complete linkage disequilibrium and moderately correlated (D' = 1.0 and $r^2 = 0.78$, respectively), they are likely reporters of the same causative variant. *PTEN* rs701848 and rs1903858 did not interact significantly with coal use or smoking; however, there was some evidence that *PTEN* rs701848 and rs1903858 may play an important role in COPD risk among heavy smokers (Table 4).

Discussion

Through an exploratory gene-based analysis, 22 genes were identified as significantly associated with COPD susceptibility, of which *PTEN* was the most significant gene after accounting for multiple comparisons. *PTEN* rs701848 and rs1903858, which were moderately correlated, were associated with a significantly decreased risk of COPD.

PTEN, or phosphatase and tensin homolog, is an important regulator of cell cycle progression and cellular survival via the AKT signaling pathway (12;13). In fact, PTEN deletion is the most common mechanism of inappropriate AKT activation in human malignancy (14). PTEN negatively regulates the AKT pathway and is essential to homeostatis (15;16). As such, AKT and PTEN expression have been observed to be inversely correlated in lung cancer tissue

samples (17). While this is the first report, to the best of our knowledge, of specific *PTEN* SNPs associated with COPD, we previously reported the association between *PTEN* rs1903858 and lung cancer risk, in our parallel study in Xuanwei, which utilized the same controls (18). The inverse relationship observed in our studies between COPD and lung cancer and *PTEN* rs1903858 suggests that the variant may play an important role in respiratory diseases.

Smoke-induced mutations have been hypothesized to be associated with chronic inflammation of the airways and lung that accompanies COPD (19). *PTEN* is one such gene that is frequently mutated or deleted in the epithelium of smokers (20). In mice, PTEN expression play a crucial role in mediating airway inflammation and responsiveness for asthma (21). Further, PTEN overexpression also reduced airway hyperresponsiveness in mice (22).

Although smoking is an established risk factor for COPD, it was only slightly associated with increased risk of COPD in our analyses. Our results are similar to a cohort study in Xuanwei, which also observed comparable smoking habits between cases and the general population (23). Similarly, our lung cancer studies in Xuanwei find only a slight increased risk of lung cancer associated with smoking (24). The relatively weak effect of smoking on respiratory diseases in Xuanwei may result from the indoor air pollution levels attributed to smoky coal combustion in overwhelming the effect of tobacco smoke. Given that smoking is an established risk factor for COPD, and that smoky coal use has been associated with COPD in the cohort study in Xuanwei, it was important to explore the interactions between the significant *PTEN* SNPs and these important environmental risk factors, even though the statistical power was limited (<10%).

One of the major strengths of our population-based case-control study is the high participation rates. Further, our results are biologically plausible given that *PTEN* could contribute to COPD. Since the small sample size may lead to false positive and false negative findings (25), our findings should be viewed as hypothesis-generating. However, we adjusted the gene-based analyses for multiple comparisons to minimize the possibility of spurious findings. Due to our small sample size, we had limited power to detect modest risks at low minor allele frequencies (MAFs); however, we did have ample power (>80%) to detect risks of 2.0 and 2.5 for MAFs of ≥0.3 and ≥0.1, respectively. Specific SNP associations should be cautiously interpreted until these results are replicated, since SNP associations may be attributed to another SNP in linkage disequilibrium. Although the functionality of each SNP is not known, *PTEN* rs701848 is likely to alter *PTEN* expression and/or function provided that it is located in the 3′ translational termination codon. Further limitations of our study pertain to generalizability. As our analysis included only Asians, the generalizability of our findings to other ethnic groups is limited. The allelic frequency variation by ethnicity in HapMap found that the T allele at rs701848 is only 56% among individuals of Asian descent, compared to 81% for African descent. Similarly, the A allele at rs1903858 is only 44% among individuals of Asian descent, compared to 73% and 58% for European and African descent, respectively. Finally, our findings may not generalize to all COPD cases since disease degree of severity was not available for our analysis; however, it is likely that our study population consists of individuals with moderate to serve COPD as they sought treatment for symptoms in a hospital setting. While some controls may have had mild undiagnosed COPD, this would have underestimated our results.

In summary, our findings provide evidence of genetic variation that may be important to COPD susceptibility. Our results implicate the cell cycle and apoptosis regulatory AKT signaling pathway, particularly *PTEN*. Our results should be viewed as exploratory until they are replicated in larger studies.

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Table 1

Study participant characteristics of case-control study in Xuanwei

	Cases		Controls				
	N	%	N	%	OR*	95% CI*	p value**
Gender							0.78
Male	34	64.2	71	66.4			
Female	19	35.8	36	33.6			
Age (years)							0.05
<55	13	24.5	43	40.2			
≥55	40	75.5	64	59.8			
Smoking (Pack-Years)							0.34
Non-smokers	22	41.5	45	42.1	1.00	reference	
<25	11	20.8	32	29.9	1.12	0.28-4.39	
≥25	20	37.7	30	28.0	1.82	0.48-6.90	
Lifetime Coal Use (Tons)							0.47
<130	29	54.7	65	60.7	1.00	reference	
≥130	24	45.3	42	39.3	1.02	0.89-1.18	

Odds ratios (OR) and 95% confidence intervals (CI) are adjusted for age (<55 years, ≥55 years) and sex

** X^2 test

Table 2

Summary of the global gene p-values, permutation test, and False Disvory Rate for all genes with a permutation p ≤ 0.05

Gene	# SNPs	p-value for most signifcant SNP*	permutation p - value for gene*	False Discovery Rate
PTEN	2	<0.0001	0.0001	0.019
POLB	3	0.00010	0.00040	0.076
ARNT	9	0.00040	0.0018	0.22
IFNGR1	2	0.0023	0.0070	0.58
RGS5	1	0.0073	0.0092	0.58
ROS1	7	0.0012	0.0098	0.58
MX1	7	0.0020	0.015	0.58
AMACR	6	0.0026	0.016	0.58
CFH	5	0.0042	0.020	0.58
IL12A	1	0.018	0.022	0.58
TERF1	5	0.0052	0.026	0.58
SLC23A1	3	0.0084	0.027	0.58
BAX	1	0.027	0.031	0.58
NQO1	2	0.026	0.031	0.58
PARP1	6	0.0064	0.032	0.58
MBL2	9	0.0037	0.033	0.58
LIPC	10	0.0035	0.036	0.58
CAV1	3	0.012	0.041	0.58
SCUBE2	3	0.013	0.044	0.58
ESR1	6	0.0072	0.047	0.58
PLK1	1	0.043	0.047	0.58
XRCC3	2	0.023	0.048	0.58

* Adjusted for age (<55 years, ≥55 years), sex, smoking (0 pack-years, 0< pack-years <25, ≥25 pack-years), and lifetime coal use (<130 tons, ≥130 tons)

Table 3

***PTEN* polymorphisms and COPD risk**

SNP	Genotype	Cases	Controls	OR*	95% CI*	p
rs701848	CC	31	29			
	CT	19	53	0.31	0.14-0.67	0.0030
	TT	3	22	0.12	0.03-0.47	0.0020
	CT + TT	22	75	0.25	0.12-0.53	0.00026
	trend					0.00021
rs1903858	GG	24	22			
	AG	23	55	0.32	0.14-0.71	0.0054
	AA	4	20	0.13	0.04-0.44	0.0010
	AG + AA	27	84	0.25	0.12-0.55	0.00055
	trend					0.00031

*Odds ratios (OR) and 95% confidence intervals (CI) are adjusted for age (<55 years, ≥55 years), sex, smoking (0 pack-years, 0< pack-years <25, ≥25 pack-years), and lifetime coal use(<130 tons, ≥130 tons).

Table 4

***PTEN* polymorphisms and COPD risk, by smoking status**

SNP	Genotype	Non-smokers	<25 pack-years	≥25 pack-years	p-interaction
		OR (95% CI)*	OR (95% CI)*	OR (95% CI)*	
rs701848	GG	1.00 (reference)	1.00 (reference)	1.00 (reference)	
	GA+AA	0.40 (0.13-1.23)	0.14 (0.01-1.43)	0.15 (0.04-0.51)	0.24
rs1903858	AA	1.00 (reference)	1.00 (reference)	1.00 (reference)	
	AG+GG	0.38 (0.11-1.26)	0.36 (0.05-2.52)	0.15 (0.04-0.55)	0.35

* Odds ratios (OR) and 95% confidence intervals (CI) are adjusted for age (<55 years, ≥55 years), sex, and lifetime coal use(<130 tons, ≥130 tons).