CHRNA3 Variant for Lung Cancer Is Associated with Chronic Obstructive Pulmonary Disease in Korea

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Key Words

CHRNA3 • Chronic obstructive pulmonary disease • Genetic association

Abstract

Background: Genome-wide association studies have identified *CHRNA3* as a lung cancer and chronic obstructive pulmonary disease (COPD) candidate gene in non-Hispanic Caucasian cohorts. However, there are differences in minor allele frequencies among ethnic groups, and limited data exists for Asian populations. **Objectives:** The aim of this case-control study was to determine whether there is an association between COPD and genetic variation in *CHRNA3* in the Korean population. In addition, we investigated the association of *CHRNA3* with intermediate disease phenotypes including emphysema and lung function in COPD subjects. **Methods:** Two single-nucleotide polymorphisms (SNPs) in

CHRNA3 (rs660652 and rs12910984) were genotyped in 219 COPD subjects registered in the Korean Obstructive Lung Disease cohort study and in 305 control subjects. Volumetric computed tomography was performed in all COPD subjects. Emphysema severity was measured quantitatively by determining the volume fraction of the lung below –950 Hounsfield units. Logistic regression analysis for case-control analysis and linear regression modeling for quantitative analysis were performed using SAS. **Results:** This case-control analysis of 219 COPD patients and 305 control participants identified a significant association between an SNP of *CHRNA3* (rs12910984) and COPD ($p = 0.049$). Analysis in COPD subjects revealed that genetic variations were not associated with FEV₁. There was no association between SNPs and emphysema severity. However, both SNPs were significantly associated with DLCO. **Conclusion:** Genetic variations in *CHRNA3* are associated with COPD in the Korean population.

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Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by airflow limitation that is not fully reversible [1] and has a high worldwide disease prevalence [2]. Although cigarette smoking is a major risk factor, the pathophysiology of COPD is not fully understood. Recently, several studies suggested that exposure to other environmental factors, including biomass smoke [3], may also present important risk factors.

Genetic factors of COPD are also suggested because this disease develops in a subgroup of the population that has environmental exposure [4]. Recently, genome-wide association studies identified significant associations between COPD and novel genes that may be related to the pathophysiology of the disease [5]. Interestingly, the *CHRNA3/5* gene region on 15q24/25, which was repeatedly reported to be associated with lung cancer in genome-wide association studies [6, 7], is also associated with COPD [8, 9]. This region also contains *IREB2*, which has also been shown to be associated with COPD on the basis of results of a gene expression analysis of lung tissues of COPD patients and a genetic association study [10]. In addition, this region is also associated with emphysema [11]. These common genetic susceptibility results for COPD and lung cancer indicate possible common pathogenesis or common risk factors, such as smoking.

Few reports on the genetic associations of COPD exist in Asia. A previous report identified a genetic association between *CHRNA* and lung cancer in the Chinese population [12], but no reports on COPD genetic associations have been published in an Asian population. Therefore, we investigated whether an association exists between COPD and chromosome 15q25 *CHRNA3* in the Korean population. We also investigated the association between *CHRNA3* and intermediate disease phenotypes, including emphysema and lung function, in COPD subjects.

Methods

Study Design and Population

Individuals with COPD were recruited from the Korean Obstructive Lung Disease (KOLD) cohort, a longitudinal prospective study of COPD beginning in June 2005 [13]. Complete computed tomography (CT) scanning data, blood tests, and other clinical information were obtained from all patients. Spirometry was performed using a Vmax22 (SensorMedics, Yorba Linda, Calif., USA) or a PFDX (MedGraphics, St. Paul, Minn., USA). Diffusing capacity for carbon monoxide (DLCO) was measured by the single-breath method using a Vmax229D (SensorMedics) or a Masterlab Body (JaegerAB, Würzburg, Germany). All pulmonary

function tests were performed as recommended by the American Thoracic Society [14]. All COPD subjects included in the study had post-bronchodilator FEV₁/FVC values <0.7 and had >10 pack-years of smoking history. Control subjects consisted of 305 smokers or ex-smokers with normal lung function selected from the Korean Genome Epidemiology Study (KoGES) [15]. Institutional Review Board approval at all sites and informed consent from all patients were obtained.

CT Scanning

Volumetric CT scans were performed on all patients at full inspiration and expiration. Using in-house software, images of the whole lung were extracted automatically and calculated. Emphysema severity was measured quantitatively by determining the volume fraction of the lung below -950 Hounsfield units.

Genotyping

Two tagging single-nucleotide polymorphisms (SNPs) were chosen using the tagger program in Haploview [16]. We used pairwise linkage disequilibrium tagging with a minimum minor allele frequency of 0.10 and an r^2 threshold of 0.8 based on phase 2 genotype data in the Asian population of the HapMap Project (fig. 1). Two SNPs (rs660652 and rs12910984) out of 13 SNPs in the *CHRNA3* gene were selected as tagging SNPs. Genomic DNA was prepared from blood samples from all patients and SNPs were genotyped by the TaqMan method using an ABI Prism 7300 (Applied Biosystems, Foster City, Calif., USA) system and predesigned SNP genotyping assays according to the manufacturer's instructions.

Statistical Analysis

Hardy-Weinberg equilibrium was assessed using the goodness-of-fit test. Baseline characteristics were analyzed using a t test for quantitative traits and a χ^2 test for binary traits. Logistic regression was used to estimate the association with COPD adjusting for age, sex, and smoking amount. Associations between the quantitative phenotypes and SNP genotypes were tested using linear regression models adjusted for age, sex, height, and pack-years of cigarette smoking using SAS 9.1 (SAS Institute, Cary, N.C., USA).

Results

Demographic Characteristics

We analyzed 219 subjects with COPD (mean age 66.5 years) registered in the KOLD study and 305 control subjects (mean age 60.5 years). The mean FEV₁ of the COPD subjects and the control subjects were 1.46 and 2.76 liters, respectively (table 1). The age and pack-years of cigarette smoking were higher in the study group than in the control group. We adjusted age and smoking intensity in our analysis for associations.

Association between COPD Susceptibility and SNPs

Two SNPs (rs660652 and rs12910984) were chosen for genotyping. They were in Hardy-Weinberg equilibrium. A case-control analysis adjusting for age, sex, and pack-

Fig. 1. Linkage disequilibrium structure of SNPs in the *CHRNA3* region based on r^2 in phase 2 genotype data in the Asian population of the HapMap Project.

Table 1. Demographic characteristics of analyzed COPD subjects from the KOLD cohort and control subjects from the KoGES

	COPD	Control	p value
Number	219	305	
Males, n (%)	210 (96)	258 (85)	<0.01
Age, years	66.2 ± 7.3	60.5 ± 6.7	<0.01
Smoking, pack-years	47.7 ± 27.1	33.7 ± 20.2	<0.01
FEV ₁ , liters	1.46 ± 0.5	2.76 ± 0.3	<0.01
FEV ₁ , % of predicted	48.0 ± 15.6	113.5 ± 15.5	<0.01
DLCO	15.3 ± 6.08	–	
DLCO/VA	2.99 ± 1.06	–	
Emphysema severity	24.0 ± 16.1	–	

Data represent the means (±SD) unless otherwise noted.

years of smoking showed a significant association between rs12910984 and COPD ($p = 0.049$; table 2), but no such association was identified for rs660652.

Association with Lung Function in COPD Subjects

Linear regression analysis of COPD subjects adjusting for age, sex, pack-years of smoking, and height revealed that rs660652 was not associated with pre- or postbron-

chodilator FEV₁ ($p = 0.10$ and $p = 0.34$, respectively). Rs12910984 was also not associated with pre- or post-bronchodilator FEV₁ ($p = 0.25$ and $p = 0.48$, respectively). DLCO data was available in 195 COPD subjects. Both SNPs were significantly associated with DLCO and DLCO/VA (table 3). The mean DLCO was lower in the risk allele of rs12910984 for COPD (fig. 2).

Association with Emphysema and Smoking Amount in COPD Subjects

Emphysema data was available in 197 COPD subjects. The mean emphysema index was 24% in COPD patients (table 1). There was no association between SNPs and emphysema severity after adjustments for age, sex, height, and smoking amount ($p = 0.78$ and 0.30 , respectively). There was also no association between SNPs and smoking amount after separate adjustments for age and sex ($p = 0.42$ and $p = 0.72$, respectively).

Discussion

The association between *CHRNA3* polymorphisms and the development of COPD in Asian cohorts has not been analyzed. In this case-control study, we investigated

Table 2. Association analyses between SNPs in the *CHRNA3* gene and COPD

		COPD (n = 219)	Control (n = 305)	Odds ratio	p value
rs660652	GG	157	224	1.0 (reference)	
	GA	58	71	1.23 (0.79–1.93)	0.37
	AA	4	10	0.77 (0.20–2.93)	0.70
	MAF	0.151	0.149		0.60 (trend)
rs12910984	AA	63	67	1.0 (reference)	
	AG	110	160	0.67 (0.42–1.07)	0.10
	GG	46	78	0.50 (0.28–0.87)	0.02
	MAF	0.461	0.518		0.049 (trend)

Covariates in the regression models included age, sex, and pack-years of smoking.
MAF = Minor allele frequency.

Table 3. Genetic association analyses of the lung function and emphysema using linear regression in KOLD subjects

	Pre-FEV ₁		Post-FEV ₁		DLCO		DLCO/VA		Emphysema	
	estimate	p	estimate	p	estimate	p	estimate	p	estimate	p
rs660652	−0.12	0.10	−0.07	0.34	−1.67	0.03	−0.41	0.004	2.38	0.78
rs12910984	−0.06	0.25	−0.04	0.48	1.26	0.03	0.29	0.006	2.96	0.30

whether an association exists between COPD and chromosome 15q25 *CHRNA3* variants in the Korean population. We also investigated the association of *CHRNA3* with intermediate disease phenotypes, including emphysema and lung function in COPD subjects. We found that rs12910984, an SNP of *CHRNA3*, is associated with COPD, suggesting that a genetic association between *CHRNA3* and COPD could be replicated in an Asian COPD cohort at the gene level. We also found that both SNPs were associated with DLCO in the COPD cohort.

In previous reports, SNPs in chromosome 15q25 and in the region that includes rs1051730 in *CHRNA3* and rs8034191 in *LOC123688* were found to be associated with lung cancer and COPD in Caucasian populations [6, 7]. The most significant SNP in studies in Caucasian populations was rs1051730. However, the minor allele frequency of this SNP in Asians is very low. While one study reports a significant association between lung cancer and this SNP in the Japanese population [17], replication at the SNP level is difficult. In the present study, we used tagging SNPs in an Asian database using HapMap. In a previous lung cancer association study in China, risk

Fig. 2. DLCO by *CHRNA3* genotype (rs1910984) in the KOLD cohort. Mean values (±SEM) for DLCO are shown.

SNPs identified in Caucasians were not significantly associated with lung cancer risk in Chinese populations. However, commonly identified SNPs in the Chinese population, including rs12910984, were associated with lung cancer [12]. Interestingly, the A allele of rs12910984 was

previously identified as a risk allele for lung cancer, and here we show that the A allele of rs12910984 is also a risk allele for COPD in a Korean population. Another study in a Chinese population identified common SNPs in *CHRNA3*, one of which showed a significant association with lung cancer risk and prognosis [18]. One report suggests a genetic association between rs1051730 and emphysema and pulmonary function [11]. In this study, SNPs were not associated with FEV₁ or the emphysema index; however, they were significantly associated with DLCO. It is interesting that the risk allele of rs12910984 for COPD was associated with a lower DLCO, which may be associated with emphysema.

Interestingly, there are several genetic risk genes common to both COPD and lung cancer [19]. In the present study, rs12910984 was identified as a risk factor for COPD, showing the same trend as in a recent lung cancer study in the Chinese population. Although COPD and lung cancer can be initiated by a common environmental factor of cigarette smoking, it remains unclear whether they have a common pathogenic mechanism [20]. Our finding may reflect that *CHRNA3* can be a common susceptibility gene; however, this may be due to a possible confounding effect [8]. The amount an individual smoked was significantly associated with *CHRNA3/5* expression in COPD subjects in Caucasian and Asian populations [21–23]. In another study, rs660652 in *CHRNA3* was significantly associated with smoking dependence [24]. However, genetic variation in the *CHRNA3* gene was not associated with smoking amount in the current study. The association between *CHRNA3* and smoking behavior and addiction is controversial [25]. In this study, a variant of rs660652, which was associated with more lifetime smoking, showed an association with lower DLCO after adjusting for smoking amount. It is not clear whether multiple functional genes mediate different phenotypes including lung function, smoking, and emphysema [21] or share the same pathogenesis. *CHRNA3* is a subunit of the nicotine cholinergic receptor. The cholinergic system is expressed not only in cholinergic neuronal cells but also in bron-

chial epithelial cells [26] and airway inflammatory cells [27]. The expression of this receptor in the airways may be modified by nicotine in cigarette smoke and could potentially influence the pathogenesis of lung cancer and COPD.

There are several limitations to this study. First, there were significant differences in the demographics of the case and control subjects, as well as in the pack-years of cigarette smoking between the two groups. Specifically, like in many COPD case-control studies, age and smoking amount were higher in the study group [28]. Therefore, we adjusted for age and smoking amount in our statistical analysis. After stratification of control for smoking amount and age, we found that the results were the same. Second, the number of cohort subjects is relatively small, and our results showed only marginally significant *p* values. We evaluated a few SNPs within a limited genetic region, so common functional variants in Korean patients may not have been tagged. However, considering that SNPs in the chromosome 15q25 region were associated with COPD and lung function in other populations, our results for the Asian population are likely to be meaningful. Functional data for this variant are currently lacking. However, rs12910984 is in tight linkage disequilibrium with rs6495309, which showed a significant association with lung cancer and increased *CHRNA3* expression [12]. This may also have a functional effect in COPD pathogenesis. Further studies are needed to evaluate the role of *CHRNA3* in COPD.

In conclusion, genetic variation in *CHRNA3* is associated with COPD and DLCO in the Korean population.

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