Association Between Single-Nucleotide Polymorphisms in *Interleukin-12A* and Risk of Chronic Obstructive Pulmonary Disease

En-yin Wang, Wei-bo Liang,* and Lin Zhang*

Chronic obstructive pulmonary disease (COPD) is characterized by airflow obstruction due to chronic bronchitis, emphysema, and/or disease of small airways. It has been reported that the genetic variation may play a role in the development and severity of COPD. The purpose of this study was to investigate whether single-nucleotide polymorphisms (SNP) in interleukin (*IL*)-12A and *IL*-12B were associated with COPD in a Chinese population. The *IL*-12A rs2243115 and *IL*-12B rs3212227 polymorphisms were genotyped by performing polymerase chain reaction–restriction fragment length polymorphism in 298 patients with COPD and 346 healthy controls. We observed that the frequencies of GT and GT + GG of *IL*-12A rs2243115 were significantly different from TT in the COPD group and the control group (GT vs. TT: odds ratio [OR] = 2.35, 95% confidence interval [CI] = 1.55–3.57, $p < 0.001$; GT + GG vs. TT: OR = 2.46, 95% CI = 1.63–3.71, $p < 0.001$). These data suggest that the *IL*-12A rs2243115 polymorphism may contribute to genetic susceptibility to COPD in a Chinese population.

Introduction

CHRONIC OBSTRUCTIVE PULMONARY disease (COPD), as a major and increasing health problem in developing countries, is predicted to become the third most important cause of death and the fifth most common cause of disability throughout the world by 2020 (Lopez and Murray, 1998). It is estimated by a World Health Organization (WHO)/World Bank study that the worldwide prevalence of COPD was 9.33/1000 in men and 7.33/1000 in women in 1990 (Murray and Lopez, 1996a, 1996b). COPD ranks the fourth leading cause of death in the United States (Hoyert *et al.*, 1999), by characteristic of airflow obstruction due to chronic bronchitis, emphysema, and/or disease of small airways (Pauwels *et al.*, 2001). COPD is also an increasingly common problem in China, with a prevalence rate about 3% (Su *et al.*, 2005). Obviously, COPD is associated with huge economic cost, and the exacerbation of COPD gives a substantial burden to the health care system in the world. In the United States, for example, the estimated annual costs of COPD were over \$30 billion (National Institutes of Health, 2007). In the European Union, ~6% of the total health care budget, which COPD accounts for 56% of the cost, is spent to care the respiratory disease patients every year (European Respiratory Society Journals, 2003). In China, many

people have suffered from this disease, and a lots of people die prematurely because of its complications. Therefore, it is necessary and urgent to prevent and treat this devastating lung disease through better understanding of its pathogenesis.

COPD is a chronic inflammatory disease of the lower airways, which become worse and worse during exacerbations, and most patients with COPD have three typical pathologic conditions (chronic obstructive bronchiolitis, emphysema, and mucus plugging) (Caramori and Adcock, 2003). Although cigarette smoking is by far the most important risk factor for COPD, there is a wide range in disease severity, irrespective of the number of pack-years of smoking. Furthermore, only a minority of smokers develop the disease (Bascom, 1991), suggesting that besides smoking, other factors such as genetic variation may play a role in the development and severity of COPD.

COPD is characterized by inflammation in the airways, with an increasing in the total number of T cells in lung parenchyma, peripheral, and central airways (Hodge *et al.*, 2004); this suggests the important role of T cell in the pathogenesis of COPD. *Interleukin* (*IL*)-12 is a 70-kDa heterodimer composed of two disulfide-linked subunits of 35 kDa (*IL*-12A) and 40 kDa (*IL*-12B) (Ma *et al.*, 1996; Maes *et al.*, 1999; Commins *et al.*, 2010). As an important

Department of Forensic Biology, West China School of Preclinical and Forensic Medicine, Sichuan University, Chengdu, China.

*These two authors contributed equally to this work.

immunoregulatory cytokine with an antagonistic effect of the Th1/Th2 cytokine balance, IL-12 provides a functional link between innate and acquired immune responses (Miteva and Stanilova, 2008), and it has been reported that IL-12 expression increased in tissues derived from a variety of inflammatory and autoimmune disorders, including COPD (Gessner *et al.*, 2005). These suggested that *IL-12* may be an important factor in causing inflammatory diseases and be one of the candidate genes in the pathogenesis of COPD. Previous studies have indicated that the *IL-12B* single-nucleotide polymorphism (SNP; rs3212227) was significantly associated with the risk of a wide range of autoimmune and inflammatory diseases such as asthma (Chen *et al.*, 2011), asthma severity phenotype in Caucasians (Randolph *et al.*, 2004), type 1 diabetes (Morahan *et al.*, 2001), Crohn's disease (Zwiers *et al.*, 2004), psoriasis (Cargill *et al.*, 2007), and rheumatoid arthritis (Orozco *et al.*, 2005). However, few studies up to date have examined the association between the *IL-12B* gene polymorphism and the risk of COPD in a Chinese population. Therefore, the *IL-12B* polymorphism rs3212227 was selected to investigate whether its polymorphism was related to a COPD risk. Additionally, because of the complexity of COPD, gene-gene interaction may play an important role in COPD development. *IL-12A* rs2243115 was also chosen to detect its polymorphism on the risk of COPD.

Based on these literatures, we hypothesized that *IL-12* polymorphisms may be associated with the risk of COPD. The aim of this study was to test the hypothesis in a Chinese population.

Materials and Methods

Subjects

A total of 298 patients with COPD (157 men and 141 women) and 346 (183 men and 163 women) non-COPD control subjects were recruited for this study. The subjects in both groups were unrelated Chinese people who were selected from the same population living in China between July 2006 and November 2009. The case group was consecutively recruited from the West China Hospital, Sichuan University. The recruited COPD patients have a firm clinical diagnosis of stable COPD, who had histories of chronic or recurrent productive coughs more than 2 years and lasting for at least 3 months each year. Pulmonary function tests (CHESTAC-33-8800) were performed to determine forced vital capacity (FVC), forced expiratory volume in one second (FEV1), FEV1% predicted, and FEV1/FVC. The gold standard for diagnosis was FEV1/FVC <70%, and FEV1 predicted <80% after inhalation of salbutamol. Patients were excluded from the study if they had other significant respiratory diseases, such as lung cancer, pulmonary tuberculosis, cystic fibrosis, or bronchial asthma.

All controls have no evidence of airflow obstruction (FEV1 and FVC ≥80%, and FEV1/FVCN70%). Individuals were excluded if they had a history of chronic respiratory disease, atopy, and an acute pulmonary infection in the 4 weeks before assessment for this study, or a family history of COPD.

The 298 patients (157 men and 141 women) had a mean (SD) age of 63.43±6.31 years. The mean (SD) age of the

control group (183 men and 163 women) was 64.54±8.32 years. No significant difference in sex, age, smoking history, body mass index, fasting plasma glucose, or blood pressure was observed between the COPD cases and controls (Table 1). Written informed consent was obtained from all the subjects, and the research protocol was reviewed and approved by the Ethics Committee of Sichuan University.

Blood sample and DNA isolation

A 2-mL peripheral venous blood sample was drawn from each individual by standard venopuncture. Blood samples were collected in sterile tubes with EDTA-Na₂ anticoagulants and stored at -20°C. Genomic DNA was isolated from the stored blood using a commercial extraction kit (Bioteke Corporation, Beijing, China) according to the manufacturer's instructions.

Genotyping

A polymerase chain reaction (PCR)-restriction fragment length polymorphism assay was used to acquire the genotypes of rs2243115 in *IL-12A* and rs3212227 in *IL-12B*. The primers for rs2243115 and rs3212227 restriction enzymes (New England BioLabs, Inc., Beverly, MA) have been described previously (Chen *et al.*, 2011). Restriction fragments were distinguished on a 5% polyacrylamide gel and visualized by silver staining to determine the genotypes. To confirm the accuracy of the method, the PCR products of the 2 SNPs with different genotypes were analyzed by the direct sequencing method.

Statistical analysis

The associations between *IL-12A* and *IL-12B* genotypes and the risk of COPD were estimated by computing the odds ratios (ORs) and their 95% confidence intervals (95% CIs). The Hardy-Weinberg equilibrium was tested by a goodness-of-fit χ^2 test to compare the observed genotype frequency among the control subjects with the expected ones. Statistical

TABLE 1. CHARACTERISTICS OF THE STUDY POPULATION

Variables	COPD patients	Non-COPD controls
Subject (n)	298	346
Age (years)	63.43±6.31	64.54±8.32
Gender, M/F (n)	157/141	183/163
Smoking/non-smoking	166/132	188/158
FEV1 observed (L)	0.73±0.50	2.50±0.46
FVC observed (L)	1.58±0.94	3.25±0.50
FEV1% predicted	64.20±11.03	93.82±9.60
FEV1/FVC (%)	64.34±6.43	85.43±7.82
Clinical stages		
Stage I (n)	54	—
Stage II (n)	87	—
Stage III (n)	96	—
Stage IV (n)	61	—

Data are presented as mean±SD.

n, number of subjects; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; COPD, chronic obstructive pulmonary disease.

significance was set at the $p < 0.05$ level. All data were analyzed using SPSS for Windows software package version 13.0.

Results

The genotype and allele frequency distributions of *IL-12A* rs2243115 and *IL-12B* rs3212227 in the cases and controls are shown in Table 2. The observed genotype frequencies for the 2 polymorphisms in the controls agreed with the Hardy-Weinberg equilibrium. The frequencies of the TT, GT, and GG genotypes of *IL-12A* rs2243115 were 87.6%, 12.1%, and 0.3% in controls, and 74.2%, 24.2%, and 1.7% in COPD patients, respectively. The frequencies of GT and GT+GG of *IL-12A* rs2243115 were significantly different from TT in the COPD group and the control group (GT vs. TT: OR=2.35, 95% CI=1.55–3.57, $p < 0.001$; GT+GG vs. TT: OR=2.46, 95% CI=1.63–3.71, $p < 0.001$). The frequencies of T and G alleles of *IL-12A* rs2243115 were 93.6% and 6.4% in controls, and 83.2% and 13.8% in cases, respectively. The G allele of rs2243115 was associated with a significantly increased risk of COPD compared with the T allele (OR=2.35, 95% CI=1.60–3.45, $p < 0.001$).

We then analyzed the frequencies of the *IL-12B* rs3212227 genotype and the allele between COPD patients and controls. The frequencies of the AA, AC, and CC genotypes of *IL-12B* rs3212227 were 36.7%, 49.7%, and 13.6% in controls, and 36.6%, 44.6%, and 18.8% with COPD, respectively. The frequencies of A and C alleles of *IL-12B* rs3212227 were 61.6% and 38.4% in controls, and 58.9% and 41.1% in COPD group, respectively (Table 3). However, no significant association was observed between the *IL-12B* rs3212227 A/C polymorphism and the risk of COPD (AC vs. AA: OR=0.90, 95% CI=0.64–1.27, $p=0.55$; CC vs. AA: OR=1.39, 95% CI=0.87–2.21, $p=0.17$, respectively).

Discussion

COPD is a complex disease and a leading cause of mortality and morbidity both in developed and developing countries. Inflammation of the airways and lung paren-

TABLE 3. ALLELE FREQUENCY OF TWO SINGLE-NUCLEOTIDE POLYMORPHISMS IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND CONTROLS

SNPs	Alleles	Controls (n=346) n (%)	Patients (n=298) n (%)	OR (95% CI)	p-Value
<i>IL-12A</i> rs2243115					
	T	648 (93.6)	514 (86.2)	1.0 (ref)	
	G	44 (6.4)	82 (13.8)	2.35 (1.60–3.45)	<0.001
<i>IL-12B</i> rs3212227					
	A	426 (61.6)	351 (58.9)	1.0 (ref)	
	C	266 (38.4)	245 (41.1)	1.12 (0.89–1.40)	0.33

SNP, single-nucleotide polymorphisms.

chyma plays an important role in the pathogenesis of COPD (Sutherland and Martin, 2003). Although the pathogenesis of COPD remains unknown, the risk of developing COPD was significantly associated with a complex interaction among multiple genes and environmental factors.

IL-12 is an important mediator of immune-mediated inflammatory diseases, which induces the production of interferon- γ and favors the differentiation of Th1 cells in the early phases of the immune responses (Trinchieri, 2003); it may play a relatively minor immunoregulatory role in late-stage inflammation (Arthur and Scott, 2005). It had been observed that increased expression of IL-12 was observed in lung epithelium cells and exhaled breath condensate of a COPD patient (Gessner *et al.*, 2005). Thus, IL-12 may be an important factor in causing inflammatory diseases and be one of the candidate genes in the pathogenesis of COPD.

IL-12 is composed of p35 and p40 subunits (encoded by the *IL-12A* and *IL-12B* genes, respectively), which are located on separate chromosomes (3p12-q13.2 and 5q31-33). Previous studies have indicated the association of *IL-12A* and *IL-12B* polymorphisms with inflammatory diseases (Morahan *et al.*, 2001; Randolph *et al.*, 2004; Zwiers *et al.*, 2004; Orozco *et al.*, 2005; Cargill *et al.*, 2007). Based on the literatures, the *IL-12A* gene rs2243115 and the *IL-12B* gene rs3212227 were selected to investigate whether the polymorphisms were associated with the risk of COPD. Results in the present study showed that the G allele and the TG genotype of the *IL-12A* rs2243115 increased significantly in the COPD group, which indicates that patients carrying the rs2243115 G allele of *IL-12A* would have an increasing risk of developing COPD. To our knowledge, this is the first study to investigate the association between *IL-12A* rs2243115 and COPD.

Chromosome 5q31 contains numerous immunoregulatory genes that could influence atopy phenotypes in many genetic studies (Meyers *et al.*, 1994; Rosenwasser *et al.*, 1995; Cookson and Moffatt, 2000; Walley *et al.*, 2001). *IL-12B* is located in this region. However, no significant association was observed in this study between the *IL-12B* gene rs3212227 A/C polymorphism and the risk of COPD. The possible reason maybe as following: (1) the *IL-12B* gene rs3212227 A/C polymorphism is not totally associated with the risk of COPD; (2) the different study design and different sample source are most likely responsible for the result; (3)

TABLE 2. GENOTYPE FREQUENCIES OF TWO SINGLE-NUCLEOTIDE POLYMORPHISMS IN *IL-12A* AND *IL-12B* BETWEEN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND CONTROLS

Polymorphisms	Controls (n=346) n (%)	Patients (n=298) n (%)	OR (95% CI)	p-Value
<i>IL-12A</i> rs2243115				
TT	303 (87.6)	221 (74.2)	1.0 (ref)	
GT	42 (12.1)	72 (24.2)	2.35 (1.55–3.57)	<0.001
GG	1 (0.3)	5 (1.7)	—	
GT/GG	43 (12.4)	77 (25.8)	2.46 (1.63–3.71)	<0.001
<i>IL-12B</i> rs3212227				
AA	127 (36.7)	109 (36.6)	1.0 (ref)	
AC	172 (49.7)	133 (44.6)	0.90 (0.64–1.27)	0.55
CC	47 (13.6)	56 (18.8)	1.39 (0.87–2.21)	0.17
AC/CC	219 (63.3)	189 (63.4)	1.01 (0.73–1.39)	0.97

n, number of subjects; %, frequency of the genotype; ref, reference; OR, odds ratio; CI, confidence interval; IL, interleukin.

environmental factors may contribute to the pathogenesis of COPD; thus, replicated studies are warranted to confirm this finding.

There are some limitations in this study. For example, the study subjects were all ethnic Han Chinese, and the results maybe not extended to other populations. Thus, our findings need to be interpreted cautiously, and the associations need to be replicated in larger, preferably population-based studies.

In conclusion, we found that *IL-12A* rs2243115 rather than *IL-12B* rs3212227 is associated with the risk of COPD in the ethnic Han Chinese population. Additional studies are needed to better understand the factors contributed to these associations (especially the environmental factors). Furthermore, these findings should be further warranted by a larger and, preferably, prospective study with more diverse ethnic groups.

Acknowledgments

This study was financially supported by the Base Programs for Application of Sichuan Province under the Grant No. 04JY029-002.

Disclosure Statement

No competing financial interests exist.

References

- Arthur, M.B., and Scott, E.P. (2005). The interleukin-12 family of cytokines: therapeutic targets for inflammatory disease mediation. *Clin Appl Immunol* **5**, 225–240.
- Bascom, R. (1991). Differential susceptibility to tobacco smoke: possible mechanisms. *Pharmacogenetics* **1**, 102–106.
- Caramori, G., and Adcock, I. (2003). Pharmacology of airway inflammation in asthma and COPD. *Pulm Pharmacol Ther* **16**, 247–277.
- Cargill, M., Schrod, S.J., Chang, M., Garcia, V.E., Brandon, R., Callis, K.P., Matsunami, N., Ardlie, K.G., Civello, D., Catane, J.J., Leong, D.U., Panko, J.M., McAllister, L.B., Hansen, C.B., Papenfuss, J., Prescott, S.M., White, T.J., Leppert, M.F., Krueger, G.G., and Begovich, A.B. (2007). A large-scale genetic association study confirms *IL12B* and leads to the identification of *IL23R* as psoriasis-risk genes. *Am J Hum Genet* **80**, 273–290.
- Chen, T., Liang, W., Gao, L., Wang, Y., Liu, Y., Zhang, L., and Zhang, L. (2011). Association of single nucleotide polymorphisms in interleukin 12 (*IL-12A* and *-B*) with asthma in a Chinese population. *Hum Immunol* **72**, 603–606.
- Commins, S.P., Borish, L., and Steinke, J.W. (2010). Immunologic messenger molecules: cytokines, interferons, and chemokines. *J Allergy Clin Immunol* **125**, S53–S72.
- Cookson, W.O., and Moffatt, M.F. (2000). Genetics of asthma and allergic disease. *Hum Mol Genet* **9**, 2359–2364.
- European Respiratory Society Journals. (2003). *European Lung White Book* (Huddersfield, United Kingdom).
- Gessner, C., Scheibe, R., Wotzel, M., Hammerschmidt, S., Kuhn, H., Engelmann, L., Hoheisel, G., Gillissen, A., Sack, U., and Wirtz, H. (2005). Exhaled breath condensate cytokine patterns in chronic obstructive pulmonary disease. *Respir Med* **99**, 1229–1240.
- Hodge, S.J., Hodge, G.L., Holmes, M., and Reynolds, P.N. (2004). Flow cytometric characterization of cell populations in bronchoalveolar lavage and bronchial brushings from patients with chronic obstructive pulmonary disease. *Cytometry B Clin Cytom* **61**, 27–34.
- Hoyert, D.L., Kochanek, K.D., and Murphy, S.L. (1999). Deaths: final data for 1997. *Natl Vital Stat Rep* **47**, 1–104.
- Lopez, A.D., and Murray, C.C. (1998). The global burden of disease, 1990–2020. *Nat Med* **4**, 1241–1243.
- Ma, X., Aste-Amezaga, M., and Trinchieri, G. (1996). Regulation of interleukin-12 production. *Ann N Y Acad Sci* **795**, 13–25.
- Maes, M., Song, C., Lin, A.H., Bonaccorso, S., Kenis, G., De Jongh, R., Bosmans, E., and Scharpe, S. (1999). Negative immunoregulatory effects of antidepressants: inhibition of interferon-gamma and stimulation of interleukin-10 secretion. *Neuropsychopharmacology* **20**, 370–379.
- Meyers, D.A., Postma, D.S., Panhuysen, C.I., Xu, J., Amelung, P.J., Levitt, R.C., and Bleeker, E.R. (1994). Evidence for a locus regulating total serum IgE levels mapping to chromosome 5. *Genomics* **23**, 464–470.
- Miteva, L., and Stanilova, S. (2008). The combined effect of interleukin (IL)-10 and IL-12 polymorphisms on induced cytokine production. *Hum Immunol* **69**, 562–566.
- Morahan, G., Huang, D., Ymer, S.I., Cancilla, M.R., Stephen, K., Dabadghao, P., Werther, G., Tait, B.D., Harrison, L.C., and Colman, P.G. (2001). Linkage disequilibrium of a type 1 diabetes susceptibility locus with a regulatory *IL12B* allele. *Nat Genet* **27**, 218–221.
- Murray, C., and Lopez, A. (1996a). *The Global Burden of Disease: A Comprehensive Assessment of Mortality and Disability from Diseases, Injuries and Risk Factors in 1990 and Projected to 2020* (Harvard University Press, Cambridge, MA).
- Murray, C.J., and Lopez, A.D. (1996b). Evidence-based health policy—lessons from the Global Burden of Disease Study. *Science* **274**, 740–743.
- National Institutes of Health. (2007). *2004 NHLBI Morbidity and Mortality Chartbook on Cardiovascular, Lung and blood Diseases* (US Department of Health and Human Services, Health Service, National Institutes of Health, Bethesda, MD).
- Orozco, G., Gonzalez-Gay, M.A., Paco, L., Lopez-Nevot, M.A., Guzman, M., Pascual-Salcedo, D., Balsa, A., and Martin, J. (2005). Interleukin 12 (*IL12B*) and interleukin 12 receptor (*IL12RB1*) gene polymorphisms in rheumatoid arthritis. *Hum Immunol* **66**, 710–715.
- Pauwels, R.A., Buist, A.S., Calverley, P.M., Jenkins, C.R., and Hurd, S.S. (2001). Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop summary. *Am J Respir Crit Care Med* **163**, 1256–1276.
- Randolph, A.G., Lange, C., Silverman, E.K., Lazarus, R., Silverman, E.S., Raby, B., Brown, A., Ozonoff, A., Richter, B., and Weiss, S.T. (2004). The *IL12B* gene is associated with asthma. *Am J Hum Genet* **75**, 709–715.
- Rosenwasser, L.J., Klemm, D.J., Dresback, J.K., Inamura, H., Mascali, J.J., Klennert, M., and Borish, L. (1995). Promoter polymorphisms in the chromosome 5 gene cluster in asthma and atopy. *Clin Exp Allergy* **25 Suppl 2**, 74–78; discussion 95–76.
- Su, Z.G., Wen, F.Q., Feng, Y.L., Xiao, M., and Wu, X.L. (2005). Transforming growth factor-beta1 gene polymorphisms associated with chronic obstructive pulmonary disease in Chinese population. *Acta Pharmacol Sin* **26**, 714–720.
- Sutherland, E.R., and Martin, R.J. (2003). Airway inflammation in chronic obstructive pulmonary disease: comparisons with asthma. *J Allergy Clin Immunol* **112**, 819–827; quiz 828.

- Trinchieri, G. (2003). Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nat Rev Immunol* **3**, 133–146.
- Walley, A.J., Wiltshire, S., Ellis, C.M., and Cookson, W.O. (2001). Linkage and allelic association of chromosome 5 cytokine cluster genetic markers with atopy and asthma associated traits. *Genomics* **72**, 15–20.
- Zwiers, A., Seegers, D., Heijmans, R., Koch, A., Hampe, J., Nikolaus, S., Pena, A.S., Schreiber, S., and Bouma, G. (2004). Definition of polymorphisms and haplotypes in the interleukin-12B gene: association with IL-12 production but not with Crohn's disease. *Genes Immun* **5**, 675–677.

Address correspondence to:

Wei-bo Liang, Ph.D.

Department of Forensic Biology

West China School of Preclinical and Forensic Medicine

Sichuan University

Chengdu 410040

China

E-mail: liangweibo@scu.edu.cn

Lin Zhang, Ph.D.

Department of Forensic Biology

West China School of Preclinical and Forensic Medicine

Sichuan University

Chengdu 410040

China

E-mail: zhanglin@scu.edu.cn

Received for publication April 12, 2012; received in revised form May 1, 2012; accepted May 18, 2012.