The Journal of International Medical Research
2010; 38: 1093 – 1098

Association of Vitamin D Binding Protein Variants with Susceptibility to Chronic Obstructive Pulmonary Disease

L-H Shen[1], X-M Zhang[2], D-J Su[1], S-P Yao[3], B-Q Yu[1], H-W Wang[1] and F-Z Lu[1]

[1]Department of Respiratory Medicine, The Second Affiliated Hospital of Harbin Medical University, Harbin, China; [2]Department of Histology and Embryology, and [3]Department of Public Health, Harbin Medical University, Harbin, China

Chronic obstructive pulmonary disease (COPD) is characterized by chronic airflow limitation and it is thought that neutrophils play a major role in the disease pathogenesis. Genetic polymorphism of the *vitamin-D-binding protein* (*VDBP*) gene is considered one of the candidates for variation in susceptibility to COPD. To evaluate the potential influences of *VDBP* gene polymorphisms on COPD, a case–control study was conducted in the Han population of north-east China. The *VDBP* polymorphic site was genotyped in 100 COPD patients and 100 controls. Genotypes were determined by polymerase chain reaction–restriction fragment length polymorphism. A significantly higher proportion of *VDBP*-1F homozygosity was found in COPD patients, while the frequency of *VDBP*-2 homozygosity was significantly lower in COPD patients, which seemed to suggest that *VDBP*-2 homozygocity provided a protective effect. These data suggest that the *VDBP* gene may be involved in COPD susceptibility in Chinese Han population.

KEY WORDS: Chronic obstructive pulmonary disease; Vitamin D binding protein; Polymorphism; PCR–RFLP; Genetic study

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by expiratory airflow obstruction and hyperinflation, and is becoming the most prevalent respiratory disease in China.[1] Tobacco smoking is the greatest risk factor for the development of COPD, although approximately 85% of smokers do not develop clinically relevant airflow obstruction.[2] Several epidemiological studies have suggested that genetic factors are likely to have a role in determining an individual's susceptibility to COPD.[3 – 6] Polymorphisms in several genes have been shown to be involved in the development of COPD and one such candidate is the *vitamin D binding protein* (*VDBP*) gene.[7]

Vitamin D binding protein is a multifunctional, polymorphic 55 kDa protein that acts both as a precursor of macrophage-activating factor and as a co-chemotaxin for phagocytic cells,[8 – 10] indicating a role in chronic inflammatory responses in the lung. The *VDBP* gene has been localized to human chromosome 4q11-q13,[11] and exhibits considerable genetic

variability due to its three polymorphic alleles (1F, 1S and 2) and > 124 rarer alleles.[12] An important factor in investigating the role of VDBP is the variation in allele frequency between populations. The aim of the present study was, therefore, to evaluate any relationship that may exist between *VDBP* allele frequency and the risk for COPD in the Han Chinese population.

Patients and methods

PATIENTS

The study group consisted of adults who were diagnosed with COPD (according to the guidelines of The American Thoracic Society[13]) at the Second Affiliated Hospital of Harbin Medical University, Harbin, China. Unrelated healthy Han Chinese adults with no history of COPD were enrolled as controls. Patients and healthy controls were both recruited from the Heilongjiang Province of China between January and October 2009. The study was approved by the Ethics Committee of Harbin Medical University and adhered to the tenets of the Helsinki Declaration. Additionally, written informed consent was obtained from patients and controls.

LUNG FUNCTION TESTS

Lung function tests were carried out for all patients and controls and the use of bronchodilators was prohibited for at least 12 h before the tests were performed. The following pulmonary function data were calculated: (i) the percentage of predicted forced expiratory volume in 1 s (FEV_1 %pred) and forced vital capacity (FVC); (ii) the residual volume divided by the total lung capacity; and (iii) the diffusing capacity of the lung for carbon monoxide taken with patients in a stable condition (not at a time of exacerbation of COPD). The existence of COPD was confirmed with FEV_1 < 80%pred and FEV_1/FVC < 70%; healthy controls were required to have FEV_1 > 85%pred and FEV_1/FVC > 75%.

DNA EXTRACTION AND GENOTYPING

Genomic DNA was extracted from 5 ml of frozen whole blood using a DNA extraction kit (Tiangen Biotech Co. Ltd, Beijing, China) according to the manufacturer's protocol. Primers for the polymerase chain reaction (PCR) were designed from the published *VDBP* gene sequence,[14] and the *VDBP* gene was amplified using a commercially available PCR reaction mix (Takara Bio Inc., Dalian, Japan), according to the manufacturer's instructions. The primer pairs used to amplify the *VDBP* gene were: upstream, 5′-TAATGAGCAAATGAAAGAAG-3′; downstream, 5′-AATCACAGTAAAGAGG AGGT-3′. The cycling programme involved preliminary denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 48 °C for 30 s, and elongation at 72 °C for 30 s, followed by a final elongation step at 72 °C for 7 min.

The PCR product was genotyped by restriction fragment length polymorphism analysis, involving overnight restriction enzyme digestion at 37 °C with the HaeIII and StyI restriction enzymes. If the *VDBP*-1S allele is present, the HaeIII enzyme produces bands of 295 and 93 bp, whereas if the *VDBP*-2 allele is present the StyI enzyme produces bands of 304 and 84 bp; the *VDBP*-1F variant remains uncut with both enzyme digests. The digested DNA fragments were then resolved on a 2.0% agarose gel with 0.2 µg/ml ethidium bromide.

STATISTICAL ANALYSES

All statistical analyses were carried out using the SPSS® statistical package, version 13.0

(SPSS Inc., Chicago, IL, USA) for Windows®. The genotype and allele frequencies for each patient and control were obtained by direct counting of bands resulting from the restriction enzyme digests. Comparisons of the distributions of the allele and genotype frequencies were performed using the χ^2-test. The relative risk associated with rare alleles was estimated as an odds ratio (OR) with a 95% confidence interval (CI). A *P*-value < 0.05 was considered to be statistically significant.

Results

In total, 100 patients with COPD and 100 healthy control subjects were recruited into the study. Lung function test results are shown in Table 1. The groups were matched in terms of age and gender. The distribution of the six major genotypes of *VDBP* in the control and COPD groups is presented in Table 2. The proportion of *VDBP*-1F homozygotes was significantly higher in COPD patients than control subjects (*P* = 0.0003; OR (95% CI) versus all other genotypes of 3.08 [1.498, 6.347]). The proportion of *VDBP*-2 homozygotes was significantly lower in COPD patients than in control subjects (*P* = 0.0017; OR [95% CI] versus all other genotypes of 0.215 [0.060, 0.772]). The distribution frequency of the three alleles is shown in Table 3. In the COPD group of patients, the frequency of the *VDBP*-1F allele was significantly higher (*P* = 0.0035) and the frequency of *VDBP*-2 was significantly lower (*P* = 0.0003) than for the healthy control group. There were no significant between-group differences in the frequency distribution of the *VDBP*-1S allele.

TABLE 1:
Baseline characteristics and lung function test results for patients with chronic obstructive pulmonary disease (COPD) (*n* = 100) and for control subjects (*n* = 10)

Subjects	Men/Women (*n*)	Age (years)	Smoking history (pack years)	FEV_1 (l)	FEV_1/FVC (%)	RV/TLC (%)	DL_{CO} (%)
COPD patients	72/28	62.3 ± 9.7	123.5 ± 29.7	1.28 ± 0.43	43.5 ± 12.8	49.5 ± 10.3	41.3 ± 16.8
Control subjects	66/34	60.9 ± 8.6	25.6 ± 13.3	3.57 ± 0.65	92.8 ± 5.8	27.3 ± 16.7	75.5 ± 13.3
Statistical significance	NS[a]	NS[b]	$P < 0.0001$[b]	$P < 0.0001$[b]	$P < 0.0001$[b]	$P < 0.0001$[b]	$P < 0.0001$[b]

Data are presented as mean ± SD unless otherwise indicated.
[a]χ^2-test; [b]*t*-test.
FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; RV/TLC, residual volume/total lung capacity; DL_{CO}, diffusing capacity of the lung for carbon monoxide; NS, not statistically significant (*P* > 0.05).

Discussion

Neutrophils have been assumed to play a key role in the pathogenesis of COPD.[15] Thus, variation in genes with a function

TABLE 2:
***Vitamin B-binding protein* (*VDBP*) genotype frequency distribution between patients with chronic obstructive pulmonary disease (COPD) and control subjects**

	VDBP genotype frequency			
VDBP genotype	COPD patients (n = 100)	Control subjects (n = 100)	Statistical significance[a]	Odds ratio (95% CI)
1F-1F	0.35	0.13	P = 0.0003	3.08 (1.498, 6.347)
1F-1S	0.20	0.26	NS	
1S-1S	0.07	0.04	NS	
2-1S	0.13	0.12	NS	
2-1F	0.22	0.29	NS	
2-2	0.03	0.16	P = 0.0017	0.215 (0.060, 0.772)

[a]χ^2-test.
NS, not statistically significant ($P > 0.05$).

TABLE 3:
***Vitamin B-binding protein* (*VDBP*) allele frequency distribution in patients with chronic obstructive pulmonary disease (COPD) and in control subjects**

	VDBP allele frequency			
SNP	COPD patients (n = 100)	Control subjects (n = 100)	Statistical significance[a]	Odds ratio (95% CI)
1F	0.560	0.405	P = 0.0035	1.886 (1.230 – 2.891)
1S	0.235	0.230	NS	0.975 (0.607 – 1.567)
2	0.095	0.220	P = 0.0003	0.345 (0.192 – 0.620)

[a]χ^2-test.
NS, not statistically significant ($P > 0.05$); SNP, single nucleotide polymorphism.

related to the chemotaxis of neutrophils may confer a risk for the development of COPD. VDBP has two different biological functions related to inflammation: it enhances the chemotactic activity of C5a and C5a des-Arg for neutrophils;[10] and it undergoes conversion to a potent macrophage-activating factor (MAF) as a result of the removal of specific glycosylated moieties.[9] It has been shown, however, that there are no differences between the three VDBP isoforms in terms of their ability to induce neutrophil chemotaxis.[16]

The present study showed that the proportion of COPD patients with the homozygous *VDBP*-1F genotype was significantly higher than for control subjects, suggesting that *VDBP*-1F homozygosity may be a risk factor for COPD. This confirms data from a study in a Japanese population.[17] Previous studies on *VDBP* gene polymorphism have shown varying results in association of this polymorphism with the risk of COPD. For example, Kueppers *et al.*[18] showed that the frequency of the homozygous *VDBP*-2 genotype was only 0.01 in patients with COPD compared with 0.05 in control subjects, but the study performed by Kauffmann *et al.*[19] failed to confirm this result. Subsequently, Horne *et al.*[7] showed that the frequency of the homozygous *VDBP*-1F genotype in patients with COPD was 0.06

and was significantly greater than in control subjects (P = 0.01; relative risk, 4.8). It has also been noted that the frequencies of the *VDBP* alleles vary with ethnicity.[7,20,21]

The present study found that VDBP-2 homozygosity seemed to exert a protective effect with respect to COPD, a result that has also been reported by Schellenberg *et al.*[16] Besides increasing neutrophil chemotaxis, when VDBP is converted to a MAF (as described above) it increases the activation of macrophages at the sites of inflammation. According to Yamamoto and Naraparaju[22] both the VDBP-1S and VDBP-1F isoforms contain identical side-chain structures, while the VDBP-2 isoform has alternate sugar moieties. The VDBP2 protein has two different forms; the less prevalent form (10%) displays a shorter side chain that can be modified to become a MAF. Most of the VDBP2 protein molecules undergo no glycosylation and are incapable of being converted to a MAF. The low glycosylation level in homozygous VDBP2 individuals could result in less MAF being produced and, therefore, less pulmonary inflammation.[22] This could perhaps provide one explanation for the protective effect of the VDBP2-2 genotype against COPD found in the present study.

In conclusion, the data of the present study suggest that the 1F allele of the *VDBP* gene may be a risk factor for COPD, while the presence of the 2 allele may be one of the protective factors against COPD.

Acknowledgements

The work was supported by the Project of Science Research Foundation of Heilongjiang Health Department (No. 2009-140) and a grant from the Youth Fund of The Second Affiliated Hospital of Harbin Medical University (No. QN2009-08).

Conflicts of interest

The authors had no conflicts of interest to declare in relation to this article.

• Received for publication 7 January 2010 • Accepted subject to revision 10 January 2010
• Revised accepted 20 May 2010

References

1 Zhong N, Wang C, Yao W, *et al:* Prevalence of chronic obstructive pulmonary disease in China. *Am J Respir Crit Care Med* 2007; **176**: 753 – 760.
2 Fletcher C, Peto R, Tinker C, *et al*: *The Natural History of Chronic Bronchitis: An Eight Year Follow-up Study of Working Men in London*. Oxford: Oxford University Press, 1976.
3 Tager I, Tishler PV, Rosner B, *et al*: Studies of the familial aggregation of chronic bronchitis and obstructive airways disease. *Int J Epidemiol* 1978; **7**: 55 – 62.
4 Khoury MJ, Beaty TH, Newill CA, *et al*: Genetic–environmental interactions in chronic airways obstruction. *Int J Epidemiol* 1986; **15**: 65 – 72.
5 Higgins M, Keller J: Familial occurrence of chronic respiratory disease and familial resemblance in ventilatory capacity. *J Chronic Dis* 1975; **28**: 239 – 251.
6 Kueppers F, Miller RD, Gordon H, *et al*: Familial prevalence of chronic obstructive pulmonary disease in a matched pair study. *Am J Med* 1977; **63**: 336 – 342.
7 Horne SL, Cockcroft DW, Dosman JA: Possible protective effect against chronic obstructive airways disease by the Gc2 allele. *Hum Hered* 1990; **40**: 173 – 176.
8 Cooke NE, Haddad JG: Vitamin D binding protein (Gc-globulin). *Endocr Rev* 1989; **10**: 294 – 307.
9 Yamamoto N, Homma S: Vitamin D_3 binding protein (group-specific component) is a precursor for the macrophage activating signal factor from lysophosphatidylcholine-treated lymphocytes. *Proc Natl Acad Sci USA* 1991; **88**: 8539 – 8543.
10 Kew RR, Webster RO: Gc-globulin (vitamin D-binding protein) enhances the neutrophil chemotactic activity of C5a and C5a des Arg. *J Clin Invest* 1988; **82**: 364 – 369.
11 Cooke NE, Willard HF, David EV, *et al*: Direct regional assignment of the gene for vitamin D binding protein (Gc-globulin) to human chromosome 4q11–q13 and identification of an associated DNA polymorphism. *Hum Genet*

1986; **73**: 225 – 229.
12 Cleve H, Constans J: The mutants of the vitamin-D-binding protein: more than 120 variants of the Gc/DBP system. *Vox Sang* 1988; **54**: 215 – 225.
13 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 – S121.
14 Braun A, Kofler A, Morawietz S, *et al*: Sequence and organization of the human vitamin D-binding protein gene. *Biochim Biophys Acta* 1993: **1216**: 385 – 394.
15 Ishil T, Keicho N, Teramoto S, *et al*: Association of Gc-globulin variation with susceptibility to COPD and diffuse panbronchiolitis. *Eur Respir J* 2001; **18**: 753 – 757.
16 Schellenberg D, Pare PD, Weir TD, *et al*: Vitamin D binding protein variants and the risk of COPD. *Am J Respir Crit Care Med* 1998; **157**: 957 – 961.
17 Ito I, Nagai S, Hoshino Y, *et al*: Risk and severity of COPD is associated with the group-specific component of serum globulin 1F allele. *Chest* 2004; **125**: 63 – 70.
18 Kueppers F, Miller RD, Gordon H, *et al*: Familial prevalence of chronic obstructive pulmonary disease in a matched pair study. *Am J Med* 1977; **63**: 336 – 342.
19 Kauffmann F, Kleisbauer JP, Cambon-de-Mouzon A, *et al*: Genetic markers in chronic airflow limitation: a genetic epidemiologic study. *Am Rev Respir Dis* 1983; **127**: 263 – 269.
20 Sandford AJ, Chagani T, Weir TD, *et al*: Susceptibility genes for rapid decline of lung function in the lung health study. *Am J Respir Crit Care Med* 2001; **163**: 469 – 473.
21 Laufs J, Andrason H, Sigvaldason A, *et al*: Association of vitamin D binding protein variants with chronic mucus hypersecretion in Iceland. *Am J Pharmacogenomics* 2004; **4**: 63 – 68.
22 Yamamoto N, Naraparaju VR: Role of vitamin D3-binding protein in activation of mouse macrophages. *J Immunol* 1996; **157**: 1744 – 1749.

Author's address for correspondence
Dr Fu-Zhen Lü
Department of Respiratory Medicine, The Second Affiliated Hospital of Harbin Medical University, 246 Xuefu Road, Nangong District, Harbin 150081, China.
E-mail: lvtongxun@yahoo.cn