Polymorphisms in the *SERPINA1* Gene and the Risk of Chronic Obstructive Pulmonary Disease in a Korean Population

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한국인에서 *SERPINA1* 유전자 다형성과 만성폐쇄성폐질환의 위험도

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연구배경: 만성폐쇄성폐질환의 대부분은 흡연과 연관되어 발생하지만 흡연자의 약 10~20%에서만 만성폐쇄성폐질환이 발생하는 현상은 질환의 발생에 개체의 유전적인 소인이 관여함을 시사한다. 저자들은 α1-antitrypsin 단백질을 암호화하는 *SERPINA1* 유전자의 다형성에 따른 만성폐쇄성폐질환의 위험도를 조사하였다.

방 법: 경북대학교병원 호흡기내과에서 만성폐쇄성폐질환으로 진단 받은 93명의 환자와 112명의 정상인을 대상으로 하였다. *SERPINA1* 유전자의 M1Val, M1Ala, M2, S와 Z 대립유전자(allele)는 중합효소연쇄반응과 restriction fragment length polymorphism을 이용하여 조사하였다.

결 과: 환자군과 대조군 모두에서 S 및 Z allele은 없었으며, M1Val allele의 빈도는 환자군에서 유의하게 낮았다 (73.6% vs. 82.7%, p=0.03). M1Val/M1Val 유전자형인 경우에 비해 M2 혹은 M1Ala allele을 갖는 유전자형인 경우 만성폐쇄성폐질환의 대응비는 1.86 (95% CI: 1.02~3.41, p=0.04)으로 유의하게 높았다. M2 allele 갖는 유전자형인 경우 대응비는 1.77 (95% CI: 0.96~3.27, p=0.07)이었으며, 연령에 따라 구분한 경우 64세 미만에서는 M2 allele을 갖는 경우 대응비가 3.09 (95% CI: 1.16~8.21, p=0.02)로 유의하게 높았다.

결 론: *SERPINA1* 유전자의 유전자형은 만성폐쇄성폐질환의 위험도를 결정하는 인자로 생각되나, 보다 많은 예를 대상으로 한 연구가 필요할 것으로 생각된다. *(Tuberc Respir Dis 2008;65:285-291)*

Key Words: *SERPINA1* gene, Polymorphism, COPD

Introduction

Cigarette smoking is the major risk factor in 80~90% of patients with chronic obstructive pulmonary disease (COPD)[1]. However, only 10~20% of cigarette smokers develop clinically significant airway obstruction, suggesting that genetic factors may play an important role in determining susceptibility to COPD[2-4]. This genetic susceptibility may result from functional polymorphisms of genes involved in antiproteolysis, xenobiotic metabolism, the inflammatory response to cigarette smoke, and mucociliary clearance[3,4].

Human α_1-antitrypsin (AAT) is a 54 kDa glycoprotein encoded by *SERPINA1*, which is part of a serine proteinase inhibitor gene cluster that includes corticosteroid binding globulin (*SERPINA6*), protein C inhibitor (*SERPINA5*), and alpha 1-antichymotrypsin (*SERPINA3*)[5]. AAT is produced by hepatocytes and mononuclear phagocytes, and functions as a major inhibitor of neutrophil elastase, an ominous protease capable of destroying most components of the lung matrix[6]. An inherited severe deficiency of AAT increases the risk of COPD, particularly in smokers[7,8].

The *SERPINA1* gene is located on chromosome 14q32, and more than 100 genetic variants have been identified. The initially discovered AAT variants were named based

on electrophoretic migration velocity, as follows: F (fast), M (medium), S (slow), or Z (very slow)[9,10]. The most common variants of AAT are the M variants, which consist of at least the following four subtypes: $M1_{Val}$, $M1_{Ala}$, M2 and M3. The four subtypes differ in their amino acid composition at codons 101, 273 and 376 as follows: $M1_{Val}$, Arg^{101}-Val^{213}-Glu^{376}; $M1_{Ala}$, Arg^{101}-Ala^{213}-Glu^{376}; M2, His^{101}-Val^{213}-Asp^{376}; and M3, Arg^{101}-Val^{213}-Asp^{376} [7-11].

The M variants are characterized by normal plasma AAT levels and are thought to be unrelated to any disease[7,12]. However, Gaillard et al.[13,14] reported that the $M1_{Ala}$ and M2 alleles had a significantly lower elastase inhibitory capacity compared to the $M1_{Val}$ allele. In addition, a number of studies have observed an increased prevalence of the $M1_{Ala}$ and M2 allele in asthmatics[13-15]. These results suggest that the $M1_{Ala}$ and M2 alleles may play a role in the inflammatory reaction and/or the elastase-antielastase balance, which are important in the pathogenesis of COPD. We conducted a case-control study to evaluate the potential association between *SERPINA1* genotypes ($M1_{Val}$, $M1_{Ala}$, S and Z) and the risk of COPD.

Materials and Methods

1. Study population

The patient group consisted of 89 male patients who were diagnosed with COPD at the Kyungpook National University Hospital according to the criteria established by the NHLBI/WHO Global Initiative for COPD (GOLD)[16]. The inclusion criteria for COPD were as follows: chronic respiratory symptoms and signs such as cough and dyspnea; post-bronchodilator $FEV_1 < 80\%$ of the predicted value; $FEV_1/FVC < 70\%$; and FEV_1 reversibility after inhalation of 200 μg salbutamol $< 12\%$ of the pre-bronchodilator FEV_1. The severity of COPD was classified by the guidelines of the GOLD in terms of the percentage predicted FEV_1, as follows: mild ($>80\%$), moderate ($50 \sim 80\%$), severe ($30 \sim 50\%$), or very severe ($<30\%$). Control subjects (n=112) were selected from a pool of healthy men who visited the general health check-up center. The enrollment criteria for the controls were as follows: male gender, age >45 years, no known disease and no history

of any disease, and no airflow limitation. All of the cases and the controls were ethnic Koreans that resided in Daegu City or in the surrounding regions. A trained interviewer completed detailed questionnaires for each patient and each control subject. This study was approved by the Institutional Review Board of the Kyungpook National University Hospital, and written informed consent was obtained from each participant.

2. Genotyping

Genomic DNA was extracted from peripheral blood lymphocytes by proteinase K digestion and phenol/chloroform extraction. Genotypes were determined by PCR-RFLP assay as described previously[15,17]. The PCR primers for the $Ala^{213}Val$ (M1), $Arg^{101}His$ (M2), $Glu^{264}Val$ (S) and $Glu^{342}Lys$ (Z) variants were 5'-CCCACCTTCCCCTC TCTCCAGGCAAATGGG-3' (forward) and 5'-GGGCCTCA GTCCCAACATGGCTAAGAGGTG-3' (reverse); 5'-GCAGG ACAATGCCGTCTTCTGTCTC-3' (forward) and 5'-CCACTA GCTTCAGGCCCTCGCTGAG-3' (reverse); 5'-TGAGGGG AAACTACAGCACCTCG-3' (forward) and 5'-AGGTGTGG CAGCTTCTTGGTCA-3' (reverse); and 5'-ATAAGGCTGT GCTGACCATCGTC-3' (forward) and 5'-TTGGGTGGGAT TCACCACTTTTC-3' (reverse), respectively. The PCR reactions were performed in a total volume of 20 μl that contained 200 ng genomic DNA, 10 pM of each primer, 4 mM dNTPs, 10 mM Tris-HCl (pH 8.3), 50mM KCl, 1.5 mM $MgCl_2$, and 1 unit of Taq polymerase (Takara Shuzo Co., Otsu, Shiga, Japan). The PCR cycle conditions consisted of an initial denaturation step at 94°C for 5 min followed by 35 cycles for 30 s at 94°C, 20 s at 67°C for the M1 and M2 variants, and 55°C for the S and Z variants, 30 s at 72°C, and a final elongation step at 72°C for 10 min.

The restriction enzyme, *BstE*II (New England BioLabs, Beverly, MA, USA), was used to distinguish the M1 variant in which the gain of a *BstE*II restriction site in addition to a restriction site at codon 288 occurs in the Val allele. Thus, the Val allele yields three bands (228, 83 and 49 bp) and the Ala allele yields two bands (311 and 49 bp). The restriction enzyme *Rsa*I (New England BioLabs, Beverly, MA, USA) was used to distinguish the

M2 variants in which the loss of the *Rsa*I restriction site occurs in the His allele. The Arg allele yields two bands (383 and 79 bp) and the His allele has only one band representing the entire 462 bp fragment. The restriction enzyme, *Taq*I (New England BioLabs, Beverly, MA, USA), was used to distinguish the S and Z variations. Using the primers for the S variant, the S allele has a band at 121 bp whereas the M allele has a band at 157 bp. With the primers for the Z variant, the Z allele has a band at 179 bp and the M allele has a band at 157 bp. Five microliters of the PCR products were digested over-night with 5 U *Bst*EII at 60°C, 5 U *Rsa*I at 37°C or 10 U *Taq*I at 65°C. The digestion products were separated on 2.5% agarose gel for the M1 and M2 variants and on 8% acrylamide gel for the S and Z variants.

3. Statistical analysis

The cases and controls were compared using the Student's *t*-test for continuous variables and a χ^2 test for categorical variables. Unconditional logistic regression analyses were used to calculate the odds ratios (ORs) and 95% confidence intervals (CIs), with adjustment for possible confounders (age and pack-years of smoking as continuous variables). In addition to the overall associ-ation analysis, we performed a stratified analysis accord-ing to age (median age,<62 years/≥ 62 years), smoking status and severity of COPD (GOLD I-II/GOLD III-IV) to further explore the association between genotypes and the risk of COPD in each stratum. The homogeneity test was done to compare the difference between geno-type/diplotype-related ORs of different groups. p-values from the analyses results<0.05 were considered as stat-istically significant. All of the analyses were performed using Statistical Analysis Software for Windows, version 9.1.3 (SAS Institute, Cary, NC, USA).

Results

The baseline characteristics of the cases and controls enrolled in this study are shown in Table 1. There was a significant difference in the mean age between the cases and controls (65.4±7.2 vs. 60.8±8.7 years, p

Table 1. Characteristics of the study population

Variable	COPD (n=93)	Control (n=112)	p-value
Age (years)	65.4±7.2	60.8±8.7	<0.001*
Smoking status			0.57[†]
Current	63 (67.7)[†]	80 (71.4)	
Former	30 (32.3)	32 (28.6)	
Pack-years	47.2±23.8	35.8±15.4	<0.001*
FEV1 (% Predicted)	56.3±23.2	107.5±16.1	<0.001*
FEV1/FVC (%)	45.2±11.8	78.5±6.3	<0.001*

FEV1: forced expiratory volume in 1 second; FVC: forced vital capacity.
*t-test, [†]χ^2 test, [†]Numbers in parenthesis, column percentage.

<0.001). In addition, the number of pack-years in the smokers was significantly higher in the cases than in the controls (47.2±23.8 vs. 35.8±15.4 pack-years; p <0.001). These differences were controlled for in the subsequent multivariate analyses. The FEV_1 and FEV_1/FVC ratio were significantly lower in the case group than in the controls (both, p<0.001).

The distributions of *SERPINA1* genotypes among the cases and controls are shown in Table 2. The S and Z alleles were not observed in the cases and controls. The frequency of the M1$_{Val}$, M1$_{Ala}$, and M2 alleles among the healthy controls were 0.83, 0.005, and 0.17, re-spectively, which were similar to the healthy Koreans (0.81, 0.003, and 0.19, respectively) in our previous study[18], but significantly different from Caucasians (0.44 ~0.49, 0.20~0.23, and 0.14~0.19, respectively)[10]. The M1$_{Val}$ allele was significantly less frequent in the cases than in the controls (73.6% vs. 82.7%, p=0.03). The M1$_{Val}$/M1$_{Val}$ genotype was less frequent in the cases (53.9%) than in the controls (69.2%), whereas the M1$_{Val}$/M1$_{Ala}$, M1$_{Val}$/M2, and M2/M2 genotypes were more frequent in the cases (3.4, 36.0, and 6.7%, respectively) than in the controls (1.0, 26.0, and 3.8%, respectively). These findings suggest that the M1$_{Val}$/M1$_{Ala}$, M1$_{Val}$/M2 and M2/M2 genotypes, which carry the M1$_{Ala}$ or M2 al-leles, might be risk genotypes for COPD. Compared with the M1$_{Val}$/M1$_{Val}$ genotype, the M1$_{Val}$/M1$_{Ala}$, M1$_{Val}$/M2 or M2/M2 genotypes were associated with a sig-nificantly increased risk of COPD (adjusted OR: 1.86,

Table 2. Frequency distribution of *SERPINA1* genotypes in COPD cases and controls

Genotype	Cases (n=93)	Controls (n=112)	p*	Crude OR (95% CI)	p-value	Adjusted[†] OR (95% CI)	p-value
M1$_{Val}$/M1$_{Val}$	51 (54.8)	78 (69.6)	0.14	1.00		1.00	
M1$_{Val}$/M1$_{Ala}$	3 (3.2)	1 (0.9)		4.59 (0.46~45.33)	0.19	4.88 (0.46~51.37)	0.19
M1$_{Val}$/M2	34 (36.6)	29 (25.9)		1.79 (0.98~3.29)	0.06	1.74 (0.92~3.30)	0.09
M2/M2	5 (5.4)	4 (3.6)		1.91 (0.49~7.46)	0.35	2.00 (0.46~8.70)	0.35
M1$_{Val}$/M1$_{Val}$	51 (54.8)	78 (69.6)	0.03	1.00		1.00	
Others	42 (45.2)	34 (30.4)		1.89 (1.07~3.35)	0.03	1.86 (1.02~3.41)	0.04
M1$_{Val}$/M1$_{Val}$	51 (56.7)	78 (70.3)	0.045	1.00		1.00	
M1$_{Val}$/M2+M2/M2	39 (43.3)	33 (29.7)		1.81 (1.01~3.24)	0.046	1.77 (0.96~3.27)	0.07

*Two-sided chi-square test for genotype distributions between the cases and controls, [†]Adjusted for age and pack-years of smoking.

Table 3. Association between *SERPINA1* genotypes and COPD risk according to age, smoking status and severity of COPD

Variables	Cases (n=90)		Controls (n=111)		p[†]	Adjusted OR (95% CI) for M2(+)	p	p$_H$[‡]
	M1$_{Val}$/M1$_{Val}$	M2 (+)*	M1$_{Val}$/M1$_{Val}$	M2 (+)*				
Age (years)								
<64	18 (54.5)	15 (45.5)	48 (75.0)	16 (25.0)	0.04	3.09 (1.16~8.21)[§]	0.02	0.04
≥64	33 (57.9)	24 (42.1)	30 (63.8)	17 (36.2)	0.54	1.24 (0.56~2.76)[§]	0.60	
Smoking status								
Current	36 (58.1)	26 (41.9)	57 (72.2)	22 (27.8)	0.08	1.80 (0.86~3.75)[¶]	0.12	0.80
Former	15 (53.6)	13 (46.3)	21 (65.6)	11 (34.4)	0.34	1.62 (0.57~4.63)[¶]	0.37	
Pack-years of smoking								
<40	10 (43.5)	13 (56.5)	42 (68.9)	19 (31.1)	0.03	2.69 (0.96~7.52)[¶]	0.06	0.26
≥ 40	41 (61.2)	26 (38.8)	36 (72.0)	14 (28.0)	0.22	1.60 (0.72~3.53)[¶]	0.25	
Severity of COPD								
Gold I-II	28 (59.6)	19 (40.4)	78 (70.3)	33 (29.7)	0.19	1.50 (0.72~3.10)**	0.28	0.54
Gold III-IV	23 (53.5)	20 (46.5)	78 (70.3)	33 (29.7)	0.049	1.91 (0.86~4.25)**	0.11	
DLCO[††]								
≥ 70% pred.	32 (59.3)	22 (40.7)	78 (70.3)	33 (29.7)	0.16	1.47 (0.73~2.99)**	0.28	0.29
< 70% pred.	16 (51.6)	15 (48.4)	78 (70.3)	33 (29.7)	0.05	2.25 (0.95~5.35)**	0.07	

*M1$_{Val}$/M2 or M2/M2, [†]Two-sided chi-square test for genotype distribution between the cases and controls, [‡]Test for homogeneity. Odds ratios (95% confidence intervals) and their corresponding p-values were calculated by unconditional logistic regression analysis, [§]adjusted for pack-yearsof smoking; [¶]for age; and **for age and pack-years of smoking, [††]Diffusing capacity of the lung for carbon monoxide.

95% CI: 1.02~3.41, p=0.04). To examine the effect of the M2 allele on the risk of COPD, we combined the M1$_{Val}$/M2 genotype with the M2/M2 genotype into one susceptible group and compared it with the M1$_{Val}$/M1$_{Ala}$ genotype. Individuals with at least one M2 allele were at a borderline significantly increased risk of COPD compared with those with the M1^{Val}/M1^{Val} genotype

(adjusted OR: 1.77, 95% CI: 0.96~3.27, p=0.07).

The association between the M2 allele and the risk of COPD was further examined after stratifying the subjects according to age, smoking status, COPD severity and diffusing capacity of the lung for carbon monoxide (DL$_{CO}$). When stratified by median age, the effect of the combined M1$_{Val}$/M2 and M2/M2 genotype on the risk

of COPD was more pronounced in the subgroup <64 years than in the subgroup ≥64 years (adjusted OR: 3.09, 95% CI: 1.16~8.21, p=0.02 versus adjusted OR: 1.24, 95% CI: 0.56~2.76, p=0.60; p in test for homogeneity [p_H]=0.04; Table 3). When the subjects were stratified according to the smoking status, the effect of the combined $M1_{Val}$/M2 and M2/M2 genotype on the risk of COPD was similar in current and former smokers (adjusted OR: 1.80, 95% CI: 0.86~3.75; and adjusted OR: 1.62, 95% CI: 0.57~4.63, respectively, p_H=0.80). However, when the subjects were dichotomized by median pack-years of smoking, the combined $M1_{Val}$/M2 and M2/M2 genotype was associated with a borderline significantly increased risk of COPD in lighter smokers (adjusted OR: 2.69, 95% CI: 0.96~7.52, p=0.06), whereas there was no significant association in heavier smokers (adjusted OR: 1.60, 95% CI: 0.72~3.53, p=0.25). When the COPD cases were categorized based on disease severity, the effect of the combined $M1_{Val}$/M2 and M2/M2 genotype on the risk of COPD was similar in individuals with mild-to-moderate COPD (adjusted OR: 1.50, 95% CI: 0.72~3.10) and those with severe COPD (adjusted OR: 1.91, 95% CI: 0.86~4.25, p_H=0.54). When the COPD cases were categorized based on the DLCO, the combined $M1_{Val}$/M2 and M2/M2 genotype was associated with a borderline significantly increased risk of COPD in individuals with a DL_{CO} less than 70% of the predicted value (adjusted OR: 2.25, 95% CI: 0.95~5.35, p=0.07), whereas there was no significant association in those with a DL_{CO} ≥70% of the predicted value (adjusted OR: 1.47, 95% CI: 0.73~2.99, p=0.28).

Discussion

We investigated the association between the *SERPINA1* genotypes and the risk of COPD in a Korean population. The $M1_{Val}$ allele was significantly less frequent in the COPD cases than in the controls. Individuals with the M2 or $M1_{Ala}$ allele were at a significantly increased risk of COPD compared to those with the $M1_{Val}$/$M1_{Val}$ genotype. The association of the *SERPINA1* genotypes with the risk of COPD was more evident in the subgroup <64 years. These results suggest that the *SERPINA1* genotypes contribute to genetic susceptibility to COPD in Koreans.

In the present study, the M2 allele had a more pronounced effect on the risk of COPD in the subgroup <64 years than in the subgroup ≥64 years, which is consistent with the notation that genetic factors can play a major role in the early onset of disease[19,20]. Individuals with aberrant AAT activity may be prone to developing COPD at a younger age, thus the association would be more clearly observed in younger patients with COPD. Another interesting finding of this study was that the effect of the M2 allele on the risk of COPD was significant in lighter smokers, yet not significant in heavier smokers. This finding is also biologically plausible, as genetic effect on the risk may be smaller at high levels of tobacco exposure when environmental influences may overpower any genetic predisposition[21-23].

The mechanism responsible for the association of the M2 allele with the risk of COPD remains to be elucidated. However, it has been observed that the M2 variant has lower elastase inhibitory capacity compared to the $M1_{Val}$ variant[13,14]. Therefore, it is possible that individuals with the M2 variant have lower elastase inhibitory capacity, and are thus prone to develop COPD. Another possible mechanism is that the M2 allele may be in linkage disequilibrium with other functional polymorphisms in the *SERPINA1* gene (not the Z or S mutations) or in neighboring genes. Because the *SERPINA1* gene is located within a cluster of serine protease inhibitor genes that include *SERPINA6*, *SERPINA5*, *SERPINA3*, and *Kallistatin*[24,25], the observed association between the M2 allele and COPD risk may be resulted from linkage disequilibrium with polymorphisms of these adjacent genes.

Several studies have investigated the association between AAT variants and the risk of COPD[7,8,26]. In contrast to the finding in the present study, the M2 variant was not associated with the risk of COPD in these previous studies. This discrepancy may be due to the different ethnicity of the study populations. The allele and genotype frequencies of the *SERPINA1* polymorphisms, as

well as the LD status with other variants in neighboring genes, vary greatly between ethnic groups. Therefore, the genetic effects of *SERPINA1* polymorphisms may be different in different ethnic populations. The discrepancy may arise from differences in the methods of assigning AAT status. In the previous studies[7,8,26], the AAT phenotype was only determined by isoelectric focusing of serum and the serum AAT levels without measuring functional activity of the AAT protein. Although the M2 variant does not affect serum AAT levels, it may alter the functional activity of the AAT protein[13,14], and thus predispose to the development of COPD.

In conclusion, the M2 allele of the *SERPINA1* gene was significantly associated with the risk of COPD in Koreans. The effect of the M2 allele on the risk of COPD was more pronounced in the subgroup < 64 years. These results suggest that *SERPINA1* polymorphisms may contribute to a genetic predisposition for COPD. However, it is possible that our findings are attributable to chance because of the relatively small numbers of cases. Therefore, additional studies with larger sample sizes will be required to confirm our findings. Moreover, further study is needed to define a functional role of the M1 allele in the development of COPD.

Summary

Background: We conducted a case-control study to evaluate the potential association between *SERPINA1* genotypes (M1$_{Val}$, M1$_{Ala}$, S, and Z) and the risk COPD.

Methods: The study population consisted of 93 patients with COPD and 112 healthy controls. The polymerase chain reaction and restriction fragment length polymorphism for detecting the *SERPINA1* variants.

Results: The M2 allele of the *SERPINA1* gene was significantly associated with the risk of COPD in Koreans. The effect of the M2 allele on the risk of COPD was more pronounced in the subgroup < 64 years.

Conclusion: These results suggest that *SERPINA1* polymorphisms may contribute to a genetic predisposition for COPD. However, additional studies with larger sample sizes are required to confirm our findings.

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