SMAD4 rs10502913 is Significantly Associated with Chronic Obstructive Pulmonary Disease in a Chinese Han Population: A Case-Control Study

Zhifei Hou1,2, Zhihui Yuan3, Hao Wang4, Kang Chang5, Yong Gao1

1Department of Pulmonary and Critical Care Medicine, Sinopharm Tongmei General Hospital, Datong, People’s Republic of China; 2China-Japan Friendship Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, People’s Republic of China; 3Department of Emergency, Chinese PLA General Hospital -Fourth Medical Center, Beijing, People’s Republic of China; 4School of Population Medicine and Public Health, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, People’s Republic of China; 5China-Japan Friendship Hospital, National Clinical Research Center for Respiratory Diseases, Clinical Center for Pulmonary Infections, Capital Medical University, Beijing, People’s Republic of China

Correspondence: Yong Gao; Zhifei Hou, Email tmzyyhx@163.com; houzhifei1898@126.com

Background: COPD is a respiratory disease caused by a combination of genetic and environmental factors. Polymorphism, as a genetic factor, can affect the susceptibility of the disease of COPD. In this study, we assessed the relationship between the polymorphisms of three genes and COPD risk in a Chinese Han population.

Patients and Methods: A total of 376 patients diagnosed with COPD and 284 control subjects were enrolled in this study. Multivariate logistic regression analysis was used to analyze the association between three polymorphisms (SMAD4 rs10502913, IL-4 rs2070874, HSPA1L rs2227956) and COPD susceptibility.

Results: The SMAD4 rs10502913 GG and AG genotype significantly increased COPD risk (adjusted OR = 2.235, 95% CI 1.198–4.104; adjusted OR = 2.218, 95% CI 1.204–4.151, respectively) compared with the AA genotype. In the stratification analyses, the GG genotype significantly increased the risk of COPD in subjects aged 60 and over (adjusted OR = 2.519, 95% CI 1.266–5.015) and with a smoking history of less than 30 years (p = 0.009; adjusted OR = 3.751; 95% CI 1.398–10.062). This increased risk was more pronounced in the group of GOLD I and GOLD II (adjusted OR = 3.628, 95% CI 1.022–12.885; adjusted OR = 2.394, 95% CI 1.004–5.710, respectively). In addition, AG genotype was associated with an increased COPD risk in subjects aged 60 and over (adjusted OR = 2.599, 95% CI 1.304–5.176) and in smokers (p = 0.021; adjusted OR = 2.269; 95% CI 1.132–4.548). This increased risk was more obvious in the group of GOLD III COPD (p = 0.047; adjusted OR = 2.532; 95% CI 1.012–6.336).

Conclusion: Our present study indicated that the genotype GG and AG of SMAD4 rs10502913 are associated with an increased risk of COPD in a Chinese Han population. Further validation studies with large-scale populations are needed to confirm our findings.

Keywords: COPD, chronic obstructive pulmonary disease, polymorphism, SMAD4 rs10502913, similar to mother against decapentaplegic 4, risk

Introduction

Chronic obstructive pulmonary disease (COPD), which includes chronic obstructive bronchitis and emphysema, is a chronic inflammatory respiratory disease that can be prevented and treated. It is characterized by persistent respiratory symptoms and incomplete reversible airflow limitations.1–3 COPD is currently one of the major burdens on global health, and the prevalence of the disease among the elderly is about 10%, which is likely to become the third leading cause of death by 2030 in the world.4,5 The pathogenesis of this disease involved in complex interactions among several factors which are mainly caused by long-term smoking, chronic infection, inhalation of industrial dust, and lacking of the body’s own alpha-1-anti-trypsin.6 Although smoking is a recognized risk factor for COPD, only a fraction of people who smoke end up with COPD.7 In addition, a small percentage of people who have never smoked also develop COPD, and some people diagnosed with respiratory limitations in childhood may also develop COPD later in life.7 Furthermore, several studies have demonstrated the onset of
COPD is associated with familial aggregation, proving that in addition to environmental factors, genetic factors are also involved in the onset of COPD. Therefore, the discovery of new susceptibility factors of COPD will be conducive to the early prevention, early diagnosis and early treatment of this disease.

It is particularly interesting to note that SMAD4 (Similar to mother against decapentaplegic 4) plays an important role in the development of a variety of diseases. The transforming growth factor-beta (TGF-β)/SMAD4 signaling pathway affects cellular processes by regulating signal transduction, including proliferation, differentiation, apoptosis, and disease initiation and progression. SMAD4 which participates in canonical TGF-β signaling pathway, plays a key role in tumorigenesis. Several evidences reveal that SMAD4 is a tumor suppressor, and its mutations have been found in at least 26 types of cancer, with a higher frequency in esophageal, gastric, pancreatic, colorectal and lung cancers. Another study showed that SMAD4 rs12455792 CT or CT+TT genotype was significantly associated with the risk of thoracic aortic aneurysm and dissection (TAAD). Compared with allele C, allele T was significantly associated with the risk of developing TAAD. A recent study suggested that the AG and GG genotypes of SMAD4 rs10502913 did not increase the risk of coal workers' pneumoconiosis (CWP) compared with the AA genotype. Furthermore, SMAD4 rs10502913 genotypes had no interaction with CWP risk in a stratified analysis of smokers and never-smokers. However, the functionality of SMAD4 rs10502913 SNP in COPD has not yet been reported.

Interleukin-4 (IL-4) is a key regulator of inflammatory pathways, which produced by the T-cell thymocyte populations and mast cell precursors. It is important for B-cell activation, proliferation and differentiation. Interleukin-4 has been shown to play an important role in type 2 immune responses characterized by the production of immunoglobulin G1 (IgG1) and immunoglobulin E (IgE). Several studies have shown that IL-4 single nucleotide polymorphisms (SNPs) are also significantly associated with susceptibility to different diseases. A meta-analysis revealed that the IL-4 rs2070874 polymorphism was associated with gastrointestinal cancer susceptibility, and the T allele was significantly increased the risk of gastrointestinal cancer. Another study suggested that the T allele of IL-4 rs2070874 decreased the risk of esophageal squamous cell carcinoma. IL-4 rs2070874 had a significant association with phenotype of impaired limb mobility and fluid accumulation, and the TT genotype was increased the risk of phenotype of impaired limb mobility.

Heat shock proteins (HSPs) play an important role in protecting cells against oxidative damage, apoptosis and genetic mutation. The 70-kDa heat shock proteins (HSP70s) are the most studied human heat shock proteins. The HSP70 gene family mainly includes three subtypes, namely HSP70-1 (HSPA1A), HSP70-2 (HSPA1B) and HSP70-Hom (HSPA1L). Although both HSPA1A and HSPA1B are heat-inducible protein, HSPA1L is not heat-inducible protein and which encodes a protein that is highly related to HSPA1A. HSPA1L polymorphisms are associated with the risk of several diseases. A previous study revealed that HSPA1L rs2227956 polymorphism had a significant association with male infertility in Iranian population, and the C allele increased the risk of idiopathic male infertility. The AG genotype of HSPA1L rs2227956 had a significant association with decreased the risk of idiopathic pulmonary fibrosis.

In our study, we examined the three single nucleotide polymorphisms of different genes (SMAD4 rs10502913, IL-4 rs2070874, HSPA1L rs2227956) in COPD patients and healthy control individuals and investigated whether these SNPs would be associated with the risk of COPD in a Chinese Han population.

Materials and Methods

Study Population

A total of 376 male Chinese Han COPD patients were recruited from November 2018 to June 2021, who were diagnosed by the Sinopharm Tongmei General Hospital in Shanxi Province, and the diagnostic criteria are based on WHO Global initiative for chronic Obstructive Lung Disease (GOLD). The inclusion criteria for COPD were as follows: chronic respiratory symptoms and signs; post bronchodilator FEV1 <80% of the predicted value, FEV1/FVC <70%, and FEV1 reversibility after inhalation of 200 mg salbutamol <12% of the pre-bronchodilator FEV1. The severity of COPD was classified by the guidelines of the GOLD in terms of the percentage predicted FEV1: mild (>80%), moderate (50–80%), severe (30–50%) or very severe (<30%). The exclusion criteria for COPD patients were as follows: 1) The patient could not be tested for lung function; 2) The patient had other significant respiratory diseases such as asthma, lung cancer, congestive heart failure, tuberculosis, and cystic fibrosis; 3) The patients had previous history of chemotherapy.
radiotherapy or other cancers. Participants were chosen without limitations of age, smoking status, or disease stage. We recruited 284 male control subjects, who visited the outpatient or health check-up center. Individuals with any organic disease were excluded by clinical examination, chest X-ray examination, and laboratory examination. Trained interviewers completed detailed questionnaires for each subject. After the interview, approximately 5mL of venous blood was obtained from each participant. All participants in this study signed informed consent and agreed to use their biological samples for research purposes. This study was approved by the Ethics Committee of Sinopharm Tomei general hospital (NO.201902) and conducted in accordance with the Helsinki declaration.

Genotyping
Genomic DNA was isolated from venous blood using standard procedures. Extracted DNA samples from two groups were randomly segmented into 96-well plates. According to the manufacturer’s instructions (Applied Biosystem, USA), the TaqMan method and the ABI 7900HT Real Time PCR system were to genotype. However, due to DNA quality, several samples failed genotyping, which were excluded in further analyses. For IL-4 rs2070874, 3 DNA samples failed genotyping, including 1 COPD patient and 2 control subjects. For HSPA1L rs2227956, 1 COPD patient’s DNA samples failed genotyping. For SMAD4 rs10502913, no DNA samples failed genotyping. To ensure quality control, genotyping was performed by two laboratory staff in a double-blind manner.

Statistical Analysis
SPSS 26.0 software (SPSS Incorporation, USA) and Microsoft Excel were used for statistical analyses. Frequency of genotypes of COPD cases and controls was assessed for Hardy-Weinberg equilibrium by goodness-of-fit chi-square test ($\chi^2$ test). The Student’s $t$-test for continuous variable and Pearson’s chi-square test for categorical variable were used to calculate differences in the distribution of demographic characteristics between two groups. Logistic regression analysis was used to assess crude odds ratios (ORs), adjusted ORs and 95% confidence interval (CI) for COPD risk and SNPs. Adjustment of multivariate logistic regression model to smoking years and smoking status was done. The significance of all statistical tests is two-sided and achieved in $p < 0.05$.

Results
Demographic Characteristics
A total of 660 participants all of which are Chinese Han descents, including 376 COPD cases and 284 controls. As shown in Table 1, Case subjects on average were older than control subjects (aged 69.74 years versus 62.00 years, $p < 0.001$), and there was a statistically significant difference in the smoking status ($p<0.001$) and smoking index ($p=0.027$) between the two groups. The proportion of never smokers in the control group was higher than that in the COPD group (25.4% versus 10.4%). However, there were no significant differences in body mass index (BMI) between the two groups ($p = 0.127$). Because of the study design, the COPD cases had worse pulmonary function (FEV1/FVC) than the control subjects.

Allelic Frequencies and Genotype Distributions of 3 Genes Polymorphism
The detailed information of candidate SNPs in 3 genes is demonstrated in Table 2. The distribution of all genotypes in two groups is consistent with Hardy-Weinberg equilibrium ($p > 0.05$). The minor allele frequency (MAF) for all three SNPs was consistent with the reported in the HapMap database.

The Association Between SMAD4 rs10502913, IL-4 rs2070874, HSPA1L rs2227956 Polymorphism and COPD Risk
As shown in Table 3, while there was no significant difference between A allele and G allele ($P = 0.104$), the genotype frequency of SMAD4 rs10502913 polymorphism was significantly different between the cases and the controls ($p = 0.022$). Multivariate logistic regression revealed that SMAD4 rs10502913 GG and AG genotype significantly increased COPD risk (adjusted OR = 2.235, 95% CI 1.198–4.104; adjusted OR = 2.218, 95% CI 1.204–4.151, respectively) compared with the AA genotype. Therefore, it is suggested that SMAD4 rs10502913 polymorphism affected the susceptibility of COPD. However,
Table 1 Demographic and Selected Variables Among the COPD Cases and Control Subjects

| Variables          | COPD (N=376) | Control (284) | P    |
|-------------------|--------------|---------------|------|
| Age (years)       | 69.74±10.40  | 62.00±10.91   | <0.001|
| BMI               | 22.8±3.40    | 23.3±3.50     | 0.127 |
| Smoking index     | 34.61±26.46  | 22.86±21.76   | 0.027 |
| Smoking status    |              |               |      |
| Former            | 216          | 99            | <0.001|
| Current           | 121          | 113           |       |
| Never             | 39           | 72            |       |
| Pulmonary function|              |               |      |
| FVC observed (L)  | 2.61±0.89    | 3.58±0.81     |      |
| FEV1 observed (L) | 1.47±0.69    | 2.74±0.66     |      |
| FEV1,% predicted  | 54.28±22.68  | 89.29±16.07   |      |
| FEV1/FVC (%)      | 54.29±11.09  | 76.53±4.60    |      |

Note: Data are presented as mean ±SD.

Abbreviations: N, number of subjects; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; COPD, chronic obstructive pulmonary disease; BMI, body mass index.

Table 2 Primary Information of Genotyped SNPs

| Gene    | SNP        | Chromosomal Position | Base     | MAF    | HWE P |
|---------|------------|----------------------|----------|--------|-------|
|         |            |                      | Cases    | Controls |       |
| SMAD4   | rs10502913 | chr18:51041901       | G>A      | 0.286  | 0.327 |
| IL-4    | rs2070874  | chr5:132674018       | C>T      | 0.239  | 0.204 |
| HSPA1L  | rs2227956  | chr6:31810495        | G>A      | 0.159  | 0.188 |

Abbreviations: HWE, Hardy-Weinberg equilibrium; SNP, single nucleotide polymorphism; MAF, minor allele frequency.

Table 3 Distributions of Genotypes of Different Genes Their Associations with Risk of COPD

| Variables          | COPD       | Controls   | P    | COR (95% CI) | AOR (95% CI) |
|--------------------|------------|------------|------|--------------|--------------|
|                    | N          | %          | N    | %            |              |
| SMAD4 rs10502913   | 376        | 284        |      |              |              |
| AA                 | 23         | 6.1        | 35   | 12.3         | 0.022        |
| AG                 | 169        | 44.9       | 116  | 40.8         | 0.007        |
| GG                 | 184        | 48.9       | 133  | 46.8         | 0.011        |
| A allele           | 215        | 57.4       | 186  | 57.4         | 0.082        |
| G allele           | 537        | 71.4       | 382  | 71.4         | 0.097        |
| IL-4 rs2070874     | 375        | 282        |      |              |              |
| CC                 | 20         | 5.3        | 11   | 3.9          | 0.321        |
| CT                 | 139        | 37.1       | 93   | 33.3         | 0.023        |
| TT                 | 216        | 57.6       | 178  | 63.1         | 0.029        |
| C allele           | 179        | 23.9       | 115  | 23.9         | 0.124        |
| T allele           | 571        | 76.1       | 449  | 76.1         | 0.082        |
| HSPA1L rs2227956   | 375        | 284        |      |              |              |
| AA                 | 267        | 71.2       | 185  | 65.1         | 0.220        |
| AG                 | 97         | 25.9       | 91   | 32.0         | 0.082        |
| GG                 | 11         | 2.9        | 8    | 2.8          | 0.142        |
| A allele           | 631        | 84.1       | 461  | 84.1         | 0.156        |
| G allele           | 119        | 15.9       | 107  | 15.9         | 0.081        |

Note: P-values were calculated with Pearson’s χ² tests.

Abbreviations: COPD, chronic obstructive pulmonary disease; COR, crude odds ratio; AOR, adjusted odds ratio; CI, confidence interval; N, number of subjects.
other polymorphisms (IL-4 rs2070874, HSPA1L rs2227956) examined in this study were not significantly associated with the development of COPD.

**Stratification Analyses of SMAD4 rs10502913 Genotype and COPD Risk**

Further stratification analyses of SMAD4 rs10502913 polymorphism by multivariate logistic regression are shown in Table 4. When using the genotype AA as a reference, we found that only AG genotype was associated with an increased COPD risk in smokers ($p=0.021$; adjusted OR = 2.269; 95% CI 1.132–4.548), and GG genotype significantly increased the risk of COPD in subjects with a smoking history of less than 30 years ($p=0.009$; adjusted OR = 3.751; 95% CI 1.398–10.062) and smoking index less than 20 ($p=0.003$; adjusted OR = 4.003; 95% CI 1.597–10.032). Interestingly, AG and GG genotypes significantly increased the risk of COPD compared with AA genotypes of people aged 60 and over (adjusted OR = 2.519, 95% CI 1.266–5.015, respectively).

Analyses of the genotypes of SMAD4 rs10502913 polymorphism and COPD risk stratified by severity are observed in Table 5. There was a significant interaction between genotype GG and GOLD I ($p = 0.046$), subjects who belonged to GOLD I and carried GG genotype had an adjusted OR of 3.628 (95% CI 1.022–12.885). This increased risk was also more pronounced.

**Table 4 Analyses the Genotypes of SMAD4 rs10502913 Polymorphism and COPD Risk Stratified by Smoking Status and Age**

| Variables      | Controls/Cases | Genotypes (Controls/Cases) | P* | AOR (95% CI) | Pb | AOR (95% CI) |
|----------------|---------------|-----------------------------|----|--------------|----|--------------|
|                | AG            | GG                         | AA |               |    |              |
|                | N %           | N %                        | N % |              |    |              |
| Total          | 284/376       | 116/169                    | 133/184 | 46.8/48.9 | 35/23 | 12.3/6.1 | 0.011 | 2.218 (1.204–4.151) | 0.011 | 2.235 (1.198–4.104) |
| Smoking status |               |                            |    |              |    |              |
| NO             | 72/39         | 36/14                      | 50.0/35.9 | 23/22 | 31.9/56.4 | 13/3 | 18.1/7.7 | 0.587 | 1.498 (0.349–6.431) | 0.063 | 3.949 (0.930–16.771) |
| YES            | 212/337       | 80/155                     | 37.7/46.0 | 110/162 | 51.9/48.1 | 22/20 | 10.4/5.9 | 0.021 | 2.269 (1.132–4.548) | 0.076 | 1.862 (0.936–3.703) |
| Smoking years  |               |                            |    |              |    |              |
| <30            | 151/105       | 70/41                      | 46.4/39.0 | 54/57 | 35.8/54.3 | 27/7 | 17.9/6.7 | 0.135 | 2.125 (0.791–5.714) | 0.009 | 3.751 (1.398–10.602) |
| ≥30            | 133/271       | 46/128                     | 34.6/47.2 | 75/127 | 56.4/46.9 | 12/16 | 9.0/5.9 | 0.077 | 2.131 (0.922–4.922) | 0.382 | 1.442 (0.634–3.280) |
| Smoking index  |               |                            |    |              |    |              |
| ≤20            | 173/124       | 79/45                      | 45.7/36.3 | 68/71 | 39.3/57.3 | 26/8 | 15.0/6.4 | 0.124 | 2.078 (0.817–5.284) | 0.003 | 4.003 (1.597–10.032) |
| >20            | 111/252       | 37/124                     | 33.3/49.2 | 65/113 | 58.6/44.8 | 9/15 | 8.1/6.0 | 0.101 | 2.156 (0.860–5.402) | 0.616 | 1.263 (0.508–3.124) |
| Age            |               |                            |    |              |    |              |
| <60            | 130/69        | 51/25                      | 39.2/36.2 | 67/40 | 51.5/58.0 | 12/4 | 9.2/5.8 | 0.055 | 1.332 (0.378–4.693) | 0.557 | 1.446 (0.422–4.951) |
| ≥60            | 154/307       | 65/144                     | 42.2/46.9 | 66/144 | 42.9/46.9 | 23/19 | 14.9/6.2 | 0.007 | 2.599 (1.304–5.176) | 0.009 | 2.519 (1.266–5.015) |

Notes: P-values were calculated by unconditional logistic regression adjusted for age, smoking status and smoking years. P*Statistically significant difference between AA and AG; PbStatistically significant difference between AA and GG; YES: including current smoking and former smoking.

Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; N, number of subjects.

**Table 5 Analyses the Genotypes of SMAD4 rs10502913 Polymorphism and COPD Risk Stratified by Severity**

| Variables | Cases | Genotypes (Controls/Cases) | P* | AOR (95% CI) | Pb | AOR (95% CI) |
|-----------|-------|---------------------------|----|--------------|----|--------------|
|           | AG    | GG                        | AA |               |    |              |
|           | N     | N                         | N  |              |    |              |
| Total     | 376   | 116/169                   | 133/184 | 35/23 | 0.011 | 2.218 (1.204–4.151) | 0.011 | 2.235 (1.198–4.104) |
| GOLD I    | 66    | 116/26                    | 133/37 | 35/3 | 0.103 | 2.921 (0.806–10.579) | 0.046 | 3.628 (1.022–12.885) |
| GOLD II   | 139   | 116/60                    | 133/71 | 35/8 | 0.050 | 2.370 (0.986–5.685) | 0.049 | 2.394 (1.004–5.710) |
| GOLD III  | 108   | 116/54                    | 133/47 | 35/7 | 0.047 | 2.532 (1.012–6.336) | 0.178 | 1.886 (0.749–4.749) |
| GOLD IV   | 63    | 116/29                    | 133/29 | 35/5 | 0.261 | 1.833 (0.637–5.276) | 0.476 | 1.470 (0.509–4.243) |

Notes: P-values were calculated by unconditional logistic regression adjusted for age, smoking status and smoking years. P*Statistically significant difference between AA and AG; PbStatistically significant difference between AA and GG.

Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; N, number of subjects.
in the GOLD II group with GG genotype (p = 0.049; adjusted OR = 2.394; 95% CI 1.004–5.710). Moreover, a significantly increased risk for GOLD III COPD was found in genotype AG of SMAD4 rs10502913 (p = 0.047; adjusted OR = 2.532; 95% CI 1.012–6.336).

Discussion

It has been shown that several genetic polymorphisms are associated with susceptibility to COPD, which is thought to be the simplest and the most common reason to affect the gene base sequence and to lead to genetic information changes. In order to understand the influence factors of COPD risk in a Chinese Han population, we assessed three candidate inflammatory related genetic polymorphisms in our study. An excellent result was shown that SMAD4 rs10502913 polymorphism was significantly associated with COPD risk, and the association was more evident in older patients with a smoking history for less than 30 years who belonged to GOLD I to III. However, no significant association with COPD was identified for the other polymorphisms examined in this study.

Genome-wide linkage and genetic association studies have identified some genes that may be involved in the development of COPD. Currently, an increasing number of genetic association studies on COPD risk focus on understanding the individual effects of different SNPs and their interactions on the disease. Studies have suggested that several genetic polymorphisms are associated with the development of COPD. CYP2B6 rs4803420 G/T was associated with a decreased COPD risk compared to GG genotype, and rs1038376 A/T was related to an increased COPD risk compared with the AA genotype in the co-dominant and dominant models. In the study by Korytina et al demonstrated that rs2787094 and rs2280091 GG haplotypes of ADAM33 gene significantly increased the incidence of COPD. The study of Ding et al found that the genotype TT of IREB2 rs13180 significantly decreased the COPD risk, but failed to find the association between the SNPs with COPD risk in a Han population in the further stratification analysis. A study by Du et al demonstrated that the polymorphisms of GSTP1, HO-1, and SOD3 are correlated with the onset of COPD, but there is no association with the different stages of COPD. Moreover, it is indicated that the incidence of COPD in GSTP1-exon5 SNP and HO-1 (GT)n SNP are high-risk factors for COPD. Although several genetic polymorphisms have been reported to be associated with the risk of COPD, there is little study on the role of SMAD4 rs10502913 polymorphism and COPD.

In this study, the most striking finding was an association between SMAD4 rs10502913 and COPD risk. The results revealed that the genotype frequency of SMAD4 rs10502913 polymorphism was significantly different between the cases and the controls, indicating that the polymorphism of SMAD4 rs10502913 was correlated with the onset of COPD. When compared with the AA genotype, SMAD4 rs10502913 GG genotype significantly increased COPD risk, as well as AG genotype. It revealed that genetic polymorphisms may play an important role in the development of COPD susceptibility. One possible explanation is that the G allele may result in increased pro-inflammatory cytokine production or as a pro-oxidative stress factor favoring the lung injury. But it is so interesting that there was no remarkable difference between A allele and G allele between COPD patients and controls. We also found that GG genotype greatly increased the risk of COPD in subjects who are over 60 years old and with a smoking history of less than 30 years, but there was no significant risk between GG genotype of subjects who are younger than 60 years old or with a smoking history of more than 30 years. This increased risk was more evident in mild to moderate COPD with GG genotype but not in severe to very severe COPD, suggesting that the discrepancies may arise from differences in the severity of the COPD cases across different studies. In addition, the AG genotype was associated with the risk of COPD patients who are over 60 years old with a history of smoking, but not in those who are less than 60 years old or without a history of smoking. Moreover, a significantly increased risk for severe COPD was found in genotype AG but not mild, moderate and very severe COPD. The reason for the inconsistency between AG and GG genotypes in COPD severity is not clear. One of the reasons may be that there is a complex relationship between pulmonary ventilation dysfunction and genotype, and the specific mechanism needs further study.

Genetic polymorphisms play an important role in the pathogenesis of different diseases. SMAD4 is a class of highly polymorphic gene, and mutations in this gene is often observed in several diseases. Mutation or deletion of SMAD4 is a common feature of some human malignancies and is associated with the onset and progression of malignancies. A previous case-control study showed SMAD4 rs10502913 polymorphism increases the risk of colorectal cancer in men and decreases the risk of colorectal cancer in women. But Wosiak et al have shown no significant association between
SMAD4 rs10502913 polymorphism and the risk of developing colorectal cancer. It is well known that differences in ethnicity and region are important factors affecting genetic polymorphism. Therefore, racial differences may be one of the reasons for the inconsistency of the results of the two studies. Although SMAD4 rs10502913 polymorphism has been widely studied in malignant tumors, there are also reports about the association between SMAD4 rs10502913 polymorphism and other diseases. A case-control study indicated that the G allele of SMAD4 rs10502913 did not alter the risk of CWP compared with the A allele, and the GG genotypes did not increase the CWP risk compared with the AA genotype.13 Another study based on the Health Professionals Follow-up and Nurses’ Health Study in the United States showed there is no association between SMAD4 rs10502913 polymorphism and type 2 diabetes risk.35 However, it is still unclear which signaling pathways or target genes SMAD4 rs10502913 acts through, and further studies are needed to clarify its molecular mechanism.

In summary, to the best of our knowledge, this study is the first to demonstrate an association between SMAD4 rs10502913 and COPD susceptibility, and to confirm that AG and GG genotypes increase the risk of developing COPD. These findings will contribute to a better understanding of genetic polymorphisms at risk for COPD and provide a new perspective for the study of biomarkers for genetic susceptibility to COPD in humans. Therefore, early screening for SMAD4 rs10502913 genotype may help reduce the incidence of COPD in the Chinese Han population, although this is an ideal situation. However, this may help personalize medicine or prevent the development of COPD, and SMAD4 may become an intervention target for COPD prevention and treatment.

There are several limitations in our study. First, the sample size of this study is relatively small, so it cannot fully represent the whole population and other differences between groups cannot be fully reflected. Second, this study was a case-control study, so selection bias may exist. Third, although this study revealed a significant correlation between SMAD4 rs10502913 polymorphism and COPD susceptibility, functional and mechanism studies need to be further clarified. Finally, cross-sectional study designs cannot assess the effect of time on disease. Therefore, we were unable to assess the dynamic changes of lung function, which may lead to a biased diagnosis of COPD classification in some subjects.

Conclusion
In conclusion, our present study first indicated that the genotype GG and AG of SMAD4 rs10502913 are associated with an increased risk of COPD in a Chinese Han population. Thus, we thought that SMAD4 rs10502913 genotypes may be a potential candidate biomarker for predicting COPD development. Further validation studies with large-scale populations are needed to confirm our findings.

Acknowledgments
We are grateful to all people who participated in the data collecting in this experiment (Duo Li, Guoxi Feng, Songyue Liu and Zhenhua Li).

Funding
This work was supported by the Sinopharm Tongmei General Hospital, Science and Technology Research Projects (NO.20190002).

Disclosure
The authors report no conflicts of interest in this work.

References
1. Lee JM, Kang YR, Park SH, et al. Polymorphisms in interleukin-1B and its receptor antagonist genes and the risk of chronic obstructive pulmonary disease in a Korean population: a case-control study. *Respir Med*. 2008;102(9):1311–1320. doi:10.1016/j.rmed.2008.03.026
2. Barnes PJ. Chronic obstructive pulmonary disease. *N Engl J Med*. 2000;343(4):269–280. doi:10.1056/NEJM200007273430407
3. Zhou X, Zhang Y, Zhang Y, et al. LINC01414/LINC00824 genetic polymorphisms in association with the susceptibility of chronic obstructive pulmonary disease. *BMC Pulm Med*. 2021;21(1):213. doi:10.1186/s12890-021-01579-3
4. Gao SL, Wang YH, Li CY, et al. A highly significant association between Cathepsin S gene polymorphisms rs12068264 and chronic obstructive pulmonary disease susceptibility in Han Chinese population. *Biosci Rep*. 2018;38(4). doi:10.1042/BSR20180410
5. Shergis JL, Di YM, Zhang AL, et al. Therapeutic potential of Panax ginseng and ginsenosides in the treatment of chronic obstructive pulmonary disease. *Complement Ther Med*. 2014;22(5):944–953. doi:10.1016/j.ctm.2014.08.006

6. Wang J, Dai N, Cheng DH, et al. STAT3 gene polymorphism in chronic obstructive pulmonary disease. *Eur Rev Med Pharmacol Sci*. 2020;24(18):9618–9625. doi:10.26355/eurrev/202009.23050

7. Yuan C, Chang D, Lu G, et al. Genetic polymorphism and chronic obstructive pulmonary disease. *Int J Chron Obstruct Pulmon Dis*. 2017;12:1385–1393. doi:10.2147/COPD.S134161

8. Khoury MJ, Beatty TH, Tockman MS, et al. Familial aggregation in chronic obstructive pulmonary disease: use of the loglinear model to analyze intermediate environmental and genetic risk factors. *Genet Epidemiol*. 1985;2(2):155–166. doi:10.1002/gepi.1370020206

9. Yu S, Xue M, Yan Z, et al. Correlation between TNF-α −308 and +489 gene polymorphism and acute exacerbation of chronic obstructive pulmonary diseases. *Respir Med*. 2021;66(12):1618–1628. doi:10.1155/2021/6661281

10. Zhao M, Mishra L, Deng CX. The role of TGF-β/SMAD4 signaling in cancer. *Int J Biol Sci*. 2018;14(2):111–123. doi:10.7150/ijbs.23230

11. Du X, Pan Z, Li Q, et al. SMAD4 feedback regulates the canonical TGF-β signaling pathway to control granulosa cell apoptosis. *Cell Death Dis*. 2018;9(2):151. doi:10.1038/s41419-017-0205-2

12. Wang Y, Huang HY, Bian GL, et al. A functional variant of SMAD4 enhances thoracic aortic aneurysm and dissection risk through promoting smooth muscle cell apoptosis and proteoglycan degradation. *Ebiomedicine*. 2017;21:197–205. doi:10.1016/j.ebiom.2017.06.022

13. Zhao H, Huang Y, Wang H, et al. Associations of SMAD4 rs10502913 and NLRP3 rs1539019 polymorphisms with risk of coal workers’ pneumoconiosis susceptibility in Chinese Han Population. *Pharmgenomics Pers Med*. 2022;15:167–175. doi:10.2147/PGPM.S51658

14. Chen S, Cao R, Liu C, et al. Investigation of IL-4, IL-10, and HVEM polymorphisms with esophageal squamous cell carcinoma: a case–control study involving 1929 participants. *Biosci Rep*. 2020;40(8). doi:10.1042/BSR20193895

15. Meteoukki W, Fodil M, Negaz NA, et al. Association of IL4 rs2070874, Fox3 rs3761548 Polymorphisms with Keratoconus in Algeria. *J Ophthalmic Vis Res*. 2021;16(4):558–565. doi:10.18502/jovr/v16i4.9745

16. Cho YA, Kim J. Association of IL4, IL13, and IL4R polymorphisms with gastrointestinal cancer risk: a meta-analysis. *J Epidemiol*. 2017;27(5):215–220. doi:10.1016/j.je.2016.06.002

17. Fu MR, Conley YP, Axelrod D, et al. Precision assessment of heterogeneity of lymphedema phenotype, genotypes and risk prediction. *Breast*. 2016;29(3):231–240. doi:10.1016/j.breast.2016.06.023

18. Zheng JP, Lyu Y, Li RF, et al. Interaction of heat shock protein 70 (HSP70) polymorphisms and occupational hazards increases the risk of hypertension in coke oven workers. *Occup Environ Med*. 2018;75(11):807–813. doi:10.1136/oemed-2018-105160

19. Chang NC, Yang HL, Dai CY, et al. The association of heat shock protein genetic polymorphisms with age-related hearing impairment in Taiwan. *J Otolaryngol Head Neck Surg*. 2021;50(1):31. doi:10.1186/s40463-021-00512-2

20. Kohan I, Tabeei O, Sepahi N. HSPA1L and HSPA1B gene polymorphisms and haplotypes are associated with idiopathic male infertility in Iranian population. *Eur J Obest Gynecol Reprod Biol*. 2019;240:57–61. doi:10.1016/j.ejogr.2019.06.014

21. Aquino-Gálvez A, González-ávila G, Pérez-Rodríguez M, et al. Analysis of heat shock protein 70 gene polymorphisms Mexican patients with idiopathic pulmonary fibrosis. *BMC Pulm Med*. 2015;15:129. doi:10.1186/s12890-015-0127-7

22. Almutairi M, Almutairi B, Almutairi M, et al. Human beta-defensin-1 rs2738047 polymorphism is associated with shisha smoking risk among Saudi population. *Environ Sci Pollut Res Int*. 2021;28(1):1–18. doi:10.1007/s11356-020-11060-z

23. Gao X, Wang X, Jiao N, et al. Association of VEGFA polymorphisms with chronic obstructive pulmonary disease in Chinese Han and Mongolian populations. *Exp Physiol*. 2021;106(8):1839–1848. doi:10.1113/EP089523

24. Faiz A, Rathnayake SNH, Ten NHIT, et al. Single-nucleotide polymorphism rs2070600 regulates AGER splicing and the spumon level of the COPD biomarker soluble receptor for advanced glycation end-products. *ERJ Open Res*. 2021;7(2). doi:10.1183/23120541.00947-2020

25. Du Y, Zhang H, Xu Y, et al. Association among genetic polymorphisms of GSTP1, HO-1, and SOD-3 and chronic obstructive pulmonary disease susceptibility. *Int J Chron Obstruct Pulmon Dis*. 2019;14:2081–2088. doi:10.2147/COPD.S213364

26. Bchir S, Boumiza S, Ben Nasr H, et al. Impact of cathepsin D activity and C224T polymorphism (rs17571) on chronic obstructive pulmonary disease. *Sci Rep*. 2015;5:1–8. doi:10.1038/srep10238

27. Bchir S, Boumiza S, Ben Nasr H, et al. Mild catalytic defects of tert rs61748181 polymorphism affect the clinical presentation of chronic obstructive pulmonary disease. *Sci Rep*. 2021;11(1):4333. doi:10.1038/s41598-021-83686-z

28. Ding Y, Li Q, Feng Q, CYP2B6 genetic polymorphisms influence chronic obstructive pulmonary disease susceptibility in the Hainan population. *Int J Chron Obstruct Pulmon Dis*. 2019;14:2103–2115. doi:10.2147/COPD.S214961

29. Korytina GF, Tselousova OS, Akhmadishina LZ, et al. Association of the MMP3, MMP9, ADAM33 and TIMP3 genes polymorphic markers with development and progression of chronic obstructive pulmonary disease. *Mol Biol*. 2012;46(3):487–499. doi:10.1134/S0026893312020082

30. Ding Y, Yang D, Xun X. Association of genetic polymorphisms with chronic obstructive pulmonary disease in the Hainan population: a case-control study. *Int J Chron Obstruct Pulmon Dis*. 2015;10:7–13. doi:10.2147/COPD.S73042

31. Wang LH, Kim SH, Lee JH, et al. Inactivation of SMAD4 tumor suppressor gene during gastric carcinoma progression. *Clin Cancer Res*. 2007;13(1):101–110. doi:10.1158/1078-0432.CCR-06-1467

32. Slattery ML, Lundgreen A, Herrick JS, et al. Associations between genetic variation in RUNX1, RUNX2, RUNX3, MAPK1 and eIF4E and risk of colon and rectal cancer: additional support for a TGF-β-signaling pathway. *Carcinogenesis*. 2011;32(3):318–326. doi:10.1093/carcin/bgr245

33. Wosiak A, Wodziński D, Michalska K, et al. Assessment of the role of selected SMAD3 and SMAD4 genes polymorphisms in the development of colorectal cancer: preliminary research. *Pharmgenomics Pers Med*. 2021;14:167–178. doi:10.2147/PGPM.S21958

34. Pasquale LR, Loomis SI, Aschard H, et al. Exploring genome-wide - dietary heme iron intake interactions and the risk of type 2 diabetes. *Front Genet*. 2013;4:7. doi:10.3389/fgene.2013.00007
