Respiratory Research

RESEARCH

Open Access

Impact of HHIP gene polymorphisms on phenotypes, serum IL-17 and IL-18 in COPD patients of the Chinese Han population

Jiajun Zhang[1,5], Di Zhao[1], Lili Zhang[1,5], Xueyan Feng[1], Beibei Li[1], Hui Dong[2], Yanchao Qi[3], Zun Jia[4], Fuyun Liu[4], Shaohui Zhao[4] and Jin Zhang[5*]

Abstract

Background Genetic factors, including the Hedgehog Interacting Protein (HHIP) gene, play a crucial role in Chronic Obstructive Pulmonary Disease (COPD) susceptibility. This study examines the association between HHIP gene polymorphisms and COPD susceptibility, phenotypes, and serum IL-17 and IL-18 levels in a Han Chinese population.

Methods A case-control study was conducted with 300 COPD patients and 300 healthy controls in Chinese Han population. Participants underwent genotyping for HHIP gene polymorphisms, pulmonary function tests, and quantitative CT scans. DNA samples were sequenced using a custom chip targeting the HHIP gene. Serum IL-17 and IL-18 levels were measured by enzyme-linked immunosorbent assay. Associations between SNPs, COPD susceptibility, and phenotypes were analyzed using logistic and multiple linear regression models, adjusting for confounders.

Results Our study identified the rs11100865 polymorphism in the HHIP gene as significantly associated with COPD susceptibility (OR 2.479, 95% CI 1.527–4.024, $P = 2.39E\text{-}04$) after screening 114 SNPs through rigorous quality control. Stratified analyses further indicated this association was particularly in individuals aged 60 or older. Serum levels of IL-17 and IL-18 were significantly elevated in COPD patients compared to controls, with rs11100865 showing a notable association with IL-18 levels ($B = 49.654$, $SE = 19.627$, $P = 0.012$). However, no significant associations were observed between rs11100865 and serum IL-17 levels, COPD-related imaging parameters, or clinical phenotypes.

Conclusion This study identified a significant association between HHIP gene polymorphisms and COPD susceptibility in a Han Chinese population, with connections to inflammation, but found no significant associations between this SNP and COPD-related imaging or clinical phenotypes.

Trial registration www.chictr.org.cn ID: ChiCTR2300071579 2023-05-18.

Keywords Hedgehog interacting protein, COPD, Single nucleotide polymorphism, Phenotype, IL-17, IL-18

*Correspondence:
Jin Zhang
2140148928@qq.com
[1]School of Clinical Medicine, Ningxia Medical University, Yinchuan, Ningxia 750004, People's Republic of China
[2]Center of Research Equipment Management, General Hospital of Ningxia Medical University, Yinchuan 750004, People's Republic of China
[3]Department of Respiratory and Critical Care Medicine, The Second People's Hospital of Shizuishan, Shizuishan 753000, People's Republic of China
[4]Department of Respiratory and Critical Care Medicine, The Fifth People's Hospital of Ningxia, Shizuishan 753000, People's Republic of China
[5]Department of Respiratory and Critical Care Medicine, General Hospital of Ningxia Medical University, 804 Shengli South Street, Xingqing District, Yinchuan 750004, People's Republic of China

Introduction

Chronic Obstructive Pulmonary Disease (COPD) is a chronic respiratory condition characterized by persistent airflow limitation caused by airway and alveolar damage. A 2018 survey reported that the prevalence of COPD among individuals aged 40 and above in China was 13.7% [1]. COPD is believed to involve interactions between genetic, environmental, and individual factors [2–4]. Cigarette smoke is widely recognized as a primary cause [5]. However, only about 20% of active smokers develop the disease, and it is estimated that 25–45% of COPD patients have never smoked [6, 7]. Besides environmental factors such as biomass exposure and occupational hazards [8, 9], COPD exhibits familial clustering, and the characteristics of lung function are also hereditary in patients suggest that genetic susceptibility plays a significant role in its pathogenesis [10, 11].

Genome-wide association studies (GWAS) are a powerful tool for detecting genetic variations associated with traits using genome-wide linkage and association methods. GWAS offers high significance standards, large sample sizes, and strong statistical power [12, 13]. The first GWAS for COPD was published in 2009 and identified a locus near the Hedgehog Interacting Protein (HHIP) gene on 4q31 associated with COPD susceptibility [14]. Many countries have studied the relationship between HHIP gene polymorphisms and COPD in different ethnic groups, revealing associations between HHIP single nucleotide polymorphisms (SNPs) and COPD susceptibility [15, 16]. Several studies have discovered multiple SNPs near the HHIP gene on 4q31 that are significantly associated with disease severity, emphysema, and lung function [17, 18]. The HHIP gene, located at chromosome 4q31.21-31.3, encodes a transmembrane glycoprotein that is a transcriptional target of the Hedgehog (HH) signaling pathway. The highly conserved HH signaling pathway plays a crucial role in various physiological and pathological processes in the lungs and other organs, acting as a key factor in the pathway's negative feedback regulation [19]. Animal studies indicate that HHIP is involved in airway development, reduces airway lumen enlargement, improves lung compliance, and protects cells from oxidative stress caused by environmental factors [20–22].

Inflammation is a central feature of COPD, involving intricate cytokine networks. Among these, interleukin-17 (IL-17) and interleukin-18 (IL-18) have emerged as key mediators. IL-17, predominantly produced by Th17 cells, promotes neutrophil activation and recruitment, thereby exacerbating chronic inflammation—a hallmark of COPD. Elevated IL-17 levels in COPD patients correlate with disease severity and exacerbation risk, highlighting its potential as a biomarker and therapeutic target [23]. On the other hand, IL-18, known for its multifaceted roles in inflammatory responses, demonstrates elevated levels in COPD patients, correlating with worsened lung function and indicating its involvement in disease progression [24, 25]. Recent study revealed that genetic variation in the HHIP gene exacerbated lymphocytic inflammation in COPD by enhancing interaction between lung fibroblasts and $CD8^+$ T cells, particularly through upregulated IL-18 pathway genes, leading to increased Interferon gamma (IFN-γ) production [26].

In recent years, the number of GWAS related to COPD has significantly decreased, and the results have varied considerably among different ethnic groups. Most current findings apply primarily to Caucasian populations, and many of the loci associated with COPD, despite showing statistical associations, lack sufficient clinical validation. Ningxia, located in the northwest of China, is a region primarily inhabited by Chinese Han and Hui ethnic groups. As of 2020, the Han population reached 7.20 million. A survey conducted in 2013 indicated a prevalence of COPD at 8.9% in Ningxia, with the prevalence among the Han population at 9.4%, which is higher than the 8.0% rate observed in the Hui population [27]. To investigate the relationship between HHIP gene polymorphisms and COPD genetic susceptibility in a local Han Chinese population, we conducted a case-control study, collecting patient lung function data and quantitative CT parameters. Additionally, we aimed to explore whether these genetic susceptibility loci are associated with COPD-related phenotypes and serum level of IL-17 and IL-18.

Methods

Study subjects

We collected COPD patients of Han Chinese ethnicity who visited the outpatient departments of General Hospital of Ningxia Medical University, Yinchuan in the northern Ningxia Hui Autonomous Region, China. These patients were collected between January 1, 2021, and January 31, 2024. Additionally, Han Chinese individuals who underwent physical examinations at the Physical Examination Center of General Hospital of Ningxia Medical University during the same period served as the healthy control group. This study was approved by the Ethics Committee of General Hospital of Ningxia Medical University, and all participants signed informed consent forms. Ethical approval number: 2020−786.

Inclusion and exclusion criteria

The inclusion criteria for the study involved patients diagnosed with stable COPD according to the GOLD guidelines [2], which include symptoms such as dyspnea, chronic cough, sputum production, wheezing, and a history of exposure to COPD risk factors, with a post-bronchodilator FEV1/FVC<0.70 on pulmonary

function test. Additionally, these patients were required to be in a stable phase of COPD with stable or mild clinical symptoms for at least one month without any changes in treatment due to symptom exacerbation and be of Han Chinese ethnicity. The healthy control group criteria included individuals with no significant abnormalities in pulmonary function tests. Exclusion criteria encompassed the non-native population, lung abnormalities on chest CT and presence of other pulmonary diseases such as bronchiectasis, interstitial lung disease, active pulmonary tuberculosis, pleural effusion, lung tumors, or a history of lung resection, as well as comorbidities like heart failure, severe liver or kidney dysfunction, infectious diseases, mental or cognitive impairment, and hearing or communication disorders. Additionally, participants with poor compliance, refusal to complete tests, or unwillingness to participate in the study, as well as those with multiple quality control failures of DNA samples resulting in unsuccessful genotype determination after repeated submissions, were excluded.

Phenotype information collection

Data on age, gender, body mass index (BMI) and smoking history were collected from all study subjects. All subjects underwent pulmonary function tests using a spirometer (Jaeger MasterScreen SeS, Germany), recording FEV1, FVC, FEV1/FVC, and FEV1% predicted. Each subject underwent at least two acceptable tests, with repeated measurement differences not exceeding 0.20 L. Multislice spiral CT (GE MEDICAL SYSTEMS, USA) was used for scans. Prior to the scan, subjects received breath-holding training and were positioned supine with arms raised above the head. Scans were performed from the thoracic inlet to the level of the adrenal glands, covering the entire lungs during deep inspiration. Scans were reconstructed using a high-resolution algorithm with 1.25 mm slice thickness, a window width of 1200 Hounsfield units (HU), and a window level of -600 HU. Mediastinal windows were reconstructed with 1.25 mm slice thickness, a window width of 350 HU, and a window level of 40 HU. Images were saved in DICOM format for quantitative analysis. Using the 3D Slicer image computing platform 5.61 (https://www.slicer.org/), DICOM format files were analyzed to measure airway and emphysema parameters [28]. The Chest Imaging Platform module's AirwayInspector was used to measure airway wall area and total airway area in the segmental bronchi of the upper lobes and lower lobes of the left and right lungs, calculating the airway wall area percentage (3-WA%). The formula for airway wall area percentage is airway wall area percentage = (airway wall area / total airway area) × 100%. The Lung CT Analyzer module of the Chest Imaging Platform measured the percentage low attenuation area using a threshold of -950HU (%LAA_{-950}) in the lungs, defined as regions with attenuation values below −950 HU at the end of deep inspiration.

Based on literature reports [29, 30], %LAA_{-950}≥6% is defined as emphysema; however, there is no established standard for the 3-WA% parameter. Therefore, we calculated the upper limit of normal (ULN) for bilateral 95% reference ranges using the formula: mean+1.96 × standard deviation. Quantitative CT parameters were then used to classify COPD imaging into four types: (1) Airway-dominant type (A type): %LAA_{-950} < 6% and 3-WA% ≥ ULN of the control group; (2) Emphysema-dominant type (E type): %LAA_{-950} ≥ 6% and 3-WA% < ULN of the control group; (3) Mixed type (M type): %LAA_{-950} ≥ 6% and 3-WA% ≥ ULN of the control group; and (4) Mild type: %LAA_{-950} < 6% and 3-WA% < ULN of the control group. Additionally, following the GOLD and Spanish COPD guidelines [2, 31], we classified the clinical phenotypes of COPD into the following categories: (1) Non-exacerbator (NE): patients with no more than two acute exacerbations in the past year; (2) Exacerbator with emphysema (EE): patients with more than two acute exacerbations in the past year and %LAA_{-950}≥6%; (3) Exacerbator with chronic bronchitis (ECB): patients with more than two acute exacerbations in the past year, accompanied by symptoms of cough, sputum production, or wheezing that persist for at least 3 months each year for two consecutive years or longer, excluding other diseases that could cause these symptoms. (4) Positive bronchodilator test (PBD) phenotype was defined as an increase in FEV1 of more than 200 mL and an increase of more than 12% after inhaling a bronchodilator.

DNA extraction and SNPs detection

All participants in the study provided blood samples upon obtaining informed consent. Blood samples (4 ml) were drawn from all subjects in the early morning after fasting and placed in EDTA anticoagulant tubes. The samples were stored at -80 °C and will be sent for unified testing at a later stage. Peripheral blood was collected from routine blood tests and processed for DNA extraction. Initially, blood samples were centrifuged at room temperature at 2500 g for 10 min to separate plasma. The blood cells were then collected into 1.5 ml EP tubes and stored at -80 °C until DNA extraction. Before extraction, EP tubes were thawed at room temperature for at least 15 min, and samples were thoroughly mixed. DNA was extracted using the QIAamp DNA Mini Kits (Germany). Prior to sequencing, the purity and integrity of DNA samples were assessed. Qualified DNA samples underwent target sequencing using a custom solution-based chip (iGeneTech Ltd, Beijing, China) to capture the HHIP gene. Specific probes on the chip hybridized with the target DNA sequences, capturing and enriching the target regions. High-throughput sequencing was

performed using the Illumina sequencing platform. The sequencing data were aligned to the reference genome GRCh38, and quality control measures were applied to remove sequencing adapters and low-quality sequences. After passing quality control, SNPs were identified, annotated, and recorded in VCF files. Samples with missing genotypes underwent resequencing for those specific individuals. SNP data were further analyzed using PLINK software. Quality control steps included filtering out SNPs with a minor allele frequency (MAF) less than 0.05 and those not in Hardy-Weinberg equilibrium (HWE, $P<0.05$). The relationship between genotypes and phenotypes was then assessed using generalized linear models in PLINK.

Stratified analysis

To investigate whether the association between COPD susceptibility and HHIP SNP varies across specific populations, we performed stratified analyses based on age, sex, BMI, and smoking status. Age was stratified at 60 years, as COPD prevalence is highest in individuals aged 60 and above [2]; BMI was stratified at 24, following the Chinese obesity-related guidelines, where a BMI≥24 is defined as overweight [32].

ELISA

Serum IL-17 levels were analyzed using The Human IL-17 Quantikine HS ELISA Kit HS170 from R&D Systems; Serum IL-18 levels were analyzed using Human Total IL-18/IL-1F4 Quantikine ELISA Kit from R&D Systems. Both interleukins were analyzed according to the protocol. We used a manually operated ELISA kit for the measurements. To minimize experimental error, the interleukin levels were measured twice, and we reduced the number of testing batches as much as possible. Additionally, all measurements were conducted by the same person to further control for variability.

Data analysis

A case-control study was conducted to statistically describe the baseline characteristics. Continuous variables were expressed as mean±standard deviation, and categorical variables as percentages. The t-test was used for comparing means between two groups with normally distributed data, while one-way ANOVA, followed by the Student-Newman-Keuls test, was employed for multiple group comparisons. For comparisons against the healthy control group, Dunnett's t-test was used. If the data do not meet the assumption of normal distribution, the Kruskal-Wallis H test was applied, followed by Dunn's test for multiple comparisons. The chi-square test was used to compare categorical variables, Hardy-Weinberg equilibrium, allele frequencies, and genotypes between groups. Binary logistic regression, adjusting for sex, age, BMI, smoking status, and genetic principal components, was performed to compare allele frequencies and genotypes. Multiple linear regression was employed to assess the association between SNP genotype and pulmonary function and imaging parameters, adjusting for age, sex, and smoking history. Multivariable logistic regression, adjusted for age, sex, BMI, and pack years, was used to examine the association between SNP genotype and COPD-related phenotypes. A significance threshold of $\alpha=0.05$ divided by the number of SNPs compared was used for screening significant SNPs, while a P-value of less than 0.05 was considered statistically significant for all other tests. All analyses were conducted using SPSS version 27.0 software, GraphPad Prism version 10.1.2 for graphing, PLINK version 1.90 for sequencing data analysis, LocusZoom website(http://locuszoom.sph.umich.edu) for graphing region plot [33], G*Power Version 3.1.9.7 software for calculating statistical power.

Results

Patients selection

From January 1, 2021, to January 31, 2024, a total of 695 participants were initially enrolled in the study, with 312 patients in the COPD group and 383 healthy individuals in the control group. 12 participants were excluded from the COPD group due to additional lung lesions identified on CT ($n=9$), unquantified DNA samples ($n=2$), and comorbidities meeting exclusion criteria ($n=1$). Similarly, 83 participants were excluded from the control group for reasons including abnormal pulmonary function tests ($n=33$), lung abnormalities on CT ($n=35$), lipemia ($n=1$), non-native population ($n=12$), and comorbidities meeting exclusion criteria ($n=2$). Ultimately, 300 participants from each group proceeded to HHIP gene sequencing (Fig. 1).

Characteristics of enrolled participants

The baseline characteristics of the study showed significant differences between the COPD and control groups. The COPD group was older (67.28 vs. 57.91 years, $P<0.001$), with a higher proportion of males (70% vs. 61%, $P=0.026$). The COPD group had a lower BMI (24.43 vs. 25.45 kg/m², $P<0.001$), a higher smoking history (13.28 vs. 4.02pack years, $P<0.001$). Additionally, the COPD group had higher $\%LAA_{-950}$ (7.77% vs. 1.40%, $P<0.001$) and 3-WA% (67.29% vs. 63.31%, $P<0.001$). In terms of pulmonary function parameters, the control group had an FEV1%predicted of 115.64% and an FEV1/FVC of 80.62%. In the COPD group, after inhaling a bronchodilator, the FEV1%predicted was 69.34% and the FEV1/FVC was 57.28. Additionally, the CAT score for the COPD group was 10.87±7.14, with an acute exacerbation frequency of 1.09±1.48 over the past year. According to GOLD classification, there were 248 patients in grades

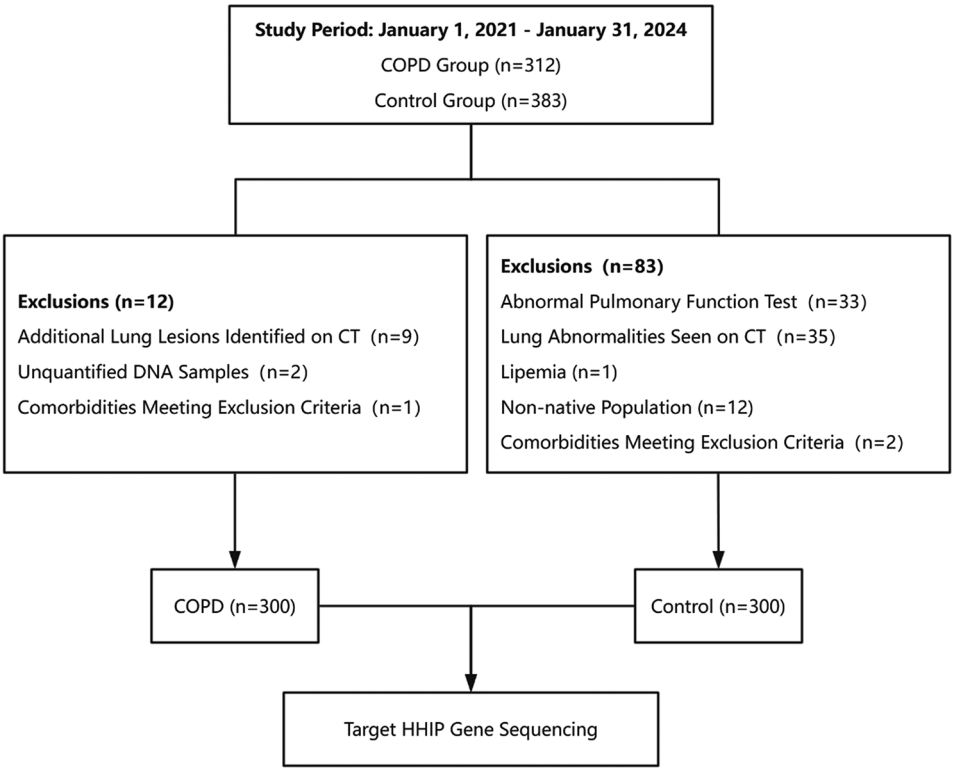

Fig. 1 Flow diagram of the participants exclusion process in the study. *COPD* chronic obstructive pulmonary disease, *CT* computer tomography, *HHIP* Hedgehog Interacting Protein

Table 1 Characteristics of enrolled COPD patients and healthy control

Variable	COPD($n=300$)	Control($n=300$)	*P*
Age(years)	67.28±8.61	57.91±10.09	**<0.001**
Sex(%)			**0.026**
Male	210(70.00)	183(61.00)	
Female	90(30.00)	117(39.00)	
BMI(kg/m^2)	24.43±3.84	25.45±3.40	**<0.001**
Pack years	13.28±19.80	4.02±9.36	**<0.001**
Smoking status(%)			**<0.001**
No	171(57.00)	229(76.33)	
Yes	129(43.00)	71(23.67)	
PFT			
FEV1%predicted(%)	69.34±19.80	/	
FEV1/FVC(%)	57.28±10.35	/	
QCT			
%LAA$_{-950}$(%)	7.77±9.78	1.40±1.37	**<0.001**
3-WA%(%)	67.29±6.85	63.31±3.86	**<0.001**
CAT	10.87±7.14	/	
Exacerbation frequency	1.09±1.48	/	
GOLD stage(%)			
GOLD 1–2	248(82.67)	/	
GOLD 3–4	52(17.33)	/	

COPD chronic obstructive pulmonary disease, *PFT* pulmonary function test, *FEV1* forced expiratory volume in 1 s, *FVC* forced vital capacity, *QCT* quantitative computer tomography, *%LAA$_{-950}$* percentage low attenuation area using a threshold of -950 HU, *WA* wall area for the segmental bronchus, *CAT* COPD assessment test, *GOLD* Global Initiative Chronic Obstructive Lung Disease

1–2, accounting for 82.67%, and 52 patients in grades 3–4, accounting for 17.33%. Detailed information is provided in Table 1.

Serum IL-17 and IL-18 in different stage of COPD patients and healthy control

The serum levels of IL-17 and IL-18 were significantly elevated in COPD patients compared to the 250 healthy control group ($P<0.001$ for both interleukins). Specifically, IL-17 levels were highest in the GOLD 3–4 stage COPD patients (0.78±0.25pg/mL), followed by those in the GOLD 1–2 stage (0.68±0.24pg/mL), and were lowest in the control group (0.21±0.04pg/mL). Similarly, IL-18 levels were significantly higher in GOLD 3–4 stage COPD patients (461.84±255.93pg/mL) than in GOLD 1–2 stage patients (372.84±237.98pg/mL), with the control group having the lowest levels (126.50±72.11pg/mL). The differences between all groups were statistically significant. Detailed information is provided in Table 2; Fig. 2.

Genotyping and SNPs selection

Sequencing of the HHIP gene was performed for all study subjects, and variations were annotated based on the reference genome (GRCh38), resulting in the identification of 789 SNP loci within the HHIP gene. After quality control using Plink software, 671 SNPs with

Table 2 Serum IL-17 and IL-18 in different stage of COPD patients and healthy control

Interleukin	GOLD 1–2 (*n* = 248)	GOLD 3–4 (*n* = 52)	Control (*n* = 250)	*P*
IL-17 (pg/mL)	0.68 ± 0.24[a]	0.78 ± 0.25[b]	0.21 ± 0.04[c]	<0.001
IL-18 (pg/mL)	372.84 ± 237.98[a]	461.84 ± 255.93[b]	126.50 ± 72.11[c]	<0.001

a, b, c: The lower-case letters in the superscripts indicate the results of multiple comparisons. When rows share the same letter, the differences between their data are not statistically significant

MAF < 0.05, 3 SNPs with HWE $P < 0.05$ and 1 multiallelic SNP were excluded, leaving 114 SNPs for further association analysis.

Association with COPD susceptibility

Comparing the allele frequencies of these 114 SNPs between the COPD and healthy control groups revealed the association between SNPs within the HHIP gene and COPD susceptibility. Using a threshold of $P = 4.4E\text{-}04$ ($P = 0.05/114$), rs11100865 were found to be significantly associated with COPD susceptibility using additive genetic model adjusted by age, sex, BMI, pack years and genetic principal components (OR 2.479, 95% CI 1.527–4.024, $P = 2.39E\text{-}04$). Detailed results are presented in Table 3; Fig. 3.

We further explored the relationship between rs11100865 and COPD susceptibility under different genetic models. The A allele is associated with an increased risk of COPD (OR = 1.607, 95% CI: 1.140–2.266, $P = 0.007$). In the codominant model, the GA genotype (OR = 2.787, 95% CI: 1.558–4.983, $P < 0.001$) and the AA genotype (OR = 5.799, 95% CI: 2.171–15.489, $P < 0.001$) show a significant association with increased risk. The dominant model indicates a higher risk for the GA-AA genotype compared to GG (OR = 2.455, 95% CI: 1.398–4.309, $P = 0.002$). However, the recessive model does not show a significant association (OR = 1.636, 95% CI: 0.847–3.159, $P = 0.143$). All results are adjusted for

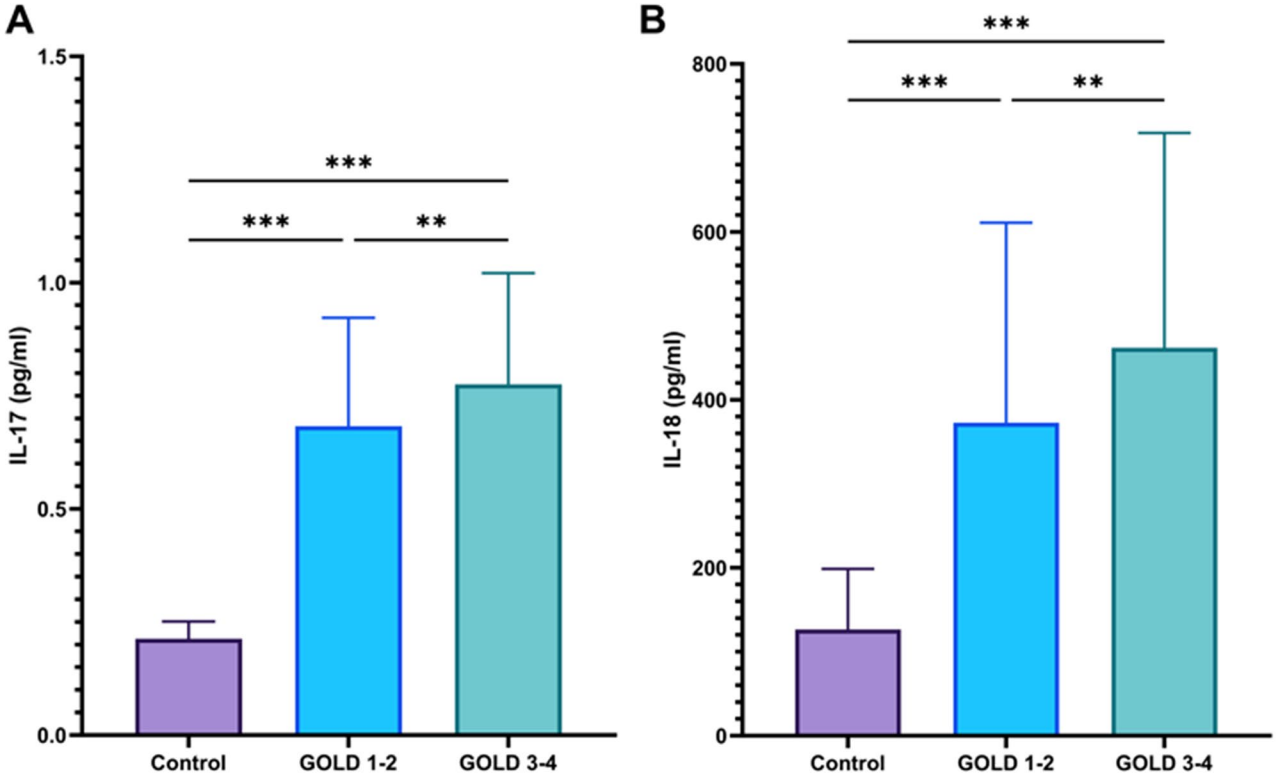

Fig. 2 Serum IL-17 and IL-18 in COPD patients and healthy control. (**A**) IL-17, (**B**) IL-18. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$

Table 3 Genetic association between selected HHIP gene SNP and COPD susceptibility

SNP ID	Function	Position	Allele	$P_{HWE-ALL}$	MAF		OR(95%CI)	*P*
					COPD	Control		
rs11100865	Intron Variant	chr4:144729995	G> A	0.115	0.493	0.380	2.479 (1.527–4.024)	**2.39E-04**

Using additive genetic model adjusted by age, sex, BMI, pack years and genetic principal components. *SNP* single nucleotide polymorphism, *HWE* Hardy-Weinberg Equilibrium, *MAF* Minor allele frequency, *COPD* chronic obstructive pulmonary disease, *OR* odds ratio, *CI* confidence interval

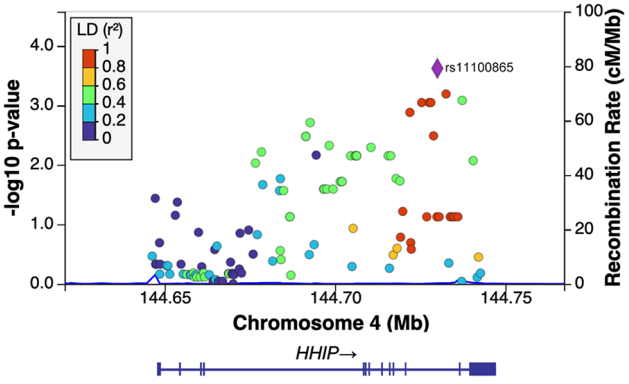

Fig. 3 Region plot of 114 SNPs in Hedgehog Interacting Protein gene and their relationships with COPD susceptibility. *LD* linkage disequilibrium

age, sex, BMI, smoking pack years, and genetic principal components. Detailed results are presented in Table 4.

Association between HHIP gene and COPD susceptibility stratified by age, sex, BMI and smoking status

The stratified analysis results show varied associations between the rs11100865 polymorphism in the HHIP gene and COPD susceptibility across different subgroups. All analyses were performed using binary logistic regression models adjusted for confounding factors, including age, sex, BMI, pack-years of smoking, and genetic principal components (excluding the stratification factor itself).

In the age-stratified analysis, no significant association was observed in individuals under 60 years old (OR 1.465, 95%CI 0.817–2.624, $P=0.200$). In the codominant model, using the GG genotype as a reference, the GA genotype was significantly associated with COPD in individuals aged 60 and above (OR 2.183, 95%CI 1.166–4.084, $P=0.015$). Similarly, the AA genotype was also significantly associated with COPD in the same age group (OR 3.398, 95%CI 1.133–10.191, $P=0.029$). This effect was also observed in the dominant model (OR 2.090, 95%CI 1.125–3.883, $P=0.020$) and the additive model (OR 1.943, 95%CI 1.145–3.298, $P=0.014$). In the recessive model, no significant association between specific genotypes and COPD susceptibility was found in different age strata. Detailed results are presented in Table 5.

In the gender-stratified analysis, compared to the G allele, the A allele was significantly associated with an increased risk of COPD in women (OR 1.856, 95%CI 1.072–3.213, $P=0.027$). In the codominant model, using the GG genotype as a reference, the GA genotype was significantly associated with COPD in women (OR 4.200, 95%CI 1.607–10.979, $P=0.003$), while the AA genotype posed a higher risk (OR 10.729, 95%CI 2.014–57.143, $P=0.005$). Similar effects were observed in the dominant model (OR 3.502, 95%CI 1.408–8.709, $P=0.007$) and the additive model (OR 3.481, 95%CI 1.532–7.911, $P=0.003$). In men, the AA genotype was associated with a higher risk in the codominant model (OR 3.511, 95%CI 1.010-12.198, $P=0.048$), and the additive model also indicated an increased risk (OR 1.892, 95%CI 1.022–3.503, $P=0.042$). In the recessive model, no significant association between specific genotypes and COPD susceptibility was found across different gender strata. Detailed results are presented in Table 6.

In the BMI-stratified analysis, for individuals with BMI ≥ 24, the A allele was significantly associated with an increased risk of COPD (OR 1.815, 95%CI 1.166–2.823, $P=0.008$). In the codominant model, using the GG genotype as a reference, the GA genotype was significantly associated with COPD in individuals with BMI ≥ 24 (OR 2.988, 95%CI 1.449–6.202, $P=0.003$); the AA genotype was also significantly associated with COPD in this group (OR 7.912, 95%CI 2.336–26.795, $P<0.001$). Similar effects were observed in the dominant model (OR 2.625, 95%CI 1.296–5.317, $P=0.007$) and the additive model (OR 2.850, 95%CI 1.563–5.317, $P<0.001$). In the recessive model, no significant association between specific genotypes and COPD susceptibility was observed across different BMI strata. Although no significant association between rs11100865 and COPD susceptibility was observed across different genetic models in COPD patients with BMI < 24, the statistical power calculations revealed that

Table 4 rs11100865 in HHIP gene associated with susceptibility of COPD

Model	Genotype	COPD($N=300$)	Control($N=300$)	OR(95% CI)	*P*
Allele	G	304(50.67)	372(62.00)	-	
	A	296(49.33)	228(38.00)	1.607(1.140–2.266)	**0.007**
Codominant	GG	78(26.00)	122(40.67)	-	
	GA	148(49.33)	128(42.67)	2.787(1.558–4.983)	**<0.001**
	AA	74(24.67)	50(16.66)	5.799(2.171–15.489)	**<0.001**
Recessive	GG-GA	226(75.33)	250(83.33)	-	
	AA	74(24.67)	50(16.67)	1.636(0.847–3.159)	0.143
Dominant	GG	78(26.00)	122(40.67)	-	
	GA-AA	222(74.00)	178(59.33)	2.455(1.398–4.309)	**0.002**

Adjusted by age, sex, BMI, pack years and genetic principal components. *SNP* single nucleotide polymorphism, *COPD* chronic obstructive pulmonary disease, *OR* odds ratio, *CI* confidence interval

Table 5 rs11100865 in HHIP gene associated with COPD susceptibility stratified by age

Model	Genotype	Age ≥ 60(*N* = 368)					Age<60(*N* = 232)					
		COPD(*N* = 239)	Control(*N* = 129)	OR (95%CI)	*P*	R^2	Power	COPD(*N* = 61)	Control(*N* = 171)	OR (95%CI)	*P*	R^2	Power
Allele	G	244(51.05)	173(67.05)	-		0.119	0.590	60(49.18)	199(58.19)	-		0.125	0.355
	A	234(48.95)	85(32.95)	1.473(0.987–2.198)	0.058			62(50.82)	143(41.81)	1.465(0.817–2.624)	0.200		
Codominant	GG	62(25.94)	61(47.29)	-		0.130	1.000	16(26.23)	61(35.67)	-		0.138	1.000
	GA	120(50.21)	51(39.53)	2.183(1.166–4.084)	**0.015**			28(45.90)	77(45.03)	2.240(0.695–7.211)	0.177		
	AA	57(23.85)	17(13.18)	3.398(1.133–10.191)	**0.029**			17(27.87)	33(19.30)	6.874(0.991–47.682)	0.051		
Recessive	GG-GA	182(76.15)	112(86.82)	-		0.116	0.241	44(72.13)	138(80.70)	-		0.131	0.703
	AA	57(23.85)	17(13.18)	1.363(0.605–3.071)	0.454			17(27.87)	33(19.30)	2.298(0.799–6.608)	0.123		
Dominant	GG	62(25.94)	61(47.29)	-		0.128	0.805	16(26.23)	61(35.67)	-		0.124	0.215
	GA-AA	177(74.06)	68(52.71)	2.090(1.125–3.883)	**0.020**			45(73.77)	110(64.33)	1.480(0.524–4.180)	0.459		
Log-additive	-	-	-	1.943(1.145–3.298)	**0.014**	0.129	0.947	-	-	2.633(0.997–6.956)	0.051	0.137	0.997

Binary logistic regression adjusted by confounding factors: sex, BMI, pack years and genetic principal components

all statistical power values were low. Detailed results are presented in Table 7.

Among the 300 stable COPD patients included in the study, 129 were smokers, accounting for 43% of the total COPD cohort. The mean smoking pack years for smoking COPD patients was 30.89±19.18, with a median of 30.00 pack years. In the stratified analysis by smoking status, a significant association was observed in the non-smoking group between allele A and COPD susceptibility compared to allele G (OR 1.774, 95%CI 1.187–2.651, $P=0.005$). In the codominant model, using the GG genotype as the reference, the GA genotype was significantly associated with COPD in non-smokers (OR 3.384, 95%CI 1.712–6.689, $P<0.001$), as was the AA genotype (OR 7.900, 95%CI 2.517–24.801, $P<0.001$). Similar trends were observed in the dominant model (OR 2.940, 95%CI 1.522–5.677, $P=0.001$) and the additive model (OR 2.912, 95%CI 1.655–5.123, $P<0.001$). No significant association was found between any specific genotype and COPD susceptibility in the recessive model across different subgroups. Although no significant association between rs11100865 and COPD susceptibility was observed across different genetic models in COPD patients with smoking, the statistical power calculations revealed that all statistical power values were low. Detailed results are presented in Table 8.

Association between rs11100865 and COPD phenotypes and serum interleukin

According to genotype classification, the patients were categorized into rs11100865 GG (78 cases), GA (148 cases), and AA (74 cases), with detailed values shown in Table 9. Using the GG genotype as the reference, no statistically significant differences were observed in COPD patients with GA or AA genotypes across age, gender, BMI, smoking status, pack-years of smoking, lung function parameters (FEV1% predicted and FEV1/FVC), CAT scores, frequency of acute exacerbations in the previous year, imaging parameters ($\%LAA_{-950}$ and 3-WA%). For serum interleukins, using the GG genotype as a reference, no significant differences were found in serum IL-17 levels between the GA and AA genotypes (GA vs. GG, $P=0.998$; AA vs. GG, $P=0.741$). Both the GA and AA genotypes showed significantly elevated serum IL-18 levels compared to the GG genotype (GA vs. GG, 414.39 vs. 317.13pg/ml, $P=0.010$; AA vs. GG, 411.00 vs. 317.13pg/ml, $P=0.002$). In the control group of 250 individuals, no significant differences were observed in serum IL-17 and IL-18 levels across different genotypes.

To determine whether rs11100865 are associated with COPD-related phenotypes and serum interleukin, multiple linear regression was performed, adjusting for age, gender, BMI, and smoking status. No significant associations were observed between the

Table 6 rs11100865 in HHIP gene associated with COPD susceptibility stratified by sex

Model	Genotype	Male(*N* = 393)						Female(*N* = 207)					
		COPD(*N* = 210)	Control(*N* = 183)	OR (95%CI)	*P*	R^2	Power	COPD(*N* = 90)	Control(*N* = 117)	OR (95%CI)	*P*	R^2	Power
Allele	G	216(51.43)	225(61.48)	-		0.344	0.470	88(48.89)	147(62.82)	-		0.204	0.792
	A	204(48.57)	141(38.52)	1.401(0.898–2.185)	0.138			92(51.11)	87(37.18)	1.856(1.072–3.213)	**0.027**		
Codominant	GG	56(26.67)	74(40.44)	-		0.349	0.996	22(24.44)	48(41.03)	-		0.234	1.000
	GA	104(49.52)	77(42.07)	1.982(0.939–4.183)	0.073			44(48.89)	51(43.59)	4.200(1.607–10.979)	**0.003**		
	AA	50(23.81)	32(17.49)	3.511(1.010-12.198)	**0.048**			24(26.67)	18(15.38)	10.729(2.014–57.143)	**0.005**		
Recessive	GG-GA	160(76.19)	151(82.51)	-		0.344	0.380	66(73.33)	99(84.62)	-		0.199	0.395
	AA	50(23.81)	32(17.49)	1.523(0.655–3.543)	0.329			24(26.67)	18(15.38)	1.732(0.589–5.093)	0.318		
Dominant	GG	56(26.67)	74(40.44)	-		0.347	0.996	22(24.44)	48(41.03)	-		0.224	0.969
	GA-AA	154(73.33)	109(59.56)	1.799(0.868–3.730)	0.114			68(75.56)	69(58.97)	3.502(1.408–8.709)	**0.007**		
Log-additive	-	-	-	1.892(1.022–3.503)	**0.042**	0.349	0.923	-	-	3.481(1.532–7.911)	**0.003**	0.232	0.999

Binary logistic regression adjusted by confounding factors: age, BMI, pack years and genetic principal components

genotype distributions of rs11100865 and lung function or quantitative CT parameters ($P>0.05$). For interleukins, the analysis revealed a significant association between the genotype distribution of rs11100865 and serum IL-18 level (B = 49.654, SE = 19.627, $P=0.012$). However, no significant associations were observed between the genotype distributions and IL-17 ($P>0.05$). Detailed results are presented in Table 10; Fig. 4.

Association of rs11100865 genotype with imaging-based and clinical phenotypes in COPD patients

After performing quantitative CT analysis on all study subjects, the $\%LAA_{-950}$ and 3-WA% parameters were obtained. The upper limit of the 3-WA% reference range was calculated based on the mean + 1.96s from the 300 healthy controls. Using a $\%LAA_{-950}$ threshold of greater than 6, COPD patients were classified into four types: mild type (109 cases), airway-dominant type (82 cases), emphysema-dominant type (83 cases), and mixed type (26 cases). Additionally, based on the clinical data, lung function parameters, and $\%LAA_{-950}$ parameter, the patients were classified into NE type (204 cases), EE type (43 cases), ECB type (39 cases), and PBD type (14 cases).

The association analysis between the rs11100865 genotype and different COPD phenotypes, both imaging-based and clinical, showed no statistically significant differences across the genotypes. All analyses were adjusted by age, sex, BMI, and pack years. For imaging-based phenotypes, the comparison between the GG genotype and GA or AA genotypes did not show significant associations: emphysema-dominant type (OR 1.245, 95% CI 0.827–1.874, $P=0.294$), airway-dominant type (OR 1.178, 95% CI 0.783–1.773, $P=0.431$), and mix type (OR 1.534, 95% CI 0.824–2.855, $P=0.177$). For clinical phenotypes, the comparisons also did not reach statistical significance: exacerbator with emphysema (EE) (OR 1.360, 95% CI 0.843–2.193, $P=0.207$), exacerbator with chronic bronchitis (ECB) (OR 1.357, 95% CI 0.827–2.226, $P=0.227$), and positive bronchodilator test (PBD) (OR 1.593, 95% CI 0.694–3.657, $P=0.272$). Detailed results are presented in Table 11.

Discussion

This study demonstrated a significant association between the rs11100865 SNP in the HHIP gene and increased susceptibility to COPD, with the A allele being particularly implicated in heightened disease risk. The association was most pronounced in additive genetic models, where the A allele conferred a substantially higher likelihood of developing COPD. Stratified analyses further revealed that this risk was more significant in individuals aged 60 or older, suggesting that the genetic influence of rs11100865 may vary across different population. Although no significant association was observed

Table 7 rs11100865 in HHIP gene associated with COPD susceptibility stratified by BMI

Model	Genotype	BMI ≥ 24(*N* = 358)						BMI<24(*N* = 242)					
		COPD(*N* = 159)	Control(*N* = 199)	OR (95%CI)	*P*	R^2	Power	COPD(*N* = 141)	Control(*N* = 101)	OR (95%CI)	*P*	R^2	Power
Allele	G	164(51.57)	244(61.31)	-		0.268	0.922	140(49.65)	128(63.37)	-		0.315	0.293
	A	154(48.43)	154(38.69)	1.815(1.166–2.823)	**0.008**			142(50.35)	74(36.63)	1.293(0.741–2.257)	0.368		
Codominant	GG	45(28.30)	80(40.20)	-		0.286	1.000	33(23.40)	42(41.59)	-		0.321	0.967
	GA	74(46.54)	84(42.21)	2.998(1.449–6.202)	**0.003**			74(52.49)	44(43.56)	2.206(0.818–5.945)	0.118		
	AA	40(25.16)	35(17.59)	7.912(2.336–26.795)	**<0.001**			34(24.11)	15(14.85)	2.849(0.520-15.625)	0.228		
Recessive	GG-GA	119(74.84)	164(82.41)	-		0.267	0.858	107(75.89)	86(85.15)	-		0.314	0.050
	AA	40(25.16)	35(17.59)	2.144(0.927–4.960)	0.075			34(24.11)	15(14.85)	1.007(0.339–2.990)	0.591		
Dominant	GG	45(28.30)	80(40.20)	-		0.276	0.967	33(23.40)	42(41.58)	-		0.321	0.647
	GA-AA	114(71.70)	119(59.80)	2.625(1.296–5.317)	**0.007**			108(76.60)	59(58.42)	2.074(0.802–5.365)	0.133		
Log-additive	-	-	-	2.850(1.563–5.196)	**<0.001**	0.285	1.000	-	-	1.777(0.767–4.118)	0.180	0.319	0.640

Binary logistic regression adjusted by confounding factors: age, sex, pack years and genetic principal components

between rs11100865 and COPD susceptibility in COPD patients with a BMI < 24 or in smokers, the low statistical power suggests that the lack of significance may be due to a small sample size, potentially leading to false-negative results. Despite the strong link between rs11100865 and overall COPD susceptibility, our study did not uncover significant associations between this SNP and specific imaging-based or clinical phenotypes, including mild type, emphysema-dominant, airway-dominant, and mixed type, or clinical subtypes like NE, EE, ECB, PBD. However, a significant association was found between the rs11100865 genotype and serum IL-18 levels, with higher IL-18 levels observed in individuals carrying the A allele. No significant relationship was found with IL-17 levels, suggesting that while rs11100865 may influence certain inflammatory pathways in COPD, its role in driving specific phenotypic expressions of the disease remains unclear. This highlights the complexity of COPD pathogenesis and suggests that other genetic, environmental, or epigenetic factors may be involved in determining the specific clinical and imaging manifestations of the disease.

In China, several scholars have studied the association between HHIP gene SNPs and COPD in the Han Chinese population. Several studies conducted in the Han Chinese population have shown that certain polymorphic loci in the HHIP gene are associated with the incidence of COPD. However, the specific SNPs identified differ between studies, and even the same loci may yield different results within the same population. Zhang et al. found that the HHIP polymorphic loci rs12509311, rs13118928, and rs182859 were not associated with COPD susceptibility in southern Han Chinese patients [34]. Xu et al. discovered that the HHIP SNPs rs12504628, rs1828591, and rs13118928 could reduce the risk of COPD in a Han Chinese population in Inner Mongolia [35]. Xie et al. identified two new polymorphic loci within the HHIP gene, rs11100865 and rs7654947, in a Han Chinese population in Hubei, and noted that HHIP rs12504628 and rs13118928 were not associated with increased COPD risk [36]. In our study, we also found that the polymorphic locus rs11100865 was associated with COPD susceptibility in the Han Chinese population of Ningxia, suggesting that this locus may play a significant role in COPD development among Han Chinese individuals. We also found that the association between the rs11100865 genotype and COPD susceptibility in older adults. In studies using $HHIP^{+/-}$ mouse models, it was observed that these mice spontaneously develop age-related emphysema. Yun et al. discovered an accumulation of $CD8^+$ lymphocytes in the lung tissue of these mice, which increased with age. These findings are consistent with the results of this study, supporting the notion that alteration in HHIP gene expression led to increased susceptibility

Table 8 rs11100865 in HHIP gene associated with COPD susceptibility stratified by smoking status

Model	Genotype	Smoking(*N*=200)						No smoking(*N*=400)					
		COPD(*N*=129)	Control(*N*=71)	OR (95%CI)	*P*	R^2	Power	COPD(*N*=171)	Control(*N*=229)	OR (95%CI)	*P*	R^2	Power
Allele	G	142(55.04)	89(62.68)	-		0.341	0.056	162(47.37)	283(61.79)	-		0.210	0.945
	A	116(44.96)	53(37.32)	1.082(0.576-2.034)	0.806			180(52.63)	175(38.21)	1.774(1.187-2.651)	**0.005**		
Codominant	GG	38(29.46)	28(39.44)	-		0.343	0.247	40(23.39)	94(41.05)	-		0.231	1.000
	GA	66(51.16)	33(46.48)	1.437(0.477-4.323)	0.519			82(47.96)	95(41.48)	3.384(1.712-6.689)	**<0.001**		
	AA	25(19.38)	10(14.08)	1.262(0.195-8.178)	0.807			49(28.65)	40(17.47)	7.900(2.517-24.801)	**<0.001**		
Recessive	GG-GA	104(80.62)	61(85.92)	-		0.342	0.087	122(71.35)	189(82.53)	-		0.206	0.156
	AA	25(19.38)	10(14.08)	0.803(0.225-2.867)	0.735			49(28.65)	40(17.47)	1.785(0.835-3.818)	0.135		
Dominant	GG	38(29.46)	28(39.44)	-		0.343	0.169	40(23.39)	94(41.05)	-		0.223	0.996
	GA-AA	91(70.54)	43(60.56)	1.470(0.500-4.316)	0.484			131(76.61)	135(58.95)	2.940(1.522-5.677)	**0.001**		
Log-additive	-	-	-	1.183(0.471-2.969)	0.720	0.343	0.095	-	-	2.912(1.655-5.123)	**<0.001**	0.230	1.000

Binary logistic regression adjusted by confounding factors: age, sex, BMI and genetic principal components

to COPD-related pathological and inflammatory changes as individuals age [21, 37].

This study demonstrates that serum levels of IL-17 and IL-18 are elevated in patients with stable COPD compared to the control group, with a marked increase observed in those with GOLD stage 3–4 COPD. These findings are consistent with previous studies, all indicating that IL-17 and IL-18 play a role in the pathogenesis of COPD [25, 38]. We also found that genetic variation at the HHIP gene polymorphism rs11100865 correlates with interleukin-18 levels. This finding is consistent with results reported by Yun et al., who identified an association between HHIP gene polymorphism rs1032296 alleles and IL-18 levels [26]. Using single-cell sequencing, their team detailed how genetic variations influence inflammation levels. Their research suggests elevated IL-18 levels may induce increased interferon-gamma production, ultimately leading to $CD8^+$ lymphocyte aggregation and involvement in COPD inflammation. Furthermore, we did not find susceptibility loci associated with COPD to correlate with IL-17 levels. This may suggest that IL-17 is involved in other complex pathways contributing to COPD pathogenesis.

This study has several limitations. First, the sample size for the stratified analysis remains relatively small, requiring the inclusion of more COPD cases to strengthen the conclusions and enhance the robustness of the findings. Additionally, factors that could influence COPD, such as biomass exposure, occupational hazards, and secondhand smoking, were not fully collected. These factors may interact with genetic variations, jointly influencing COPD pathogenesis. Furthermore, the COPD severity in the patients involved in this study was relatively mild, which could impact the relationship between the gene and lung function. Future studies should include more patients with GOLD stage 3–4 COPD. While this study identified an association between HHIP gene polymorphisms and COPD susceptibility, further functional experiments are needed to verify the role of the HHIP gene in COPD.

Conclusion

This study identified a significant association between HHIP gene polymorphisms and COPD susceptibility in a Han Chinese population. The findings also suggested a connection between HHIP gene variants and inflammation in COPD, providing insights into the genetic underpinnings of the disease and potential targets for future research and therapeutic interventions. However, there was no significant associations between this SNP and COPD-related imaging or clinical phenotypes.

Table 9 Association between rs11100865 and COPD phenotypes and serum interleukins

Variables	rs11100865		
	GG(*n* = 78)	GA(*n* = 148)	AA(*n* = 74)
Age(years)	68.33 ± 8.24	68.33 ± 8.24	68.33 ± 8.24
Sex (%)			
Male	56(71.79)	104(70.27)	50(67.57)
Female	22(28.21)	44(29.73)	24(32.43)
BMI(kg/m^2)	24.60 ± 3.58	24.13 ± 3.84	24.85 ± 4.09
Smoking status(%)			
No	40(51.28)	82(55.41)	49(66.22)
Yes	38(48.72)	66(44.59)	25(33.78)
Pack years	13.27 ± 17.68	14.66 ± 21.23	10.53 ± 18.89
PFT			
FEV1%predicted(%)	71.60 ± 18.74	69.08 ± 20.25	67.46 ± 19.80
FEV1/FVC(%)	58.34 ± 9.28	56.55 ± 10.84	57.59 ± 10.41
CAT	9.73 ± 6.82	11.23 ± 7.18	11.35 ± 7.37
Exacerbation frequency	0.95 ± 1.46	1.19 ± 1.52	1.05 ± 1.40
QCT			
%LAA$_{-950}$(%)	5.99 ± 7.74	8.76 ± 10.79	7.67 ± 9.42
3-WA%(%)	67.63 ± 6.70	66.93 ± 6.71	67.65 ± 7.31
Interleukin			
IL-17(pg/ml)	0.69 ± 0.20	0.69 ± 0.23	0.72 ± 0.30
IL-18(pg/ml)	317.13 ± 197.00	414.39 ± 277.54*	411.00 ± 196.72**

Using the GG genotype as the reference, multiple comparisons were performed across genotypes. *, *P*<0.05;**, *P*<0.01. *PFT* pulmonary function test, *CAT* COPD assessment test, *QCT* quantitative computer tomography, *FEV1* forced expiratory volume in 1 s, *FVC* forced vital capacity, *%LAA*$_{-950}$ percentage low attenuation area using a threshold of -950 HU, WA wall area for the segmental bronchus

Table 10 Association between rs11100865 and parameters of pulmonary function test, quantitative computer tomography and serum interleukin level in COPD patients

Variables	rs11100865		
	B	SE	*P*
PFT			
FEV1%pred	-2.244	1.606	0.163
FEV1/FVC	-0.664	0.795	0.405
CAT	0.941	0.574	0.102
Exacerbation frequency	0.080	0.118	0.497
QCT			
%LAA$_{-950}$	1.062	0.767	0.167
3-WA%	-0.043	0.557	0.938
Interleukin			
IL-17	0.016	0.020	0.409
IL-18	49.654	19.627	**0.012**

Adjusted by age, sex, BMI and smoking status. *PFT* pulmonary function test, *QCT* quantitative computer tomography, *FEV1* forced expiratory volume in 1 s, *FVC* forced vital capacity, *%LAA*$_{-950}$ percentage low attenuation area using a threshold of -950 HU, *WA* wall area for the segmental bronchus

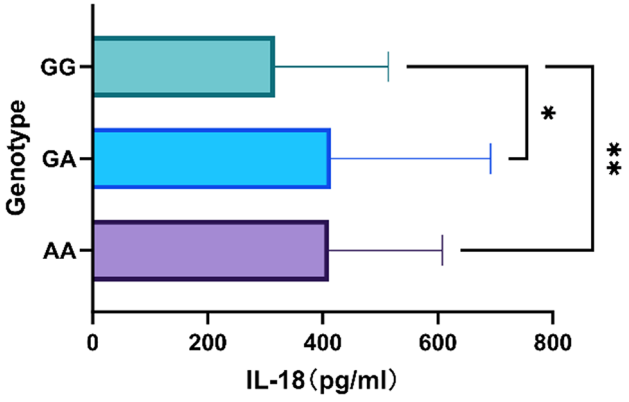

Fig. 4 Serum IL-18 levels among different HHIP polymorphism genotypes in COPD patients. *, $P < 0.05$, **, $P < 0.01$

Table 11 Association between rs11100865 genotype and imaging-based phenotypes and clinical phenotypes in COPD patients

COPD phenotypes	*N*	rs11100865			OR	*P*
		GG	GA	AA		
Imaging-based phenotypes						
Mild type	109	30(27.52)	58(53.21)	21(19.27)	-	
Emphysema-dominant type	83	21(25.30)	40(48.19)	22(26.51)	1.245(0.827–1.874)	0.294
Airway-dominant type	82	23(28.05)	35(42.68)	24(29.27)	1.178(0.783–1.773)	0.431
Mix type	26	4(15.38)	15(57.70)	7(26.92)	1.534(0.824–2.855)	0.177
Clinical phenotypes						
NE	204	60(29.41)	97(47.55)	47(23.04)	-	
EE	43	9(20.93)	22(51.16)	12(27.91)	1.360(0.843–2.193)	0.207
ECB	39	7(17.95)	22(56.41)	10(25.64)	1.357(0.827–2.226)	0.227
PBD	14	2(14.29)	7(50.00)	5(35.71)	1.593(0.694–3.657)	0.272

Multivariable logistic regression adjusted by age, sex, BMI, pack years. *NE* non-exacerbator, *EE* exacerbator with emphysema, *ECB* exacerbator with chronic bronchitis, *PBD* positive bronchodilator test

Abbreviations

COPD	chronic obstructive pulmonary disease
PFT	pulmonary function test
FEV1	forced expiratory volume in 1 s
FVC	forced vital capacity
QCT	quantitative computer tomography
$\%LAA_{-950}$	percentage low attenuation area using a threshold of -950 HU
WA	wall area for the segmental bronchus
CAT	COPD assessment test
GOLD	Global Initiative Chronic Obstructive Lung Disease
SNP	single nucleotide polymorphism
HWE	Hardy-Weinberg Equilibrium
MAF	Minor allele frequency
LD	linkage disequilibrium
NE	non-exacerbator
EE	exacerbator with emphysema
ECB	exacerbator with chronic bronchitis
PBD	positive bronchodilator test
OR	odds ratio
CI	confidence interval

Acknowledgements
We appreciate all participants contributed to this study.

Author contributions
JZ: Patient collection, data analysis, blood sample processing, manuscript writing; DZ: Patient collection, data analysis, blood sample processing; LZ: Blood sample processing; XF, BL, HD: Experimental guidance; YQ, ZJ, FL, SZ: Patient collection, blood sample processing; JZ: Experimental design, supervision, manuscript review.

Funding
Supported by grants from the Science and Technology Key Research Program of Ningxia, China (2021BEG02031) and the National Key Research and Development Program of China (2018YFC1313704).

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethical approval and consent to participate
We promise that our research was performed in accordance with the Declaration of Helsinki and all methods were carried out in accordance with relevant guidelines and regulations. All experimental protocols were approved by the Ethics Committee of General Hospital of Ningxia Medical University, and all participants signed informed consent forms. Ethical approval number: 2020 – 786.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 30 August 2024 / Accepted: 21 October 2024
Published online: 28 October 2024

References

1. Wang C, Xu J, Yang L, Xu Y, Zhang X, Bai C, Kang J, Ran P, Shen H, Wen F, et al. Prevalence and risk factors of chronic obstructive pulmonary disease in China (the China Pulmonary Health [CPH] study): a national cross-sectional study. Lancet. 2018;391:1706–17.
2. GLOBAL STRATEGY FOR PREVENTION, DIAGNOSIS AND MANAGEMENT OF COPD. 2024 Report [https://goldcopd.org/2024-gold-report/].
3. Mei F, Dalmartello M, Bonifazi M, Bertuccio P, Levi F, Boffetta P, Negri E, La Vecchia C, Malvezzi M. Chronic obstructive pulmonary disease (COPD) mortality trends worldwide: an update to 2019. Respirology. 2022;27:941–50.
4. Christenson SA, Smith BM, Bafadhel M, Putcha N. Chronic obstructive pulmonary disease. Lancet. 2022;399:2227–42.
5. Gershon AS, Warner L, Cascagnette P, Victor JC, To T. Lifetime risk of developing chronic obstructive pulmonary disease: a longitudinal population study. Lancet. 2011;378:991–6.
6. Yang IA, Jenkins CR, Salvi SS. Chronic obstructive pulmonary disease in never-smokers: risk factors, pathogenesis, and implications for prevention and treatment. Lancet Respir Med. 2022;10:497–511.
7. Salvi SS, Barnes PJ. Chronic obstructive pulmonary disease in non-smokers. Lancet. 2009;374:733–43.
8. Postma DS, Bush A, van den Berge M. Risk factors and early origins of chronic obstructive pulmonary disease. Lancet. 2015;385:899–909.
9. Murgia N, Gambelunghe A. Occupational COPD-The most under-recognized occupational lung disease? Respirology. 2022;27:399–410.
10. McCloskey SC, Patel BD, Hinchliffe SJ, Reid ED, Wareham NJ, Lomas DA. Siblings of patients with severe chronic obstructive pulmonary disease have a significant risk of airflow obstruction. Am J Respir Crit Care Med. 2001;164:1419–24.
11. Polderman TJ, Benyamin B, de Leeuw CA, Sullivan PF, van Bochoven A, Visscher PM, Posthuma D. Meta-analysis of the heritability of human traits based on fifty years of twin studies. Nat Genet. 2015;47:702–9.
12. Manolio TA. Genomewide association studies and assessment of the risk of disease. N Engl J Med. 2010;363:166–76.
13. Silverman EK. Genetics of COPD. Annu Rev Physiol. 2020;82:413–31.
14. Sakornsakolpat P, Prokopenko D, Lamontagne M, Reeve NF, Guyatt AL, Jackson VE, Shrine N, Qiao D, Bartz TM, Kim DK, et al. Genetic landscape of chronic obstructive pulmonary disease identifies heterogeneous cell-type and phenotype associations. Nat Genet. 2019;51:494–505.
15. Ragland MF, Benway CJ, Lutz SM, Bowler RP, Hecker J, Hokanson JE, Crapo JD, Castaldi PJ, DeMeo DL, Hersh CP, et al. Genetic advances in Chronic Obstructive Pulmonary Disease. Insights from COPDGene. Am J Respir Crit Care Med. 2019;200:677–90.

16. Ortega-Martinez A, Perez-Rubio G, Ramirez-Venegas A, Ramirez-Diaz ME, Cruz-Vicente F, Martinez-Gomez ML, Ramos-Martinez E, Abarca-Rojano E, Falfan-Valencia R. Participation of HHIP Gene variants in COPD susceptibility, lung function, and serum and Sputum Protein Levels in women exposed to Biomass-Burning smoke. Diagnostics (Basel) 2020, 10.
17. Prokopenko D, Sakornsakolpat P, Fier HL, Qiao D, Parker MM, McDonald MN, Manichaikul A, Rich SS, Barr RG, Williams CJ, et al. Whole-genome sequencing in severe chronic obstructive Pulmonary Disease. Am J Respir Cell Mol Biol. 2018;59:614–22.
18. Bartholo TP, Porto LC, Pozzan R, Nascimento A, Da Costa CH. Evaluation of HHIP polymorphisms and their Relationship with Chronic Obstructive Pulmonary Disease Phenotypes. Int J Chron Obstruct Pulmon Dis. 2019;14:2267–72.
19. Werder RB, Zhou X, Cho MH, Wilson AA. Breathing new life into the study of COPD with genes identified from genome-wide association studies. Eur Respir Rev 2024, 33.
20. Lahmar Z, Ahmed E, Fort A, Vachier I, Bourdin A, Bergougnoux A. Hedgehog pathway and its inhibitors in chronic obstructive pulmonary disease (COPD). Pharmacol Ther. 2022;240:108295.
21. Lao T, Jiang Z, Yun J, Qiu W, Guo F, Huang C, Mancini JD, Gupta K, Laucho-Contreras ME, Naing ZZ, et al. Hhip haploinsufficiency sensitizes mice to age-related emphysema. Proc Natl Acad Sci U S A. 2016;113:E4681–4687.
22. Lao T, Glass K, Qiu W, Polverino F, Gupta K, Morrow J, Mancini JD, Vuong L, Perrella MA, Hersh CP, et al. Haploinsufficiency of hedgehog interacting protein causes increased emphysema induced by cigarette smoke through network rewiring. Genome Med. 2015;7:12.
23. Ritzmann F, Lunding LP, Bals R, Wegmann M, Beisswenger C. IL-17 cytokines and Chronic Lung diseases. Cells 2022, 11.
24. Kang MJ, Choi JM, Kim BH, Lee CM, Cho WK, Choe G, Kim DH, Lee CG, Elias JA. IL-18 induces emphysema and airway and vascular remodeling via IFN-gamma, IL-17A, and IL-13. Am J Respir Crit Care Med. 2012;185:1205–17.
25. Kubysheva N, Boldina M, Eliseeva T, Soodaeva S, Klimanov I, Khaletskaya A, Bayrasheva V, Solovyev V, Villa-Vargas LA, Ramirez-Salinas MA et al. Relationship of Serum Levels of IL-17, IL-18, TNF-alpha, and Lung Function Parameters in Patients with COPD, Asthma-COPD Overlap, and Bronchial Asthma. *Mediators Inflamm* 2020, 2020:4652898.
26. Yun JH, Lee C, Liu T, Liu S, Kim EY, Xu S, Curtis JL, Pinello L, Bowler RP, Silverman EK et al. Hedgehog interacting protein-expressing lung fibroblasts suppress lymphocytic inflammation in mice. JCI Insight 2021, 6.
27. Qiu J, Zhang YN, Chen J, Luo T, Yu XH, Wang JC, Tan H, Lu XL, Zhang J. [Prevalence of chronic obstructive pulmonary disease in Ningxia Hui Autonomous Region of China]. Zhonghua Jie He He Hu Xi Za Zhi. 2013;36:265–8.
28. Fedorov A, Beichel R, Kalpathy-Cramer J, Finet J, Fillion-Robin JC, Pujol S, Bauer C, Jennings D, Fennessy F, Sonka M, et al. 3D slicer as an image computing platform for the quantitative Imaging Network. Magn Reson Imaging. 2012;30:1323–41.
29. Lynch DA, Austin JH, Hogg JC, Grenier PA, Kauczor HU, Bankier AA, Barr RG, Colby TV, Galvin JR, Gevenois PA, et al. CT-Definable subtypes of Chronic Obstructive Pulmonary Disease: A Statement of the Fleischner Society. Radiology. 2015;277:192–205.
30. Subramanian DR, Gupta S, Burggraf D, Vom Silberberg SJ, Heimbeck I, Heiss-Neumann MS, Haeussinger K, Newby C, Hargadon B, Raj V, et al. Emphysema- and airway-dominant COPD phenotypes defined by standardised quantitative computed tomography. Eur Respir J. 2016;48:92–103.
31. Calle Rubio M, Casamor R, Miravitlles M. Identification and distribution of COPD phenotypes in clinical practice according to Spanish COPD guidelines: the FENEPOC study. Int J Chron Obstruct Pulmon Dis. 2017;12:2373–83.
32. Chinese Nutrition Society, Obesity P, Control S, Chinese Nutrition Society Clinical, Nutrition S, Chinese Preventive Medicine Association Behavioral Health S, Chinese Preventive Medicine, Association S, Health S. [Expert Consensus on Obesity Prevention and Treatment in China]. Zhonghua Liu Xing Bing Xue Za Zhi. 2022;43:609–26.
33. Boughton AP, Welch RP, Flickinger M, VandeHaar P, Taliun D, Abecasis GR, Boehnke M. LocusZoom.js: interactive and embeddable visualization of genetic association study results. Bioinformatics. 2021;37:3017–8.
34. Zhang Z, Wang J, Zheng Z, Chen X, Zeng X, Zhang Y, Li D, Shu J, Yang K, Lai N, et al. Genetic variants in the Hedgehog Interacting Protein Gene Are Associated with the FEV1/FVC ratio in Southern Han Chinese subjects with chronic obstructive Pulmonary Disease. Biomed Res Int. 2017;2017:2756726.
35. Xu G, Gao X, Zhang S, Wang Y, Ding M, Liu W, Shen J, Sun D. Comparison of the role of HHIP SNPs in susceptibility to chronic obstructive pulmonary disease between Chinese Han and Mongolian populations. Gene. 2017;637:50–6.
36. Xie J, Wu H, Xu Y, Wu X, Liu X, Shang J, Zhao J, Zhao J, Wang J, Dela Cruz CS, et al. Gene susceptibility identification in a longitudinal study confirms new loci in the development of chronic obstructive pulmonary disease and influences lung function decline. Respir Res. 2015;16:49.
37. Yun JH, Morrow J, Owen CA, Qiu W, Glass K, Lao T, Jiang Z, Perrella MA, Silverman EK, Zhou X, Hersh CP. Transcriptomic Analysis of Lung Tissue from cigarette smoke-Induced Emphysema Murine models and Human Chronic Obstructive Pulmonary Disease Show Shared and distinct pathways. Am J Respir Cell Mol Biol. 2017;57:47–58.
38. Ma R, Su H, Jiao K, Liu J. Association between IL-17 and Chronic Obstructive Pulmonary Disease: a systematic review and Meta-analysis. Int J Chron Obstruct Pulmon Dis. 2023;18:1681–90.

Publisher's note