Peer Review File

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Reviewer A

Comment 1: The characteristics of COPD and Asthma patients should be enriched with CAT, ACT, cigarette smoking, exacerbations/year, treatment...

Reply 1: We sincerely thank the Reviewer's comment. We have supplemented the data about cigarette smoking status in COPD and asthma patients, please kindly see in revised **Table 1**.

Because the main aim of this study was the association between these selected SNPs and overall genetic susceptibility to the two diseases. We included the sujective data to reflect the phenotypes of the diseases, including blood Eos, seum TIgE, and lung function measures. These measurements are widely adopted by the majority of genetic studies performed in asthma and COPD.

CAT, ACT, exacerbations/year, and treatment were recalled by the patients and reported to be biased. All these would cause the statistical incorrections in multiple gene diseases, such as asthma and COPD. Thus, we didn't include these data in this cross-sectional study.

Sentences in **Method, Results**, and **Table 1** were also revised. Please kindly see the revised manuscript.

Changes in the text:

1. Method：

Line 140: "We collected the following variables: age, gender, and smoking status."

Line 144-146: "According to the GINA, participants were stratified into three groups based on age: (1) age between 18 and 40 years; (2) age between 40 and 65 years; (3) and age ≥ 65 years."

2. Results:

Line 176-177: "The percentage of total smokers (ex-smokers and current smokers) in COPD patients was 71.3%, higher than those in asthma patients (12.9%)."

Comment 2: The Figure demonstrates the correlation between phenotype gene mutation and COPD /Asthma features should be done.

Reply 2: Thank you so much for your comment. The figure has been supplemented and the revised sentences were shown as follows (Please kindly see Line 231-233, Page 12):

Changes in the text:

Results:

"Overall, FECR2 was associated with blood eosinophils, FEV1/FVC, FEV1% predicted, and serum IgE levels in asthma patients, while FECR2 was associated with blood eosinophils, FEV1/FVC and FEV1% predicted (Figure 1).

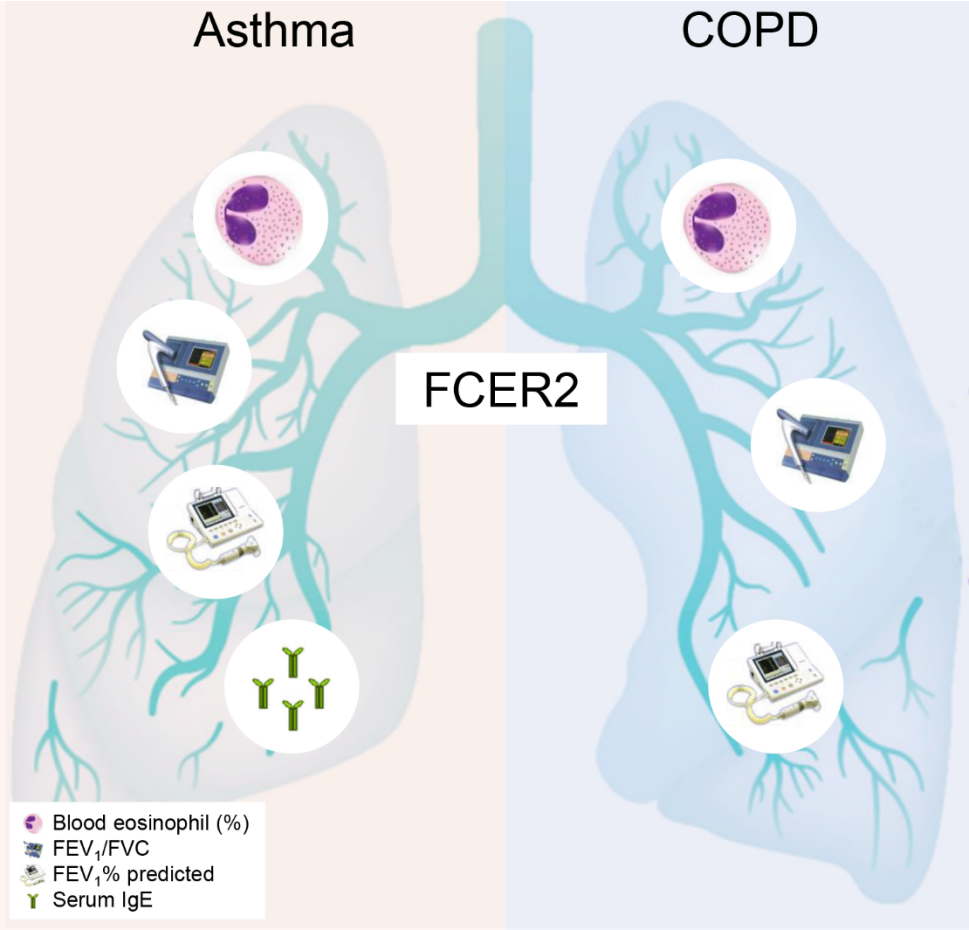

Figure 1. *FCER2* was correlated with COPD/asthma features."

Reviewer B

Comment 1: The authors used GINA and GOLD classification; however, they never mentioned the severity of the disease and the adherence to treatment, how many are severe?

Reply 1: Thank you so much for this comment. The severity of COPD patients based on the GOLD stages has been supplemented in **Table 1**.

Because the main aim of this study was the association between these selected SNPs and overall genetic susceptibility to the two diseases. We included the sujective data to reflect the phenotypes of the diseases, including blood Eos, seum TIgE, and lung function measures. These measurements are widely adopted by the majority of genetic studies performed in asthma and COPD.

Evaluation of asthma based on the control level and treatment adherence were recalled by the patients themselves and reported to be biased. All these would cause the statistical incorrections in multiple gene diseases, such as asthma and COPD. Thus, we didn't include these data in our cross-sectional study.

Comment 2: In table 1 there is no information about the asthma cohort. Despite the fact that it is not critical for the study FEV1 difference with age should be compared with appropriate controls.

Reply 2: Thank you very much for carefully reviewing our paper. We have supplemented this information in **Table 1**:

Changes in the text:

"Table 1. Baseline characteristics of all participants involved in the study

Variables	Controls	COPD		Asthma	
	n=632	n=251	p*	n=597	p*
Age (years)	37.9 ± 11.7	68.9 ± 10.1	$p <$ 0.001	43.9 ± 13.07	$p <$ 0.001
≥18, < 40	390 (61.7)	1 (0.4)	$p <$ 0.001	218 (36.5)	$p <$ 0.001
≥40, < 65	215 (34.0)	88 (35.1)	p =0.769	233 (39.0)	p =0.068
≥65	27 (4.3)	162 (64.5)	$p <$ 0.001	146 (24.5)	$p <$ 0.001
Gender (male, %)	284 (42.5)	212 (84.5)	$p <$ 0.001	214 (35.8)	$p =$ 0.606

Smoking status (%)					
Non-smokers	N/A	72 (28.7)	N/A	520 (87.1)	N/A
Ex-smokers	N/A	53 (21.1)	N/A	24 (4.0)	N/A
Current smokers	N/A	126 (50.2)	N/A	53 (8.9)	N/A
Blood Eso (%)	2.28 ± 1.45	2.24 ± 1.99	p = 0.78	5.72 ± 4.54	p < 0.001
Serum IgE (U/L)	48.33 ± 56.12	132 ± 152.52	p < 0.001	275 ± 385.90	p < 0.001
FEV1/FVC (%)	84.9 ± 8.54	49.64 ± 15.28	p < 0.001	65.59 ± 14.17	p < 0.001
FEV1%pred	102.97 ± 13.40	52.01 ± 17.79	p< 0.001	70.55 ± 23.63	p< 0.001
≥18, < 40	101.6±13.4	65.6	N/A	77.6±22.4	p < 0.001
≥40, < 65	107.3±124	51.6±17.8	p< 0.001	65.2±23.4	p< 0.001
≥65	N/A	52.2±17.7	N/A	65.0±20.7	N/A
The severity of airflow limitation(%)					
GOLD 1	N/A	73 (29.1)	N/A	N/A	N/A
GOLD 2	N/A	99 (39.4)	N/A	N/A	N/A
GOLD 3	N/A	65 (25.9)	N/A	N/A	N/A
GOLD 4	N/A	14 (5.6)	N/A	N/A	N/A

* Relative to controls.

Abbreviation: COPD, chronic obstructive pulmonary disease; Eos: eosinophil; FEV1: forced expiratory volume in one second; FVC: forced vital capacity; pred: predicted; N/A: not applicable; GOLD: The Global Initiative for Chronic Obstructive Lung Disease."

Comment 3: The possibility of some of the patients with COPD had the overlap asthma/COPD syndrome is high, they differ from pure COPD.

Reply 3: Thank you very much for your comment. We agree to the reviewer's point that the patients with asthma/COPD syndrome are different from those with pure COPD. We have realized this impotant point, subjects with ACO syndrome were excluded as

possible when recruitment. The revised sentences in the **Method** section were shown below:

Changes in text:

"Individuals were excluded from the study if they (a) were diagnosed with asthma-COPD overlap syndrome; (b) had a suspected acute inflammatory or infectious disease; (c) had a history of stroke or acute coronary syndrome; (d) experienced venous thromboembolism; (e) received anticoagulant therapy; (f) were diagnosed with cancer within the last 5 years; (g) were pregnant or under hormone-replacement therapy."

Comment 4: Were the controls smokers too? Furthermore, a general view of the treatment is important since patients with overlap syndrome require different treatment as compared to pure COPD. Why table 4 does not include asthmatic patients? What is the data of the whole group without correction?

Reply 4: Thank you very much for your comment. The smoking status of controls was not included because this study didn't aim to check the interactions between genes and environments. Whereas the main object of this comparative study was to see the genetic ground of these two similar airway diseases. However, lung function measurements were normal in controls.

Changes in text:

Table 4 Distributions of haplotypes (frequency > 3%) in the three SNPs in FCER2 between patients and healthy controls

Haplotype (%) *	Controls	COPD				Asthma			
		Patients	OR (95%CI)	p	p**	Patients	OR (95%CI)	p	p**
C-A-C	28.41	27.49	1			26.63	1		
C-G-C	24.92	25.90	1.07 (0.72-1.60)	0.727	0.258	22.61	0.97 (0.71-1.33)	0.839	0.607
C-G-T	9.52	11.16	1.21 (0.71-2.05)	0.477	0.187	11.89	1.33 (0.89-2.00)	0.164	0.224

T-A-C	17.46	15.14	0.90 (0.57-1.42)	0.642	0.248	16.58	1.01 (0.71-1.42)	0.966	0.914
T-G-C	15.40	15.54	1.04 (0.66-1.66)	0.859	0.175	15.24	1.06 (0.74-1.51)	0.764	0.770
T-G-T	3.17	4.78	1.56 (0.72-3.35)	0.256	0.493	6.70	2.25 (1.26-4.01)	**0.005**	**0.006**

* Alleles in each haplotype were appointed in the order of rs28364072, rs2228137, and rs3760687, respectively.
** P value adjusted for age and gender with binary logistic regression.
Abbreviations: COPD, chronic obstructive pulmonary disease.
Bold represents significant value.

Comment 5: The discussion is a little vague if there is no information on the allergic conditions of the individuals, exposure to the antigen, therapy, and adherence.

Reply 5: Thank you so much for your comments.

Because the main aim of this study was the association between these selected SNPs and overall genetic susceptibility to the two diseases. We included the sujective data to reflect the phenotypes of the diseases, including blood Eos, seum TIgE, and lung function measures. These measurements are widely adopted by the majority of genetic studies performed in asthma and COPD.

Because treatment adherence were recalled by the patients themselves and reported to be biased. All these would cause the statistical incorrections in multiple gene diseases, such as asthma and COPD. Thus, we didn't include these data in our cross-sectional study. A brief discussion of this under limitations was added in the revised manuscript as follows (please kindly see Line 313-318 in the revised manuscript):

Changes in text:

"Fourth, because this study was a case-control investigation aiming to compare the similarities and differences in genetic background in two common airway diseases, we didn't include data regarding the allergic status, antigen exposure, therapy intensification, and adherence to therapy, when recruitment. Future studies with

focusing on how the environmental factors interacting with the genes influence the disease expression are needed."

Reviewer C

Comment 1: The abstract, "we detected ten single nucleotide polymorphisms 12 (SNPs) of seven genes (FCER1A, FCGR2A, FCGR2B, CHI3L1, ADRB2, STAT6, and 13 FCER2) using SNaPshot……". These are results and not methods. This should not be included in the method section.

Reply 1*:* We sincerely thank the reviewer for this comment. We have corrected this error, and the revised sentences are shown as follows:

Changes in text:

1. Line 39-40 in the revised manuscript:

"In this case-control study, single nucleotide polymorphisms (SNPs) were genotyped using SNaPshot."

2. Line 44-47 in the revised manuscript:

"We detected ten single nucleotide polymorphisms (SNPs) of seven genes (FCER1A, FCGR2A, FCGR2B, CHI3L1, ADRB2, STAT6, and FCER2) expressed by airway epithelial cells. We detected genotypes and allele distributions in 251 COPD patients, 597 asthma patients, and 632 healthy controls."

Comment 2 : Line 44-45, "Asthma and chronic obstructive pulmonary disease (COPD) are the major health problems worldwide, affecting individuals of all ages (1, 2)". I don't think this is a fair statement since asthma is more common among children and adolescents than adults.

Reply 2: Thank you very much for your careful review of our paper. We have corrected the mistakes and the revised version of the sentence is shown as follows (please kindly see Line 72-73 in the revised manuscript):

Changes in the text:

"Asthma and chronic obstructive pulmonary disease (COPD) are the major health problems worldwide (1, 2)"

Comment 3：Detailed information on how the study participants were recruited is needed. Information on inclusion and exclusion is needed.

Reply 3*:* We sincerely thank the reviewer for this comment. Detailed information about the inclusion criteria was shown in the **Method** section (Line 122-128). Additionally, sentences about the exclusion criteria in the revised version were added as follows (please kindly see Line 134-138 in the revised manuscript):

Changes in the text:

"Individuals were excluded from the study if they (a) were diagnosed with asthma-COPD overlap syndrome; (b) had a suspected acute inflammatory or infectious disease; (c) had a history of stroke or acute coronary syndrome; (d) experienced venous thromboembolism; (e) received anticoagulant therapy; (f) were diagnosed with cancer within the last 5 years; (g) were pregnant or under hormone-replacement therapy."

Comment 4: How and Why the 10 SNPs within 7 genes were selected? Criteria of selection?

Reply 4: Thank you so much for your comment. The airway epithelium is considered the first line of defense and plays an essential role in initiating host defense and controlling immune responses in both asthma and COPD. We focused on the genes expressed by airway epithelium, and 10 SNPs of interest 7 genes, which are mainly in airways, were selected after a comprehensive literature search and conclusion. Revised sentences in the **Method** section are shown as follows (please kindly see Line 149-153 in the revised manuscript)：

Changes in the text:

"After a comprehensive literature search and conclusion, a total of 10 SNPs of interest within 7 genes which are mainly expressed in airway epithelial cells, including FCER1A (rs2427837), FCGR2A (rs1801274), FCGR2B(rs1050501), CHI3L1(rs4950928), ADRB2 (rs1042713 and rs1042714), STAT6 (rs12368672) and FCER2 (rs28364072, rs2228137 and rs3760687), were selected."

Comment 5: Test of Hardy-Weinberg equilibrium?

Reply 5: Thank you so much for your comment. Test of Hardy-Weinberg equilibrium was finished and a new table will be uploaded as **Supplementary** for the manuscript as follows:

1. Line 156-157 in the Method section:

"Hardy-Weinberg equilibrium was tested as shown in Supplementary Table 1 (p cutoff-value = 0.05).

Supplementary

Table 1 Test of Hardy-Weinberg equilibrium of 10 SNPs

SNP name	Chromosome	Ref./Alt.*	HWpval[a]
rs2427837	1	G/A	1
rs1801274	1	T/C	0.7042
rs1050501	1	T/C	<0.0001
rs4950928	1	C/G	0.1422
rs1042713	5	A/G	0.1555
rs1042714	5	C/G	0.5271
rs12368672	12	C/G	0.09883
rs28364072	19	A/G	0.1768
rs2228137	19	C/T	0.3513
rs3760687	19	C/T	0.04289

* Ref./Alt.: Reference/Alternative.

[a] HWpval: Hardy-Weinberg equilibrium *p*-value."

Comment 6: Why SNK-q test was used?

Reply 6*:* Thank you so much for your comments. SNK-q is a post hoc test for differences in means and has been used in pairwise comparison of mean between multiple groups [1][2]. In Table 3, statistical analysis was performed by one-way ANOVA followed by the SNK-q post hoc test, because each SNP contains 3 genotypes.

[1] Zhang J, Yi Y, Wang C, et al. Effect of Acid-Etching Duration on the Adhesive Performance of Printed Polyetheretherketone to Veneering Resin. Polymers (Basel). 2021 Oct 13;13(20):3509.

[2] Underwood, A.J. 1997. Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variance. Cambridge University Press, Cambridge.

Comment 7: Adjusting for multiple comparison?

Reply 7: Thank you so much for your comment. To analyze the distributions of haplotypes (Table 4) and the association of phenotype with haplotypes in COPD and

asthma (Table 5), multiple factors ANOVA was used with adjusting for age and sex (See Line 160-163 in the revised manuscript).

Comment 8: How haplotypes were derived?

Reply 8: Thank you so much for your comment. As we mentioned in the **Method** section (Line 149-153), briefly, all the possible combinations of haplotypes using three SNPs (rs28364072, rs2228137, and rs3760687) of the FCER2 gene were taken into consideration, and haplotypes with frequencies ≥ 3% were used for "haplotype-disease analysis".

Comment 9: Possible population stratification?

Reply 9: Thank you so much for your comments. As we showed in **Table 1**, the sample size was small in each group according to stratification with age, which may cause potential bias in subgroup analysis. Therefore, subgroup analysis was not performed in the following analysis.

Comment 10: The manuscript is not easy to follow and the writing needs to be significantly improved. A lot of information is missing.

Reply 10: We sincerely thank the reviewer for this comment. Those tables with missing information were revised. We have invited Dr. Youming Zhang, an established Respiratory Genetics, of Imperial College London to critically read this manuscript. Some revised sentences were as follows:

Changes in the text:

Line in the revised version	Original version	Revised version
62-65	Our data suggest that *FCER2* SNP rs28364072 might associate with the genetic predisposition to asthma and COPD, while *FCER2* haplotypes were associated with lung function and	Our data suggest that a SNP rs28364072 in FCER2 gene might associate with the genetic predisposition to asthma and COPD, while FCER2 haplotypes were associated with pulmonary function

	eosinophils counts in both diseases.	measurements and eosinophils counts in both diseases.
76-80	Despite the differences in pathogenic factors and endotypes (4, 5), the two diseases share many phenotypic similarities. Typically, chronic inflammation in asthma is dominated by CD4+ lymphocytes and eosinophils, while CD8+ lymphocytes, macrophages, and neutrophils are elevated in COPD (6).	Despite the differences in pathogenic factors and endotypes (4, 5), the two diseases showed many phenotypic similarities. Typically, chronic inflammation in asthmatic airways is featured by infiltration of CD4+ lymphocytes and eosinophils, while CD8+ lymphocytes, macrophages, and neutrophils are elevated in COPD airways (6).
84-88	In 1961, Orie and colleagues proposed the “Dutch hypothesis” stipulating that asthma and COPD are two different manifestations of one disease entity called "chronic non-specific lung disease" (CNSLD), which resulted from the interactions between genetic predisposition and exposure to similar environmental factors (10, 11).	“Dutch hypothesis” was proposed by Orie and colleague that asthma and COPD are two different manifestations of one disease entity called "chronic non-specific lung disease" (CNSLD), which resulted from the interactions between genetic predisposition and exposure to similar environmental factors, further leading to the clinical presentations of the disease (10, 11).
91-92	In recent years, the Dutch hypothesis was revisited and supported by growing evidence showing the commonalities	In recent years, growing evidence supported the Dutch hypothesis by showing the commonalities between asthma and COPD (12-14).

	between asthma and COPD (12-14).	
187-191	Genotype analyses showed that, of 10 variants within 7 genes, only one SNP rs28364072 (*FCER2*) was significantly different between COPD patients and controls (p = 0.009), while 3 SNPs [rs1801274 (*FCGR2A*), rs12368672 (*STAT6*), rs2228137 (*FCER2*)] differed significantly between asthma and controls (p = 0.004, 0.007 and 0.01, respectively).	Genotype analyses showed that, of 10 variants within 7 genes, only one SNP rs28364072 (*FCER2*) was significantly different between COPD patients and controls (p = 0.009), while 3 SNPs (rs1801274 in *FCGR2A*, rs12368672 in *STAT6*, rs2228137 in *FCER2*) differed significantly between asthma and controls (p = 0.004, 0.007 and 0.01, respectively).
210-211	To facilitate the identification of the combinatorial effects of these three polymorphisms of the *FCER2* gene on the risk of COPD or asthma, we performed haplotype analysis.	To identify the combination effects of these three polymorphisms of the *FCER2* gene on the risk of COPD or asthma, we performed haplotype analysis.
240-246	Haplotype analyses suggested that FCER2 haplotypes C-A-C and C-G-T were associated with blood eosinophils in both diseases, while a haplotype T-A-C correlated with FEV1% predicted in COPD and FEV1/FVC ration in asthma, haplotype T-G-C correlated with FEV1/FVC ratio in COPD and FEV1% predicted in asthma. Our results suggested	Haplotype analyses suggested that *FCER2* haplotypes C-A-C and C-G-T were associated with blood eosinophils in both diseases, while a haplotype T-A-C correlated with FEV_1% predicted in COPD and FEV_1/FVC ratio in asthma, haplotype T-G-C correlated with FEV_1/FVC ratio in COPD and FEV1% predicted in asthma. Our results suggested that

	that *FCER2* polymorphisms may genetically play a role in both asthma and COPD mechanisms, supporting the Dutch hypothesis that asthma and COPD share common genetic backgrounds.	*FCER2* polymorphisms may genetically play a role in both overall asthma and COPD susceptibility, partly supporting the Dutch hypothesis that asthma and COPD share common genetic backgrounds.
248-250	CD23, a type II transmembrane glycoprotein, was expressed in many cells, such as T lymphocytes, B lymphocytes, eosinophils, neutrophils, and other cell types (32). CD23 interacts with IgE with low affinity, playing a dual role in regulating IgE synthesis in activated B lymphocytes and facilitating allergen-specific activation in T lymphocytes (33, 34). Additionally, CD23 can be released as soluble CD23 (sCD23) proteins, which also showed a dual function in IgE synthesis by its oligomerization state. Monomeric sCD23 suppressed IgE synthesis in activated B cells, while trimeric sCD23 molecules enhance IgE production (32).	CD23 interacts with IgE with low affinity, playing a dual role in regulating IgE synthesis in activated B lymphocytes and facilitating allergen-specific activation in T lymphocytes (33, 34).