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The association study of *Apolipoprotein E* polymorphisms and chronic obstructive pulmonary disease in the Chinese population

A case-control study

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Abstract

Chronic obstructive pulmonary disease (COPD) patients have increased cardiovascular morbidity and mortality. *Apolipoprotein E (ApoE)* is involved in chronic inflammation which is the common characteristic of emphysema and cardiovascular disease. *ApoE* polymorphisms are associated with cardiovascular disorders and atherosclerosis. There is no report about the association between *ApoE* polymorphism and COPD.

A total of 480 COPD patients and 322 controls who were unrelated Chinese Han individuals were enrolled. Rs429358 and rs7412 were genotyped and the associations between *ApoE* polymorphisms and COPD risk were analyzed by logistic regression analysis. Online software SHEsis were applied to perform linkage disequilibrium (LD) and haplotypes analysis. The interactions of *ApoE* and environmental factor on COPD susceptibility was analyzed by software MDR3.0.2.

No significant association was found between rs429358, rs7412 and COPD under different genetic models. Rs429358 and smoking formed the best model in the MDR analysis. The frequency of E2/E2 phenotype was the lowest in 2 groups. E3/E3 was the most common phenotype, accounting for 69.8% of COPD patients and 68.9% of controls. No statistically difference was identified between the cases and controls under different phenotypes.

This was the first genetic association study between *ApoE* and COPD. No positive association was found in the Chinese Han population. Rs429358 and smoking status existed significant interaction, indicating that both of *ApoE* and smoking may be involved in the development of COPD disease.

Abbreviations: ApoE = Apolipoprotein E, BMI = body mass index, CHB = Han Chinese in Beijing, China, COPD = chronic obstructive pulmonary disease, CVD = cardiovascular disease, GOLD = Global Initiative for Chronic Obstructive Lung Disease, HWE = Hardy-Weinberg equilibrium, LD = inkage disequilibrium, LDL = low-density lipoprotein, MAF = minor allele frequency, MDR = multifactor dimensionality reduction, MMP = matrix metalloproteinase, ORs = odds ratios, SNPs = single nucleotide polymorphisms.

Keywords: *ApoE*, association study, chronic obstructive pulmonary disease, polymorphism

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All protocols for this study were reviewed and approved by the Ethics Committee of the Faculty of Medicine, Sichuan Academy of Medical Sciences, Sichuan Provincial People's Hospital. Written informed consent was obtained from all the study participants.

The authors declare that there is no conflict of interest.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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1. Introduction

As a public health problem, chronic obstructive pulmonary disease (COPD) is characterized by persistent respiratory symptoms, progressive declined lung function, emphysema and airflow limitation that is due to an abnormal inflammatory response of airway and/or alveolar to noxious particles or gases.[1] COPD is the fourth leading cause of death in the world and brings heavy burden to the patients. In China, the overall prevalence of spirometry-defined COPD was high to 8.6% and accounting for 99.9 million people with COPD in 2015.[2]

COPD is a systemic disease and most patients suffer from COPD are prone to have concomitant chronic diseases including cardiovascular disease (CVD), which increase its morbidity and mortality.[1] Cigarette smoking contributes to increased and chronic systemic inflammation and may cause both COPD and atherosclerosis development.[1,3,4]*Apolipoprotein E (ApoE)* is an important component of all lipoproteins and its polymorphisms are associated with cardiovascular disorders and atherosclerosis, especially in allele ε4 carriers.[5–8]*ApoE* is involved in modulation of inflammation and oxidation,[9,10] by influencing macrophage proliferation which also contributes to inflammation in emphysema.[11] However, there is no report about the association between *ApoE* polymorphism and COPD. This study firstly attempt to investigate whether *ApoE* polymorphism may be linked to COPD susceptibility in the Chinese population.

2. Material and methods

2.1. Study population

Totally 480 COPD patients and 322 controls, unrelated Chinese Han individuals, were enrolled from Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital from June 2018 to March 2020 in this case and control study. The diagnosis of COPD was according to the criteria of the Global Initiative for Chronic Obstructive Lung Disease (GOLD 2018). The controls were healthy volunteers collected from the physical examination center and in-patients only diagnosed with prolapse of lumbar intervertebral disc from the Sichuan Provincial People's Hospital. The Exclusion criteria were uncontrolled hypertension and diabetes, cancer, CVD, atherosclerosis, inflammation and any organ failure. This study was approved by the Ethics Committee of the Faculty of Medicine, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital. Waive of informed consent was granted by the Ethics Committee as the blood samples were used samples collected from clinical laboratory, they were initially for blood routine examinations.

2.2. Genotype detection

Rs429358 and rs7412 in *ApoE* were selected after literature review. The minor allele frequency (MAF) of the 2 single nucleotide polymorphisms (SNPs) were 0.102 and 0.107 separately with r^2 <0.8 in Han Chinese in Beijing, China (CHB) in 1000genomes (http://grch37.ensembl.org/Homo_sapiens/Info/Index). Venous blood samples were extracted from all participants and genomic DNA was extracted from whole blood samples using the whole blood genomic DNA extraction and purification kit (Tiangen, China, Cat. No.: DP348-03). SNPs were genotyped by the Genesky Bio-Tech Co., Ltd (http://geneskybiotech.com/index.html) using SNPscan technology.[12] For quality control, 5% of random samples were repeatedly genotyped with a concordance rate of 100%.

2.3. Statistical analysis

Hardy-Weinberg equilibrium (HWE) among the controls was tested by the Chi-Squared test. All analyses were performed using the Statistical Package for the Social Sciences (SPSS, SPSS Inc., Chicago, IL, USA), version 17.0. Statistical significance was defined as $P<.05$. All data were expressed as the mean ± standard deviation. Continuous and categorical variables were analyzed using the Student *t*-test and Pearson's Chi-Squared test, respectively. Phenotype distributions were analyzed by Pearson's Chi-Squared test. Genotype distributions under different genetic models were examined by logistic regression analysis, adjusting for the confounders including age, sex, body mass index (BMI) and smoking history. Results were reported as odds ratios (ORs) with 95% confidence intervals (95% CI). Linkage disequilibrium (LD) was calculated using the SHEsis software.[13] The multifactor dimensionality reduction software (MDR3.0.2)[14] was used to identify the interactions of *ApoE* rs429358, rs7412 and environmental factor on COPD susceptibility.

3. Results

3.1. Subject demographics

Subject demographics of the 480 cases and 322 controls were presented in Table 1. The case group consisted of 252 males and 229 females while 158 males and 165 females were in the control group. The average age was 74.70 ± 10.02 and 72.85 ± 10.03 years in COPD and control group, separately. There were no statistical differences in sex, age and BMI.

3.2. ApoE SNPs association study

Firstly, we detected the association between rs429358, rs7412 and COPD susceptibility, separately. No deviation from Hardy–Weinberg equilibrium was observed and the association results are presented in Table 2. After adjusting for confounding factors including age, sex, BMI and smoking history, no significant association was found between rs429358, rs7412 and COPD under different genetic models. The LD between the above SNPs was 0.426 (Fig. 1). There was no significant difference observed in the haplotype analysis between 2 groups ($P>.05$) (Table 3).

Secondly, we detected the association between *ApoE* protein phenotypes and COPD. There are 6 protein phenotypes in *ApoE*, E2/2, E3/3, E4/4, E2/3, E2/4, and E3/4, according to different genotypes in rs429358 and rs7412 in 1 subject. *ApoE* protein genotype and frequencies in cases and controls are presented in

Table 1

Characteristics of cases and controls.

	Case	Control	*P* value
Male	251	158	.33
Female	229	164	
Age (Mean ± SD)	74.70 ± 10.02	72.85 ± 10.03	.62
BMI	23.84 ± 3.03	22.59 ± 5.07	.45
Smoker + Ex-smoker (%)	59	13	$2.01\text{x}10^{-16}$
Non-smoker (%)	41	87	

BMI = body mass index, SD = standard deviation.

Table 2

***ApoE* rs429358 and rs7412 polymorphisms in cases and controls.**

SNPs	Cases N (%)	Controls N (%)	Genetic model[b]	OR (95%CI)	P[a]	Genetic model[b]	OR (95%CI)	P[a]
rs429358 (T > C)	480	322						
TT	400 (83.3)	267 (82.9)	Add	1.285 (0.870-1.898)	.21	Hom	1.101 (0.945-1.284)	.22
TC	77 (16.1)	53 (16.5)	Dom	1.330 (0.867-2.041)	19	Het	1.246 (0.855-1.817)	.25
CC	3 (0.6)	2 (0.6)	Rec	1.349 (0.489-3.721)	.56	All	0.975 (0.685–1.387)	.89
rs7412 (C > T)	480	322						
CC	404 (84.2)	268 (83.2)	Add	1.205 (0.826–1.759)	.33	Hom	1.056 (0.901–1.238)	.50
TC	72 (15.0)	49 (15.2)	Dom	1.216 (0.788–1.874)	.38	Het	1.179 (0.833–1.668)	.35
TT	4 (0.8)	5 (1.6)	Rec	1.493 (0.591–3.771)	0.40	All	1.109 (0.780–1.578)	.56

[a] Adjusted for sex, age, BMI and smoking history with logistic regression.

[b] All, allelic model; Add = additive model; Dom = dominant model; Rec = recessive model; Hom = homozygote model; Het = heterozygote model.

Table 4. E3/E3 was the most common 1, accounting for 69.8% of COPD patients and 68.9% of controls. The frequency of E2/E2 was the lowest in 2 groups, and E2/E3 was detected in 12.7% of COPD patients and 12.4% of controls. E2/E4, E3/E4, and E4/E4 together accounted for 16.7% of COPD patients and 17.1% of controls. In addition, subjects were divided into 2 models consisting of 3 groups based on their phenotypes: *E2 (E2/E2, E2/E3, E2/E4), *E3 (E3/E3), and *E4 (E3/E4, E4/E4). Another model was also considered, which was *E2 (E2/E2, E2/E3), *E3 (E3/E3), and *E4 (E2/E4,E3/E4, E4/E4) (14). Unfortunately, there was no statistically difference between the cases and controls in the above 2 models (Table 5).

3.3. MDR analysis

ApoE rs429358, rs7412 and smoking status on COPD susceptibility in the Chinese population were analyzed by multifactor dimensionality reduction (MDR) analysis. Rs429358 and smoking formed the best model, which was statistically significant after permutation for 1000 times ($P < .05$), and it indicated that there was significant interaction between *ApoE* rs429358 and smoking (Table 6).

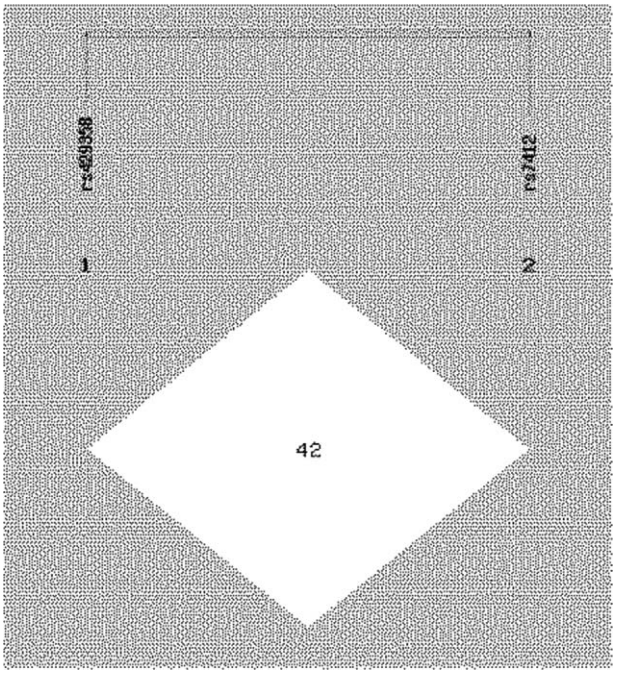

Figure 1. The Analysis of linkage disequilibrium of 2 SNPs, rs429358 and rs7412.

4. Discussion

ApoE is a 34 kDa circulating glycoprotein mainly synthesized in the liver and composed of 299 amino acids, which is essential in the catabolism of triglycerides and cholesterol.[15] The *Apo*E, located at chromosome 19q13.2, is polymorphic with the 3 most common alleles, ε2 (rs7412-allele T, rs429358- allele T), ε3 (rs7412- allele C, rs429358- allele T), and ε4 (rs7412- allele C, rs429358- allele C), which are associated with the risk of cardiovascular and neurological health.[16] The variants occur at 2 amino acids, positions 112 and 158, contributed to3 corresponding protein isoforms, *ApoE*2, E3, and E4, and 6 phenotypes, E2/2, E3/3, E4/4, E2/3, E2/4, and E3/4. In the human population, the most common allele and phenotype are ε3 and E3/3, respectively.[17] Allele ε2 carriers are more favorable for a low risk of cardiovascular disease (w), while allele ε4 is associated with increased carotid intima-media thickness[18] and higher low-density lipoprotein (LDL) cholesterol, as a strong risk factor for atherosclerosis and CVD.[19] ApoE can be expressed by the lungs, especially by $CD68^+$ alveolar macrophages in the airway.[20] It has many functions, including inflammatory effects on macrophages transformation, lymphocytes and T helper cells proliferation[21] and normal lung health modulation.[22]

COPD patients have more prominent to be accompanied by CVD, especially in smokers.[1] And atherosclerosis, the basis of CVD, is one of the leading causes of mortality in COPD.[23] Therefore, there is a mechanism link between CVD, atherosclerosis and COPD.[4] The common characteristic of emphysema and atherosclerosis is chronic and systemic inflammation which including the recruitment and infiltration of macrophages and lymphocytes into the airway and vessel wall, respectively.[24,25] Meanwhile, cigarette smoking and oxidative stress may induce both of COPD and atherosclerosis, too.[3] Cigarette smoke exposure contributes to increased inflammation,[26] protease/anti-protease imbalance,[27] apoptosis,[24] oxidative stress[28] and matrix metalloproteinases (MMPs),[29] which may cause the alveolar destruction even emphysema, while it can raise the levels of oxidized LDL cholesterol and damages vessel endothelium to cause atherosclerosis development.[30]

Apolipoprotein E-deficient ($ApoE^{-/-}$) mice is considered to be the best suitable animal model to study the CVD with emphysema.[31,32] Previous study observed enlarged airspace, increased macrophages and lymphocytes, elevated matrix metal-

Table 3

Haplotype analysis of *ApoE* in cases and controls.

Haplotype	Cases N (%)	ControlsN (%)	x^2	P^a	OR (95%CI)
C C	79.51 (0.083)	53.54 (0.083)	0.001		0.94 [0.77–1.15]
C T	3.49 (0.004)	3.46 (0.005)	-		1.23 [0.97–1.56]
T C	800.49 (0.834)	531.46 (0.825)	0.142		0.84 [0.65–1.11]
T T	76.51 (0.080)	55.54 (0.086)	0.228		
Global test	960	644	0.233	0.89	

For each haplotype, alleles were arranged in order of rs429358 - rs7412.
[a] Adjusted for sex, age, BMI and smoking history with logistic regression.
[*] The lowest frequency threshold (LFT) <0.03 were pooled in this part.

Table 4

The distribution of *ApoE* protein phenotypes in cases and controls.

N (%)	E2/ E2	E2/ E3	E3/ E3	E3/ E4	E4/ E4	E2/ E4
Cases	4 (0.8)	61 (12.7)	335 (69.8)	66 (13.8)	3 (0.6)	11 (2.3)
Controls	5 (1.6)	40 (12.4)	222 (68.9)	44 (13.7)	2 (0.6)	9 (2.8)

Table 5

Association between *ApoE* protein phenotypes and COPD.

Groups	Case N (%)	Controls N (%)	P^1	Case N (%)	Controls N (%)	P^2
*E2	76 (15.8)	54 (16.8)	.94	65 (13.5)	45 (14.0)	.97
*E3	335 (69.8)	222 (68.9)		335 (69.8)	222 (68.9)	
*E4	69 (14.4)	46 (14.3)		80 (16.7)	55 (17.1)	

P^1, *P* value of model 1: *E2 includes E2/E2, E2/E3 and E2/E4 genotypes; *E3 includes E3/E3 genotypes; *E4 includes E3/E4 and E4/E4 genotypes.
P^2, *P* value of model 2: *E2 includes E2/E2 and E2/E3 genotypes; *E3 includes E3/E3 genotypes; *E4 includes E2/E4, E3/E4 and E4/E4 genotypes.

Table 6

The MDR analysis of the interaction of *ApoE* and Smoking.

Best model[a]	Testing balanced accuracy[b]	Cross-validation consistency[c]	*P* value[*]
rs429358- smoking	0.7321	10/10	0.00

[a] the best combination of variables for each order model.
[b] the ratio of correct classifications to the total classifications in the testing set.
[c] denominator means 10-fold cross validation, numerator means number of times a combination model was selected as the best model in 10-fold cross validation.
[*] *P* value for testing balanced accuracy using 1000-fold permutation test. $P < .05$ was significant.

loproteinase-9 (MMP-9) activity and MMP-12 expression in the lungs of *Apoe*$^{-/-}$mice on a Western-type diet.[11] The authors considered that abnormal cholesterol efflux resulted in emphysema development by the TLR pathway activation in the *Apoe*$^{-/-}$mice fed with an atherogenic diet, even in the absence of smoking.[11]

Therefore, we thought that *ApoE* may be involved in the COPD development. Although many reports about the association of *ApoE* polymorphisms and CVD or atherosclerosis, there is no report referring to the relationship between *ApoE* polymorphism and COPD. In this study, we excluded the patients with CVD or atherosclerosis in order to avoid confounding factor. It was the firstly attempt to identify the genetic association between rs429358 and rs7412 of *ApoE* and the Chinese Han COPD population. Unfortunately, there was no positive result in the association analysis and it may need further study with more samples or in different ethics. However, we found the best model consist of rs429358 and smoking in the MDR analysis, which meant that there was an interaction between *ApoE* and smoking status on COPD susceptibility. It was consistent with the previous reports. The limitation of this study was that it only aimed to the Chinese Han population and further study is needed in the future.

5. Conclusions

In this case and control study, we failed to find the positive association of *ApoE* polymorphisms and COPD in the Chinese Han population and it may need further study with larger samples and in different ethics. However, rs429358 and smoking

status was found to be existed significant interaction, which meant both of *ApoE* and smoking status may be involved in the development of COPD disease.

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Author contributions

Guo Chen and Yuan Zhang: Study design, manuscript writing and revising; Lu Xiang, Meng Xiao, Jian-shu Guo: Sample collecting and data recording; Yu-tian Zhou: Experiment implementation; Xiao-hui Li: Experiment implementation and data interpretation. Jing-wei Zhang: Sample collecting. All authors have reviewed and approved the manuscripts.

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