Frontiers in Bioscience Landmark

*Front. Biosci. (Landmark Ed)* **2023**; 28(9): 215
https://doi.org/10.31083/j.fbl2809215

*Original Research*

# *EPO* rs1617640 A>C is a Protective Factor for Chronic Obstructive Pulmonary Disease: A Case Control Study

Yunchao Wang[1,†], Zhi Li[2,†], Xiaoyi Zhang[1,†], Ao Lin[2], Cuiyi Chen[3], Xiaobin Zeng[2], Yujie Pan[2], Chun Mao[2], Chenli Xie[4], Dongsheng Huang[5], Yibin Deng[6], Xuhui Zhang[7], Jiachun Lu[2,*], Xinhua Wang[1,*]

[1]Institute of Basic Medicine, Institute of Public Health, Gansu University of Chinese Medicine, 730000 Lanzhou, Gansu, China
[2]The State Key Lab of Respiratory Disease, The First Affiliated Hospital, Institute of Public Health, Guangzhou Medical University, 511436 Guangzhou, Guangdong, China
[3]Department of Respiratory Medicine, SSL Central Hospital of Dongguan City, 523326 Dongguan, Guangdong, China
[4]Department of Respiratory and Critical Care Medicine, Dongguan Binwan Central Hospital, 523903 Dongguan, Guangdong, China
[5]Department of Respiratory and Critical Care Medicine, Shenzhen Longhua District Central Hospital, 518110 Shenzhen, Guangdong, China
[6]Centre for Medical Laboratory Science, the Affiliated Hospital of Youjiang Medical University for Nationalities, 533099 Baise, Guangxi, China
[7]Department of Respiratory Medicine, Affiliated Hospital of Gansu University of Chinese Medicine, 730000 Lanzhou, Gansu, China
*Correspondence: wxh@gszy.edu.cn (Xinhua Wang); jcLu@gzhmu.edu.cn (Jiachun Lu)
[†]These authors contributed equally.

Academic Editor: Hongwei Yao
Submitted: 8 February 2023 Revised: 29 March 2023 Accepted: 11 April 2023 Published: 24 September 2023

**Abstract**

**Background**: The occurrence and development of chronic obstructive pulmonary disease (COPD) are regulated by environmental and genetic factors. In hypoxia, Erythropoietin (*EPO*) satisfies the body's need for oxygen by promoting the production of red blood cells. Hypoxia was proven to be a common physiological condition in COPD progression and associated with many complications. Some studies have found that *EPO* is involved in the development of COPD. But the mechanism has not been fully proven. **Methods**: We conducted a case-control study enrolled 1095 COPD patients and 1144 healthy controls in Guangdong Province to evaluate the association between *EPO* polymorphisms (rs1617640 A>C, rs507392 A>G, rs564449 G>T) and COPD susceptibility. 872 participants from southern Gansu Province were recruited to verify the effect of *EPO* polymorphisms on lung function. **Results**: *EPO* rs1617640 C allele reduced COPD susceptibility in southern Chinese significantly (AC vs. AA: adjusted Odds ratio (OR) = 0.805, 95% CI = 0.669–0.969; AC+CC vs. AA: adjusted OR = 0.822, 95% CI = 0.689–0.980). However, there was no association between rs507392 A>G and rs564449 G>T polymorphisms and COPD susceptibility ($p > 0.05$). We further observed that the rs1617640 C allele was associated with higher $FEV_1$ and FVC in Guangdong and Gansu populations significantly (both $p < 0.05$). In brief, the level of $FEV_1$ and FVC increased with the C allele number. We modeled the relative risk for men and women, in which the population-attributable risks chances were 0.449 (0.258–0.641) and 0.262 (0.128–0.396) respectively. In this model, smoking status, coal as fuels, education level, and rs1617640 A>C were finally retained for males, while smoking status, biomass as fuels, and1617640 A>C were retained for females. In the end, using the method developed by Gail and Bruzzi, we fitted a 10-year absolute risk model for southern Chinese with different individual relative risks, which was presented as a table. **Conclusions**: In conclusion, this study found that *EPO* rs1617640 A>C polymorphism is associated with COPD susceptibility in southern Chinese, and the C allele was associated with better lung function. In addition, it could also be considered a genetic marker associated with environmental factors to predict the absolute 10-year risk of COPD in southern Chinese.

**Keywords:** COPD; *EPO*; polymorphism; susceptibility; absolute risk

## 1. Introduction

Chronic obstructive pulmonary disease (COPD) is the most common pulmonary disease characterized by small airway lesions and chronic respiratory symptoms and continuous airflow restriction [1]. Its common pathological features include bronchiolitis and destruction of lung parenchyma (emphysema) [1]. COPD usually progresses slowly, often leading to the neglect of early treatment and prevention [2]. Thus, when the disease is brought to the patients' attention, it has usually progressed to the third or fourth stage and may be accompanied by complications [3]. It is estimated that 4.5 million deaths yearly will be attributed to COPD by 2030 [4], and it is on track to become the third leading cause of death worldwide [5]. Due to the prolonged course and the high prevalence of the disease, COPD will also place a heavy burden on the global healthcare economy.

COPD is considered to be a process of chronic hypoxia and chronic systemic inflammation with complex pathogenesis. It is reported that environmental and genetic factors affect the risk of COPD jointly [6,7]. It is well known

that smoking is a recognized risk factor for COPD, still, this proportion in long-term smokers is only 10%–20%, and there is a considerable proportion of non-smoker people diagnosed with COPD, which indicates the importance of genetic factors in COPD [3]. With the development of genome-wide association analysis (GWAS), Pillai and colleagues first found that the single nucleotide polymorphisms (SNPs) of CHRNA3/CHRNA5/IREB2 located on chromosome 15q25 were associated with COPD significantly [8]. Since then, GWAS at home and abroad have reported lots of genes influencing susceptibility to COPD successively [9–11]. However, there is still largely unknown in the genetic etiology of COPD.

Erythropoietin (*EPO*) stimulated by Hypoxia is a recognized hematopoietic cytokine that can control the oxygen-carrying capacity of blood and enhance ventilatory function under hypoxic conditions [12–14]. *EPO* was observed to play anti-inflammatory, anti-apoptosis, and antioxidant/fibrosis roles in the lung by interacting with inflammation, apoptosis, oxidative stress, or fibrosis-related pathways [15–20]. Some studies indicated that the expression of *EPO* was correlated with the severity of COPD positively, while correlated with the pulmonary function index $FEV_1$ negatively [21–23]. In addition, it has been pointed out that an increase in *EPO* levels can be observed in exacerbations of COPD [24,25]. Based on the evidence above, we hypothesized that *EPO* may be involved in the occurrence and development of COPD.

Therefore, we conducted a case-control study enrolled 1095 COPD patients and 1144 healthy controls in several hospitals in Guangdong to evaluate the association between *EPO* polymorphisms (rs1617640 A>C, rs507392 A>G, rs564449 G>T) and COPD susceptibility. 872 participants were recruited from a prevalence study performed in Gansu Province to verify the effect of *EPO* polymorphisms on lung function. Using the method developed by Gail and Bruzzi, we fitted a 10-year absolute risk model for southern Chinese.

## 2. Materials and Methods

### *2.1 Study Population*

We had performed a case-control study among Han Chinese that included 1095 COPD patients and 1144 healthy controls in several hospitals in Guangdong Province (Songshan Lake Central Hospital of Dongguan, the Dongguan Binhaiwan Central Hospital, and the Shenzhen Longhua District Central Hospital) during 2015 to 2019, to evaluate the association between *EPO* polymorphisms (rs1617640 A>C, rs507392 A>G, rs564449 G>T) and COPD susceptibility. In addition, we used the electronic medical records of the above institutions from 2016 to 2020 to conduct a clinical cohort study to calculate the incidence of COPD.

872 participants, from a prevalence study in Zhuoni County of Gannan Tibetan Autonomous Prefecture of Gansu Province in 2019, was recruited to verify the effect of EPO polymorphisms on lung function.

All subjects from case-control study and prevalence study received the lung function test and the respiratory health questionnaire (including their demographic characteristics and environmental exposure factors, like education level and smoking status) for collecting their demographic characteristics and environmental exposure information [26]. We also obtained their 5 mL peripheral blood for genotyping after they signed informed consent.

This study was reviewed by the Ethics Committee of Guangzhou Medical University, Xi'an Jiaotong University and Gansu University of Chinese Medicine.

### *2.2 COPD's Diagnostic Criteria and Lung Function Test*

According to the Global Initiative for Chronic obstructive pulmonary disease 2019 [27], we diagnosed COPD if participants experienced respiratory symptoms like coughing, expectoration, dyspnea and wheezing in their daily lives, and the ratio of forced expiratory volume in 1 second ($FEV_1$) to forced vital capacity (FVC) after inhaling of 400 $\mu$g salbutamol for half an hour was less than 70%. The lung function was measured by the EasyOne Spirometer (NDD Medizintechnik AG, Zurich, Switzerland) following the instrument instructions.

### *2.3 SNPs Selection and Genotyping*

Potential risk SNPs are screened through the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP) based on the following criterion: SNPs were limited in the region between 2000bp upstream and downstream of *EPO*; the minor allele frequencies (MAFs) were more than 0.05 in the Chinese population; the linkage disequilibrium of the selected SNPs was low (LD, $R^2$ <0.8); they were potential functional variations or tag SNPs. The rs1617640 A>C, rs507392 A>G, and rs564449 G>T were selected for further analysis finally [28,29].

Using the TIANamp Genomic DNA Kit (Tiangen Biotech, Beijing) to extract DNA from the collected peripheral blood sample. TaqMan real-time polymerase chain reaction (PCR) was used for genotyping. **Supplementary Table 4** shows the details of primers and probes. Furthermore, to ensure the reliability of the PCR reaction, we not only set up a negative control on each plate, and 10% random samples repeat tests were carried out.

### *2.4 Statistical Analysis*

The Chi-square test was used to assess the difference in demographic characteristics and the frequency distribution of genotypes between the case and control. After adjusting for age, sex, smoking, education, coal and biomass as fuels, logistic regression analysis was used to evaluate the relationship between *EPO* polymorphisms and susceptibility to COPD. Stratified analysis was then performed by age, sex, smoking, education level, coal and biomass as fu-

els. The consistency of ORs between the layers was tested by the Breslow-day test. The effect of genotypes on lung function was compared by the Kruskal-Wallis test.

The 10-year absolute risk was calculated by the method of Gail and Bruzzi [30–33]. Based on a case-control study in Guangdong, the risk factors were screened by the backward stepwise logistic regression model. The relative risk and population-attributable risk were estimated by IRAP 2.2.0 (Bethesda, Maryland, USA).

IBM SPSS® Statistics 26.0 software (IBM Corp., Chicago, IL, USA) was used to analyze the data. The Odds ratio (OR) and 95% confidence interval (95% CI) were used as evaluation indexes. All analyses were two-sided with a significance level of 0.05.

## 3. Results

### *3.1 Demographic Characteristics*

**Supplementary Table 1** shows the Demographic characteristics of the case-control study in Guangdong with 1095 COPD patients and 1144 healthy controls. The distribution of Education level, Smoking status, Coal as fuels, and Biomass as fuels showed significant statistical differences between cases and controls (all $p < 0.001$).

**Supplementary Table 2** shows the Demographic characteristics of the prevalence study in Gansu with 872 participants. There were 252 cases >60 years old (28.9%) and 620 cases ≤60 years old (71.1%); 395 males (45.3%) and 477 females (54.7%); 551 Tibetan (63.2%) and 321 Han Chinese (36.8%); 465 cases (53.3%) of rs1617640 genotype AA, 340 cases (39.0%) of AC and 67 cases (7.7%) of CC genotype. The median of Pre-$FEV_1$ (L), Pre-FVC (L), Pre-$FEV_1$/Pre-FVC (%) were 2.44 (2.05, 2.91), 3.25 (2.81, 3.92) and 74.88 (69.13, 79.24) respectively.

### *3.2 Association Analysis between EPO SNPs and COPD Risk*

We adjusted for sex, age, smoking status, education, coal as fuel and biomass as fuel at a significance level of 0.05. As shown in Table 1, *EPO* rs1617640 C allele reduced COPD risk in southern Chinese significantly (AC vs. AA: adjusted OR = 0.805, 95% CI = 0.669–0.969, $p = 0.022$; AC+CC vs. AA: adjusted OR = 0.822, 95% CI = 0.689–0.980, $p = 0.029$). Unfortunately, we could not observe the association between the other two SNPs (rs507392 A>G and rs564449 G>T) and the risk of COPD ($p > 0.05$).

### *3.3 Stratification and Interaction Analysis between EPO rs1617640 A>C and COPD Risk*

The results of stratification and interaction analysis are shown in Table 2. Compared with *EPO* rs1617640 AA genotypes, AC and CC genotypes remained protective in the strata of non-smokers (OR = 0.782, 95% CI = 0.616–0.992), avoiding coal and biofuels (OR = 0.812, 95% CI = 0.674–0.979; OR = 0.829, 95% CI = 0.687–0.999). The homogeneity test showed no differences among the sublayers ($p_{homo} > 0.05$). Furthermore, there was no multiplicative interaction between the stratified factors and rs1617640 A>C on the risk of COPD ($p_{inter} > 0.05$).

### *3.4 Effect of EPO RS1617640 A>C on Lung Function*

The effect of rs1617640 A>C polymorphism on premedication lung function was analyzed furtherly. We found that participants in Guangdong with different genotypes showed significant differences in $FEV_1$ and FVC. The values of $FEV_1$ and FVC may be positive with the increase of C allele number [Fig. 1, $FEV_1$ AA, AC, CC: 1.965 (1.500, 2.510), 2.020 (1.630, 2.530), 2.210 (1.760, 2.570), $p = 0.0117$; FVC AA, AC, CC: 2.605 (2.070, 3.330), 2.670 (2.175, 3.305), 2.820 (2.350, 3.460), $p = 0.040$]. The result was further verified in Gansu [Fig. 2, $FEV_1$ AA, AC, CC: 2.380 (2.000, 2.880), 2.460 (2.093, 2.948), 2.640 (2.240, 3.060), $p = 0.0163$; FVC AA, AC, CC: 3.180 (2.755, 3.910), 3.270 (2.843, 3.893), 3.560 (3.130, 4.030), $p = 0.0302$].

### *3.5 Incidence of COPD Based on a Clinical Cohort*

We calculated the incidence of COPD from a clinical cohort constructed by electronic medical records from 2016 to 2020 in several hospitals in Guangdong Province (see **Supplementary Table 3**). The incidence of the 40-50 age group was 1436.73/100,000 person-years in males and 697.82/100,000 person-years in females. In the 50-60 age group was 2972.24/100000 person-years in males and 1321.07/100000 person-years in females. In the 60-70 years group was 5795.21/100,000 person-years in males and 2260.12/100,000 person-years in females.

### *3.6 The 10-Year Absolute Risk of COPD in Southern Chinese*

A backward stepwise logistic regression model was fitted (Table 3), which finally retained four factors, including education, smoking status, coal as fuels, and rs1617640 A>C for males, while three factors as smoking status, biomass as fuels, and rs1617640 A>C were retained for females. The population-attributable risk for males and females were 0.449 (0.258–0.641) and 0.262 (0.128–0.396) respectively.

Combined with the retention factors, tables were developed to show the 10-year absolute risk of COPD for men (Table 4) and women (Table 5) at different individual relative risks [32,33]. For example, a 45-year-old woman wants to know her risk of developing COPD in 10 years. It is known that the woman with the rs1617640 AA genotype does not smoke, and has a history of biomass as fuels. According to Table 3, the individual relative risk of this woman is 2.922 ($r = RR_{\text{non-smoking}} \times RR_{\text{biomass as fuels}} \times RR_{\text{rs1617640 AA}} = 1 \times 2.219 \times 1.317$). Thus, As demonstrated in Table 5, the 10-year absolute risk of the woman with COPD is about 0.142.

**Table 1. Frequency distributions of *EPO* gene polymorphisms and the associations with COPD susceptibility.**

| Models | Genotypes | Case (n = 1095) | Control (n = 1144) | Adjusted OR (95% CI)[a] | $p^a$ | AIC |
|---|---|---|---|---|---|---|
| | | n (%) | n (%) | | | |
| **rs1617640 A>C** | AA | 725 (66.2) | 701 (61.3) | 1.000 (ref.) | | |
| | AC | 317 (28.9) | 387 (33.8) | 0.805 (0.669–0.969) | 0.022 | |
| Codominant | CC | 53 (4.8) | 56 (4.9) | 0.935 (0.628–1.392) | 0.742 | 3018.001 |
| Additive | *p* trend | | | 0.875 (0.757–1.012) | 0.073 | 3020.055 |
| Dominant | AC+CC vs. AA | 370 (33.8) | 443 (38.7) | 0.822 (0.689–0.980) | 0.029 | 3018.506 |
| Recessive | CC vs. AA+AC | 53 (4.8) | 56 (4.9) | 1.004 (0.678–1.488) | 0.984 | 3023.279 |
| **rs507392 A>G** | AA | 697 (63.7) | 682 (59.6) | 1.000 (ref.) | | |
| | AG | 341 (31.1) | 398 (34.8) | 0.849 (0.707–1.020) | 0.080 | |
| Codominant | GG | 57 (5.2) | 64 (5.6) | 0.877 (0.600–1.283) | 0.500 | 3020.052 |
| Additive | *p* trend | | | 0.890 (0.771–1.026) | 0.108 | 3020.688 |
| Dominant | AG+GG vs. AA | 398 (36.3) | 462 (40.2) | 0.853 (0.717–1.015) | 0.074 | 3020.078 |
| Recessive | GG vs. AA+AG | 57 (5.2) | 64 (5.6) | 0.928 (0.638–1.351) | 0.697 | 3023.128 |
| **rs564449 G>T** | GG | 915 (83.6) | 957 (83.7) | 1.000 (ref.) | | |
| | GT | 172 (15.7) | 172 (15.0) | 1.051 (0.832–1.330) | 0.670 | |
| Codominant | TT | 8 (0.7) | 15 (1.3) | 0.557 (0.230–1.346) | 0.222 | 3021.279 |
| Additive | *p* trend | | | 0.976 (0.792–1.202) | 0.817 | 3023.226 |
| Dominant | GT+TT vs. GG | 180 (16.4) | 187 (16.3) | 1.013 (0.806–1.273) | 0.912 | 3023.267 |
| Recessive | TT vs. GG+GT | 8 (0.7) | 15 (1.3) | 0.552 (0.229–1.334) | 0.187 | 3021.460 |

OR, odds ratio; CI, confidence interval; AIC, Akaike Information Criterion.

[a]ORs were adjusted for gender, age, smoking status, education level, coal or biomass as fuels by the logistic regression model.

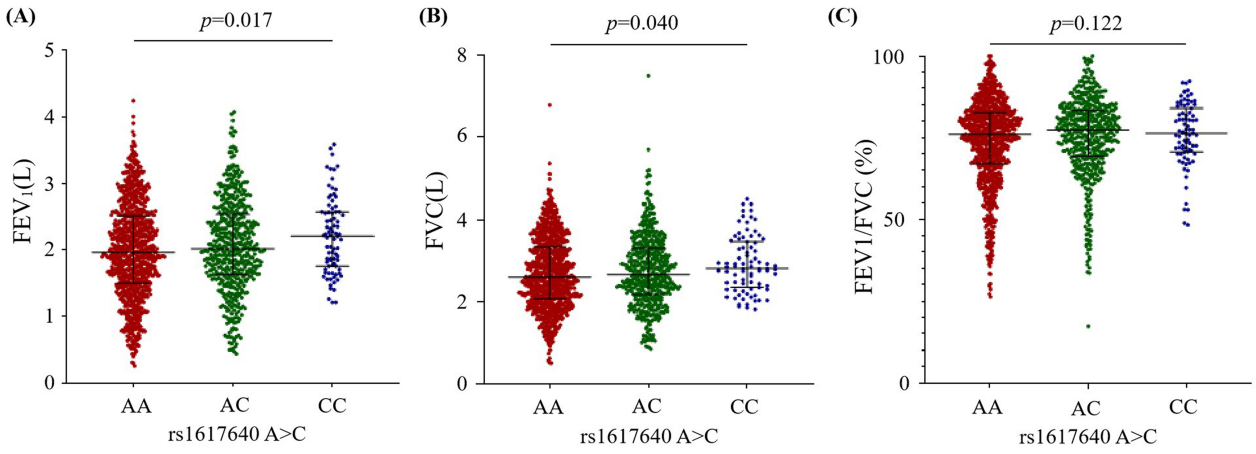


**Fig. 1. The effect of the EPO rs1617640 A>C on pulmonary function in Guangdong population was estimated by the Kruskal-Wallis test.** (A) On pre-forced expiratory volume in 1s (pre-$FEV_1$) [AA, AC, CC: 1.965 (1.500, 2.510), 2.020 (1.630, 2.530), 2.210 (1.760,2.570), $p$ = 0.017]. (B) On pre-forced vital capacity ratio (pre-FVC) [AA, AC, CC: 2.605 (2.070, 3.330), 2.670 (2.175, 3.305), 2.820 (2.350, 3.460), $p$ = 0.040]. (C) On pre-$FEV_1$/FVC [AA, AC, CC: 75.99 (66.91, 82.71), 77.34 (69.29, 83.25), 76.34 (70.63, 83.99), $p$ = 0.122].

## 4. Discussion

To reveal the role of *EPO* polymorphisms on COPD, we investigated the relationship between three SNPs of EPO (rs1617640 A>C, rs507392 A>G, and rs564449 G>T) and the risk of COPD in southern Chinese in this study. We found that the C allele of *EPO* rs1617640 A>C was associated with a reduced risk of COPD and an increase in $FEV_1$ and FVC in Chinese significantly. Meanwhile, compared with *EPO* rs1617640 AA genotype, AC and CC genotypes retained protective effects in the strata of non-smokers, avoiding coal and biofuels ($p < 0.05$).

*EPO*, a pleiotropic factor, can affect the occurrence and progression of many diseases [34,35]. *EPO* was reported that expressed in the lung and responded to Hypoxia by promoting erythropoiesis and neovascularization [13,36–38]. In addition, in acute lung injury, *EPO* could activate the proliferation of pulmonary endothelial cells and reduce the infiltration of inflammatory cells, which plays a

**Table 2. Stratification analysis of the association between *EPO* rs1617640 A>C and COPD susceptibility.**

| Variables | Case (n = 1095) | | | Control (n = 1144) | | | AC+CC vs. AA | $p_{homo}$ [b] | $p_{inter}$ [c] |
|---|---|---|---|---|---|---|---|---|---|
| | AA | AC | CC | AA | AC | CC | OR (95% CI) [a] | | |
| | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | | | |
| Age | | | | | | | | | |
| ≤60 | 288 (64.6) | 132 (29.6) | 26 (5.8) | 303 (59.2) | 187 (36.5) | 22 (4.3) | 0.763 (0.579–1.005) | 0.836 | 0.738 |
| >60 | 437 (67.3) | 185 (28.5) | 27 (4.2) | 398 (63.0) | 200 (31.6) | 34 (5.4) | 0.856 (0.673–1.090) | | |
| Sex | | | | | | | | | |
| Male | 456 (67.2) | 192 (28.3) | 31 (4.6) | 461 (62.9) | 229 (31.2) | 43 (5.9) | 0.851 (0.677–1.070) | 0.670 | 0.772 |
| Female | 269 (64.7) | 125 (30.0) | 22 (5.3) | 240 (58.4) | 158 (38.4) | 13 (3.2) | 0.765 (0.571–1.025) | | |
| Education level | | | | | | | | | |
| Primary school or below | 340 (66.9) | 145 (28.5) | 23 (4.5) | 272 (62.7) | 143 (32.9) | 19 (4.4) | 0.849 (0.644–1.118) | 0.982 | 0.846 |
| Middle school or High school | 345 (65.2) | 157 (29.7) | 27 (5.1) | 388 (60.1) | 224 (34.7) | 34 (5.3) | 0.802 (0.629–1.021) | | |
| College, undergraduate or above | 40 (69.0) | 15 (25.9) | 3 (5.2) | 41 (64.1) | 20 (31.2) | 3 (4.7) | 0.707 (0.301–1.663) | | |
| Smoking status | | | | | | | | | |
| No | 353 (64.5) | 168 (30.7) | 26 (4.8) | 402 (59.2) | 246 (36.2) | 31 (4.6) | 0.782 (0.616–0.992) | 0.710 | 0.688 |
| Yes | 372 (67.9) | 149 (27.2) | 27 (4.9) | 299 (64.3) | 141 (30.3) | 25 (5.4) | 0.888 (0.677–1.165) | | |
| Coal as fuels | | | | | | | | | |
| No | 629 (66.7) | 268 (28.4) | 46 (4.9) | 650 (61.7) | 352 (33.4) | 52 (4.9) | 0.812 (0.674–0.979) | 0.857 | 0.909 |
| Yes | 96 (63.2) | 49 (32.2) | 7 (4.6) | 51 (56.7) | 35 (38.9) | 4 (4.4) | 0.788 (0.439–1.414) | | |
| Biomass as fuels | | | | | | | | | |
| No | 596 (65.6) | 268 (29.5) | 44 (4.8) | 628 (61.1) | 350 (34.0) | 50 (4.9) | 0.829 (0.687–0.999) | 0.781 | 0.885 |
| Yes | 129 (69.0) | 49 (26.2) | 9 (4.8) | 72 (62.9) | 37 (31.9) | 6 (5.2) | 0.721 (0.426–1.221) | | |

OR, odds ratio; CI, confidence interval.

[a] ORs were adjusted for age, gender, education level, smoking status, coal or biomass as fuels by the logistic regression model.

[b] *p*-value of the Breslow-Day homogeneity test for the ORs between strata.

[c] *p*-value of test for the multiplicative interaction between *EPAS1* rs13419896 genotypes and the factors.

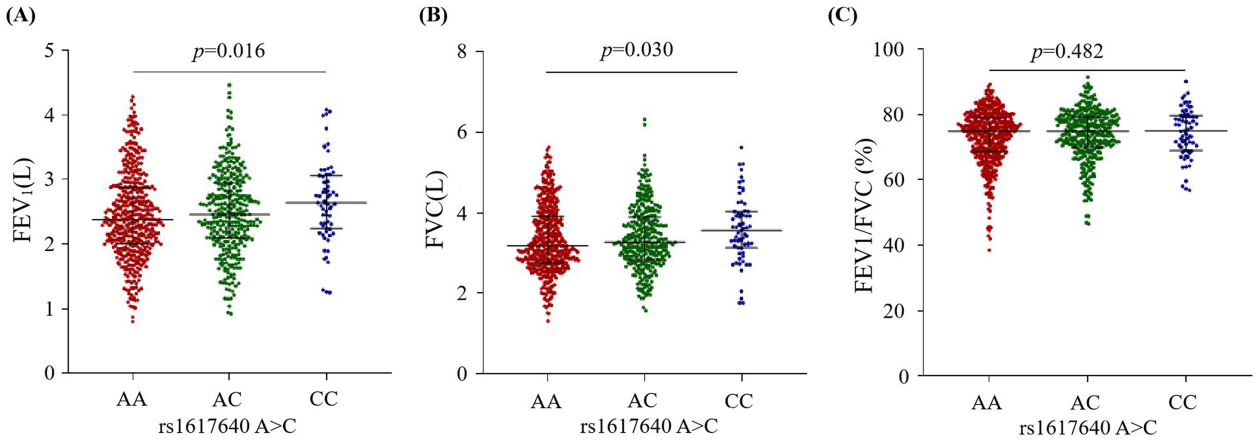


**Fig. 2. The effect of the EPO rs1617640 A>C on pulmonary function in Gansu population was estimated by the Kruskal-Wallis test.** (A) On pre-forced expiratory volume in 1s (pre-$FEV_1$) [AA, AC, CC: 2.380 (2.000, 2.880), 2.460 (2.093, 2.948), 2.640 (2.240, 3.060), $p$ = 0.016]. (B) On pre-forced vital capacity ratio (pre-FVC) [AA, AC, CC: 3.180 (2.755, 3.910), 3.270 (2.843, 3.893), 3.560 (3.130, 4.030), $p$ = 0.030]. (C) On pre-$FEV_1$/FVC [AA, AC, CC: 74.87 (68.72, 79.03), 74.80 (70.05, 79.33), 75.07(68.98, 79.70), $p$ = 0.482].

key role in controlling pulmonary ventilation. Some studies have shown that the inflammatory response and hypoxemia caused by COPD change the expression of *EPO*. For example, COPD patients have up-regulated *EPO* expres-

**Table 3. Estimates relative risk based on case-control studies.**

| Factors | RR (95% CI)[a] |
|---|---|
| Male | |
| Education level | |
| College, undergraduate or above | 1.000 (ref.) |
| Middle school or High school | 1.218 (0.766–1.937) |
| Primary school or below | 1.793 (1.111–2.894) |
| Smoking status | |
| No | 1.000 (ref.) |
| Yes | 1.973 (1.559–2.495) |
| Coal as fuels | |
| No | 1.000 (ref.) |
| Yes | 2.058 (1.436–2.951) |
| rs1617640 (Dominant) | |
| AC+CC | 1.000 (ref.) |
| AA | 1.163 (0.925–1.463) |
| Population–attributable risk | 0.449 (0.258–0.641) |
| Female | |
| Smoking status | |
| No | 1.000 (ref.) |
| Yes | 1.809 (1.039–3.150) |
| Biomass as fuels | |
| No | 1.000 (ref.) |
| Yes | 2.219 (1.440–3.418) |
| rs1617640 (Dominant) | |
| AC+CC | 1.000 (ref.) |
| AA | 1.317 (0.987–1.758) |
| Population–attributable risk | 0.262 (0.128–0.396) |

RR, relative risk.

[a]backward logistic regression, adjusting for age.

sion due to increased inflammatory factors and decreased erythrocytes in the body [39,40]. Thus, an increase in hemoglobin concentration by upregulation of *EPO*'s expression can correct hypoxemia seen in COPD patients commonly [22,41,42]. Existing researches indicate that *EPO* may participate in the development of COPD. However, no study has explored whether *EPO* polymorphisms modify the susceptibility to COPD.

We finally retained three SNPs (rs1617640 A>C, rs507392 A>G, and rs564449 G>T) to conduct the research. Online web tools (NCBI, Ensembl and SNPinfo Web Server) were used to find the location and functional information of SNPs. Rs1617640 A>C is a transcription factor located in upstream of the *EPO* transcription start site (Chr 7:100719675), as well as impact the potential transcription factors such as AP1\STAT5\STAT3\GATA-2 and so on. In addition, it has been shown to be strongly association with a variety of diseases including complications of diabetic, hepatitis C, myelodysplasia, erythropoietic and so on in the present studies [43–46]. Rs507392 A>G, located at Chr 7:100722313, is an intron variant of the *EPO*, considering the current studies reported it associated with diabetic retinopathy [47,48], so we decided to keep the SNP for further analysis after consulting the relevant experts. Rs564449 G>T is a miRNA binding site located in 3′-UTR of the *EPO* (Chr 7:100723515) and no research has reported that it is associated with any disease.

In this study, we first discovered that the variation of *EPO* rs1617640 A>C was associated with COPD risk, and compared with AA genotype, patients with AC/CC genotypes had a lower incidence of COPD. Unfortunately, no association was found between the other two SNPs (rs507392 A>G, and rs564449 G>T) and the risk of COPD. Rs1617640 A>C is located in the upstream promoter region of *EPO*. The promoter SNP can cause abnormal gene expression by affecting the binding of transcription factors to templates [49]. In the present studies, *EPO* rs1617640 polymorphism was shown to be associated with erythropoietin expression and level [43], CC genotype can promote the expression of erythropoietin [45], which leads to the increasing of Hb level, hematocrit and erythrocyte count [50]. In other words, *EPO* rs1617640 polymorphism can cause abnormal expression of *EPO* and affect the hematopoietic function of the body [50]. Moreover, we found a correlation between the *EPO* rs1617640 A>C genetic variant and the level of pre-drug lung function indexes in both Guangdong and Gannan. Similar to our results, Wang L found that serum *EPO* levels in COPD patients were correlated with $FEV_1$% significantly and negatively through a clinical study ($p < 0.05$) [21]. *EPO* levels may, to some extent, reflect the condition and prognosis of COPD patients. Therefore, we concluded that rs1617640 might affect the risk of COPD by influencing the binding of transcription factors to DNA fragments and regulating the expression level of target genes.

Our clinical cohort found that the incidence of COPD and the mortality without COPD differed between men and women, both indicators were higher in males than in females in all age strata (see **Supplementary Table 3**). This is consistent with the findings of Garcia Rodriguez in the incidence of COPD [51]. Hence, we decided to fit a 10-year absolute risk model for COPD in southern Chinese by sex. We found the factors retained in the models of males and females were slightly different (see Table 3), which may be related to the reduction of sample size after stratification. At the same time, the differences in the social division of labor and lifestyles between men and women may also lead to differences in risk factors [52,53]. In the model for the female, the population attributable risk was 0.262 (0.128–0.396), indicating that additional risk factors need to be considered to predict the risk of COPD accurately [54].

There are some limitations in this study. First, this was a case-control study, and there was a certain degree of recall bias when obtaining environmental exposure information. Secondly, the participants in this study were mainly Han Chinese, which may restrict the extrapolation of conclusions to other populations. Moreover, this study clarified the association between *EPO* polymorphisms and COPD

**Table 4. The 10-year absolute risk of COPD in southern China for men.**

| Initial age (years) | Individual relative risk | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0.00 | 1.00 | 2.00 | 3.00 | 4.00 | 5.00 | 6.00 | 7.00 | 8.00 |
| 40–49 | 0.000 | 0.075 | 0.145 | 0.209 | 0.269 | 0.324 | 0.375 | 0.421 | 0.465 |
| 50–59 | 0.000 | 0.147 | 0.272 | 0.378 | 0.468 | 0.545 | 0.611 | 0.666 | 0.714 |
| 60–69 | 0.000 | 0.258 | 0.447 | 0.586 | 0.687 | 0.762 | 0.818 | 0.858 | 0.889 |

**Table 5. The 10-year absolute risk of COPD in southern China for women.**

| Initial age (years) | Individual relative risk | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0.00 | 1.00 | 2.00 | 3.00 | 4.00 | 5.00 | 6.00 |
| 40–49 | 0.000 | 0.050 | 0.097 | 0.142 | 0.185 | 0.226 | 0.265 |
| 50–59 | 0.000 | 0.092 | 0.175 | 0.251 | 0.319 | 0.382 | 0.438 |
| 60–69 | 0.000 | 0.150 | 0.276 | 0.384 | 0.475 | 0.552 | 0.618 |

using epidemiological methods, which needed further verification by cellular and molecular experiments. Finally, this study did not conduct whole genome sequencing and analysis, it's a potential limitation of the study.

## 5. Conclusions

In conclusion, this study reported for the first time that the *EPO* rs1617640 A>C polymorphism was associated with COPD susceptibility, and the C allele was associated with better lung function in southern Chinese. On this basis, the study suggests that *EPO* rs1617640 A>C can be used to predict the 10-year absolute risk for COPD among southern Chinese as a genetic marker when combined with environmental factors.

## Abbreviations

COPD, chronic obstructive pulmonary disease; EPO, Erythropoietin; GWAS, genome-wide association analysis; SNPs, the single nucleotide polymorphisms; $FEV_1$, forced expiratory volume in 1 second; FVC, forced vital capacity; MAFs, the minor allele frequencies; PCR, TaqMan real-time polymerase chain reaction.

## Availability of Data and Materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Author Contributions

YW and ZL conceived and designed the study with support from XW and JL as well as wrote the manuscript; XZ conducted the experiment; YW, ZL, AL and XZ preformed the statistical analysis of the results; YP, CM, CC, CX, DH, YD, and XZ participated in sample collection for the case-control study and prevalence study, as well as the electronic medical record entry. XW and JL were responsible for the design and quality control of the whole research and the guidance of the methodology. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

## Ethics Approval and Consent to Participate

This study was performed under the Declaration of Helsinki and approbated by the institutional review boards of Xi'an Jiaotong University Health Science Center (approval: XJTU 2016-411) and Guangzhou Medical University (approval: GZMC2007-07-0676).

In addition, we explained the purpose of the study to all participants at the time of the study and obtained their signed informed consent.


## Acknowledgment

Thanks to the Institute of Public Health, Guangzhou Medical University. Thanks to the researchers of this laboratory for their guidance on experimental technology.

## Funding

This study was supported by the following grants: National Key Research and Development Project of China 2017YFC0907202 (Xinhua Wang); National Natural Science Foundation of China 81872694, 82173609 (Jiachun Lu); Local Innovative and Research Teams Project of Guangdong Pearl River Talents Program 2017BT01S155 (Jiachun Lu); Guangdong Medical Science and Technology Research Fund Project A2020324 (Cuiyi Chen); National Natural Science Foundation of China (81460123) and Guangxi Natural Foundation (2018GXNSFAA281187) (Yibin Deng); National Natural Science Foundation of China (82260889) (Xuhui Zhang); the Science and Technology Project of Guangzhou (No.202103000073).

## Conflict of Interest

The authors declare no conflict of interest.

## Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.fbl2809215.

## References


[1] Olloquequi J, Silva O R. Biomass smoke as a risk factor for chronic obstructive pulmonary disease: effects on innate immunity. Innate Immunity. 2016; 22: 373–381.

[2] Raherison C, Girodet PO. Epidemiology of COPD. European Respiratory Review: an Official Journal of the European Respiratory Society. 2009; 18: 213–221.

[3] Yuan C, Chang D, Lu G, Deng X. Genetic polymorphism and chronic obstructive pulmonary disease. International Journal of Chronic Obstructive Pulmonary Disease. 2017; 12: 1385–1393.

[4] Gupta N, Agrawal S, Chakrabarti S, Ish P. COPD 2020 Guidelines - what is new and why? Advances in Respiratory Medicine. 2020; 88: 38–40.

[5] Cosentino J, Behsaz B, Alipanahi B, McCaw ZR, Hill D, Schwantes-An TH, *et al*. Inference of chronic obstructive pulmonary disease with deep learning on raw spirograms identifies new genetic loci and improves risk models. Nature Genetics. 2023; 55: 787–795.

[6] Silverman EK. Genetics of COPD. Annual Review of Physiology. 2020; 82: 413–431.

[7] Dijkstra AE, Postma DS, van Ginneken B, Wielpütz MO, Schmidt M, Becker N, *et al*. Novel genes for airway wall thickness identified with combined genome-wide association and expression analyses. American Journal of Respiratory and Critical Care Medicine. 2015; 191: 547–556.

[8] Pillai SG, Ge D, Zhu G, Kong X, Shianna KV, Need AC, *et al*. A genome-wide association study in chronic obstructive pulmonary disease (COPD): identification of two major susceptibility loci. PLoS Genetics. 2009; 5: e1000421.

[9] Hancock DB, Eijgelsheim M, Wilk JB, Gharib SA, Loehr LR, Marciante KD, *et al*. Meta-analyses of genome-wide association studies identify multiple loci associated with pulmonary function. Nature Genetics. 2010; 42: 45–52.

[10] Repapi E, Sayers I, Wain LV, Burton PR, Johnson T, Obeidat M, *et al*. Genome-wide association study identifies five loci associated with lung function. Nature Genetics. 2010; 42: 36–44.

[11] Cho MH, McDonald MLN, Zhou X, Mattheisen M, Castaldi PJ, Hersh CP, *et al*. Risk loci for chronic obstructive pulmonary disease: a genome-wide association study and meta-analysis. The Lancet. Respiratory Medicine. 2014; 2: 214–225.

[12] Jelkmann W. Regulation of erythropoietin production. The Journal of Physiology. 2011; 589: 1251–1258.

[13] Oshima N, Onimaru H, Yamagata A, Itoh S, Matsubara H, Imakiire T, *et al*. Erythropoietin, a putative neurotransmitter during hypoxia, is produced in RVLM neurons and activates them in neonatal Wistar rats. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology. 2018; 314: R700–R708.

[14] Semenza GL. Involvement of oxygen-sensing pathways in physiologic and pathologic erythropoiesis. Blood. 2009; 114: 2015–2019.

[15] Zhu M, Wang L, Yang J, Xie K, Zhu M, Liu S, *et al*. Erythropoietin Ameliorates Lung Injury by Accelerating Pulmonary Endothelium Cell Proliferation via Janus Kinase-Signal Transducer and Activator of Transcription 3 Pathway After Kidney Ischemia and Reperfusion Injury. Transplantation Proceedings. 2019; 51: 972–978.

[16] He Q, Zhao X, Bi S, Cao Y. Pretreatment with Erythropoietin Attenuates Lung Ischemia/Reperfusion Injury via Toll-Like Receptor-4/Nuclear Factor-κB (TLR4/NF-κB) Pathway. Medical Science Monitor: International Medical Journal of Experimental and Clinical Research. 2018; 24: 1251–1257.

[17] Shang Y, Jiang YX, Xu SP, Wu Y, Wu ZY, Yuan SY, *et al*. Reduction of pulmonary inflammatory response by erythropoietin in a rat model of endotoxaemia. Chinese Medical Journal. 2009; 122: 834–838.

[18] Ardalan MR, Estakhri R, Hajipour B, Ansarin K, Asl NA, Nasirizade MR, *et al*. Erythropoietin ameliorates oxidative stress and tissue injury following renal ischemia/reperfusion in rat kidney and lung. Medical Principles and Practice: International Journal of the Kuwait University, Health Science Centre. 2013; 22: 70–74.

[19] Jin W, Wu J, Wang H, Kong J, Ni H, Liang W. Erythropoietin administration modulates pulmonary Nrf2 signaling pathway after traumatic brain injury in mice. The Journal of Trauma. 2011; 71: 680–686.

[20] MacRedmond R, Singhera GK, Dorscheid DR. Erythropoietin inhibits respiratory epithelial cell apoptosis in a model of acute lung injury. The European Respiratory Journal. 2009; 33: 1403–1414.

[21] Wang L, Yang H, Gu W. Value of erythrocyte sedimentation rate and serum epo levels in evaluating the condition and prognosis of COPD in the elderly. Pakistan Journal of Pharmaceutical Sciences. 2021; 34: 435–439.

[22] Sosnin DY, Khovaeva YB, Podyanova AI, Syromyatnikova TN, Nenasheva OY. Eritropoetin as laboratory index of the degree of respiratory insufficiency in chronic obstructive pulmonary diseases. Klinicheskaia Laboratornaia Diagnostika. 2018; 63: 691–695.

[23] Graudal N, Galløe AM, Nielsen OJ. Erythropoietin in chronic obstructive pulmonary disease. Relationship between serum erythropoietin, blood hemoglobin and lung function–effect of the calcium antagonist isradipine on serum erythropoietin. Respiration; International Review of Thoracic Diseases. 1991; 58: 141–144.

[24] Markoulaki D, Kostikas K, Papatheodorou G, Koutsokera A, Alchanatis M, Bakakos P, *et al*. Hemoglobin, erythropoietin and systemic inflammation in exacerbations of chronic obstructive pulmonary disease. European Journal of Internal Medicine. 2011; 22: 103–107.

[25] Tandara L, Grubisic TZ, Ivan G, Jurisic Z, Tandara M, Gugo K, *et al*. Systemic inflammation up-regulates serum hepcidin in exacerbations and stabile chronic obstructive pulmonary disease. Clinical Biochemistry. 2015; 48: 1252–1257.

[26] Yang L, Lu X, Deng J, Zhou Y, Huang D, Qiu F, *et al*. Risk factors shared by COPD and lung cancer and mediation effect of COPD: two center case-control studies. Cancer Causes & Control: CCC. 2015; 26: 11–24.

[27] Singh D, Agusti A, Anzueto A, Barnes PJ, Bourbeau J, Celli BR, *et al*. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease: the GOLD science committee report 2019. The European Respiratory Journal. 2019; 53: 1900164.

[28] Harlow CE, Gandawijaya J, Bamford RA, Martin ER, Wood AR, van der Most PJ, *et al*. Identification and single-base gene-editing functional validation of a cis-EPO variant as a genetic predictor for EPO-increasing therapies. American Journal of Human Genetics. 2022; 109: 1638–1652.

[29] Montesanto A, Bonfigli AR, De Luca M, Crocco P, Garagnani P, Marasco E, *et al*. Erythropoietin (EPO) haplotype associated with all-cause mortality in a cohort of Italian patients with Type-2 Diabetes. Scientific Reports. 2019; 9: 10395.

[30] Fears TR, Guerry D, 4th, Pfeiffer RM, Sagebiel RW, Elder DE, Halpern A, *et al*. Identifying individuals at high risk of melanoma: a practical predictor of absolute risk. Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology. 2006; 24: 3590–3596.
[31] Xin J, Du M, Gu D, Ge Y, Li S, Chu H, *et al*. Combinations of single nucleotide polymorphisms identified in genome-wide association studies determine risk for colorectal cancer. International Journal of Cancer. 2019; 145: 2661–2669.
[32] Gail MH, Brinton LA, Byar DP, Corle DK, Green SB, Schairer C, *et al*. Projecting individualized probabilities of developing breast cancer for white females who are being examined annually. Journal of the National Cancer Institute. 1989; 81: 1879–1886.
[33] Bruzzi P, Green SB, Byar DP, Brinton LA, Schairer C. Estimating the population attributable risk for multiple risk factors using case-control data. American Journal of Epidemiology. 1985; 122: 904–914.
[34] Kimáková P, Solár P, Solárová Z, Komel R, Debeljak N. Erythropoietin and Its Angiogenic Activity. International Journal of Molecular Sciences. 2017; 18: 1519.
[35] Dey S, Lee J, Noguchi CT. Erythropoietin Non-hematopoietic Tissue Response and Regulation of Metabolism During Diet Induced Obesity. Frontiers in Pharmacology. 2021; 12: 725734.
[36] Weidemann A, Johnson RS. Nonrenal regulation of EPO synthesis. Kidney International. 2009; 75: 682–688.
[37] Dempke W. Preclinical and clinical effects of erythropoietin in the management of anaemia in patients with non-small cell lung cancer. Anticancer Research. 2007; 27: 1745–1757.
[38] Liu X, Tufman A, Behr J, Kiefl R, Goldmann T, Huber RM. Role of the erythropoietin receptor in Lung Cancer cells: erythropoietin exhibits angiogenic potential. Journal of Cancer. 2020; 11: 6090–6100.
[39] Crosato M, Steinborn W, Anker SD. Anemia in chronic congestive heart failure: frequency, prognosis, and treatment. Heart Failure Monitor. 2003; 4: 2–6.
[40] Weiss G, Goodnough LT. Anemia of chronic disease. The New England Journal of Medicine. 2005; 352: 1011–1023.
[41] Fernández-Plata R, Thirion-Romero I, Nava-Quiroz KJ, Pérez-Rubio G, Rodríguez-Llamazares S, Pérez-Kawabe M, *et al*. Clinical Markers of Chronic Hypoxemia in Respiratory Patients Residing at Moderate Altitude. Life (Basel, Switzerland). 2021; 11: 428.
[42] Pavlisa G, Vrbanic V, Kusec V, Jaksic B. Erythropoietin response after correction of severe hypoxaemia due to acute respiratory failure in chronic obstructive pulmonary disease patients. Clinical Science (London, England: 1979). 2004; 106: 43–51.
[43] Tong Z, Yang Z, Patel S, Chen H, Gibbs D, Yang X, *et al*. Promoter polymorphism of the erythropoietin gene in severe diabetic eye and kidney complications. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105: 6998–7003.
[44] Fan Y, Fu YY, Chen Z, Hu YY, Shen J. Gene-gene interaction of erythropoietin gene polymorphisms and diabetic retinopathy in Chinese Han. Experimental Biology and Medicine (Maywood, N.J.). 2016; 241: 1524–1530.
[45] Amanzada A, Goralczyk AD, Reinhardt L, Moriconi F, Cameron S, Mihm S. Erythropoietin rs1617640 G allele associates with an attenuated rise of serum erythropoietin and a marked decline of hemoglobin in hepatitis C patients undergoing antiviral therapy. BMC Infectious Diseases. 2014; 14: 503.
[46] Ma W, Kantarjian H, Zhang K, Zhang X, Wang X, Chen C, *et al*. Significant association between polymorphism of the erythropoietin gene promoter and myelodysplastic syndrome. BMC Medical Genetics. 2010; 11: 163.
[47] Abhary S, Burdon KP, Casson RJ, Goggin M, Petrovsky NP, Craig JE. Association between erythropoietin gene polymorphisms and diabetic retinopathy. Archives of Ophthalmology (Chicago, Ill.: 1960). 2010; 128: 102–106.
[48] Kaur N, Singh IR, Vanita V. Association of Erythropoietin Gene Polymorphisms with Type 2 Diabetic Retinopathy in Adult Patients From Northern India. Canadian Journal of Diabetes. 2021; 45: 785–791.
[49] Lin A, Zhou M, Hua RX, Zhang J, Zhou H, Li S, *et al*. METTL3 polymorphisms and Wilms tumor susceptibility in Chinese children: A five-center case-control study. The Journal of Gene Medicine. 2020; 22: e3255.
[50] Renner W, Kaiser M, Khuen S, Trummer O, Mangge H, Langsenlehner T. The Erythropoetin *rs1617640* Gene Polymorphism Associates with Hemoglobin Levels, Hematocrit and Red Blood Cell Count in Patients with Peripheral Arterial Disease. Genes. 2020; 11: 1305.
[51] García Rodríguez LA, Wallander MA, Tolosa LB, Johansson S. Chronic obstructive pulmonary disease in UK primary care: incidence and risk factors. COPD. 2009; 6: 369–379.
[52] GBD Chronic Respiratory Disease Collaborators. Prevalence and attributable health burden of chronic respiratory diseases, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet. Respiratory Medicine. 2020; 8: 585–596.
[53] Sezer H, Akkurt I, Guler N, Marakoğlu K, Berk S. A case-control study on the effect of exposure to different substances on the development of COPD. Annals of Epidemiology. 2006; 16: 59–62.
[54] Burney P, Patel J, Minelli C, Gnatiuc L, Amaral AFS, Kocabaş A, *et al*. Prevalence and Population-Attributable Risk for Chronic Airflow Obstruction in a Large Multinational Study. American Journal of Respiratory and Critical Care Medicine. 2021; 203: 1353–1365.