Original Research

A Case-Control Study of the Associations between *EGLN1* Gene Polymorphisms and COPD

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Abstract

**Background:** Environmental and genetic factors are jointly involved in the development of chronic obstructive pulmonary disease (COPD). The *EGLN1* gene is a major factor in upstream regulation of the hypoxia-inducible pathway. *EGLN1* negatively regulates the hypoxia-inducible factors HIF-1α and HIF-2α by regulating the concentration of oxygen, mainly in a hypoxic environment. Hypoxia is a common physiologic condition during the progression of COPD, and several studies have identified genetic variants in *EGLN1* as a key factor in the adaptation to hypoxic environments. However, it is still unclear whether there is an association between *EGLN1* variants and the risk of developing COPD. **Methods:** A case-control study was conducted in the Gannan Tibetan Autonomous Prefecture, Gansu Province. A total of 292 COPD patients and 297 healthy controls were enrolled to assess the association of *EGLN1* single nucleotide polymorphisms (SNPs) (rs41303095 A>G, rs480902 C>T, rs12097901 C>G, rs2153364 G>A) with COPD susceptibility. **Results:** The *EGLN1* rs41303095 A>G, rs480902 C>T, rs12097901 C>G, and rs2153364 G>A polymorphisms were not associated with COPD susceptibility (p > 0.05). **Conclusions:** The *EGLN1* rs41303095 A>G, rs480902 C>T, rs12097901 C>G and rs2153364 G>A polymorphisms were found in this study not to be associated with susceptibility to COPD in Gannan Tibetans.

**Keywords:** COPD; *EGLN1*; polymorphism; susceptibility

1. Introduction

The clinical manifestations of chronic obstructive pulmonary disease (COPD) are chronic respiratory symptoms (dyspnea, cough, sputum, acute exacerbations) due to airflow (bronchitis, bronchiectasis) and/or alveolar anomalies (emphysema) that cause persistent and progressive exacerbation of airflow limitation [1]. Early COPD is asymptomatic or mildly symptomatic, meaning that diagnosis and treatment are easily delayed. Lung function damage such as fine bronchitis and destruction of the lung parenchyma (emphysema) has often already occurred when the diagnosis is confirmed [2,3]. COPD is therefore a significant public health problem worldwide [4] and a major cause of mortality [5]. It presents a serious threat to patient survival and quality of life, as well as being a great burden to society [2].

COPD is the result of interactions between genes and the environment over time [6]. Epidemiologic investigations have shown a clear familial aggregation in some COPD patients [7,8], thus revealing the importance of genetic factors. Genome-wide association studies (GWAS) have identified many candidate genes related to COPD pathogenesis [9], reinforcing the notion that genetic factors may play an important role in the development of COPD. Raguso et al. [10] found some similarities between chronic exposure to high altitude hypoxia in healthy people and chronic hypoxia in COPD patients, again suggesting that hypoxia has an important role in the development of COPD [11]. However, most of the current studies on candidate genes for COPD have focused on inflammation-related genes [12], with oxygen-sensitive genes deserving more in-depth investigation.

Egl-9 family hypoxia-inducible factor (*EGLN1*) acts as an oxygen sensor and catalyzes prolyl hydroxylation of the transcription factor hypoxia-inducible factor-1α under normoxic conditions, leading to its proteasomal degradation. *EGLN1* therefore has a central role in the hypoxia-inducible factor-mediated hypoxia signaling pathway [13]. Tibetans have lived at high altitudes for generations and can adapt to environmental hypoxia. The gene products from some regions of their genome may be associated with high altitude adaptation, and *EGLN1* is significantly associated with a reduced hemoglobin phenotype that is characteristic of plateau populations [14–16]. At high altitudes especially, chronic hypoxia causes *EGLN1* to play an important
regulatory role in the development of COPD [17–19]. However, it is not known whether genetic variations in EGLN1 are associated with the risk of developing COPD.

The Gannan Tibetan Autonomous Prefecture is located on the northeastern edge of the Qinghai-Tibetan Plateau [20] and is in a high elevation region [21]. A previous study by our group found that the prevalence of COPD in Gannan was 23.4% (20.7%–26.4%) [22]. Here, we conducted a case-control study in this Prefecture to investigate possible associations between EGLN1 single nucleotide polymorphisms (SNPs) (rs41303095 A>G, rs12097901 C>T, rs12097901 C>G, and rs2153364 G>A) and COPD susceptibility, and to validate the effect of EGLN1 polymorphisms on lung function.

2. Materials and Methods

2.1 Study Population

A case-control study was conducted to assess the association between four EGLN1 SNPs (rs41303095 A>G, rs480902 C>T, rs12097901 C>G, rs2153364 G>A) and COPD susceptibility. The study included 292 patients with COPD and 297 healthy controls recruited in 2019 from Zhuoni County, Gannan Tibetan Autonomous Prefecture, Gansu Province, China. The inclusion criteria were: ① Tibetans living in Gannan for more than three generations, with no history of intermarriage with other ethnic groups; ② normal mental health and intellectual ability to fill in the questionnaire; ③ informed about the study and participated voluntarily. The exclusion criteria were: ① Non-Tibetan origin, or history of intermarriage with other ethnic groups in the three previous generations; ② restrictive ventilatory dysfunction, including active tuberculosis, thoracic deformity, combined pleural effusion, bronchopulmonary carcinoma, etc.; ③ other diseases leading to obstructive ventilation dysfunction, such as tuberculosis leading to disfiguring of the lungs, bronchiectasis, etc.; ④ a history of tumors in various systems.

All participants in this case-control study underwent pulmonary function tests and respiratory health questionnaires to collect information on their demographic characteristics and environmental exposures such as education, smoking and drinking status. After signing the informed consent form, 5 mL of peripheral blood was collected for genotyping.

The study was checked by the ethical committees of Xi’an Jiaotong University, Guangzhou Medical University and Gansu University of Chinese Medicine.

2.2 Diagnostic Criteria and Pulmonary Function Tests for COPD

According to the Global Initiative for Chronic Obstructive Pulmonary Disease 2023 [23], COPD was diagnosed if participants experienced respiratory symptoms such as cough, sputum, dyspnea, and wheezing in their daily lives, and if the ratio of forced expiratory volume in one second (FEV1) to forced vital capacity (FVC) was <70% at 30 min after inhalation of 400 µg of salbutamol sulfate. Lung function was measured using an EasyOne spirometer (NDD Medizintechnik AG, Zürich, Switzerland) according to the instrument instructions.

2.3 SNP Selection and Genotyping

Potential risk SNPs were identified in the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP) using the following criteria: SNPs located between 2000 bp upstream and 2000 bp downstream of EGLN1, minor allele frequency (MAF) >0.05 in the Chinese population, and a high degree of linkage disequilibrium of the selected SNP (LD, R2 >0.8) for potential functional variants or labeling SNP. Four SNPs (rs41303095 A>G, rs480902 C>T, rs12097901 C>G, and rs2153364 G>A) were selected for subsequent analysis. DNA was extracted from peripheral blood samples using the TIANamp Genomic DNA Kit (Tiangen Biotechnology, Beijing, China), and genotyping was performed using TaqMan real-time polymerase chain reaction (PCR).

2.4 Statistical Analysis

The Hardy-Weinberg equilibrium (HWE) test was performed on the observed genotype frequencies using the “SNPPasso” R package. The chi-square test was used to assess differences between cases and controls in terms of demographic characteristics and genotype frequency distribution. Associations between EGLN1 polymorphisms and COPD susceptibility were assessed using logistic regression analysis after adjusting for age, gender, BMI, literacy, hypertension, smoking and alcohol consumption. The method used for multiple comparisons was the false discovery rate (FDR) (q = 0.05). The analysis was then stratified by age, gender, BMI, literacy, hypertension, smoking and alcohol consumption. The consistency of odds ratios (ORs) between strata was examined using the Breslow-Day test. The rank sum test was used to evaluate the effect of genotype on lung function.

Data were analyzed using R 4.1.3 software (Lucent Technologies Corp., San Antonio, TX, USA). The OR and 95% confidence interval (95% CI) were used as evaluation metrics. All analyses were two-sided, with a significance level of 0.05.
Table 2. Analysis of the association between EGLN1 rs12097901 C>G and risk of COPD.

<table>
<thead>
<tr>
<th>Model</th>
<th>Genotype</th>
<th>Case (292)</th>
<th>Control (297)</th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
<th>p</th>
<th>q&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Codominant</td>
<td>CC</td>
<td>183 (63.7)</td>
<td>170 (57.2)</td>
<td>1.000 (ref.)</td>
<td>1.000 (ref.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CG</td>
<td>93 (31.8)</td>
<td>109 (36.7)</td>
<td>0.772 (0.544–1.094)</td>
<td>0.146</td>
<td>0.830</td>
<td>0.332</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>16 (5.5)</td>
<td>18 (6.1)</td>
<td>0.853 (0.414–1.749)</td>
<td>0.662</td>
<td>0.651</td>
<td>0.280</td>
</tr>
<tr>
<td>Dominant</td>
<td>CC</td>
<td>183 (62.7)</td>
<td>170 (57.2)</td>
<td>1.000 (ref.)</td>
<td>1.000 (ref.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CG+GG</td>
<td>109 (37.3)</td>
<td>127 (42.8)</td>
<td>0.783 (0.561–1.092)</td>
<td>0.150</td>
<td>0.801</td>
<td>0.227</td>
</tr>
<tr>
<td>Recessive</td>
<td>CC+CG</td>
<td>276 (94.5)</td>
<td>279 (93.9)</td>
<td>1.000 (ref.)</td>
<td>1.000 (ref.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>16 (5.5)</td>
<td>18 (6.1)</td>
<td>0.938 (0.460–1.901)</td>
<td>0.858</td>
<td>0.694</td>
<td>0.352</td>
</tr>
<tr>
<td>Additive</td>
<td>CC&gt;C&gt;GG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.173</td>
<td>0.274</td>
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<tr>
<td></td>
<td>GG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.537</td>
<td>0.352</td>
</tr>
</tbody>
</table>

*a. Adjustment for multiple comparisons using false discovery rate. COPD, chronic obstructive pulmonary disease; OR, odds ratio; 95% CI, 95% confidence interval.*

3. Results

3.1 Demographic Characteristics

Supplementary Table 1 shows the demographic characteristics of the 589 participants in this study. Of these, 293 (49.7%) were males and 296 (50.3%) females, 248 (42.1%) were >60 years old and 341 (57.9%) were ≤60 years old, 123 (20.9%) were smokers and 466 (79.1%) non-smokers, 71 (12.1%) were alcohol drinkers and 518 (87.9%) non-alcohol drinkers, and 251 (42.6%) had hypertension and 338 (57.4%) did not. The medians (IQR) for Pre-FEV1 (L), Pre-FVC (L) and Pre-FEV/Pre-FVC (%) were 2.52 (2.16–3.11), 3.09 (2.59–3.89), and 78.30 (76.50–80.30), respectively.

Supplementary Table 2 shows the demographic characteristics of 292 COPD patients and 297 healthy controls from Zhuoni County, Gannan Tibetan Autonomous Prefecture, Gansu. The age distribution and smoking status were statistically different between the case and control groups (p < 0.05).

3.2 Hardy-Weinberg Equilibrium Test for the Four EGLN1 SNP Loci

As shown in Table 1, only rs12097901 C>G SNP conformed to HWE (p > 0.05).

3.3 Analysis of the Association between EGLN1 rs12097901 C>G and COPD Risk

Binary logistic regression was used to analyze the association between EGLN1 rs12097901 C>G and the risk of COPD in four genetic models (codominant, dominant, recessive, and additive). Correction was made for sex, age, smoking and drinking status, BMI, hypertension, and literacy, and α = 0.05 was used as the level of significance. As shown in Table 2, no association was observed between rs12097901 C>G and COPD risk (p > 0.05).

3.4 Stratification and Interaction Analysis of EGLN1 rs12097901 C>G and COPD Risk

The results of the stratification and interaction analyses are shown in Table 3. The association between rs12097901 C>G and COPD risk among subgroups was further assessed using an additive model stratified by each factor. In the male population, rs12097901 C>G was associated with the risk of COPD (OR = 0.628, 95% CI = 0.414–0.951). Similar results were obtained for the smoking and drinking populations (OR = 0.526, 95% CI = 0.281–0.983; OR = 0.318, 95% CI = 0.115–0.879, respectively). However, no statistical difference was found after FDR correction. Homogeneity tests between substrata were not statistically different (p > 0.05). No additive interaction of each stratification factor with the risk of EGLN1 rs12097901 C>G for COPD was observed (RERI 95% CI, AP 95% CI contains 0, S 95% CI contains 1).

3.5 Associations of Different of EGLN1 rs12097901 C>G Genotypes with Lung Function

Possible associations of rs12097901 C>G genotypes with premedication lung function were further analyzed. As shown in Table 4, a statistically significant difference in the Pre-FEV1/Pre-FVC was found between genotypes in the Gansu Gannan population.

4. Discussion

The development of GWAS in recent years has led to many studies showing that genetic polymorphisms are associated with the development of various diseases. This includes an association with COPD, thus providing a new direction for the study of COPD susceptibility. To study whether EGLN1 polymorphisms are involved in COPD, we investigated the association of four SNP loci in EGLN1 (rs41303095 A>G, rs480902 C>T, rs12097901 C>G, and rs2153364 G>A) with the risk of COPD in Gannan Tibetans. No significant association was found between any of the above SNPs and the risk of developing COPD in this population.

Positive selection genome-wide scans of Tibetan populations revealed the EGLN1 genetic locus encodes prolyl hydroxylase 2 (PHD2), which may allow for adaptive biological changes in humans in response to the low oxy-
<table>
<thead>
<tr>
<th>Variable</th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
<th>p</th>
<th>q^a</th>
<th>p^b</th>
<th>RERI (95% CI)</th>
<th>AP (95% CI)</th>
<th>S</th>
</tr>
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<tbody>
<tr>
<td>Age</td>
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<tr>
<td>≤60 years</td>
<td>0.821 (0.568–1.185)</td>
<td>0.808 (0.555–1.176)</td>
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<td>&gt;60 years</td>
<td>0.958 (0.611–1.504)</td>
<td>0.904 (0.568–1.439)</td>
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<tr>
<td>Sex</td>
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<tr>
<td>Male</td>
<td>0.627 (0.427–0.920)</td>
<td>0.628 (0.414–0.951)</td>
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<tr>
<td>Female</td>
<td>1.134 (0.772–1.664)</td>
<td>1.165 (0.773–1.754)</td>
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<tr>
<td>BMI</td>
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<tr>
<td>&lt;24</td>
<td>0.754 (0.523–1.088)</td>
<td>0.711 (0.481–1.050)</td>
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<tr>
<td>≥24</td>
<td>0.964 (0.647–1.435)</td>
<td>1.022 (0.659–1.582)</td>
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<td>Education level</td>
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<tr>
<td>Primary and below</td>
<td>0.878 (0.654–1.178)</td>
<td>0.886 (0.648–1.212)</td>
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<td>Junior high school and above</td>
<td>0.688 (0.346–1.368)</td>
<td>0.643 (0.296–1.398)</td>
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<td>Hypertension</td>
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<tr>
<td>Yes</td>
<td>0.835 (0.546–1.277)</td>
<td>0.774 (0.488–1.226)</td>
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<tr>
<td>No</td>
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<td>0.878 (0.605–1.273)</td>
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<tr>
<td>Smoking status</td>
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<tr>
<td>Yes</td>
<td>0.614 (0.356–1.058)</td>
<td>0.526 (0.281–0.983)</td>
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<tr>
<td>No</td>
<td>0.918 (0.671–1.254)</td>
<td>0.969 (0.695–1.351)</td>
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<tr>
<td>Drink</td>
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<tr>
<td>Yes</td>
<td>0.361 (0.149–0.878)</td>
<td>0.318 (0.115–0.879)</td>
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<tr>
<td>No</td>
<td>0.938 (0.705–1.248)</td>
<td>0.969 (0.690–1.277)</td>
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</tbody>
</table>

a. Adjustment for multiple comparisons using false discovery rate.

b. Breslow-Day heterogeneity test.

BMI, body mass index; RERI, relative excess risk of interaction; AP, attributable proportion; S, Synergy Index.

A genomic environment found in high plateaus [24]. Alterations in the hypoxia-inducible factor (HIF) pathway were shown to be the mechanism underlying this adaptive change [25–27]. Moreover, the genomic region containing EGLN1 was shown to be one of the strongest selection signals in Tibetans [14]. The EGLN family is itself a member of the larger 2-oxoglutarate and ferrous iron-dependent oxygenase family [28], which has a role in maintaining oxygen.
homeostasis in oxygen-organized metabolism in biological cellular tissues. The *EGLN1* gene is located on human chromosome 1 (1q42.2) [29], which is the actual oxygen receptor in mammals [30]. Several studies have shown that under hypoxic stimulation, *EGLN1* reduces its own activity [31–33], which means that HIF-1α is ineffectively degraded and continues to accumulate [34] and overexpress, thereby regulating related transcription factors. These factors mediate the hypoxic response and include genes that promote glucose uptake, facilitate glycolysis, stimulate angiogenesis, or regulate apoptosis and erythropoiesis [35]. They are particularly important in mediating erythropoiesis, angiogenesis, and lung development, thus contributing to the pathogenesis of diseases such as congenital erythrocytosis, pulmonary hypertension, and COPD [36]. The hypothesis that *EGLN1* is involved in COPD-related responses by regulating HIF-1α in a hypoxic environment is therefore tenable.

Studies conducted by Mishra et al. [34] and Sharma et al. [37] on an Indo-Aryan population from the Tibetan plateau region found a significant difference in expression of the *EGLN1* rs480902 variant between patients with high altitude pulmonary edema (HAPE) and healthy controls (p < 0.05). This is consistent with the findings by Wu et al. [38] on Han Chinese individuals. The study by Mishra et al. [34] also found that arterial oxygen saturation (SaO₂) levels were significantly higher in non-high-altitude pulmonary edema patients and healthy controls compared to high-altitude pulmonary edema patients. Bhandari et al. [39] found that Sherpas carrying *EGLN1* rs12097901 and rs186996510 variant alleles had lower hemoglobin levels compared to wild-type allele carriers. The above finding may be attributed to the high expression of the *EGLN1* gene. This would enhance the blocking effect of hydroxylated proline-catalyzed HIF-1α translational modification at positions 402 and 564 in the structural domain of HIF-1α oxygen-dependent degradation (ODD). This destabilizes HIF-1α, preventing its downstream genes from maintaining cellular oxygen homeostasis and thereby hindering oxygen signaling. The hemoglobin concentration then increases to compensate for the losses associated with the low oxygen environment of the high-altitude plateau.

Online web tools such as NCBI and Ensembl were used in the present study to find the SNP location and functional information for the 4 SNPs investigated here. *EGLN1* rs41303095 A>G is located at the 3′UTR end of *EGLN1* (Chr: 231364492), with no studies so far having reported an association of this polymorphism with any disease. rs480902 C>T is located in the first intronic region of *EGLN1* (Chr: 231395881), which prevents the translocation of HIF-1α to the nucleus and thus hinders oxygen signaling [40,41]. Researchers have reported associations of this locus with diseases such as plateau pulmonary edema, altitude sickness, and chronic mountain sickness [34,37,38,42–45]. rs12097901 C>G is located in exon 1 of *EGLN1* and is a missense mutation (Chr: 231421509) that causes HIF activation [46]. This SNP has been associated with high-altitude acclimatization, acute low-pressure hypoxia, and high-altitude erythropoiesis [14,18,39,46]. rs2153364 G>A is located within the binding site for the *EGLN1* 5′UTR transcription factor c-Ets (Chr: 231424474). This site may affect the expression of *EGLN1*, and therefore its regulation of HIF-1α, by influencing the binding efficiency of the c-Ets transcription factor [47]. Several studies have reported associations of this polymorphism with acute altitude sickness and hemoglobinopenia [47–49].

The prevalence of COPD in individuals aged 40 years or more in the Gannan region was previously found by our group to be 23.4% (20.7%–26.4%) [22]. This is considerably higher than the national incidence of 13.7% (12.1%–15.5%) [50]. Moreover, the current study found that *EGLN1* rs12097901 C>G was associated with the level of pre-medication lung function indexes. These may be related to overexpression of the hypoxia gene *EGLN1* in the hypoxic environment of the high plateau. Another study by our group found that the hematopoietic cytokine EPO rs1617640 G>C variant was associated with COPD susceptibility in a southern Chinese (Guangzhou) population [51], with the C allele being associated with pre-medication lung function. We also found that the hypoxia-inducible factor *EPAS1* rs13419896 G>A SNP could reduce susceptibility to COPD [52]. *EPAS1* and *EGLN1* are two of the preferred candidate genes previously reported in high altitude acclimatization studies in Tibetans [53–55].

In summary, it is reasonable to assume that *EGLN1* may be involved in COPD pathogenesis. However, this study failed to show a statistically significant association between *EGLN1* genetic variants and COPD. This may be due to the screening of only a few loci, insufficient sample size, recall bias in the case-control studies, and lack of quality control in the field work. Further studies will be conducted to overcome these shortcomings and to identify more relevant loci.

### Table 4. Association of different *EGLN1* rs12097901 C>G genotypes with lung function.

<table>
<thead>
<tr>
<th>rs12097901</th>
<th>CC</th>
<th>CG</th>
<th>GG</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-FEV1</td>
<td>2.48 (2.14–3.09)</td>
<td>2.65 (2.20–3.17)</td>
<td>2.51 (2.08–3.17)</td>
<td>0.204</td>
</tr>
<tr>
<td>Pre-FVC</td>
<td>3.03 (2.56–3.84)</td>
<td>3.20 (2.61–3.97)</td>
<td>3.02 (2.49–3.90)</td>
<td>0.278</td>
</tr>
<tr>
<td>Pre-FEV1/Pre-FVC</td>
<td>78.30 (76.60–80.50)</td>
<td>78.60 (76.50–80.30)</td>
<td>77.40 (75.95–78.78)</td>
<td>0.028</td>
</tr>
</tbody>
</table>

a. Rank sum test. FEV1, forced expiratory volume in one second; FVC, forced vital capacity.
5. Conclusions
The present study found that the EGLN1 rs41303095 A>G, rs480902 C>T, rs12097901 C>G, and rs2153364 G>A SNPs were not significantly associated with the susceptibility to COPD in the Gannan Tibetan population.

Abbreviations
COPD, chronic obstructive pulmonary disease; EGLN1, Egl-9 family of hypoxia-inducible factors; GWAS, genome-wide association analysis; SNPs, single nucleotide polymorphisms; FEV1, forceful expiratory volume in 1 second; FVC, forceful lung capacity; MAFs, 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
XZ designed the research study. XL wrote the main manuscript and revised it with JY, PZ. Statistical analysis of the results was done mainly by JY with the participation of XL. CZ, YS, XuW, HL and AL contributed to sample collection for the case-control and prevalence studies, and for electronic medical record entry. ZY provided assistance with experimental design and conduct. PZ has given a lot in the integration and utilization of literature searches. XiW and YW were responsible for the overall study design and quality control as well as directing the study 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 conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Boards of Xi’an Jiaotong University, Faculty of Medicine (Approval No.: XJTU 2016-411) and Guangzhou Medical University (Approval No.: GZMC2007-07-0676). In Furthermore, we interpreted the purpose of the study to all participants at the beginning of the study and obtained their signed informed consent.

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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.fbl2901018.

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