Int J Clin Exp Med 2017;10(7):10866-10873
www.ijcem.com /ISSN:1940-5901/IJCEM0046332

Original Article

Association of *EGLN1* and *EGLN3* single-nucleotide polymorphisms with chronic obstructive pulmonary disease risk in a Chinese population

Si Fang*, Jinfan Qiu*, Huapeng Yu, Huizhen Fan, Xiping Wu, Zekui Fang, Qixiao Shen, Shuyu Chen

*Department of Respiration, Zhujiang Hospital, Southern Medical University, Guangzhou, China. *Equal contributors.*

Received December 11, 2016; Accepted March 31, 2017; Epub July 15, 2017; Published July 30, 2017

Abstract: Hypoxia is implicated in the process of chronic inflammation, which is the characteristic pathogenesis of COPD (chronic obstructive pulmonary disease). EGLNs (EglN prolyl hydroxylase) that connect oxygen sensing to the activation of HIF-1 (hypoxia inducible factor-1) have been proven to play a role in the development of COPD. The objective of this study was to explore the role of *EGLN1* and *EGLN3* SNPs (single-nucleotide polymorphisms) in susceptibility and development of COPD in a Chinese cohort. Seven SNPs were chosen from two genes (*EGLN1* and *EGLN3*) in 217 cases and 447 controls. The relationship between SNPs and COPD risk was analyzed by using genetic model analysis. Among the seven SNPs, rs11156819 in *EGLN3* exhibited a significant association with COPD risk with an OR of 1.392 (95% CI=1.085-1.787, *P*=0.009). In the log-additive model, the minor allele T of rs11156819 in *EGLN3* significantly increased the risk of COPD (OR=1.58, 95% CI=1.14-2.19, *P*=0.006) after adjusting for gender, age and smoking status. Furthermore, the haplotypes TC (rs11156819-rs900358) was associated with increased COPD risk (OR=1.49, 95% CI=1.10-2.03, *P*=0.011). This study suggests that rs11156819 in *EGLN3* gene has a significant association with COPD susceptibility among the Chinese population.

Keywords: *EGLN1, EGLN3*, single-nucleotide polymorphisms, COPD, case-control study

Introduction

Chronic obstructive pulmonary disease (COPD) has been one of the diseases with the highest morbidity and mortality all over the world, resulting in increasing economic and social burden [1]. The pathogenesis character of COPD is the parenchymal tissues destruction and irreversible airflow limitation, which is induced by the chronic inflammatory response [2]. With pulmonary function deterioration and the disease progression, the alveolar hypoxia and hypoxemia are increasingly severe. In addition, it seems that tissue hypoxia plays a vital role in progression and comorbidities of COPD, including cardiovascular disease, lung cancer and pulmonary hypertension [3].

Chronic hypoxemia can stimulate the expressions of hypoxia inducible factor-1 (HIF-1) and a family of oxygen-dependent HIF hydroxylating dioxygenases termed EglN prolyl hydroxylase (EGLNs), both of which mediate the main response to hypoxia. HIF-1 is a pivotal player in mobilizing the cellular adaptation to low oxygen availability [4]. It consists of two subunits: HIF-1α and HIF-1β [5]. In normoxia, HIF-1α is rapidly degraded by hydroxylation pathway [6, 7]. However, theoxygen-dependent hydroxylation of HIF-1α is mediated by a family of EglN prolyl hydroxylase (EGLNs). There are three *EGLN* family members (*EGLN1, EGLN2, EGLN3*) in human and mice [8]. Under hypoxia conditions, EGLNs lose their activity and fail to hydroxylate HIF-1α, which leads to HIF-1α stabilization [9, 10]. As a consequence, HIF-1α speeds up the adaptive changes in cellular metabolism, along with the production of several pro-inflammatory factors after hypoxia [10]. In fact, hypoxia has been proven to participate in the process of chronic inflammation [11] and HIF-1 favors the development of inflammation [12, 13]. EGLNs which provide a link between sensing the concentration of intracel-

lular oxygen and the activation of HIF-1α are supposed to involve in the process of chronic inflammation and COPD.

Cigarette smoking is the most commonly recognized environmental risk factor for COPD [14]. However, not all smokers develop into COPD despite the similar smoking history. Therefore, there are some potential genetic factors which would contribute to the development and severity of COPD [15]. In order to illuminate the pathogenesis of COPD, researchershave conducted many candidate genes over the past few decades. Several novel loci at *CHRNA3/CHRNA5/IREB2* [16] and *HHIP* [17] have been proven to be the risk loci for COPD by GWAS; additional loci have also been identified through larger study at *RAB4B, EGLN2, MIA* and *CYP2A6* [18]. In addition, increasing studies performed on Chinese people have confirmed several susceptible loci associated with COPD such as *HHIP* [19], *RNF150/EGLN2* [20], *CDH13* [21].

Numerous studies have found that some susceptible loci in *EGLN2* increased the risk of COPD [20, 22]. However, few studies have explored the relationship between *EGLN1* and *EGLN3* and the risk of COPD. Therefore, we conducted a case-control study to evaluate the association between seven single-nucleotide polymorphisms (SNPs) in *EGLN1* and *EGLN3* genes and COPD in Chinese population.

Materials and methods

Study subjects

In this study, 217 patients from Zhujiang Hospital were enrolled. Inclusion criteria for participants with COPD were: age ≥40 years, forced expiratory volume in 1 second (FEV1)/forced vital capacity (FVC) <0.7. The severity of COPD was identified with the guidelines of WHO Global initiative for Chronic Obstructive Lung Disease (GOLD) [1]. Exclusion criteria were bronchial asthma, lung cancer, pulmonary tuberculosis, bronchiectasis and other pulmonary diseases. Meanwhile, 447 control subjects without established diagnosis of medical illness and COPD family history were randomly enrolled from the health centers of Zhujiang Hospital during the same period. All of the participants signed informed consent forms at the beginning of the study. This study was approved by the Ethics Committee of Zhujiang Hospital, Southern Medical University.

SNPs selection and genotyping

Seven tag SNPs (rs2009873, rs11156819, rs1680709, rs1680710, rs1750708, rs176-9601, rs900358) from *EGLN1* and *EGLN3*, with minor allele frequencies >5% in the HapMap Chinese Han Beijing population, were selected in this study. According to the previous studies, 5 ml of peripheral blood were collected from each subject and stored at -80°C [20]. According to the manufacturer's instructions, genomic DNA was extracted from whole blood samples using the FlexGene DNA purification kit (Xibao Biotech Co, Shanghai, china). SNPscan™ (Center for Human Genetics Research, Shanghai Genesky Bio-Tech Co, Ltd) was used to test the loci information of samples. The technical personnel of genotyping were blind to the case or control status of samples.

Statistical analysis

Statistical analyses were conducted in SPSS 20.0 (IBM Corporation, Armonk, NY, USA).

Continuous variables were presented as mean ± SD and analyzed by Student's *t*-test. Then, categorical variables were analyzed by χ^2 test. Each SNP was tested for deviation from Hardy-Weinberg equilibrium (HWE) using the x^2 test to compare the observed and expected genotype frequencies among the controls.

Furthermore, the association between all SNPs and the risk of COPD under different genetic models (codominant, dominant, recessive, over-dominant and log-additive) was calculated by SNP stats software [23]. Multivariate logistic regression analysis was used to assess the relationship between each SNPs and different group of COPD (mild/moderate and severe/very severe), and confounders (age, gender and smoking status).

The association between SNPs and COPD phenotypes (FEV1, FEV1/FVC) was analyzed by liner regression analysis. Haploview software (version 4.2) was used to analyze linkage disequilibrium (LD) and haplotype [24]. In all sta-

Table 1. Demographic characteristics of patients and control participants

Variables	Cases (N %)	Controls (N %)	*P*-Value
Sex			0.083[a]
Female	23 (10.6)	30 (6.7)	
Male	194 (89.4)	417 (93.3)	
Age (year)			<0.001[b]
Mean ± SD	70.13±7.88	65.14±9.46	
BMI			<0.001[b]
Mean ± SD	21.79±4.15	23.67±3.15	
Smoking status			<0.001[a]
Nonsmoker	51 (23.5)	365 (81.7)	
Smoker	166 (76.5)	82 (18.3)	
Lungfunction (mean ± SD)			<0.001[b]
FEV1	47.21±21.81	99.01±15.58	
FEV1/FVC	44.90±13.35	76.85±5.75	
Clinical stages			<0.001[b]
Stage I	20 (9.2)		
Stage II	65 (30.0)		
Stage III	73 (33.6)		
Stage IV	59 (27.2)		

P[a]: *P*-values were calculated from two-side chi-square test. *P*[b]: *P*-values were calculated by Student *t*-tests. BMI: body mass index; FEV1: forced expiratory volume in one second; FVC: forcedexpiratory volume.

tistical tests, a two-side significant level of *P* less than 0.05 was used.

Results

The demographic and baseline characteristics of the study are summarized in **Table 1**. Totally, 664 subjects were enrolled in our study, 217 patients with COPD and 447 control samples. There was no significant difference in gender (*P*=0.083) between the cases and controls. However, age, BMI and smoking status between the two groups were significantly different (*P*<0.001). Among patients with COPD (23 women and 194 men), most of them are in moderate to severe stage of this disease (63.6% in stage II-III).

Table 2 summarized the loci information and allele frequencies of all selected SNPs in both cases and controls. All seven tested SNPs were in Hardy-Weinberg equilibrium in control group (*P*>0.1). Furthermore, only rs11156819 on 14-q13.1 was associated with a higher risk of developing the disease (OR of 1.392, 95% CI= 1.085-1.787, *P*=0.009), while no significant differences were observed in other six SNPs.

Then the association between selected SNPs and the COPD risk was explored by genetic models (condominant, dominant, recessive, overdominant and log-additive). Our results showed that the minor allele T of rs11156819 in *EGLN3* (**Table 4**) was noticeably associated with an increased risk of COPD in the condominant (TC vs CC; TT vs CC) before (OR=1.48, 95% CI=1.05-2.09; OR= 1.75, 95% CI=0.99-3.488, *P*= 0.033, respectively) and after (OR= 1.80, 95% CI=1.14-2.84; OR=2.14, 95% CI=1.01-4.54, *P*=0.016, respectively) adjustment. Simultaneously, in the dominant model (T/C-T/T vs C/C), the T/C-T/T genotype was associated with an increased risk of developing COPD before (OR=1.53, 95% CI=1.10-2.12; OR=1.86, 95% CI=1.21-2.87, *P*=0.005, respectively) correcting for age, gender and smoking status. The genotype "T/C" in the over-dominant model, increased COPD risk with an OR of 1.58 (95% CI=1.02-2.45, *P*=0.038) after adjustment. Additionally, in the log-additive model, rs11156819 showed a significant association with the risk of COPD before (OR=1.38, 95% CI=1.08-1.76, *P*=0.011) and after adjustment (OR=1.58, 95% CI=1.14-2.19, *P*=0.006). Compared with *EGLN3*, there was no evidence that rs2009873 in *EGLN1* influenced the risk of COPD using genotype model both the crude analysis and the analysis for age, gender, and smoking status (**Table 3**).

As shown in **Table 5**, the frequencies of 4 SNPs between mild/moderate and sever/very sever subgroup were compared to illuminate the association between *EGLN1/EGLN3* genes and severity of COPD. However, there were no significant differences among these SNPs. Then, we analysed the relationship between 7 SNPs and COPD phenotypes under the assumption of a dominant mode of inheritance. Our data demonstrated that none of the SNPs was significantly associated with lung function among cases only or all subjects (**Table 6**).

Meanwhile, we performed the LD and haplotype analysis of the SNPs in *EGLN3* in patients

Table 2. Characteristics of candidate SNPs and their association with the risk of COPD

SNPID	Gene name	Chromosome	MAF (case)	MAF (control)	*P*-HWE	OR (95% CI)	*P*-value
rs2009873	EGLN1	1q42.1	0.475	0.468	0.808	1.029 (0.818-1.295)	0.808
rs11156819	EGLN3	14q13.1	0.332	0.263	0.315	1.392 (1.085-1.787)	0.009*
rs1680709	EGLN3	14q13.1	0.039	0.029	0.527	1.361 (0.730-2.536)	0.330
rs1680710	EGLN3	14q13.1	0.037	0.035	0.558	1.607 (0.577-1.973)	0.836
rs1750708	EGLN3	14q13.1	0.037	0.036	0.603	1.032 (0.560-1.903)	0.919
rs1769601	EGLN3	14q13.1	0.032	0.035	0.558	0.929 (0.489-1.765)	0.822
rs900358	EGLN3	14q13.1	0.247	0.244	0.914	1.015 (0.777-1.324)	0.915

*$P<0.05$ indicates statistical significance; *P*-values were calculated from two-side chi-square test; SNP: single-nucleotide polymorphisms; MAF: minor allele frequency; HWE: Hardy-weinberg equilibrium; OR: odds ratio; 95% CI: 95% confidence interval.

Table 3. Association between rs2009873 genotypes and COPD risk under different genetic models

Model	Genotype	Control	Case	Without adjustment		With adjustment	
				P^a	OR (95% CI)	P^b	OR (95% CI)
Condominant	T/T	128 (28.6%)	61 (28.1%)	0.996	1.00	0.995	1.00
	T/C	220 (49.2%)	106 (48.8%)		1.01 (0.69-1.48)		0.93 (0.56-1.53)
	C/C	99 (22.2%)	50 (23.1%)		1.06 (0.67-1.67)		0.95 (0.52-1.74)
Dominant	T/T	128 (28.6%)	61 (28.1%)	0.888	1.00	0.776	1.00
	T/C-C/C	319 (71.4%)	156 (71.9%)		1.03 (0.72-1.47)		0.93 (0.58-1.49)
Recessive	T/T-T/C	348 (77.9%)	167 (77.0%)	0.796	1.00	0.999	1.00
	C/C	99 (22.1%)	50 (23.0%)		1.05 (0.72-1.55)		1.0 (0.60-1.67)
Over-dominant	T/T-C/C	227 (50.8%)	111 (51.2%)	0.929	1.00	0.797	1.00
	T/C	220 (49.2%)	106 (48.8%)		0.99 (0.71-1.36)		0.95 (0.62-1.45)
Log-additive	-	-	-	0.81	1.031 (0.82-1.29)	0.86	0.97 (0.72-1.31)

P^a: *P*-values were calculated from two-side chi-square test; P^b: *P*-values were calculated by multivariate logistic regression analysis adjusted for age, sex and smoking status; COPD: chronic obstructive pulmonary; OR: odds ratio; 95% CI: 95% confidence interval.

Table 4. Association between rs11156819 genotypes and COPD risk under different genetic models

Model	Genotype	Control	Case	Without adjustment		With adjustment	
				P^a	OR (95% CI)	P^b	OR (95% CI)
Condominant	C/C	247 (55.3%)	97 (44.7%)	0.033*	1.00	0.016*	1.00
	T/C	165 (36.9%)	96 (44.2%)		1.48 (1.05-2.09)		1.80 (1.14-2.84)
	T/T	35 (7.8%)	24 (11.1%)		1.75 (0.99-3.09)		2.14 (1.01-4.54)
Dominant	C/C	247 (55.3%)	97 (44.7%)	0.011*	1.00	0.005*	1.00
	T/C-T/T	200 (44.7%)	120 (55.3%)		1.53 (1.10-2.12)		1.86 (1.21-2.87)
Recessive	C/C-T/C	412 (92.2%)	193 (88.9%)	0.178	1.00	0.183	1.00
	T/T	35 (7.8%)	24 (11.1%)		1.46 (0.85-2.53)		1.63 (0.96-3.34)
Over-dominant	C/C-T/T	282 (63.1%)	121 (55.8%)	0.071	1.00	0.038*	1.00
	T/C	165 (36.9%)	96 (44.2%)		1.36 (0.98-1.89)		1.58 (1.02-2.45)
Log-additive	-	-	-	0.011*	1.38 (1.08-1.76)	0.006*	1.58 (1.14-2.19)

*$P<0.05$ indicates statistical significance; P^a: *P*-values were calculated from two-side chi-square test; P^b: *P*-values were calculated by multivariate logistic regression analysis adjusted for age, sex and smoking status. COPD: chronic obstructive pulmonary; OR: odds ratio; 95% CI: 95% confidence interval.

with COPD and control samples. The values of LD between SNPs are shown in **Figure 1**. Based on LD determinations, rs900853 was in complete LD (D'=1) with the other SNPs except rs11156819. Rs1750708, rs1680710 and rs1769601 were in moderate LD (D'=95, 95 and 93 respectively). We estimated the frequencies of haplotypes constructed from phased multi-

Table 5. Genotype frequencies of SNPs and odd ratios in COPD subgroups

SNPID	Genotype	Mild/moderate (n=85)	Severe/very severe (n=132)	P^a	P^b	OR (95% CI)
rs2009873	T/T	18 (21.2%)	43 (32.6%)	0.055	0.095	1
	T/C	50 (58.8%)	56 (42.4%)			0.487 (0.239-0.989)
	C/C	17 (20.0%)	33 (25.0%)			0.812 (0.346-1.904)
rs11156819	C/C	40 (47.1%)	57 (43.2%)	0.149	0.198	1
	T/C	32 (37.6%)	64 (48.5%)			1.342 (0.728-2.475)
	T/T	13 (15.3%)	11 (8.3%)			0.581(0.227-1.487)
rs1680709	A/A	75 (88. 6%)	127 (96.2%)	0.068	0.067	1
	A/G	9 (10.2%)	4 (3.0%)			0.250 (0.072-0.866)
	G/G	1 (1.2%)	1 (0.8%)			0.474 (0.029-7.481)
rs900358	C/C	46 (54.1%)	77 (58.3%)	0.232	0.332	1
	C/A	31 (36.5%)	50 (37.9%)			0.992 (0.505-1.683)
	A/A	8 (9.4%)	5 (3.8%)			0.399 (0.117-1.359)

P^a: *P*-values were calculated from two-side chi-square test; P^b: *P*-values were calculated by multivariate logistic regression analysis adjusted for age, sex and smoking status. COPD: chronic obstructive pulmonary; OR: odds ratio; 95% CI: 95% confidence interval.

Table 6. Genetic association between SNPs and pulmonary function

SNPID	FEV1% predicted	FEV1/FVC	FEV1% predicted	FEV1/FVC
	P^a (case only)	P^b (case only)	P^a (all subjects)	P^b (all subjects)
rs2009873	0.269	0.198	0.737	0.066
rs111568119	0.330	0.077	0.797	0.183
rs1680709	0.325	0.441	0.891	0.568
rs1680710	0.325	0.441	0.524	0.662
rs1750708	0.192	0.249	0.640	0.363
rs1769601	0.085	0.063	0.767	0.478
rs900358	0.382	0.951	0.282	0.935

P^a: *P*-values were calculated by liner regression analysis adjusted for age, sex and smoking status. P^b: *P*-values were calculated including all subjects by liner regression analysis adjusted for age, sex, smoking status and COPD case/control status.

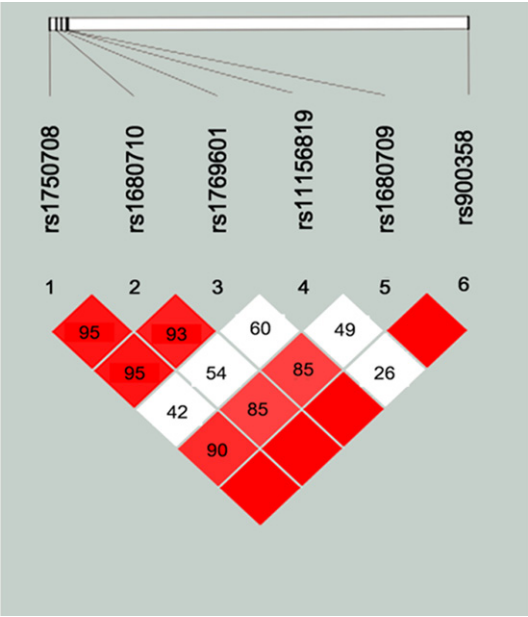

Figure 1. Linkage disequilibrium (LD) for the EGLN3 SNPs genotyped in this study. The LD plots were generated by Haploview software 4.2. LD values are shown as r^2 and LD block was defined according to the confidence intervals.

locus genotypes in *EGLN3*. As shown in **Table 7**, haplotype TC (rs11156819-rs900853) was associated with an increased risk of COPD (OR=1.49, 95% CI=1.10-2.03, *P*=0.011).

Discussion

Although the pathogenesis of COPD remains incompletely elucidated, the interaction between genetic susceptibility and exposure to environmental stimuli is well established [25]. Recently, rs3733829 in *EGLN2* has been proven to increase the risk of COPD, whereas *EGLN1* and *EGLN3* gene polymorphisms have

Table 7. EGLN3 haplotype association with COPD susceptibility

Haplotype	Freq (case)	Freq (control)	*P*-value	OR (95% CI)
rs11156819 (T/C)-rs900358 (A/C)				
CC	0.475	0.543	--	1.00
TC	0.278	0.213	0.011*	1.49 (1.10-2.03)
CA	0.193	0.194	0.43	1.15 (0.82-1.61)
TA	0.050	0.054	0.57	1.20 (0.64-2.25)

*$P<0.05$ indicates statistical significance; *P*-values were calculated from two-side chi-square test; COPD: chronic obstructive pulmonary; OR: odds ratio; 95% CI: 95% confidence interval.

yet to be explored. In this case-control study, we analyzed the association of seven SNPs (rs2009873, rs11156819, rs1680109, rs1680710, rs1750708, rs1769601, rs900358) in *EGLN1* and *EGLN3* with COPD risk in Chinese population. The results showed that rs11156819 in *EGLN3* was associated with the risk of COPD in the allelic and genetic model. Therefore, it suggested that SNPs of *EGLN3* could play a vital role in the pathogenesis of COPD.

To be specific, the minor allele T in rs11156819 was associated with a1.392-fold increment in the risk of COPD in allelic model. Under the condominant genetic model, in comparison with the C/C, the T/C or T/T model was related with an increased COPD risk. Furthermore, the similar results were also observed in the dominant and over-dominant models. In addition to the allelic and genetic approaches, we also performed haplotype analysis. The results indicated that the haplotype TC in rs11156819 and rs900358 was associated with a 49% increase in COPD risk, while the other haplotypes showed no significant difference. In general, the minor allele T in rs11156819 in *EGLN3* is a risk factor of COPD. In light of patients with COPD in different severity status in our study, further investigation was carried out to explore the correlation of these SNPs with the severity of COPD (lung function impairments). Nevertheless, we did not find associations between the selected SNPs and the severity of COPD before or after adjusting for gender, age and smoking status. At present, some researchers put forward that many COPD-related quantitative traits are more sensitive in detecting the disease status than the conventional genetic studies which use binary disease status as primary phenotypes [26]. Therefore, quantitative genetic association analysis was performed to deepen the association between candidate SNPs and FEV1% predicted or FEV1/FVC. It appears that none of these SNPs showed significant association with COPD-related quantitative traits. These contradictory results may be resulted from cross-sectional study design of our research, and lung function changing frequently.

The three HIF-prolyl-hydroxylases, termed prolyl hydroxylase domains (*PHD1*, *PHD2*, and *PHD3*), are also known as *EGLN2, EGLN1* and *EGLN3*, respectively. The study reveals that *EGLN1* is the main oxygen sensor and acts as a leading role in the regulation of HIF activity in hypoxia [27]. In our study, we failed to find the association between rs2009873 in *EGLN1* and the risk of COPD in the allelic model and genetic model. On account of the minor allele frequencies >5%, we chose only one SNP in *EGLN1*, which may not provide a comprehensive knowledge between them.

However, other study found that *EGLN3* is the isoform which plays a major role in regulation of HIF activity under chronic hypoxia [28-31] with low expression [32]. In order to compensate for the reduced activity, *EGLN3* strongly upregulates its expression and retains much of its enzymatic activity under hypoxia [33, 34]. Furthermore, *EGLN3* showed the multiple functions with a widest range of other hydroxy lation targets besides HIF [35]. In our study, rs11156819, locating in the exon of the *EGLN3* gene, was found to be associated with increased the risk of COPD in the allelic model and genetic model. The haplotype TC increased the risk of COPD by 1.49 fold. Although our study firstly reports the association between rs11156819 in EGLN3 and the pathogenesis of COPD, the underlying mechanismis not completely understood and further exploration is needed.

To our knowledge, this is the first study to estimate the relationship between *EGLN1* and

EGLN3 genes and COPD. We found the SNPs of *EGLN3* associated with the pathogenesis of COPD. Nevertheless, there are some limitations in our study. Firstly, the sample size was relatively small. The numbers of participants were insufficient to evaluate whether rs111-56819 is more significant in smokers via conducting population stratification of smokers. Secondly, age, BMI and smoking status were not completely matched between cases and controls, which might have an impact on the results. Finally, we did not estimate the rare genetic variation especially in *EGLN1*, which may be a vital contributor of susceptibility of COPD. In our future study, more patients and candidate genes should be enrolled and explored to further elucidate the genetic pathogenesis of COPD.

Acknowledgements

We acknowledge the all participants for making this work possible.

Disclosure of conflict of interest

None.

Address correspondence to: Huapeng Yu, Department of Respiration, Zhujiang Hospital of Southern Medical University, NO 253 Middle Industry Avenue, Guangzhou, China. E-mail: huapengyuzhujiang@126.com

References

[1] Vestbo J, Hurd SS, Agustí AG, Jones PW, Vogelmeier C, Anzueto A, Barnes PJ, Fabbri LM, Martinez FJ, Nishimura M, Stockley RA, Sin DD, Rodriguez-Roisin R. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2013; 187: 347-365.

[2] Hogg JC. Pathophysiology of airflow limitation in chronic obstructive pulmonary disease. Lancet 2004; 364: 709-21.

[3] Baz-Dávila R, Espinoza-Jiménez A, Rodríguez-Pérez MDC, Zulueta J, Varo N, Montejo Á, Almeida-González D, Aguirre-Jaime A, Córdoba-Lanús E, Casanova C. Role of HIF1A, VEGFA and VEGFR2 SNPs in the susceptibility and progression of COPD in a Spanish population. PLoS One 2016; 11: e0154998.

[4] Semenza GL. HIF-1: mediator of physiological and pathophysiological responses to hypoxia. J Appl Physiol (1985) 2000; 88: 1474-80.

[5] Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci U S A 1995; 92: 5510-4.

[6] Sutter CH, Laughner E, Semenza GL. Hypoxia-inducible factor 1alpha protein expression is controlled by oxygen-regulated ubiquitination that is disrupted by deletions and missense mutations. Proc Natl Acad Sci U S A 2000; 97: 4748-53.

[7] Huang LE, Gu J, Schau M, Bunn HF. Regulation of hypoxia-inducible factor 1alpha is mediated by an O2-dependent degradation domain via the ubiquitin-proteasome pathway. Proc Natl Acad Sci U S A 1998; 95: 7987-92.

[8] Zheng X, Zhai B, Koivunen P, Shin SJ, Lu G, Liu J, Geisen C, Chakraborty AA, Moslehi JJ, Smalley DM, Wei X, Chen X, Chen Z, Beres JM, Zhang J, Tsao JL, Brenner MC, Zhang Y, Fan C, DePinho RA, Paik J, Gygi SP, Kaelin WJ, Zhang Q. Prolyl hydroxylation by EglN2 destabilizes FOXO3a by blocking its interaction with the USP9x deubiquitinase. Genes Dev 2014; 28: 1429-44.

[9] Kaelin WG. Proline hydroxylation and gene expression. Annu Rev Biochem 2005; 74: 115-28.

[10] Kaelin WJ, Ratcliffe PJ. Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. Mol Cell 2008; 30: 393-402.

[11] Taylor CT. Interdependent roles for hypoxia inducible factor and nuclear factor-kappaB in hypoxic inflammation. J Physiol 2008; 586: 4055-9.

[12] Hellwig-Burgel T, Rutkowski K, Metzen E, Fandrey J, Jelkmann W. Interleukin-1beta and tumor necrosis factor-alpha stimulate DNA binding of hypoxia-inducible factor-1. Blood 1999; 94: 1561-7.

[13] Scharte M, Han X, Bertges DJ, Fink MP, Delude RL. Cytokines induce HIF-1 DNA binding and the expression of HIF-1-dependent genes in cultured rat enterocytes. Am J Physiol Gastrointest Liver Physiol 2003; 284: G373-84.

[14] Hooper R, Burney P, Vollmer WM, McBurnie MA, Gislason T, Tan WC, Jithoo A, Kocabas A, Welte T, Buist AS. Risk factors for COPD spirometrically defined from the lower limit of normal in the BOLD project. Eur Respir J 2012; 39: 1343-53.

[15] Wan ES, Silverman EK. Genetics of COPD and emphysema. Chest 2009; 136: 859-66.

[16] Ober C, Tan Z, Sun Y, Possick JD, Pan L, Nicolae R, Radford S, Parry RR, Heinzmann A, Deichmann KA, Lester LA, Gern JE, Lemanske RJ, Nicolae DL, Elias JA, Chupp GL. Effect of variation in CHI3L1 on serum YKL-40 level, risk of asthma, and lung function. N Engl J Med 2008; 358: 1682-91.

[17] Pillai SG, Ge D, Zhu G, Kong X, Shianna KV, Need AC, Feng S, Hersh CP, Bakke P, Gulsvik A, Ruppert A, Lodrup CK, Roses A, Anderson W, Rennard SI, Lomas DA, Silverman EK, Goldstein DB; ICGN Investigators. A genome-wide association study in chronic obstructive pulmonary disease (COPD): identification of two major susceptibility loci. PLoS Genet 2009; 5: e1000421.

[18] Cho MH, Castaldi PJ, Wan ES, Siedlinski M, Hersh CP, Demeo DL, Himes BE, Sylvia JS, Klanderman BJ, Ziniti JP, Lange C, Litonjua AA, Sparrow D, Regan EA, Make BJ, Hokanson JE, Murray T, Hetmanski JB, Pillai SG, Kong X, Anderson WH, Tal-Singer R, Lomas DA, Coxson HO, Edwards LD, MacNee W, Vestbo J, Yates JC, Agusti A, Calverley PM, Celli B, Crim C, Rennard S, Wouters E, Bakke P, Gulsvik A, Crapo JD, Beaty TH, Silverman EK; ICGN Investigators; ECLIPSE Investigators; COPDGene Investigators. A genome-wide association study of COPD identifies a susceptibility locus on chromosome 19q13. Hum Mol Genet 2012; 21: 947-57.

[19] Wang B, Zhou H, Yang J, Xiao J, Liang B, Li D, Zhou H, Zeng Q, Fang C, Rao Z, Yu H, Ou X, Feng Y. Association of HHIP polymorphisms with COPD and COPD-related phenotypes in a Chinese Han population. Gene 2013; 531: 101-5.

[20] Ding Y, Niu H, Yang H, Sun P, Chen Y, Duan M, Xu D, Xu J, Jin T. EGLN2 and RNF150 genetic variants are associated with chronic obstructive pulmonary disease risk in the Chinese population. Int J Chron Obstruct Pulmon Dis 2015; 10: 145-51.

[21] Yuan YM, Zhang JL, Xu SC, Ye RS, Xu D, Zhang Y, Zhang YJ, Chen YL, Liu YL, Su ZG. Genetic variants of CDH13 determine the susceptibility to chronic obstructive pulmonary disease in a Chinese population. Acta Pharmacol Sin 2016; 37: 390-7.

[22] Lamontagne M, Couture C, Postma DS, Timens W, Sin DD, Pare PD, Hogg JC, Nickle D, Laviolette M, Bosse Y. Refining susceptibility loci of chronic obstructive pulmonary disease with lung eqtls. PLoS One 2013; 8: e70220.

[23] Sole X, Guino E, Valls J, Iniesta R, Moreno V. SNPStats: a web tool for the analysis of association studies. Bioinformatics 2006; 22: 1928-9.

[24] Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005; 21: 263-5.

[25] Peter J, Barnes DS. Chronic obstructive pulmonary disease. N Engl J Med 2000; 343: 269-280.

[26] An L, Lin Y, Yang T, Hua L. Exploring the interaction among EPHX1, GSTP1, SERPINE2, and TGFB1 contributing to the quantitative traits of chronic obstructive pulmonary disease in Chinese Han population. Human Genomics 2016; 10: 3.

[27] Berra E, Benizri E, Ginouves A, Volmat V, Roux D, Pouyssegur J. HIF prolyl-hydroxylase 2 is the key oxygen sensor setting low steady-state of HIF-1 in normoxia. EMBO J 2003; 22: 4082-4090.

[28] D'Angelo G, Duplan E, Boyer N, Vigne P, Frelin C. Hypoxia up-regulates prolyl hydroxylase activity: a feedback mechanism that limits HIF-1 responses during reoxygenation. J Biol Chem 2003; 278: 38183-7.

[29] Del PL, Castellanos MC, Temes E, Martin-Puig S, Cuevas Y, Olmos G, Landazuri MO. The von Hippel Lindau/hypoxia-inducible factor (HIF) pathway regulates the transcription of the HIF-proline hydroxylase genes in response to low oxygen. J Biol Chem 2003; 278: 48690-5.

[30] Marxsen JH, Stengel P, Doege K, Heikkinen P, Jokilehto T, Wagner T, Jelkmann W, Jaakkola P, Metzen E. Hypoxia-inducible factor-1 (HIF-1) promotes its degradation by induction of HIF-alpha-prolyl-4-hydroxylases. Biochem J 2004; 381: 761-7.

[31] Aprelikova O, Chandramouli GV, Wood M, Vasselli JR, Riss J, Maranchie JK, Linehan WM, Barrett JC. Regulation of HIF prolyl hydroxylases by hypoxia-inducible factors. J Cell Biochem 2004; 92: 491-501.

[32] Appelhoff RJ, Tian YM, Raval RR, Turley H, Harris AL, Pugh CW, Ratcliffe PJ, Gleadle JM. Differential function of the prolyl hydroxylases PHD1, PHD2, and PHD3 in the regulation of hypoxia-inducible factor. J Biol Chem 2004; 279: 38458-65.

[33] Ginouves A, Ilc K, Macias N, Pouyssegur J, Berra E. PHDs overactivation during chronic hypoxia "desensitizes" HIFalpha and protects cells from necrosis. Proc Natl Acad Sci U S A 2008; 105: 4745-50.

[34] Stiehl DP, Wirthner R, Koditz J, Spielmann P, Camenisch G, Wenger RH. Increased prolyl 4-hydroxylase domain proteins compensate for decreased oxygen levels. Evidence for an autoregulatory oxygen-sensing system. J Biol Chem 2006; 281: 23482-91.

[35] Jaakkola PM, Rantanen K. The regulation, localization, and functions of oxygen-sensing prolyl hydroxylase PHD3. Biol Chem 2013; 394: 449-57.