Genetic polymorphisms of CYP2C9/CYP2C19 in chronic obstructive pulmonary disease

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Abstract

Background : Chronic obstructive pulmonary disease (COPD) has a high incidence in elderly and significantly affects the quality of life. *CYP2C9* and *CYP2C19* have an important role in tobacco-related diseases and inflammatory reaction. Thus, in this study we aims to investigate the association between *CYP2C9* / *CYP2C19* polymorphisms and the risk of COPD.

Methods: A total of 821 subjects were recruited which including 313 COPD patients and 508 healthy controls. Seven SNPs of *CYP2C9* / *CYP2C19* were selected for genotyping. The Odds ratios (ORs) and 95% confidence interval (95% CI) were calculated using a logistic regression analysis to evaluate the association between COPD risk and *CYP2C9* / *CYP2C19* polymorphisms.

Results: The rs9332220 of *CYP2C9* "A" allele was associated with reducing COPD risk (OR = 0.64, 95% CI = 0.43 – 0.94, p = 0.021). And rs11853758 "G" allele carrier could significantly decrease 0.35-fold COPD risk compared with T allele carrier (OR = 0.65, 95% CI = 0.45 – 0.96, p = 0.027). Furthermore, gender-based stratification analysis showed that rs9332220 and rs11853758 polymorphisms were associated with risk of COPD in males.

Conclusions: Our study firstly reported the effects of *CYP2C9* and *CYP2C19* genetic variants on COPD risk. We found that rs9332220 in *CYP2C9* and rs11853758 in *CYP2C19* were associated with a reduced susceptibility to COPD.

Background

Chronic obstructive pulmonary disease (COPD) has a high incidence in older people and significantly affects the quality of life. The characterized of COPD is airflow limitation. That is an incompletely reversible chronic persistent inflammatory process¹. Smoking is the major environmental risk factor². Only 10-15% of smokers end up with COPD³. The COPD is more common in people who have a COPD family history⁴. The COPD was also showed a high risk in identical twins than fetal twins⁵, which means genetic factor play a key role in COPD etiology⁶⁻⁸. Therefore, to identify the genetic factor about smoking and inflammatory in COPD is beneficial to early prevention and accurate diagnosis of COPD.

The cytochrome P450 family 2 subfamily C (*CYP2C*) is a key player in the generation of EETs, the enzymes *CYP2C9* and *CYP2C19* are the major *CYP2C* involved in epoxyeicosatrienoic acids (EETs) production^{9,10}. The *CYP2C19* metabolize arachidonic acid (AA) to produce EETs, the EETs anti-inflammatory actions possess an established protective effect on human cardiovascular system¹¹. The *CYP2C9* enzymes have been detected not only in liver-located but also in lungs, pancreas, stomach and kidneys^{12,13}. *CYP2C9* can metabolize polycyclic aromatic hydrocarbons in tobacco smoke and thereby generate disease-causing metabolites. Genetic variation in genes coding for *CYP9C9* enzymes has shown changes in enzyme activity that affect metabolite levels, resulting in a potential risk of disease, suggesting that *CYP2C9* plays a key role in tobacco-related diseases¹⁴.

In consideration of the *CYP2C9* and *CYP2C19* have an important role in tobacco-related diseases and inflammatory reaction, therefore in this study we did the association study between the *CYP2C9/CYP2C19* polymorphism and the risk of COPD. A total of four SNPs (rs10509679, rs1934967, rs1234968 and rs9332220) of *CYP2C9* and three SNPs (rs111853758, rs4494250 and rs75665761) of *CYP2C19* were enrolled in this study. This study may provide the new marker for diagnosis of COPD.

Methods

Study population

821 participants were recruited from the Hainan general hospital for genotyping, which included 313 COPD patients and 508 healthy controls. According to the related literature report¹⁵, COPD patients were diagnosed based on the multiple examinations results including the ratio of forced expiratory volume in 1 second/forced vital capacity (FEV₁/FVC ratio < 70% and FEV₁ < 80% predicted). The healthy controls were collected from physical examination center of Hainan General Hospital and all of them exhibited normal pulmonary function (FEV₁/FVC ratio > 70% and FEV₁ > 80% predicted).

This study was approved by the ethics committee of Hainan General Hospital, and all participants signed the informed consent. The characteristic information of cases and controls were showed in Table 1.

Selection and genotyping of SNPs

Based on the database of 1000 genomes project (www.internationalgenome.org/), the SNP data of *CYP2C9* and *CYP2C19* were download. Firstly, we chose the SNPs based on $r^2 > 0.8$, $MAF > 0.05$ in global population and the linkage disequilibrium of SNPs been detected by Haploview software. Secondly, the primers of the selected SNPs were designed by Agena on-line software. Four SNPs (rs10509679, rs1934967, rs1234968 and rs9332220) of *CYP2C9* and three SNPs (rs111853758, rs4494250 and rs75665761) of *CYP2C19* linkage disequilibrium and well-designed primer SNPs were chosen.

Genomic DNA of all participants was extracted from peripheral blood using GoldMag-Mini whole blood genomic DNA purification kit (GoldMag Co. Ltd. Xi'an City, China). The Agena on-line software was used to design the primers (<https://agenacx.com/online-tools/>), and the primers of this study were listed in table 2. The genotyping of each SNP was detected by Agena MassARRAY platform (Agena Bioscience, SanDiego, CA, USA).

Statistical analysis

Age was displayed as mean $\pm$ standard deviation. The number of gender and smoking status were respectively statistics in cases and controls. Hardy-Weinberg equilibrium (HWE) of each SNP was evaluated by exact test. The p value less than 0.05 were defined statistically significant. The odds ratios (ORs) and 95% confidence interval (95% CI) were calculated using a logistic regression analysis. The Microsoft Excel and PLINK software were used analysis the relationship between the COPD risk and SNPs.

Results

313 COPD patients and 508 healthy controls were enrolled in this study. The average age of cases was 60 years old and the controls were nearly 72 years old. The average age between cases and controls have a significant difference ($p < 0.001$). In order to reduce the influence of age on the results, we adjusted the age in the later analysis. We also collected the sex, smoking status, BMI index and FEV_1 /FVC information of cases and controls (Table 1).

The basic information of SNPs from *CYP2C9* and *CYP2C19* were presented in Table 3. Rs9332220 "A" allele was associated with a reduced COPD susceptibility (OR = 0.64, 95% CI = 0.43 - 0.94, $p = 0.021$), at the same time, rs11853758 "G" allele carrier with significant decreased risk 0.35-fold of COPD compared with T allele carrier (OR = 0.65, 95% CI = 0.45 - 0.96, $p = 0.027$).

The SNPs rs9332220 and rs11853758 were associated with COPD susceptibility under allele model, but the association between SNPs rs9332220 and rs11853758 and COPD risk under various genetic models were not significant (data not shown). After adjusted for age and sex, we found that the SNP loci rs1934968 AA genotype was not associated with COPD under allele model but significant associated with COPD under recessive model (OR = 0.54, 95% CI = 0.30 - 0.96, $p = 0.037$, Table 4).

As is shown in Table 5, a gender-based stratification analysis was performed. SNP loci rs9332220 of *CYP2C9* "A" allele compared with "G" allele were associated with an decreased risk of COPD in the males (OR = 0.59, 95% CI = 0.37 - 0.92, $p = 0.020$), SNP loci rs111853758 of *CYP2C19* "G" allele carriers were also associated with decreasing risk of COPD under allele model (OR = 0.62, 95% CI = 0.40 - 0.96, $p = 0.032$), but another two SNPs were not associated with COPD risk in females.

Discussion

In current study, we found that the rs9332220 "A" allele of *CYP2C9* and rs11853758 "G" allele of *CYP2C19* apparently associated with a decreased risk of COPD based on 821 participants from Hainan population. Moreover, rs9332220 and rs11853758 may influence the COPD risk of males in gender stratification analysis.

COPD is a complex disease caused by interaction of environmental and genetic factors. Several environmental risk factors for COPD have been identified ^{16,17}, While the genetic risk factors were less well understood. The cytochrome P450 proteins were catalyzed many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids ^{18,19}. In the genetic polymorphisms of

cytochrome P450 in COPD, Chen et al. found that *CYP1A1* is an independent risk factor for very severe COPD²⁰. Yang et al. also reported that *CYP1A1* polymorphisms were associated with COPD susceptibility²¹, and Wang et al meta-analysis proved that *CYP1A1* polymorphism might play a role in COPD risk among Asian population²². Seo et al. suggested that *CYP3A4* may play a role in airway injury of smokers²³. All CYP enzymes share a common structure and similar function in the same way¹¹. Thus, the association between polymorphism of *CYP2C9* and *CYP2C19* and COPD were assessed in this study.

CYP2C9 and *CYP2C19* gene were located within a cluster of cytochrome P450 genes on chromosome 10q24. Previous studies reported the polymorphisms of *CYP2C9* and *CYP2C19* and risk of coronary heart disease (CHD) in Russian population²⁴. In one study, bladder cancer decreased risk was associated with *CYP2C9* variant loci carriers²⁵. Combined with this study, *CYP2C9* not only associated with tobacco-related cancers but also associated with tobacco-related COPD. Kamata et al. noted an up-regulation of *CYP2C19* mRNA expressions in Alveolar epithelial type II (ATII) cells isolated from patients with COPD compared with smokers without COPD²⁶. Kamata et al.'s study may have important implications for the research of COPD pathogenesis. Our findings firstly revealed that rs11853758 of *CYP2C19* was obviously related to COPD susceptibility.

The CYP epoxygenases catalyze the epoxidation of the intracellular AA that gives rise to corresponding EETs²⁷. EETs have anti-inflammatory, antiapoptotic, and antioxidative activities, and autophagy is believed to be involved in the pathogenesis of COPD²⁸. EETs may protect the heart and liver by regulating autophagy²⁹. The SNPs rs9332220 and rs11853758 polymorphisms in *CYP2C9* and *CYP2C19* may regulate the epoxygenases activity and influence the EETs production. It may be protective property of COPD by inhibiting autophagy.

There are some limitations in our study. The sample size is relatively small. In the future, we need to expand the sample size to verify our results. It's necessary to study how *CYP2C9* and *CYP2C19* polymorphism influence the underlying pathogenesis of COPD for further study. Despite the above limitations, our present works provide the available evidence of *CYP2C9* and *CYP2C19* gene with COPD for the future study.

Conclusions

In conclusion, our present work indicated that rs9332220 in *CYP2C9* and rs11853758 in *CYP2C19* were associated with a reduced susceptibility to COPD.

Abbreviations

COPD: Chronic obstructive pulmonary disease; ORs: Odds ratios; 95% CI: 95% confidence interval; CYP2C: Cytochrome P450 family 2 subfamily C; EETs: Epoxyeicosatrienoic acids; AA: Arachidonic acid; FEV₁: Forced expiratory volume in 1 second; FVC: Forced vital capacity; HWE: Hardy-Weinberg equilibrium; CHD: Coronary heart disease; ATII: Alveolar epithelial type II; BMI: Body mass index.

Declarations

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Hainan General Hospital and with the 1964 Helsinki declaration. Informed consent was obtained from all individual participants included in the study.

Consent for publication

Not applicable.

Availability of data and materials

All the data regarding the findings are available within the manuscript. Anyone who is interested in the information should contact the corresponding author.

Competing interests

All authors declare that they have no competing interests.

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Authors' contributions

H L and YP: Conceived and designed the experiments. DC X, Q F, J S, QN L and J Z: Recruited and collected study samples. H N, P H and JF L: Selected the SNPs and designed primers. YX Y: Performed the data. H L: Wrote the manuscript. H L, YX Y and YP D: Contributed to data analysis and manuscript revised. All authors read and approved the final manuscript.

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References

1. Li JX, Fu WP, Zhang J, et al. A functional SNP upstream of the ADRB2 gene is associated with COPD. *International journal of chronic obstructive pulmonary disease*. 2018;13:917-925.
2. Cho MH, McDonald ML, Zhou X, et al. Risk loci for chronic obstructive pulmonary disease: a genome-wide association study and meta-analysis. *The Lancet Respiratory medicine*. 2014;2(3):214-225.
3. Wang Y, Zhou Q, Dong L, et al. The effects of CXCL10 polymorphisms on COPD susceptibility. *Molecular genetics and genomics : MGG*. 2018;293(3):649-655.
4. McCloskey SC, Patel BD, Hinchliffe SJ, Reid ED, Wareham NJ, Lomas DA. Siblings of patients with severe chronic obstructive pulmonary disease have a significant risk of airflow obstruction. *American journal of respiratory and critical care medicine*. 2001;164(8 Pt 1):1419-1424.
5. Ingebrigtsen T, Thomsen SF, Vestbo J, et al. Genetic influences on Chronic Obstructive Pulmonary Disease - a twin study. *Respiratory medicine*. 2010;104(12):1890-1895.
6. Ding Y, Niu H, Zhou L, et al. Association of multiple genetic variants with chronic obstructive pulmonary disease susceptibility in Hainan region. *The clinical respiratory journal*. 2017;11(6):727-732.
7. Ding Y, Yang D, Zhou L, et al. Variants in multiple genes polymorphism association analysis of COPD in the Chinese Li population. *International journal of chronic obstructive pulmonary disease*. 2015;10:1455-1463.
8. Ding Y, Niu H, Yang H, et al. EGLN2 and RNF150 genetic variants are associated with chronic obstructive pulmonary disease risk in the Chinese population. *International journal of chronic obstructive pulmonary disease*. 2015;10:145-151.
9. Bylund J, Ericsson J, Oliu EH. Analysis of cytochrome P450 metabolites of arachidonic and linoleic acids by liquid chromatography-mass spectrometry with ion trap MS. *Analytical biochemistry*. 1998;265(1):55-68.
10. Bertrand-Thiebault C, Berrahmoune H, Thompson A, et al. Genetic Polymorphism of CYP2C19 gene in the Stanislas cohort. A link with inflammation. *Annals of human genetics*. 2008;72(Pt 2):178-183.
11. Shahabi P, Siest G, Meyer UA, Visvikis-Siest S. Human cytochrome P450 epoxigenases: variability in expression and role in inflammation-related disorders. *Pharmacology & therapeutics*. 2014;144(2):134-161.
12. Enayetallah AE, French RA, Thibodeau MS, Grant DF. Distribution of soluble epoxide hydrolase and of cytochrome P450 2C8, 2C9, and 2J2 in human tissues. *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society*. 2004;52(4):447-454.
13. Office of the Surgeon G, Office on S, Health. Reports of the Surgeon General. In: *The Health Consequences of Smoking: A Report of the Surgeon General*. Atlanta (GA): Centers for Disease Control and Prevention (US); 2004.
14. Kaur-Knudsen D, Bojesen SE, Nordestgaard BG. Cytochrome P450 1B1 and 2C9 genotypes and risk of ischemic vascular disease, cancer, and chronic obstructive pulmonary disease. *Current vascular pharmacology*. 2012;10(4):512-520.
15. Vogelmeier CF, Criner GJ, Martinez FJ, et al. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease 2017 Report. GOLD Executive Summary. *American journal of respiratory and critical care medicine*. 2017;195(5):557-582.
16. Salvi S. Tobacco smoking and environmental risk factors for chronic obstructive pulmonary disease. *Clinics in chest medicine*. 2014;35(1):17-27.
17. Postma DS, Bush A, van den Berge M. Risk factors and early origins of chronic obstructive pulmonary disease. *Lancet (London, England)*. 2015;385(9971):899-909.
18. Guengerich FP, Waterman MR, Egli M. Recent Structural Insights into Cytochrome P450 Function. *Trends in pharmacological sciences*. 2016;37(8):625-640.

19. Bhattacharyya S, Sinha K, Sil PC. Cytochrome P450s: mechanisms and biological implications in drug metabolism and its interaction with oxidative stress. *Current drug metabolism*. 2014;15(7):719-742.
20. Cheng SL, Yu CJ, Yang PC. Genetic polymorphisms of cytochrome p450 and matrix metalloproteinase in chronic obstructive pulmonary disease. *Biochemical genetics*. 2009;47(7-8):591-601.
21. Yang L, Li F, Yan M, Su X. Association of the CYP1A1 MspI and TNFalpha-308 polymorphisms with chronic obstructive pulmonary disease in Inner Mongolia. *Genetics and molecular research : GMR*. 2014;13(2):3209-3217.
22. Wang CD, Chen N, Huang L, et al. Impact of CYP1A1 Polymorphisms on Susceptibility to Chronic Obstructive Pulmonary Disease: A Meta-Analysis. *BioMed research international*. 2015;2015:942958.
23. Seo T, Pahwa P, McDuffie HH, et al. Association between cytochrome P450 3A5 polymorphism and the lung function in Saskatchewan grain workers. *Pharmacogenetics and genomics*. 2008;18(6):487-493.
24. Polonikov A, Kharchenko A, Bykanova M, et al. Polymorphisms of CYP2C8, CYP2C9 and CYP2C19 and risk of coronary heart disease in Russian population. *Gene*. 2017;627:451-459.
25. Fortuny J, Kogevinas M, Garcia-Closas M, et al. Use of analgesics and nonsteroidal anti-inflammatory drugs, genetic predisposition, and bladder cancer risk in Spain. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2006;15(9):1696-1702.
26. Kamata S, Fujino N, Yamada M, et al. Expression of cytochrome P450 mRNAs in Type II alveolar cells from subjects with chronic obstructive pulmonary disease. *Pharmacology research & perspectives*. 2018;6(3):e00405.
27. Spector AA, Norris AW. Action of epoxyeicosatrienoic acids on cellular function. *American journal of physiology Cell physiology*. 2007;292(3):C996-1012.
28. Li Y, Yu G, Yuan S, et al. 14,15-Epoxyeicosatrienoic acid suppresses cigarette smoke condensate-induced inflammation in lung epithelial cells by inhibiting autophagy. *American journal of physiology Lung cellular and molecular physiology*. 2016;311(5):L970-980.
29. Li Y, Yu G, Yuan S, et al. Cigarette Smoke-Induced Pulmonary Inflammation and Autophagy Are Attenuated in Ephx2-Deficient Mice. *Inflammation*. 2017;40(2):497-510.

Tables

The basic information of cases and controls

Variables	Case (n=313)	Control (n=508)	p
Age	60.05 ± 6.48	71.80 ± 10.09	<0.001*
Sex			0.003*
Male	238	337	
Female	75	171	
Smoking status			0.082
Yes	147	216	
No	164	292	
BMI	24.67 ± 4.62	24.35 ± 4.58	0.587
FEV ₁ /FVC	0.56 ± 0.05	0.79 ± 0.04	

BMI: Body mass index; FEV₁: Forced expiratory volume in 1 second; FVC: Forced vital capacity

*p <0.05 indicates statistical significance

Table 2

Primers used in this study

SNP_ID	2nd-PCR	1st-PCR	UEP_DIR	UEP_SEQ
rs10509679	ACGTTGGATGAGTTGGGATCCTAGCTGATG	ACGTTGGATGGGGTATAAGTGTGATGTCTG	F	tagcgGATGAGA
rs1934967	ACGTTGGATGGGTTTGGCTACTCTTAGGTG	ACGTTGGATGTACCCTGCCAGAAATTCCA	F	TTAGTTTGTCA
rs1934968	ACGTTGGATGGCTCTCCTAGAAATTCTAGC	ACGTTGGATGGTATATTATACTGTATCTGAG	R	TTCTAGCTAAT
rs9332220	ACGTTGGATGGTTTCTTGTTCATTTGTTTC	ACGTTGGATGTACTCCCTGCACAAGTCCAC	F	gaggGTTTGCAT
rs111853758	ACGTTGGATGCTTCTGTTGGGAGAAACAC	ACGTTGGATGCTGTAGACTACACCTTTGGG	F	gcatAAGTTTCT
rs4494250	ACGTTGGATGATAATGGTCCAGAAGGACCC	ACGTTGGATGCTGATCCAGGTGAGAAATGC	R	tCCAGGCAGAC
rs75665761	ACGTTGGATGGTTTCAAGCACAAAGGAATG	ACGTTGGATGATGTGTAATGATCAAGCAGG	R	GAAAAATACTT

Table 3

Basic information of candidate SNPs in this study

SNP	Chr	Gene	Position	Allele A/B	HWE <i>p</i> value	OR (95% CI)	<i>p</i>
rs10509679	10	<i>CYP2C9</i>	94948469	A/G	1.000	0.89 (0.72 - 1.12)	0.321
rs1934967	10	<i>CYP2C9</i>	94981669	T/C	1.000	1.16 (0.90 - 1.50)	0.250
rs1934968	10	<i>CYP2C9</i>	94982060	A/G	1.000	0.98 (0.80 - 1.22)	0.884
rs9332220	10	<i>CYP2C9</i>	94984186	A/G	0.433	0.64 (0.43 - 0.94)	0.021*
rs111853758	10	<i>CYP2C19</i>	94793932	G/T	0.138	0.65 (0.45 - 0.96)	0.027*
rs4494250	10	<i>CYP2C19</i>	94804000	A/G	0.467	1.05 (0.81 - 1.35)	0.714
rs75665761	10	<i>CYP2C19</i>	94806855	A/G	0.709	1.31 (0.89 - 1.93)	0.176

SNP: single nucleotide polymorphisms; Chr: Chromosome; HWE: Hardy-Weinberg equilibrium; OR: odds ratio; 95% CI: 95% confidential interval

**p* <0.05 indicates statistical significance

Table 4

Genotype frequencies of the SNPs and their associations with risk of COPD

SNP	Gene	Model	Genotype	Case	Control	OR (95% CI)	<i>p</i>
rs1934968	<i>CYP2C9</i>	genotype	GG	96	186	1	
			AG	152	240	1.15 (0.77 - 1.71)	0.500
			AA	33	77	0.59 (0.31 - 1.09)	0.092
		dominant	GG	96	186	1	
			AG+AA	185	317	1.01 (0.69 - 1.47)	0.980
		recessive	GG+AG	248	426	1	
			AA	33	77	0.54 (0.30 - 0.96)	0.037*
		additive	—	—	—	0.86 (0.65 - 1.14)	0.295

OR: odds ratio; 95% CI: 95% confidential interval

**p* <0.05 indicates statistical significance

Table 5

The association between SNPs and COPD under gender stratification analysis

SNP	Gene	Allele	Male				Female			
			Case	Control	OR (95% CI)	<i>p</i>	Case	Control	OR (95% CI)	<i>p</i>
rs9332220	<i>CYP2C9</i>	G	447	607	1		140	313	1	
		A	29	67	0.59 (0.37 - 0.92)	0.020*	10	29	0.77 (0.37 - 1.63)	0.493
rs111853758	<i>CYP2C19</i>	T	418	602	1		136	311	1	
		G	30	70	0.62 (0.40 - 0.96)	0.032*	10	31	0.74 (0.35 - 1.55)	0.419

OR: odds ratio; 95% CI: 95% confidential interval

* $p < 0.05$ indicates statistical significance