Int J Clin Exp Pathol 2016;9(4):4397-4407
www.ijcep.com /ISSN:1936-2625/IJCEP0022698

Original Article

Smoking and multigenetic index on the risk of chronic obstructive pulmonary disease in the Chinese Li population: a case-control study

Yipeng Ding[1*], Danlei Yang[2*], Wenteng Chen[1], Peng Chen[3], Pingdong Xie[1], Hua Yang[4,5], Pei Sun[1], Huan Niu[1], Zhongjie Tian[1], Tianbo Jin[4,5]

[1]Department of Emergency, People's Hospital of Hainan Province, Haikou, Hainan, R. R. China; [2]Department of Respiratory and Critical Care Medicine, Tongji Hospital, Key Laboratory of Pulmonary Diseases of Health Ministry, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hebei, R. R. China; [3]Department of Biochemistry and Molecular Biology, Xi'an Medical University, Xi'an, Shaanxi, R. R. China; [4]School of Life Sciences, Northwest University, Xi'an, Shaanxi, R. R. China; [5]National Engineering Research Center for Miniaturized Detection Systems, Xi'an, Shaanxi, R. R. China. [*]Co-first authors.

Received December 25, 2015; Accepted March 8, 2016; Epub April 1, 2016; Published April 15, 2016

Abstract: Objective: Chronic obstructive pulmonary disease (COPD) is a complex disease in which multiple genes and their interaction with environmental factors contribute to disease development. Recent genome-wide association studies in COPD revealed several gene variants. However, there have no studies were performed in Chinese Li population, and additional studies in multiple ethnic groups are needed. We investigated genetic associations in Li population COPD and control subjects. Methods: We genotyped 25 single nucleotide polymorphisms located in 13 genes in a case-control study with 234 COPD cases and 240 controls at Hainan Province Hospital. Odds ratios and 95% confidence intervals were estimated using the chi-squared (χ^2) test, genetic model analysis, haplotype analysis, and stratification analysis. Results: We identified that the minor alleles of rs667282 and rs2823743 were associated with a 1.35-fold increased risk of COPD and the minor alleles of rs8048576 was associated with a 0.68-fold decreased risk of COPD. In the genetic model analysis, we found rs667282 and rs2823743 were associated with increased COPD risk and rs13080 was associated with decreased COPD risk. Further stratification analysis showed that rs6265 displayed a significantly increased lung cancer risk (OR=1.87) in the non-smokers. Conclusion: Our results verified that multigenetic variants of contribute to COPD susceptibility in the Chinese Li population. Additionally, we found that smoking may interact with polymorphisms to contribute to COPD susceptibility. And this is the first time that smoking and multigenetic index on the risk of COPD in the Chinese Li population has been reported.

Keywords: Multigenetic index, chronic obstructive pulmonary disease (COPD), environmental factors, case-control studies

Introduction

Chronic obstructive pulmonary disease (COPD) is the third-leading cause of worldwide mortality and is predicted to remain a major public health problem in the near future [1]. COPD is strongly associated with smoking, but only a fraction of smokers (~20%) develop the disease, suggesting that there may be unique genetic differences among individuals leading to greater susceptibility to the most adverse effects of cigarette smoke in some individuals [2]. It is recognized that COPD involves a complex interplay between genetic background and exposure to multiple environmental stimuli. Furthermore, the association of a specific genotype or genotypes with the disease is likely to vary between populations [3]. However, the specific genes responsible for enhanced risk or host differences in susceptibility to smoke exposure remain poorly understood.

Discovery genome-wide association studies for COPD have ascertained significant associations between COPD and several gene variants [4-7]. Including the iron-responsive element-

binding protein 2 (*IREB2*), cholinergic receptor, neuronal nicotinic, alpha polypeptide-5 (CHRNA5), hypoxia inducible factor 1, alpha subunit (HIF1A), ATPase, Ca^{++} transporting, type 2C (ATP2C2) and mir-99a-let-7c cluster host gene (C21orf34) etc.

It has been reported that susceptibility to COPD is not dependent on a single gene and is affected by population differences. These GWAS have been performed almost in populations of non-Asian ancestry and some has been performed in Chinese populations. The previous gene studies of the Chinese population had focused solely on the Chinese Han population. The incidence of COPD in the Li population is higher than other regions of the Peoples Republic of China and in Li population, an increasing number of people have smoking habits. However, little or nothing is known about whether these associations exist in Chinese Li populations that the interaction between SNPs and environmental factors like smoking habits.

Therefore, in this study, we chose 27 high-frequency single nucleotide polymorphisms (SNPs) of the fifteen candidate genes to study the association with COPD risk in a large case-control cohort derived from Chinese Li population and the interaction between SNPs and environmental factors.

Materials and methods

Study participants

All the patients and controls were Chinese Li minority people identity form the card information. The patients were recruited from January 2010 to December 2013 diagnosed COPD patients at Hainan Province Hospital according to the criteria established by the National Heart, Lung and Blood Institute/World Health Organization Global Initiative for Chronic Obstructive Lung Disease (GOLD).

Patients were excluded from the study if they had asthma an established diagnosis of asthma, lung cancer, chemotherapy, radiotherapy, or a history of atopy and known α1-antitrypsin deficiency Among control individuals, none had any chronic or severe endocrinological, metabolic, or nutritional diseases. Therefore, 234 cases and 240 controls were available for study. All participants were at least 40 years old and were in good mental condition.

Clinical data and demographic information

Standard physical measurements were done in duplicate, by the same examiner, on every participant: e.g., sex, age, smoking status, nationality, body mass index, education status and family history of cancer are listed in **Table 1**. Patients were classified as smokers and never smokers. All of the smokers had at least one or more per day smoking and a period or periods aggregated more than six months were defined as ever-smokers; Never smokers were defined as those who smoked less than 100 cigarettes in their lifetime (or before diagnosis for cases) and former smokers as those who quit smoking at least 1 year before the time of the survey. The case information was collected through consultation with treating physicians or by review of medical charts. All of the participants signed informed consent. The Human Research Committee for Approval of Research Involving Human Subjects, Hainan Provincial People's Hospital, approved the use of human tissue in this study and written informed consent was obtained from all subjects.

Selection of SNPs and methods of genotyping

We have selected the 25 SNPs, which located in the regions of 13 genes. The selection criteria based on the Recent genome-wide association studies (GWAS) have identified associated with COPD, including the researches of Michael et al [8], Kim et al [6], Caporaso et al [9], David et al [5], Thorgeir et al [10]. Minor allele frequencies of all SNPs were 5%, in the HapMap of the Chinese Han Beijing population.

A venous blood sample was drawn from each individual by standard venopuncture. Blood samples were collected in sterile tubes with ethylenediaminetetraacetic acid. After centrifugation, the samples were stored at -80°C until analysis. Genomic DNA was extracted from blood samples by GoldMag-Mini Whole Blood Genomic DNA Purification Kit (GoldMag Co. Ltd. Xi'an City, China) and DNA concentration was measured by NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The Multiplexed SNP MassEXTENDED assay was designed by Sequenom MassARRAY Assay Design 3.0 Software (Sequenom Inc, San Diego, CA, USA) [11]. 25 SNP genotyping was performed by Sequenom MassARRAY RS1000

Table 1. Characteristics of controls and cases

	Cases N (%)	Controls N (%)	*P*-value
Age (years)			< 0.01[a]
Mean ± SD	67.52±9.192	62.09±11.440	
Sex			0.617[b]
Male	142 (60.7)	151 (62.90)	
Female	92 (39.3)	89 (37.1)	
Smoking stats			0.162[b]
Nonsmoking	141 (60.3)	160 (66.7)	
Smoking	90 (38.5)	32.5 (32.8)	

Notes: [a]*P*-values were calculated by Student t tests. [b]*P*-values were calculated from two-sided χ^2 tests. CI, confidence interval; OR, odds ratio.

system using the standard protocol recommended by the manufacturer [11].

Statistical analysis

The SPSS 21.0 statistical packages (SPSS, Chicago, IL) were used for statistical analysis. In all analyses, the lower frequency allele was coded as the "risk" allele. All *p* values presented in this study are two sided, and we used $P \leq 0.05$ as the cutoff value for statistical significance. Fisher's exact test was used to assess the variation in each SNP frequency from Hardy-Weinberg equilibrium in the control subjects. Differences in SNP genotype distribution between cases and controls were compared by χ^2 test [12]. We tested odds ratios (ORs) and constructed 95% confidence intervals (CIs) using unconditional logistic regression analysis with adjustments for age, gender, smoking and drinking status [13].

Associations between SNPs and risk of COPD were tested using genetic models (co-dominant, dominant, recessive, over-dominant and additive) analysis with SNP Stats software, obtained from http://bioinfo.iconcologia.net (Catalan Institute of Oncology, Barcelona, Spain). For the additive model, individuals were assigned be 0, 1, or 2 representing the number of risk alleles they possessed for that SNP. For the dominant model, individuals were coded as 1 if they carried at least one risk allele and 0 otherwise; for the recessive model, individuals were coded as 1 if they were homozygous for the risk allele (two copies) and 0 otherwise. We calculated ORs and 95% CIs by unconditional logistic regression analysis adjusted for age and gender [13]. Akaike's Information Criterion and Bayesian Information Criterion were applied to estimate the best-fit model for each SNP.

We use the Haploview software version 4.2 (Dr Mark Daly's laboratory, Massachusetts Institute of Technology/Harvard Broad Institute, Cambridge, MA, USA) to analyze the association between haplotypes and COPD. Linkage disequilibrium (LD) analysis was performed using genotype data from all the subjects. The pattern of LD was analyzed using two parameters, r^2 and D^0. Statistical significance was established when $P < 0.05$.

Results

A total of 474 participants, including 234 COPD cases and 240 controls were successfully genotyped for further analysis (**Table 1**). Males were 60.7% among cases compared with 62.9% among controls. The mean age was 67.52 (±9) years for cases and 62.09 (±11) years for controls. Cases on average were older than control subjects ($P < 0.001$). There was no significant difference between groups based upon Sex ($P=0.617$) and smoking stats ($P=0.162$).

A total of ten SNPs in the *HIFIA* gene, one SNP in the *IREB2* gene, and one SNP in the *CHRNA5* gene were genotyped in COPD patients and the healthy controls. **Table 1** lists the basic characteristics of the selected variants in the study population. The average SNPs call rate was 98.5% in cases and controls. Four SNPs (rs12220777, rs10873142, rs8102683, rs7260329) were excluded at 5% HWE *p* level. Differences in frequency distributions of alleles between cases and controls we determined using the χ^2 test or Fisher's exact tests. Our study found that rs667282 and rs2823743 were significantly associated with increased COPD risk in the study population, and they respectively presented a 1.35-fold (95% CI, 0.1.04-1.75, $P=0.025$), 1.35-fold (95% CI, 1.04-1.75, $P=0.024$) COPD risk. After adjustment for age, gender, smoking, the *P*-values of the two SNPs were 0.029. In contrast, rs8048576 was significantly associated with decreased risk of COPD, with the OR of 0.68 (95% CI, 0.52-0.88; $P=0.003$). After adjustment, the *p*-value is 0.004 (**Table 2**).

We next investigated the association between SNPs and COPD risk using genetic models

Table 2. Frequency distributions of alleles and their associations with COPD

SNP ID	Band	Allele	MAF		HWE *p*	ORs	95% CI	p^b	p^c
		A[a]/B	Case	Control					
rs1678618	10q22.1	G/A	0.064	0.056	1	1.15	0.67-1.97	0.611	0.710
rs1923539	10q22.3	T/C	0.137	0.116	0.217	1.21	0.82-1.77	0.339	0.390
rs3923564	10q22.3	C/T	0.453	0.433	0.792	1.08	0.84-1.40	0.537	0.581
rs12220777	10q22.3	C/T	0.308	0.320	1.17E-16#	0.94	0.72-1.24	0.681	0.733
rs3851050	10q22.3	C/T	0.288	0.284	0.339	1.02	0.77-1.35	0.893	0.950
rs954820	10q26.3	G/A	0.276	0.271	0.145	1.03	0.77-1.37	0.862	0.920
rs6265	11p14.1	G/A	0.461	0.429	0.235	1.14	0.88-1.48	0.315	0.348
rs17157266	11q12.3	C/T	0.096	0.092	1	1.05	0.68-1.62	0.829	0.917
rs7953249	12q24.31	G/A	0.343	0.385	0.275	0.84	0.64-1.09	0.179	0.202
rs2301104	14q23.2	C/G	0.181	0.157	0.616	1.19	0.84-1.68	0.320	0.365
rs7143164	14q23.2	C/G	0.145	0.130	0.776	1.14	0.78-1.64	0.502	0.564
rs10129270	14q23.2	A/G	0.141	0.126	1	1.14	0.79-1.67	0.483	0.545
rs8005745	14q23.2	T/A	0.145	0.131	0.776	1.13	0.78-1.63	0.531	0.594
rs4899056	14q23.2	T/C	0.147	0.133	0.778	1.12	0.78-1.62	0.532	0.595
rs966824	14q23.2	T/C	0.107	0.098	0.480	1.10	0.72-1.68	0.650	0.729
rs10873142	14q23.2	C/T	0.415	0.360	2.41E-66#	1.26	0.97-1.63	0.087	0.100
rs2301112	14q23.2	C/A	0.002	0.006	1	0.34	0.03-3.29	0.634	0.634
rs2301113	14q23.2	C/A	0.284	0.307	0.204	0.90	0.67-1.20	0.465	0.511
rs4902080	14q23.2	T/C	0.101	0.092	0.702	1.11	0.72-1.71	0.632	0.712
rs13180	15q25.1	T/C	0.472	0.527	0.298	0.80	0.62-1.04	0.090	0.103
rs667282	15q25.1	C/T	0.444	0.372	0.212	1.35	1.04-1.75	0.025*	0.029*
rs8048576	16q24.1	A/G	0.353	0.447	0.512	0.68	0.52-0.88	0.003*	0.004*
rs9951925	18q22.3	A/C	0.369	0.359	0.259	1.04	0.80-1.36	0.753	0.805
rs7937	19q13.2	C/T	0.438	0.473	0.897	0.87	0.67-1.12	0.283	0.313
rs3733829	19q13.2	C/T	0.378	0.363	0.576	1.07	0.82-1.39	0.639	0.688
rs8102683	19q13.2	T/C	0.227	0.262	0.018#	0.83	0.61-1.12	0.219	0.250
rs7260329	19q13.2	G/A	0.329	0.340	5.26E-65#	0.95	0.73-1.25	0.731	0.784
rs2823743	21q21.1	C/T	0.462	0.389	0.683	1.35	1.04-1.74	0.024*	0.029*

Notes: [a]Minor allele; [b]*P*-values were calculated from two-sided chi-square tests or Fisher's exact tests for either allele frequency. [c]*P*-values were calculated by unconditional logistic regression adjusted for age, sex, smoking, and drinking status. #site with HWE *P*-value ≤ 0.05 is excluded. **P*-value ≤ 0.05 indicates statistical significance. CI, confidence interval; HWE, Hardy-Weinberg Equilibrium; MAF, minor allele frequency; ORs, odds ratios; SNP, single nucleotide polymorphism; COPD, chronic obstructive pulmonary disease.

(dominant, recessive, additive, codominant and overdominant) by unconditional logistic regression analysis with adjustments for age. The minor allele of each SNPs as a risk factor compared with the wild-type allele (**Table 3**).

The results showed that the two genetic models (rs13180 of *IREB2* , rs8048576 of *ATP2C2*) significantly decreased the risk of COPD, include: genotype "T/T" of rs13180 in co-dominant model (OR=0.57; 95% CI, 0.33-0.98, *P*=0.046) and the recessive model (OR=0.57; 95% CI, 0.37-0.89, *p*=0.013), The log-additive OR for the rs13180 risk T-allele was 0.76 (95% CI 0.58-0.99), *P*=0.043; genotype "A/A" of rs8048576 in co-dominant model (OR=0.49; 95% CI, 0.28-0.87, *P*=0.036) and genotype "A/G-A/A" of rs8048576 in dominant model (OR=0.63; 95% CI, 0.42-0.94, *P*=0.024), The additive OR for the rs8048576 risk A-allele was 0.70 (95% CI 0.53-0.89), *P*=0.01.

Additionally, other three genetic models (rs667282 of *CHRNA5*, rs2823743 of *C21orf34*) significantly increased the risk of COPD, include: genotypes "T/T" and "C/C" of rs667282 in co-dominant model, they presented a 1.71-fold (95% CI, 1.11-2.63, *P*=0.08), 2.22-fold (95% CI,

Table 3. Genotypic model analysis of relationship between SNPs and COPD

SNP (Gene)	Model	Genotype	Control	Case	Without adjustment		With adjustment	
					OR (95% CI)	P^a	OR (95% CI)	P^b
rs13180	Codominant	C/C	57 (24.1%)	64 (27.4%)	1.00	0.15	1.00	0.046*
IREB2		T/C	110 (46.4%)	119 (50.9%)	0.96 (0.62-1.50)		1.00 (0.63-1.59)	
		T/T	70 (29.5%)	51 (21.8%)	0.65 (0.39-1.08)		0.57 (0.33-0.98)	
	Dominant	C/C	57 (24.1%)	64 (27.4%)	1.00	0.41	1.00	0.39
		T/C-T/T	180 (76%)	170 (72.7%)	0.84 (0.56-1.27)		0.83 (0.54-1.28)	
	Recessive	C/C-T/C	167 (70.5%)	183 (78.2%)	1.00	0.054	1.00	0.013*
		T/T	70 (29.5%)	51 (21.8%)	0.66 (0.44-1.01)		0.57 (0.37-0.89)	
	Additive	---	---	---	0.81 (0.63-1.04)	0.094	0.76 (0.58-0.99)	0.043*
rs667282	Codominant	T/T	99 (41.4%)	73 (31.3%)	1.00	0.068	1.00	0.008*
CHRNA5		T/C	102 (42.7%)	113 (48.5%)	1.50 (1.00-2.25)		1.71 (1.11-2.63)	
		C/C	38 (15.9%)	47 (20.2%)	1.68 (0.99-2.83)		2.22 (1.26-3.89)	
	Dominant	T/T	99 (41.4%)	73 (31.3%)	1.00	0.022	1.00	0.003*
		T/C-C/C	140 (58.6%)	160 (68.7%)	1.55 (1.06-2.26)		1.83 (1.22-2.75)	
	Recessive	T/T-T/C	201 (84.1%)	186 (79.8%)	1.00	0.23	1.00	0.056
		C/C	38 (15.9%)	47 (20.2%)	1.34 (0.83-2.14)		1.63 (0.99-2.69)	
	Additive	---	---	---	1.33 (1.03-1.71)	0.029	1.52 (1.16-2.00)	0.002*
rs8048576	Codominant	G/G	75 (31.6%)	99 (42.7%)	1.00		1.00	0.036*
ATP2C2		A/G	112 (47.3%)	102 (44%)	0.69 (0.46-1.03)	0.016	0.69 (0.45-1.06)	
		A/A	50 (21.1%)	31 (13.4%)	0.47 (0.27-0.81)		0.49 (0.28-0.87)	
	Dominant	G/G	75 (31.6%)	99 (42.7%)	1.00		1.00	0.024*
		A/G-A/A	162 (68.3%)	133 (57.3%)	0.62 (0.43-0.91)	0.013	0.63 (0.42-0.94)	
	Recessive	G/G-A/G	187 (78.9%)	201 (86.6%)	1.00		1.00	0.054
		A/A	50 (21.1%)	31 (13.4%)	0.58 (0.35-0.94)	0.026	0.60 (0.36-1.01)	
	Additive	---	---	---	0.69 (0.53-0.89)	0.004	0.70 (0.53-0.92)	0.01*
rs2823743	Codominant	T/T	91 (38.1%)	69 (29.5%)	1.00		1.00	0.090
C21orf34		T/C	110 (46%)	114 (48.7%)	1.37 (0.91-2.06)	0.084	1.33 (0.86-2.05)	
		C/C	38 (15.9%)	51 (21.8%)	1.77 (1.05-2.99)		1.84 (1.06-3.20)	
	Dominant	T/T	91 (38.1%)	69 (29.5%)	1.00	0.048	1.00	0.069
		T/C-C/C	148 (61.9%)	165 (70.5%)	1.47 (1.00-2.16)		1.46 (0.97-2.19)	
	Recessive	T/T-T/C	201 (84.1%)	183 (78.2%)	1.00	0.10	1.00	0.075
		C/C	38 (15.9%)	51 (21.8%)	1.47 (0.93-2.35)		1.56 (0.95-2.55)	
	Additive	---	---	---	1.34 (1.03-1.73)	0.026*	1.35 (1.03-1.77)	0.028*

Notes: [a]*P*-values were calculated from two-sided chi-square tests or Fisher's exact tests for either allele frequency. [b]*P*-values were calculated by unconditional logistic regression adjusted for age, sex, smoking, and drinking status. **P*-value ≤ 0.05 indicates statistical significance. CI, confidence interval; ORs, odds ratios; SNP, single nucleotide polymorphism; COPD: chronic obstructive pulmonary disease. *IREB2*: iron-responsive element-binding protein 2; *CHRNA5*: cholinergic receptor, neuronal nicotinic, alpha polypeptide-5; *HIF1A*: hypoxia inducible factor 1, alpha subunit, ATPase; *ATP2C2*: Ca^{++} transporting, type 2C; C21orf34: mir-99a-let-7c cluster host gene.

1.26-3.89, *P*=0.03) COPD risk, respectively; genotype "T/C-C/C" of rs667282 in dominant model (OR=1.83; 95% CI, 1.22-2.75, *P*=0.003); the log-additive OR for the rs667282 risk T-allele was 1.52 (95% CI 1.16-2.00), *P*=0.002. Genotype "C/C" of rs2823743 in co-dominant model (OR=1.84; 95% CI, 1.06-3.20, *P*=0.009); the additive OR for the rs2823743 risk C-allele was 1.35 (95% CI 1.03-1.77, *P*=0.28). Those data have been adjustment for age, gender and drinking status.

The association of genetic polymorphisms and COPD risk was analyzed among different populations. In the genetic models analysis, genotype "G/A" of rs6265 demonstrated a significantly increased risk for lung cancer among nonsmokers (OR=1.87; 95% CI, 1.09-3.20, *P*=0.017). After adjustment for age and gender (**Table 4**).

HIF1A gene polymorphisms were further characterized using linkage disequilibrium (LD) and haplotype analyses in this study. The pattern of LD was analyzed using two parameters, r^2 and D^0. Two LD blocks were detected. Block 1 consisted of five closely linked SNPs, rs2301104, rs7143164, rs10129270, rs8005745 and rs4899056. Block 2 included four comple-tely linked SNPs, rs966824, rs2301112, rs230-

Table 4. Genotypic model analysis of relationship between SNPs and COPD in the smokers

	Genotype	Cases (n)	Controls (n)	OR (95% CI)	Genotype	Cases (n)	Controls (n)	OR (95% CI)	Genotype	Cases (n)	Controls (n)	OR (95% CI)
rs6265												
Nonsmoking	A/A	56	35	1	A/A	56	35	1	A/A-G/A	132	121	1
	G/A	76	86	1.87 (1.09-3.23)	G/A-G/G	103	106	1.73 (1.03-2.93)	G/G	27	20	0.87 (0.45-1.68)
	G/G	27	20	1.31 (0.62-2.77)								
Smoking	A/A	16	25	1	A/A	16	25	1	A/A-G/A	66	65	1
	G/A	50	40	0.58 (0.26-1.27)	G/A-G/G	62	64	0.72 (0.34-1.54)	G/G	12	24	1.91 (0.85-4.28)
	G/G	12	24	1.30 (0.49-3.47)								
P interaction				0.017*				0.060				0.140

Notes: *P*-values were calculated by unconditional logistic regression adjusted for age and sex. **P* value ≤ 0.05 indicates statistical significance. Abbreviations: CI, confidence interval; ORs, odds ratios; COPD: chronic obstructive pulmonary disease.

Table 5. Haplotypes of HIF1A and their association with COPD risk

Block	Haplotype	Freq	OR	*P*-value
1	GGAC	0.86	0.391	0.532
2	CATT	0.134	0.401	0.527

Notes: Global haplotype association *P*-value: 0.75; *P*-values were adjusted by sex, age, smoking. CI, confidence interval; OR, odd ratio.

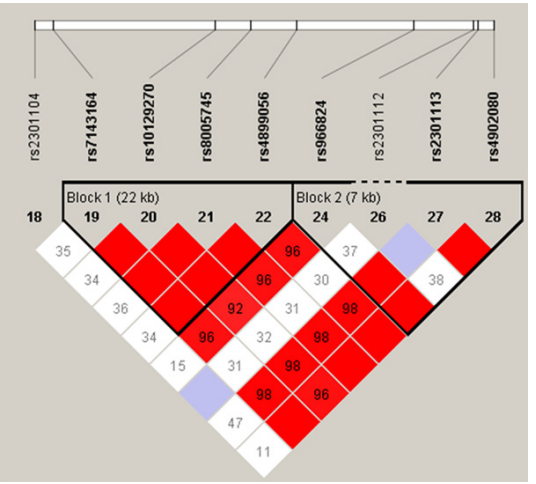

Figure 1. linkage disequilibrium of polymorphic sites in the *HIF1A* gene. A standard color scheme is used to display LD with bright red for very strong LD (LOD=2, D′=1), white for no LD (LOD < 2, D′=1), pink red (OD=2, D′=1), and blue (LOD < 2, D′=1) for intermediate LD.

1113 and rs4902080. Then, the association between inferred haplotypes and COPD risk among the individuals was analyzed. But we failed to find the association between the haplotypes and COPD risk (**Table 5** and **Figure 1**).

Discussion

A number of COPD risk variants have now been identified. Whereas each polymorphism may contribute to only a small relative risk of COPD, a combination of several responsible polymorphisms and environmental factors may be important and none study through analyses based on Chinese Li minority people. Because there are significant differences in the prevalence of COPD and the frequencies of genetic variations among different ethnic populations, it is greatly important to explore the effects of these variations in other populations. Our study has focused on the association of several candidate genetic polymorphisms, especially function-related *HIF1A* polymorphisms, with the susceptibility of COPD in Chinese Li population. To our knowledge, this is the first case-control study on the multigenetic index on the risk of COPD in the Chinese Li population.

We demonstrated that certain genetic polymorphisms, rs667282 of *CHRNA 5* gene and rs2823743 of *C21orf34* gene are associated with the increased risk of COPD. We also found rs13180 of *IREB* gene and rs8048576 of *ATP2C2* gene is associated with the decreased risk of COPD in the Chinese Li population. The association between these genetic models and COPD risk show high consistency with the results. However, none of the *HIF1A* gene variants were correlated with COPD risk. The results suggest that COPD is a complex disease with multigenetic factors.

The *CHRNA5* gene encodes nicotinic acetylcholine receptors. Differential expression of *CHRNA5* gene in human lung cells may regulate the balance between cell survival and apoptosis, cell motility and migration or wound repair of the respiratory epithelium [14]. Which may modulate cell migration and wound repair of bronchial mucosa injured by inhaled toxic substances and thus participate in lung cancer development independently of tobacco addiction [15]. Recently, several genome-wide association studies (GWAS) have identified CHRNA5 gene for COPD [16-18]. Wu et al identified rs667282, was associated with significantly increased lung cancer risk and smoking behavior in Chinese [19]. One study found rs667280 was associated with COPD in Chinese, but the other study showed no associated in Chinese Hainan population [20]. In agreement with the Chinese study, we revealed a risk factor of rs667282 in Chinese Li people. These conflicting findings might be attributed to the different ethnicity of the subjects enrolled in each study.

The *IREB2* gene is located on a region of chromosome 15q25 that is particularly compelling for investigating the genetic components of COPD. The iron-response protein (IRP) 2 encoded by this gene, together with IRP1, is involved in iron metabolism and the response to hypoxemia, and has been demonstrated to affect mitochondrial iron stores [21, 22]. In a study of Poles, *IREB2* rs13180 was found to be associated with decreased COPD risk [23]. The other study in Chinese Han population found that

rs13180 was associated with FEV1% predicted [24]. The research of Ding et al support that *IREB2* rs13180 is associated with COPD in Hainan population [20]. Consistent with the previous studies, our study confirmed that rs13180 was associated with decreased COPD risk in Chinese Li population. We found genotype "T/T" of rs13180 in co-dominant model and in the recessive model significantly decreased the risk of COPD. It could be hypothesized that the protective effect may be because the genotype T/T decreases the *IREB2* messenger ribonucleic acid expression level and reduces the oxygen consumption in the lungs; and in somehow increasing the expiratory volume, it thereby reduces pulmonary hypoxemia.

SNP of rs2823743 is a C/T single-nucleotide variation which located at the beginning of q21.1 and the protein encoded by this gene has not been characterized [25]. It has been associated with Chronic obstructive pulmonary disease-related biomarkers and *C21orf34* gene had been association with lung Cancer [26, 27]. In our study rs2823743 was significantly associated with increased COPD risk in the study population. We found genotype "C/C" of rs2823743 in co-dominant model significantly increased the risk of COPD in Chinese Li population. This may demonstrate *IREB2* as a potential gene for COPD susceptibility in Chinese Li population.

Secretory pathway Ca^{++}-ATPases (SPCAs) are responsible of pumping Ca^{++} into the Golgi stacks. There are two genes coding for human *SPCAs*, *ATP2C1* (*SPCA1*) and *ATP2C2* (*SPCA2*). The human *ATP2C2* gene was independently described by two groups in 2005. Calcium works as a second intracellular messenger in all cell types and its downstream signaling is a key pathway for many systemic functions. In the lungs, the majority of activating stimuli trigger intracellular calcium increase, which is indispensable for the normal functioning of the airways; thus, deregulation of this pathway leads to pathological conditions. A genome-wide association study (GWAS) for circulating chronic obstructive pulmonary disease (COPD) biomarkers demonstrated that rs8048576 of *ATP2C2* gene affecting surfactant protein D (SP-D) level which was nominally associated with the presence of COPD. Which is a useful approach to assess the genetic risk factors of COPD (OR=0.82, 95% CI, 0.71 -0.950) [6]. The results of this study identification firstly in Chinese Li population that rs8048576 of *ATP2C2* gene significantly associated with decreased risk of COPD.

Current literature shows that smoking is a risk factor for COPD. So, the subjects were categorized by smoking status, we found that genotype "G/A" of rs6265 demonstrated a significantly increased risk for lung cancer among nonsmokers. This result is very interesting because the general belief is that smoking increases the risk of COPD. It could be hypothesized wherein the gene influence is significant among non-smokers but not among smokers. However, this hypothesis needs to be investigated in future studies.

We attempted to estimate the relative contribution of *HIF1A* gene risk loci relative risk of COPD. Our data do not suggest that *HIF1A* gene variants correlated with COPD risk. Type II statistical error may have affected the results because of the small sample size. However, these results may suggest that the functionally activated *HIF1A* polymorphisms are not associated with COPD risk in a Chinese Li population of the People's Republic of China. However, it demonstrated the COPD is complex with contributions from multigenetic factors.

Our study provides novel findings but has some limitations that deserve comment, our sample size is still substantially smaller despite the current study possessing enough power, some limitations should be considered. Firstly, the sample size of our study was relatively small. Secondly, the exact mechanism of SNP influence has not been revealed in current study, so further work should focus on the exact relationship between multigenetic and COPD.

In conclusion, our study provides new evidence regarding the relationship between multigenetic and the risk of COPD in the Chinese Li population. Our findings suggest that the interaction of genetic variants and environmental factors may play an important role in occurrence of COPD in this population.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No

81160008). We are grateful to all study participants who made this work possible. We would also like to thank the clinicians and hospital staff who contributed to data collection for this study.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yipeng Ding, Department of Emergency, People's Hospital of Hainan Province, Haikou 570311, Hainan, P. R. China. Tel: +86-898-66222502; E-mail: dingyipenghainan@163.com; Dr. Tianbo Jin, National Engineering Research Center for Miniaturized Detection Systems, #229 North Taibai Road, Xi'an 710069, Shaanxi, R. R. China. Tel: +86-29-88305769-802; E-mail: tianbojin1973@163.com

References

[1] Murray CJ and Lopez AD. Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. Lancet 1997; 349: 1498-1504.

[2] Løkke A, Lange P, Scharling H, Fabricius P and Vestbo J. Developing COPD: a 25 year follow up study of the general population. Thorax 2006; 61: 935-939.

[3] Kleeberger SR and Peden D. Gene-environment interactions in asthma and other respiratory diseases*. Annu Rev Med 2005; 56: 383-400.

[4] 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 and Silverman EK. A genome-wide association study of COPD identifies a susceptibility locus on chromosome 19q13. Hum Mol Genet 2012; 21: 947-957.

[5] David SP, Hamidovic A, Chen GK, Bergen AW, Wessel J, Kasberger JL, Brown WM, Petruzella S, Thacker EL, Kim Y, Nalls MA, Tranah GJ, Sung YJ, Ambrosone CB, Arnett D, Bandera EV, Becker DM, Becker L, Berndt SI, Bernstein L, Blot WJ, Broeckel U, Buxbaum SG, Caporaso N, Casey G, Chanock SJ, Deming SL, Diver WR, Eaton CB, Evans DS, Evans MK, Fornage M, Franceschini N, Harris TB, Henderson BE, Hernandez DG, Hitsman B, Hu JJ, Hunt SC, Ingles SA, John EM, Kittles R, Kolb S, Kolonel LN, Le Marchand L, Liu Y, Lohman KK, McKnight B, Millikan RC, Murphy A, Neslund-Dudas C, Nyante S, Press M, Psaty BM, Rao DC, Redline S, Rodriguez-Gil JL, Rybicki BA, Signorello LB, Singleton AB, Smoller J, Snively B, Spring B, Stanford JL, Strom SS, Swan GE, Taylor KD, Thun MJ, Wilson AF, Witte JS, Yamamura Y, Yanek LR, Yu K, Zheng W, Ziegler RG, Zonderman AB, Jorgenson E, Haiman CA and Furberg H. Genome-wide meta-analyses of smoking behaviors in African Americans. Transl Psychiatry 2012; 2: e119.

[6] Kim DK, Cho MH, Hersh CP, Lomas DA, Miller BE, Kong X, Bakke P, Gulsvik A, Agusti A, Wouters E, Celli B, Coxson H, Vestbo J, MacNee W, Yates JC, Rennard S, Litonjua A, Qiu W, Beaty TH, Crapo JD, Riley JH, Tal-Singer R and Silverman EK. Genome-wide association analysis of blood biomarkers in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2012; 186: 1238-1247.

[7] Thorgeirsson TE, Gudbjartsson DF, Surakka I, Vink JM, Amin N, Geller F, Sulem P, Rafnar T, Esko T, Walter S, Gieger C, Rawal R, Mangino M, Prokopenko I, Magi R, Keskitalo K, Gudjonsdottir IH, Gretarsdottir S, Stefansson H, Thompson JR, Aulchenko YS, Nelis M, Aben KK, den Heijer M, Dirksen A, Ashraf H, Soranzo N, Valdes AM, Steves C, Uitterlinden AG, Hofman A, Tonjes A, Kovacs P, Hottenga JJ, Willemsen G, Vogelzangs N, Doring A, Dahmen N, Nitz B, Pergadia ML, Saez B, De Diego V, Lezcano V, Garcia-Prats MD, Ripatti S, Perola M, Kettunen J, Hartikainen AL, Pouta A, Laitinen J, Isohanni M, Huei-Yi S, Allen M, Krestyaninova M, Hall AS, Jones GT, van Rij AM, Mueller T, Dieplinger B, Haltmayer M, Jonsson S, Matthiasson SE, Oskarsson H, Tyrfingsson T, Kiemeney LA, Mayordomo JI, Lindholt JS, Pedersen JH, Franklin WA, Wolf H, Montgomery GW, Heath AC, Martin NG, Madden PA, Giegling I, Rujescu D, Jarvelin MR, Salomaa V, Stumvoll M, Spector TD, Wichmann HE, Metspalu A, Samani NJ, Penninx BW, Oostra BA, Boomsma DI, Tiemeier H, van Duijn CM, Kaprio J, Gulcher JR, McCarthy MI, Peltonen L, Thorsteinsdottir U and Stefansson K. Sequence variants at CHRNB3-CHRNA6 and CYP2A6 affect smoking behavior. Nat Genet 2010; 42: 448-453.

[8] 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, Investigators

I, Investigators E and Investigators CO. A genome-wide association study of COPD identifies a susceptibility locus on chromosome 19q13. Hum Mol Genet 2012; 21: 947-957.

[9] Caporaso N, Gu F, Chatterjee N, Sheng-Chih J, Yu K, Yeager M, Chen C, Jacobs K, Wheeler W, Landi MT, Ziegler RG, Hunter DJ, Chanock S, Hankinson S, Kraft P and Bergen AW. Genome-wide and candidate gene association study of cigarette smoking behaviors. PLoS One 2009; 4: e4653.

[10] Thorgeirsson TE, Gudbjartsson DF, Surakka I, Vink JM, Amin N, Geller F, Sulem P, Rafnar T, Esko T, Walter S, Gieger C, Rawal R, Mangino M, Prokopenko I, Magi R, Keskitalo K, Gudjonsdottir IH, Gretarsdottir S, Stefansson H, Thompson JR, Aulchenko YS, Nelis M, Aben KK, den Heijer M, Dirksen A, Ashraf H, Soranzo N, Valdes AM, Steves C, Uitterlinden AG, Hofman A, Tonjes A, Kovacs P, Hottenga JJ, Willemsen G, Vogelzangs N, Doring A, Dahmen N, Nitz B, Pergadia ML, Saez B, De Diego V, Lezcano V, Garcia-Prats MD, Ripatti S, Perola M, Kettunen J, Hartikainen AL, Pouta A, Laitinen J, Isohanni M, Huei-Yi S, Allen M, Krestyaninova M, Hall AS, Jones GT, van Rij AM, Mueller T, Dieplinger B, Haltmayer M, Jonsson S, Matthiasson SE, Oskarsson H, Tyrfingsson T, Kiemeney LA, Mayordomo JI, Lindholt JS, Pedersen JH, Franklin WA, Wolf H, Montgomery GW, Heath AC, Martin NG, Madden PA, Giegling I, Rujescu D, Jarvelin MR, Salomaa V, Stumvoll M, Spector TD, Wichmann HE, Metspalu A, Samani NJ, Penninx BW, Oostra BA, Boomsma DI, Tiemeier H, van Duijn CM, Kaprio J, Gulcher JR, Consortium E, McCarthy MI, Peltonen L, Thorsteinsdottir U and Stefansson K. Sequence variants at CHRNB3-CHRNA6 and CYP2A6 affect smoking behavior. Nat Genet 2010; 42: 448-453.

[11] Gabriel S, Ziaugra L and Tabbaa D. SNP genotyping using the Sequenom MassARRAY iPLEX platform. Curr Protoc Hum Genet 2009; 2.12. 11-12.12. 16.

[12] Adamec C. [Example of the use of the nonparametric test. Test X2 for comparison of 2 independent examples]. Cesk Zdrav 1964; 12: 613-619.

[13] Bland JM and Altman DG. The odds ratio. BMJ 2000; 320: 1468.

[14] Smith RM, Alachkar H, Papp AC, Wang D, Mash DC, Wang JC, Bierut LJ and Sadee W. Nicotinic alpha5 receptor subunit mRNA expression is associated with distant 5' upstream polymorphisms. Eur J Hum Genet 2011; 19: 76-83.

[15] Tekpli X, Landvik NE, Skaug V, Gulsvik A, Haugen A and Zienolddiny S. Functional effect of polymorphisms in 15q25 locus on CHRNA5 mRNA, bulky DNA adducts and TP53 mutations. Int J Cancer 2013; 132: 1811-1820.

[16] Houghton AM. Mechanistic links between COPD and lung cancer. Nat Rev Cancer 2013; 13: 233-245.

[17] Siedlinski M, Cho MH, Bakke P, Gulsvik A, Lomas DA, Anderson W, Kong X, Rennard SI, Beaty TH, Hokanson JE, Crapo JD and Silverman EK. Genome-wide association study of smoking behaviours in patients with COPD. Thorax 2011; 66: 894-902.

[18] Wilk JB, Shrine NR, Loehr LR, Zhao JH, Manichaikul A, Lopez LM, Smith AV, Heckbert SR, Smolonska J, Tang W, Loth DW, Curjuric I, Hui J, Cho MH, Latourelle JC, Henry AP, Aldrich M, Bakke P, Beaty TH, Bentley AR, Borecki IB, Brusselle GG, Burkart KM, Chen TH, Couper D, Crapo JD, Davies G, Dupuis J, Franceschini N, Gulsvik A, Hancock DB, Harris TB, Hofman A, Imboden M, James AL, Khaw KT, Lahousse L, Launer LJ, Litonjua A, Liu Y, Lohman KK, Lomas DA, Lumley T, Marciante KD, McArdle WL, Meibohm B, Morrison AC, Musk AW, Myers RH, North KE, Postma DS, Psaty BM, Rich SS, Rivadeneira F, Rochat T, Rotter JI, Artigas MS, Starr JM, Uitterlinden AG, Wareham NJ, Wijmenga C, Zanen P, Province MA, Silverman EK, Deary IJ, Palmer LJ, Cassano PA, Gudnason V, Barr RG, Loos RJ, Strachan DP, London SJ, Boezen HM, Probst-Hensch N, Gharib SA, Hall IP, O'Connor GT, Tobin MD and Stricker BH. Genome-wide association studies identify CHRNA5/3 and HTR4 in the development of airflow obstruction. Am J Respir Crit Care Med 2012; 186: 622-632.

[19] Wu C, Hu Z, Yu D, Huang L, Jin G, Liang J, Guo H, Tan W, Zhang M and Qian J. Genetic variants on chromosome 15q25 associated with lung cancer risk in Chinese populations. Cancer Res 2009; 69: 5065-5072.

[20] Ding Y, Yang D, Xun X, Wang Z, Sun P, Xu D, He P, Niu H and Jin T. Association of genetic polymorphisms with chronic obstructive pulmonary disease in the Hainan population: a case-control study. Int J Chron Obstruct Pulmon Dis 2015; 10: 7-13.

[21] LaVaute T, Smith S, Cooperman S, Iwai K, Land W, Meyron-Holtz E, Drake SK, Miller G, Abu-Asab M and Tsokos M. Targeted deletion of the gene encoding iron regulatory protein-2 causes misregulation of iron metabolism and neurodegenerative disease in mice. Nat Genet 2001; 27: 209-214.

[22] Leipuviene R and Theil E. The family of iron responsive RNA structures regulated by changes in cellular iron and oxygen. Cell Mol Life Sci 2007; 64: 2945-2955.

[23] Hardin M, Zielinski J, Wan ES, Hersh CP, Castaldi PJ, Schwinder E, Hawrylkiewicz I, Sliwinski P, Cho MH and Silverman EK. CHRNA3/5, IREB2, and ADCY2 are associated with severe chronic obstructive pulmonary dis-

ease in Poland. Am J Respir Cell Mol Biol 2012; 47: 203-208.

[24] Zhou H, Yang J, Li D, Xiao J, Wang B, Wang L, Ma C, Xu S, Ou X and Feng Y. Association of IREB2 and CHRNA3/5 polymorphisms with COPD and COPD-related phenotypes in a Chinese Han population. J Hum Genet 2012; 57: 738-746.

[25] Kuhn DE, Nuovo GJ, Martin MM, Malana GE, Pleister AP, Jiang J, Schmittgen TD, Terry AV Jr, Gardiner K, Head E, Feldman DS and Elton TS. Human chromosome 21-derived miRNAs are overexpressed in down syndrome brains and hearts. Biochem Biophys Res Commun 2008; 370: 473-477.

[26] Poirier JG, Brennan P, McKay JD, Spitz MR, Bickeboller H, Risch A, Liu G, Le Marchand L, Tworoger S, McLaughlin J, Rosenberger A, Heinrich J, Bruske I, Muley T, Henderson BE, Wilkens LR, Zong X, Li Y, Hao K, Timens W, Bosse Y, Sin DD, Obeidat M, Amos CI and Hung RJ. Informed genome-wide association analysis with family history as a secondary phenotype identifies novel Loci of lung cancer. Genet Epidemiol 2015; 39: 197-206.

[27] Kim DK, Cho MH, Hersh CP, Lomas DA, Miller BE, Kong X, Bakke P, Gulsvik A, Agustí A and Wouters E. Genome-wide association analysis of blood biomarkers in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2012; 186: 1238-1247.