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Original Article

Genetic variants of *CDH13* determine the susceptibility to chronic obstructive pulmonary disease in a Chinese population

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Aim: Adiponectin has been implicated in the development of chronic obstructive pulmonary disease (COPD). The *CDH13* gene encodes T-cadherin that is an adiponectin receptor, and genetic variants of *CDH13* determine blood adiponectin levels. The aim of this study was to investigate the effects of *CDH13* variants on COPD susceptibility in a Chinese population.
Methods: Ten single-nucleotide polymorphisms (SNP) in *CDH13* were screened using the SNaPshot method in 279 COPD patients and 367 control subjects. Association of genotypes or haplotypes constructed from these loci with COPD was analyzed in different genetic models.
Results: Among the 10 SNPs tested, rs4783244 and rs12922394 exhibited significant differences in allele or genotype frequencies between COPD patients and control subjects, whereas 8 other SNPs did not. The minor allele T was associated with decreased risk of COPD in the recessive model at rs4783244 (OR=0.42, *P*=0.023) and in the dominant model at rs12922394 (OR=0.70, *P*=0.022). The genotype TT at either rs4783244 or rs12922394 was associated with a significantly low level of plasma adiponectin when compared to genotypes GG and CC (*P*<0.05). Haplotypes GC in block 1 (rs4783244-rs12922394) as well as GTAC and ATGT in block 3 (rs4783266-rs11640522-rs11646849-rs11860282) significantly increased the risk of COPD, whereas haplotypes TT in block 1, TG in block 2 (rs11646011- rs11640875) and ATGC in block 3 were protective against COPD.
Conclusion: *CDH13* genetic variants determine Chinese individuals' susceptibility to COPD and thus are efficient genetic biomarkers for early detection of COPD.

Keywords: *CDH13*; adiponectin; chronic obstructive pulmonary disease; biomarker; genetic polymorphism; haplotype; Chinese individuals

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Introduction

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality worldwide[1], and it is estimated to affect nearly 8.2% of the adult Chinese population[2]. COPD is a heterogeneous disease. In addition to its effects in the lung, COPD is also fraught with a considerable amount of extrapulmonary complications and comorbidities, such as osteoporosis, anemia, weight loss, depression, cardiovascular disease, and cancer[3]. Although lung function, as measured by forced expiratory volume in 1 second (FEV1), is a useful indicator of poor health outcomes in COPD, it is limited in that the extrapulmonary dimensions of COPD cannot be easily captured with this one measurement. In response, there are large, ongoing initiatives to identify biomarkers that can complement FEV1 in accurately identifying those patients with COPD that are at risk for extrapulmonary complications. Recent work on peripheral blood biomarkers has suggested that there may be biomarker signatures in blood that are associated with COPD phenotypes[4, 5]. Serum adiponectin levels are a valuable diagnostic and prognostic marker of COPD.

Adiponectin is a secretory 30 kDa protein synthesized by adipocytes in healthy subjects. Adiponectin is highly abundant in plasma and is a key cytokine in energy homeostasis,

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regulating both glucose and lipid metabolism. In humans, down regulation of adiponectin and its receptors is associated with systemic inflammation and extrapulmonary effects, such as weight loss, osteoporosis, adipose atrophy, and skeletal muscle atrophy associated with age[6]. Recent human studies have demonstrated a significant increase in serum adiponectin levels in COPD and a direct correlation of these levels to the severity of the disease[7, 8]. Plasma adiponectin is increased in underweight patients with COPD and is inversely associated with body mass index, degree of airflow limitation, and adverse cardiovascular outcomes. Elevation of plasma adiponectin has been associated with an accelerated decline in lung function[9-11]. Adiponectin also contributes to the development of emphysema[7]. Moreover, genetic variants in the adiponectin gene have been associated with plasma adiponectin levels and COPD risk in a Chinese population[12].

Twin and family studies have demonstrated an estimated 30%–70% heritability for circulating adiponectin levels[13]. Recently, genome-wide association study (GWAS) methodology has been widely used to identify candidate genes that influence plasma adiponectin levels, and *CDH13* encoding T-cadherin is one of the identified genes. Several single nucleotide polymorphisms (SNPs) in the *CDH13* gene have been shown to be determinants of blood adiponectin levels in multi-ethnic populations[14-18]. T-cadherin has been reported to be an adiponectin receptor and was discovered after adipoR1 and adipoR2; it mainly binds to hexameric and high molecular weight (HMW) isoforms of adiponectin[19], which are the most abundant isoforms in the lung lining fluid[20]. The aim of the current study was to evaluate the association of variants in the *CDH13* gene with COPD in a Han Chinese population.

Materials and methods

Subjects

The subjects in this study were recruited for our previous studies[12, 21, 22]. Briefly, we investigated 646 unrelated ethnic Han Chinese individuals including 279 COPD patients and 367 age-matched control individuals. COPD was diagnosed according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria[23]. Patients were excluded if they had any other comorbidities or significant respiratory diseases, such as bronchial asthma, bronchiectasis, lung cancer, or pulmonary tuberculosis. Among the age-matched non-COPD control individuals, none had a history of chronic lung disease, atopy, acute pulmonary infection in the 4 weeks before assessment for this study, or a family history of COPD.

The use of human tissue and the protocol in this study strictly conformed to the principles expressed in the Declaration of Helsinki and were approved by the Ethical Committee of the West China Hospital of Sichuan University. Written informed consent was obtained from all subjects before their participation in the study.

Biochemical measurements

Blood samples were collected at baseline from patients and controls after an overnight fast. Separated plasma was used for lipid, glucose and adiponectin analyses. The plasma levels of total cholesterol, triglycerides and glucose were determined with an enzymatic kit (Boehringer Mannheim, Mannheim, Germany). Circulating total adiponectin levels were measured by the enzyme-linked immunosorbent assay method (Quantikine, R&D Systems, Minneapolis, MN, USA).

SNP selection and genotyping

CDH13 is located on chromosome 16q and spans ~1.17 megabases (*CDH13*: ENSG00000140945) (http://www.ensembl.org/index.html). Genotype data of the Chinese population for the *CDH13* region were obtained from the HapMap website (http://www.hapmap.org/), and ten tag single-nucleotide polymorphisms (SNPs) in intron 1 ranging from 81 218 244 bp to 81 449 467 bp (NCBI build 37.3) were selected using the Tagger software implemented in the Haploview software[24], with an r^2 threshold of 0.8 and minor allele frequencies of 0.1 (supplementary Table S1).

Genomic DNA was extracted from peripheral blood leukocytes using a commercial extraction kit (Bioteke Corporation, Beijing, China) according to the manufacturer's instructions. SNPs were genotyped using the ABI SNaPshot method (Applied Biosystems, Foster City, CA, USA) as described previously[12, 22]. Briefly, the PCR products (see supplementary Table S1 for primers) were purified by incubating with shrimp alkaline phosphatase and exo-nuclease I. Then, the purified PCR products were used as the templates for a SNaPshot reaction using specific SNaPshot primers (supplementary Table S1). The SNaPshot reaction products were analyzed on an ABI 3130 Genetic Analyzer (Applied Biosystems, Carlsbad, CA, USA). The data were analyzed by GeneMapper 4.0 software (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Statistical analyses were performed in SPSS version 17.0 (SPSS Inc, Chicago, IL, USA) and Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). The demographic and clinical data of the COPD patients and the control subjects were compared using the χ^2 test and Student's *t*-test. A multiple logistic regression analysis using BMI and glucose as covariates was performed to correct the significant *P*-value of adiponectin. A two-sided significance level of $P<0.05$ was used for all significant tests.

The Hardy-Weinberg equilibrium (HWE) test using two-sided χ^2 analysis was performed for each SNP among COPD cases and controls. The frequency of each allele at each SNP locus was obtained by adding the frequency of its homozygote to half of the frequency of the heterozygote in which it appears. Differences in the distribution of genotypes or alleles under different genetic models (including dominant, recessive and additive models) between the COPD patients and the controls were estimated by using the χ^2 test, and the best genetic model for each SNP was determined using Akaike's information criterion (AIC). The model with the smallest AIC value corresponds to the minimal expected entropy. Odds ratios (ORs) and 95% CIs were calculated by unconditional logistic

regression analyses. Linear regression was used to study the association of SNPs with two COPD phenotypes (FEV1 and FEV1/FVC ratio). Multiple testing corrections were carried out using a false discovery rate (FDR)[25]. *Q*-values (=corrected *P*-values) were adjusted with subject demographics, such as gender, age, BMI and smoking history. The significance level was set at $Q<0.05$. FDR calculations were performed using the R language package fdrtool[26].

Pairwise linkage disequilibrium (LD) estimation and haplotype reconstruction were performed using SHEsis (http://analysis.bio-x.cn). For haplotype analysis, only haplotypes with a frequency >3% in at least one group were tested. We also used Haploview 4.2[24] to estimate LD.

Results

General characteristics of the subjects

The baseline characteristics, the biochemical features and the results of the pulmonary function tests of the subjects in this study have been reported in our previous articles[12, 21, 22] and are presented in supplementary Table S2. All patients had FEV1 values <80% of predicted, and thus were diagnosed with moderate to severe COPD according to the Global Initiative for Chronic Obstructive Lung Disease[23] (classification of severity: mild=FEV1≥80% of predicted; moderate=FEV1≥50% to <80% of predicted; severe=FEV1≥30% to <50% of predicted; and very severe=FEV1<30% of predicted). The COPD cases and control subjects did not significantly differ in sex, age or smoking history ($P>0.05$). Compared to control individuals, the COPD patients had worse pulmonary function with significantly decreased FEV1, FEV1/predicted and FEV1/FVC ratios ($P<0.01$). In addition, the COPD patients had statistically higher glucose concentrations (5.86±0.16 mmol/L *vs* 5.13±0.12 mmol/L, $P<0.01$), higher adiponectin levels (8.54±0.66 μg/mL *vs* 6.12±0.57 μg/mL, $P<0.01$), and a significantly lower BMI (22.03±2.27 kg/m^2 *vs* 23.91±2.48 kg/m^2, $P<0.01$).

Distribution of the SNPs in the CDH13 gene between COPD patients and controls

Frequencies of alleles and genotypes in the population are fundamental quantities in human statistical genetics, which form the basis of association studies between SNPs and common diseases. Table 1 summarizes the genotype and allele frequencies of each SNP in both COPD patients and controls. All genotype distributions of the tested SNPs were in Hardy-Weinberg equilibrium (HWE) in patients and control subjects (all $P>0.05$), illustrating that our subjects presented the source population well. SNPs rs4783244 and rs12922394 exhibited significant differences in allele or genotype frequencies between COPD patients and control subjects, whereas other SNPs did not. For rs4783244, compared to the allele G, the allele T was associated with a significantly decreased risk of COPD (OR=0.70, 95% CI: 0.56–0.91, $P=0.007$, FDR $Q=0.022$). For rs12922394, compared to the allele C, the allele T is associated with a significantly decreased risk of COPD (OR=0.72, 95% CI: 0.56–0.93, $P=0.013$, FDR $Q=0.031$). The genotypes of both rs4783244 and rs12922394 were distributed significantly differently between COPD patients and control subjects ($P<0.05$).

Association of genotypes with COPD under different genetic models

The maximum power in genetic association studies is reached when concordance is observed between the "true" model of inheritance of disease susceptibility loci and the genetic model used in the analysis. We compared the genotype frequencies of every polymorphism between groups under the dominant, recessive and additive genetic models. The best genetic model for each SNP was determined using Akaike's information criterion. For each SNP, if one allele's frequency was relatively lower than another's, it was recognized as the minor allele (Table 1), which was assumed to be a risk allele compared to a wild type allele. As shown in Table 2, the minor allele T at rs4783244 was associated with a decreased COPD risk under a recessive model (TT *vs* GT+GG: OR=0.42, 95% CI: 0.19–0.91, $P=0.023$, FDR $Q=0.042$), whereas the minor allele T at rs12922394 was associated with a decreased COPD risk under a dominant model (CT+TT *vs* CC: OR=0.70, 95% CI: 0.51–0.95, $P=0.022$, FDR $Q=0.039$).

Association between SNPs rs4783244 and rs12922394 and lung function

To determine whether SNPs rs4783244 and rs12922394 influence COPD phenotypes, we assessed their associations with the FEV1 and FEV1/FVC ratio in the entire population, including 279 COPD patients and 367 controls (Table 3). The T allele at rs4783244 showed significant associations with both increased FEV1 ($P=0.017$) and FEV1/FVC ratio ($P=0.024$) under a recessive model (TT *vs* GT+GG), and the association remained significant even after correction for multiple testing (FDR $Q=0.031$ and 0.042 for FEV1 and FEV1/FVC ratio, respectively). The minor allele T at rs12922394 was again associated with both increased FEV1 ($P=0.028$) and FEV1/FVC ratio ($P=0.030$) under a dominant model (CT+TT *vs* CC), although this did not meet the threshold for multiple testing correction (FDR $Q>0.05$).

Association between SNPs rs4783244 and rs12922394 and plasma adiponectin levels

The relationships between SNPs rs4783244 and rs12922394 and plasma adiponectin levels were examined in both COPD patients and control subjects (Figure 1). Consistently with findings in a previous study[17, 18, 27], after adjusting for BMI and glucose levels, the subjects with homozygous TT at either rs4783244 (5.62±0.49 μg/mL in controls and 7.73±0.41 μg/mL in COPD patients) or rs12922394 (5.57±0.52 μg/mL in controls and 7.62±0.47 μg/mL in COPD patients) exhibited a significant decrease in plasma adiponectin levels when compared with the subjects with homozygous GG at rs4783244 (6.63±0.41 μg/mL in controls and 8.91±0.44 μg/mL in COPD patients) or CC at rs12922394 (6.39±0.42 μg/mL in controls and 8.72±0.43 μg/mL in COPD patients), respectively (all $P<0.05$).

Table 1. Distributions of the *CDH13* SNPs in COPD patients and controls.

SNP	Group	Genotype					HWE	Allele				
		Number			*P*	FDR *Q*	*P*	Freq (%)		*P*	FDR *Q*	OR [95% CI]
rs4783244		GG	GT	TT				G	T			
	Control	179	161	27	**0.005***	**0.019***	0.258	70.7	29.3	**0.007***	**0.022***	**0.70 [0.56–0.91]***
	COPD	162	108	9			0.074	77.1	22.9			
rs12922394		CC	CT	TT				C	T			
	Control	192	155	20	**0.009***	**0.026***	0.114	73.4	26.6	**0.013***	**0.031***	**0.72 [0.56–0.93]***
	COPD	171	101	7			0.076	79.2	20.8			
rs11646011		TT	TC	CC				T	C			
	Control	206	132	29	0.268	0.317	0.230	74.1	25.9	0.253	0.306	1.16 [0.90–1.50]
	COPD	168	93	18			0.298	76.9	23.1			
rs11640875		AA	AG	GG				A	G			
	Control	137	170	60	0.185	0.225	0.554	60.5	39.5	0.183	0.222	0.86 [0.69–1.07]
	COPD	89	139	51			0.799	56.8	43.2			
rs1870843		GG	GA	AA				G	A			
	Control	169	148	50	0.310	0.403	0.059	66.2	33.8	0.290	0.367	1.14 [0.90–1.44]
	COPD	137	111	31			0.242	69.0	31.0			
rs4783266		GG	GA	AA				G	A			
	Control	230	118	19	0.408	0.483	0.450	78.7	21.3	0.402	0.479	1.13 [0.86–1.48]
	COPD	182	86	11			0.833	80.6	19.4			
rs11640522		CC	CT	TT				C	T			
	Control	217	128	22	0.233	0.288	0.591	76.6	23.4	0.226	0.271	1.18 [0.90–1.54]
	COPD	177	89	13			0.674	79.4	20.6			
rs11646849		GG	GA	AA				G	A			
	Control	142	171	54	0.203	0.253	0.829	62.0	38.0	0.194	0.231	0.86 [0.69–1.08]
	COPD	101	124	54			0.155	58.4	41.6			
rs11860282		TT	TC	CC				T	C			
	Control	170	163	34	0.711	0.837	0.570	68.5	31.5	0.712	0.838	0.96 [0.76–1.21]
	COPD	129	119	31			0.654	67.6	32.4			
rs2549151		GG	GT	TT				G	T			
	Control	238	117	12	0.167	0.198	0.604	80.8	19.2	0.178	0.211	1.22 [0.91–1.63]
	COPD	192	83	4			0.134	83.7	16.3			

HWE: Hardy-Weinberg equilibrium; FDR: false discovery rate; OR (95% CI): odd ratio (95% confidence interval).
*Statistically significant at $P<0.05$.

Table 2. Association between *CDH13* SNPs and COPD risk under different genetic models.

SNP	Genetic model[a]		*P*	FDR *Q*	OR [95% CI]
rs4783244	Recessive	TT *vs* (GT+GG)	**0.023***	**0.042***	**0.42 [0.19–0.91]***
rs12922394	Dominant	(CT+TT) *vs* CC	**0.022***	**0.039***	**0.70 [0.51–0.95]***
rs11646011	Dominant	(TC+CC) *vs* TT	0.298	0.323	1.18 [0.86–1.62]
rs11640875	Dominant	(AG+GG) *vs* AA	1.152	1.154	0.79 [0.57–1.09]
rs1870843	Recessive	AA *vs* (AG+GG)	0.294	0.301	1.31 [0.79–2.16]
rs4783266	Recessive	AA *vs* (AG+GG)	0.423	0.432	1.37 [0.63–2.95]
rs11640522	Dominant	(CT+TT) *vs* CC	0.333	0.401	1.20 [0.87–1.65]
rs11646849	Recessive	AA *vs* (AG+GG)	0.062	0.094	0.71 [0.45–1.12]
rs11860282	Additive	CT *vs* TT	0.818	0.819	1.04 [0.75–1.44]
		CC *vs* TT	0.503	0.517	0.83 [0.49–1.43]
rs2549151	Recessive	TT *vs* (GT+GG)	0.120	0.139	2.42 [0.77–7.62]

[a]Only the estimates for the best genetic model for each SNP, as determined by AIC, are provided.
OR (95% CI): odd ratio (95% confidence interval); FDR: false discovery rate.
*Statistically significant at $P<0.05$.

Table 3. Associations between polymorphisms rs4783244 and rs12922394 and COPD phenotypes.

		FEV1			FEV1/FVC		
Genotype	Number	%predicted	*P*	FDR Q	Ratio	*P*	FDR Q
rs4783244			TT *vs* (GG+GT)			TT *vs* (GG+GT)	
GG	342	71.2±3.4			64.3±3.1		
GT	269	73.6±3.8	**0.017***	**0.031***	65.1±2.9	**0.024***	**0.042***
TT	36	82.2±3.1			71.9±2.1		
rs12922394			(CT+TT) *vs* CC			(CT+TT) *vs* CC	
CC	363	72.2±3.6			63.1±3.2		
CT	256	79.4±3.3	**0.028***	0.051	68.9±2.6	**0.030***	0.057
TT	27	81.3±3.9			70.5±3.6		

FEV1: forced expiratory volume in one second; FVC: forced vital capacity.
*Statistically significant at $P<0.05$ in bold.

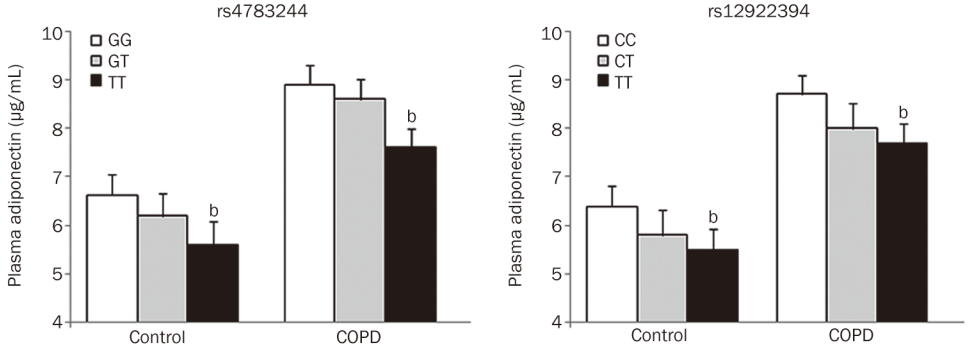

Figure 1. The allele effects of rs4783244 and rs12922394 on plasma adiponectin levels. Mean±SD. $^bP<0.05$ *vs* subjects with genotypes GG at rs4783244 and CC at rs12922394.

Linkage disequilibrium (LD) between SNPs and haplotype analysis

In HapMap populations, there is a substantive difference in LD structure at the *CDH13* locus between European and Asian subjects (supplementary Figure S1). We then assessed the LD in pairwise combinations of alleles in different SNPs by means of maximum likelihood from the genotype frequency in the COPD and control groups. Pairwise LD between SNPs is shown in supplementary Table S3 and Figure 2. Based on LD determinations, three blocks with moderate LD were detected: block 1, composed of rs4783244 and rs12922394; block 2, composed of rs11646011 and rs11640875, and block 3, composed of rs4783266, rs11640522, rs11646849, and rs11860282.

We estimated the frequencies of haplotypes constructed from phased multi-locus genotypes in *CDH13*. The haplotypes with a frequency higher than 3% in at least one group were used in the haplotype analysis. As shown in Table 4, haplotype GC in block 1 was associated with an increased risk of COPD (OR=2.21, 95% CI=1.44–3.37, $P=1.92\times10^{-4}$), whereas haplotype TT was protective against COPD (OR=0.79, 95% CI=0.63–0.99, $P=0.041$). In block 3, two haplotypes were associated with an increased risk of COPD (GTAC: OR=2.68, 95% CI=1.45–4.95, $P=0.001$; ATGT: OR=2.05, 95% CI=1.38–3.03,

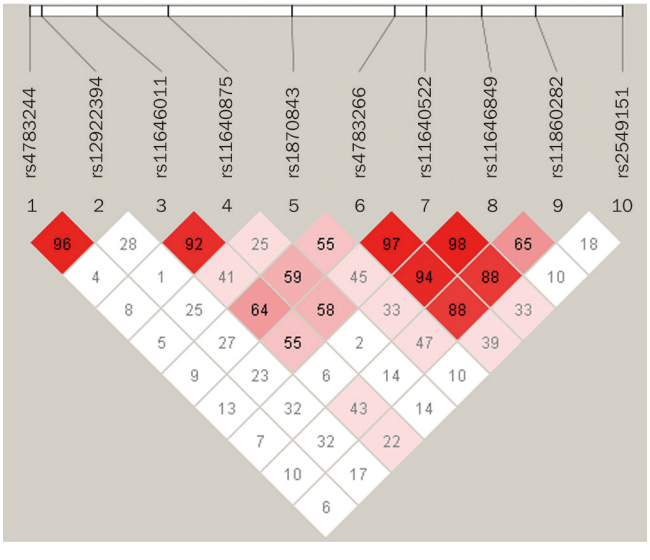

Figure 2. Linkage disequilibrium (LD) plots for *CDH13*. The LD plots were generated by Haploview 4.2. Polymorphisms are identified by their dbSNP rs numbers, and their relative positions are marked by vertical lines within the white horizontal bar. The numbers within squares indicate the D' value, expressed as a percentile.

Table 4. Frequencies of pairwise haplotype constructed by SNPs in *CDH13*.

Haplotype[a]	Freq (case)	Freq (control)	Fisher's *P*	OR [95% CI][b]
Block 1: rs4783244[G/T] - rs12922394[C/T]				
GC	0.615	0.559	**0.041***	**1.27 [1.01–1.59]***
GT	0.228	0.205	0.317	
TC	0.107	0.129	0.233	
TT	0.051	0.106	**1.92×10^{-4}***	**0.45 [0.30–0.69]***
Block 2: rs11646011[T/C] - rs11640875[A/G]				
TA	0.144	0.121	0.217	
CA	0.100	0.096	0.822	
TG	0.643	0.700	**0.031***	**0.77 [0.61–0.98]**
CG	0.114	0.084	0.074	
Block 3: rs4783266[G/A] - rs11640522[C/T] - rs11646849[G/A] - rs11860282[T/C]				
GCGT	0.029	0.032	0.482	
GCGC	0.040	0.031	0.264	
GCAT	0.025	0.029	0.478	
GTGT	0.037	0.027	0.252	
GTGC	0.076	0.069	0.323	
GTAC	0.051	0.018	**0.001***	**2.68 [1.45–4.95]***
ATGT	0.048	0.012	**3.09×10^{-4}***	**2.05 [1.38–3.03]***
ATGC	0.050	0.019	**0.003***	**0.70 [0.55–0.89]***
ATAT	0.092	0.080	0.238	

[a]Only haplotypes with a frequency >3% in at least one group were listed; [b]Only haplotypes distributed significantly differently were calculated.
OR (95% CI): odd ratio (95% confidence interval).
*Statistically significant at $P<0.05$.

$P=3.09\times10^{-4}$), whereas haplotype ATGC was protective against COPD (OR=0.70, 95% CI=0.55–0.89, $P=0.003$).

Discussion

Adiponectin, although a secreted protein of fat cells, is also expressed in the airway epithelium and BAL fluid in the emphysematous form of COPD[28]. Adiponectin has important roles in insulin sensitization, cardioprotection, and anti-inflammatory processes. There have been several clinical studies reporting on the relationship between circulating adiponectin and COPD, and plasma adiponectin level is elevated in patients with stable and acute exacerbation of COPD[7, 10, 11]. The most bioactive form of adiponectin is a 400 kDa HMW complex, which is also dramatically increased in COPD patients[29]. The *CDH13* gene encodes the T-cadherin protein, which is a receptor for hexameric and HMW forms of adiponectin[19].

In this case-control study in a Han Chinese population, we carried out the first investigation of a possible association between SNPs in the *CDH13* gene and COPD risk. Our current findings suggested that rs4783244 and rs12922394 were associated with a risk of COPD. In comparison with allele G at rs4783244 or allele C at rs12922394, the minor allele T at either site was associated with a decreased COPD risk. Moreover, the genotypes at rs4783244 and rs12922394 were also found to affect the plasma adiponectin levels, consistently with findings from previous studies[17, 18, 27]. However, given the high frequencies of the G allele at rs4783244 (70.7%) and the C allele at rs12922394 (73.4%) related to the elevated levels of adiponectin in control subjects, the effects of the genotypes on the susceptibility of COPD were limited. The frequency of the T allele at rs4783244 was 29.3% in our control subjects, which appears to be lower than the 36% in non-diabetic Han Chinese subjects[30]. This might be attributable to the differences between the two studies in subjects' age, gender, and smoking history. Compared to the non-diabetic subjects in Li's study[30], our control subjects are older (65±8 years *vs* 50±11 years), more likely to be male (88% *vs* 63%), and heavier smokers. Indeed, T allele frequency is approximately 29% in Han Chinese in HapMap project data, which is comparable to our results. In addition to the genotype analysis, our study also adopted a haplotype-based approach. We observed that some haplotypes with a low frequency dramatically changed the risk of COPD in the opposite direction (Table 4), which indicates the complexity of the *CDH13* gene in the development of COPD.

Both genetic and environmental factors contribute to the pathogenesis of COPD. Cigarette smoking is a major risk factor for COPD, and genetic and smoking interactions have been associated with lung function in COPD[31, 32]. It is possible that the effects of *CDH13* variants on COPD risk may be even more significant in individuals with low exposure to cigarette smoke than in moderate and heavy smokers, as recruited in the present study. Because the dose of toxic substrates was so high in heavy smokers, it overwhelmed the effects of the *CDH13* genotype. In moderate smokers, the relative effect of the *CDH13* genotype may be uncovered.

The association between *CDH13* variants, plasma adiponectin, and the risk of COPD may be explained by the func-

tion of the T-cadherin receptor that *CDH13* encodes. The amino acid motif of T-cadherin is well conserved in higher eukaryotes compared to that of E-cadherin, suggesting some biological significance. T-cadherin may act as a co-receptor along with other signaling molecules, but its physiological roles are largely unknown. rs4783244 or rs12922394 might be associated with an increased baseline level of T-cadherin in tissues. The increased amount of T-cadherin may capture the free adiponectin molecules in the plasma, resulting in a lowering of plasma HMW adiponectin levels, while simultaneously increasing the adiponectin signals in the targeting cells, augmenting the effect of adiponectin per cell. Consistently with this explanation, ablation of the T-cadherin receptor increased plasma adiponectin levels in mice[20, 33]. Therefore, *CDH13* genotypes and adiponectin genotypes may need to be taken into account if plasma adiponectin levels are used as a marker for COPD risk.

The statistically significant associated SNPs (rs4783244 and rs12922394) are in the first intron of the *CDH13* gene, which is usually removed during the gene-splicing process. Although they cause no apparent functional change, intronic SNPs may modify gene function by affecting the regulation of gene expression[34]. In addition, the SNPs associated with the statistical signal may play a role as a surrogate marker for a causal functional SNP or SNPs because the surrounding nucleotide sequence did not match the known transcription factor binding site or miRNA targeted sequence. rs4783244 or rs12922394 could be in LD with another polymorphism of the gene that may affect the *CDH13* expression level. However, it is also likely that the causal sequence change or changes in this region have yet to be identified, as suggested by the analysis of the significant haplotypes. For example, SNP rs4783266 was significantly associated with COPD risk as part of a haplotype, but not individually (Table 4). We selected SNPs with minor allele frequencies of >10% in the Han Chinese population using HapMap project data, but this is not suited for situations where genetic architecture is such that multiple rare disease-causing variants contribute significantly to disease risk. Recent studies have demonstrated that the identification of rare variants may lead to critically important insights about disease etiology through the implication of new genes and/or pathways[35, 36]. The rare variants of the *CDH13* gene should be investigated to clarify their role in susceptibility to the development of COPD.

The significant results in the current study could prove to be false positives because of the relatively small sample size and the lack of replication evidence. However, chromosome 16q, where *CDH13* is located, has been associated with the pulmonary function that is used in the diagnosis of COPD[37]. Further studies using larger populations are needed. However, even with a larger sample, the functional and biological impacts of the described polymorphisms would require further study. Given the number of processes and pathways in which CDH13 functions and the significance of association between *CDH13* and COPD, it is unlikely that CDH13 alone contributes to the sequelae in COPD; instead, genes regulated by CDH13, or those that regulate CDH13, may influence the disease. Therefore, the investigation of CDH13-related pathways and gene networks may lead to a better understanding of the pathophysiology of COPD.

In conclusion, our comprehensive analysis of SNPs in the *CDH13* gene suggests that *CDH13* genotypes and haplotypes may play a role in COPD development. *CDH13* encodes one of the receptors for adiponectin, and plasma adiponectin level is elevated in patients with a stable and acute exacerbation of COPD. Therefore, we may need to take into account, both *CDH13* genotypes and adiponectin genotypes, if plasma adiponectin levels are used as a marker for risk of COPD. Identifying the genetic background of patients with COPD allows us to identify risk groups in the community and develop new individual-based targets for therapy. Our results represent a key step in the development of genetic susceptible biomarkers for the detection of COPD. Because this is the first case-control study investigating the association of *CDH13* with the risk of COPD, additional studies with large patient populations are required to confirm our findings.

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Author contribution

Yi-ming YUAN, Jin-long ZHANG, Si-cheng XU, Ren-song YE, You ZHANG, Yan-Jie ZHANG, Yu-long CHEN, Dan XU, and Yu-lan LIU contributed to the acquisition and analysis of data; Zhi-guang SU contributed to the study design and data analysis and wrote the manuscript.

Supplementary information

Supplementary information is available at the Acta Pharmacologica Sinica's website.

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