Association of Polymorphisms of the Receptor for Advanced Glycation End Products Gene with COPD in the Chinese Population

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The receptor for advanced glycation end products (RAGE) is a cell surface molecule of the immunoglobulin superfamily that binds diverse endogenous ligands involved in the development of chronic diseases and inflammatory damage. A growing body of evidence has suggested that RAGE is involved in the development and progression of chronic obstructive pulmonary disease (COPD). The present study investigated the existence of an association among three polymorphisms (–374T/A, –429T/C, and G82S) of the *RAGE* gene with the risk of COPD in the Chinese population. The *RAGE* genotypes were determined by polymerase chain reaction–restriction fragment length polymorphism in 216 patients with COPD and 239 age-matched healthy individuals. Our study demonstrated that the frequencies of the GS genotype and the S allele in the G82S mutation were significantly higher in COPD patients than in controls (odds ratios [OR] = 1.70, 95% confidence interval [CI]: 1.15–2.50, $p=0.0098$ and OR = 1.42, 95% CI: 1.06–1.91, $p=0.023$, respectively). Further stratification analysis by smoking status revealed that the presence of the GS genotype conferred a higher risk of developing COPD in current smokers ($p=0.044$). In contrast, mutations at –374T/A and –429T/C did not demonstrate any association with COPD, even after taking into account the patients' smoking history. Our study provides preliminary evidence that the G82S polymorphism in the *RAGE* gene is associated with an increased risk of COPD and that the GS genotype of the G82S variant is a risk factor for COPD in the Chinese population.

Introduction

CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) is the fourth leading cause of death worldwide, and estimates by the World Bank/World Health Organization project that the disease burden of COPD will rank fifth worldwide by the year 2020 (Rabe *et al.*, 2007). Thus, a better understanding of the etiology and pathogenesis of COPD is important for reducing the risk of death from the disease and developing new therapeutic approaches. COPD is thought to be a multifactorial disorder in which genetic susceptibility, environmental factors, and tobacco exposure may all be implicated (Decramer *et al.*, 2012). Recent evidence suggests that inflammatory processes, such as an increase in cytokines, chemokines, and acute-phase reactant proteins in response to environmental stimuli, particularly smoking, may play important roles in the development of COPD (Kardos and Keenan, 2006). Therefore, numerous

genes involved in inflammatory processes have been considered to be associated with COPD (Provinciali *et al.*, 2011).

Receptor for advanced glycation end products (RAGE), a member of the immunoglobulin (Ig) superfamily, was demonstrated to be a multiligand receptor for advanced glycation end products (AGEs), S100/calgranulins, and high-mobility group box-1 proteins (HMGB1) (Chavakis *et al.*, 2004). The binding of ligands to RAGE results in the activation of the proinflammatory transcription factor NF- κ B (nuclear factor- κ B) or macrophages and the subsequent expression of proinflammatory cytokines in tissues, suggesting that these interactions influence the course of the inflammatory response (Hofmann *et al.*, 1999; Haslbeck *et al.*, 2004; Muhammad *et al.*, 2008). In addition, whereas the level of expression of RAGE is low in most healthy tissues, it is significantly elevated in type II alveolar epithelial cells, endothelial cells, and alveolar macrophages of diseased lung

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specimens (Brett *et al.*, 1993; Morbini *et al.*, 2006), which suggests that RAGE is implicated in lung homeostasis. Recent case-control studies showed striking increases of the levels of RAGE and reductions of the levels of sRAGE (a soluble isoform of RAGE that is thought to provide protection against inflammation) in COPD, which further support the potential role of the RAGE pathway in the clinical pathology of COPD (Ferhani *et al.*, 2010; Ohlmeier *et al.*, 2010; Miniati *et al.*, 2011; Wu *et al.*, 2011; Sukkar *et al.*, 2012).

The gene encoding RAGE is located on chromosome 6p21.3 near the HLA locus (Sugaya *et al.*, 1994). To date, a total of 1517 single-nucleotide polymorphisms (SNPs) have been identified in the RAGE gene (www.ncbi.nlm.nih.gov/SNP), most of which are nonsense mutations. The three functional polymorphisms most extensively studied include two SNPs in the promoter region (−429T/C and −374T/A) and one SNP in exon 3 (G82S) of the RAGE gene. The −429T/C and −374T/A polymorphisms were shown to have a marked effect on the transcriptional activity (Hudson *et al.*, 2001), and the G82S polymorphism occurring in the AGE-binding domain has been shown to have an enhanced binding and cytokine/MMP generation following ligation by a prototypic S100/calgranulin (Hofmann *et al.*, 2002). The associations identified between RAGE polymorphisms and diseases have primarily focused on inflammatory complications such as Crohn's disease, systemic lupus erythematosus, and cardiovascular diseases (Peng *et al.*, 2009; Dabritz *et al.*, 2011; Lu *et al.*, 2011; Martens *et al.*, 2012). However, whether these polymorphisms are associated with the development of COPD has not been studied. In light of the potential role of RAGE in the pathogenesis of COPD, the aim of the present study was to evaluate the existence of an association of these three polymorphisms (−374T/A, −429T/C, and G82S) with COPD in a case-control study of the Han Chinese population.

Methods

Study population

This case-control study recruited 216 inpatients from the Department of Pulmonology at the First Affiliated Hospital of Harbin Medical University who were diagnosed with COPD according to the criteria of the Global Initiative for Chronic Obstructive Lung Disease (GOLD) (Rabe *et al.*, 2007): a measurement of FEV1/FVC < 70% of the predicted value and/or FEV1 < 80% of the predicted value and without an exacerbation of the disease in the 12 weeks before recruitment. A total of 239 healthy individuals without chronic respiratory symptoms and with FEV1/FVC ≥ 70 and FEV1 ≥ 80 (% predicted) were recruited as controls from the same geographical area (Central Harbin); these participants were comparable to the patient cohort with regard to age (within 5 years), sex, and race. Individuals with a history of asthma or other airway diseases and systemic disorders, such as cardiovascular diseases, dementia, or cancers, were excluded from the study. Smoking habits were assessed during the examination by an interview. Current smokers were defined as subjects who reported current, regular use of cigarettes. Past smokers were defined as subjects who had quit smoking since at least 1 year. Never smokers were defined as subjects who reported never using

cigarettes. After informed consent was obtained from each participant, a 5 mL peripheral blood sample was drawn from each participant for genetic analysis. The RAGE promoter genotype was determined using a PCR-restriction fragment length polymorphism (RFLP)-based method. The ethnic composition of the patients with COPD and control individuals in the present study was 100% Chinese Han. This study was approved by the Ethics Committee of Harbin Medical University.

Genotyping and sequencing

Genomic DNA was isolated from the patients' white blood cells using the EZ-10 Spin Column Whole Blood Genomic DNA Isolation Kit (Sangon Biotech®, Shanghai, China) according to the manufacturer's protocols and stored at −20°C.

Genotyping of the RAGE gene for the presence of −374T/A and −429T/C mutations in the promoter region of this gene were performed by PCR-RFLP amplification using the following primers: 5'-GGG GCA GTT CTC TCC TCA-3' (forward primer) and 5'-TCG TCT TGT CAC AGG GAA-3' (reverse primer). Enzymatic digestions to identify −429T/C variants were carried out using AluI (Fermentas, Lithuania) at 37°C for 3 h. The products of enzymatic digestion were fragments of 250 bp for the wild-type allele T or 162 and 88 bp for the mutated variant C (Ng *et al.*, 2012). Enzymatic digestions were performed to identify the −374T/A variants using MfeI (Fermentas, Lithuania) at 37°C for 3 h. The products of enzymatic digestion were fragments of 215 and 35 bp for the wild-type allele T and 250 bp for the polymorphic allele A (Ng *et al.*, 2012). Genotyping of the RAGE intron 3 G82S mutation (−69 bp insertion/deletion) was also performed by PCR-RFLP using the following primers: 5'-GTA AGC GGG GCT CCT GTT GCA-3' (forward primer) and 5'-GGC CAA GGC TGG GGT TGA AGG-3' (reverse primer). After enzymatic digestion using MfeI (Fermentas, Lithuania), the following fragments were identified: 276 bp for the 82G wild-type alleles and 64 and 212 bp for the minor allele 82S (Li *et al.*, 2010).

The digestion products were all analyzed by electrophoresis on 3% agarose gel. For quality control, we sequenced 5% DNA samples directly for each SNP, and no inconsistencies were detected. The results were read and interpreted in a blinded manner without knowledge of the grouping design.

Statistical analyses

Quantitative data are expressed as mean ± SD. The allele-counting method was used to calculate the allele frequencies and genotype distribution of COPD patients and control individuals. The Hardy-Weinberg equilibrium was assessed using a χ^2 test. Allele and genotype distributions in groups were evaluated using the Fisher's exact test or the chi-square test. Odds ratios (OR) and 95% confidence intervals (CI) were calculated separately as an index of the association of the studied genotypes with COPD. Stratified analyses were performed separately for smoking and nonsmoking participants. Stata Version 11.0 software (College Station, TX) was used to perform all statistical analyses at a level of statistical significance defined as $p < 0.05$ and to determine statistical power.

Results

The demographic and clinical characteristics of the participants are shown in Table 1. The mean age of the patients (140 men and 76 women) was 60.9 ± 8.9 years as compared with 62.1 ± 10.1 years for the control group (142 men and 97 women). No significant differences were observed between the COPD and control groups with regard to age ($p=0.181$), gender ($p=0.247$), BMI ($p=0.382$), or years of education ($p=0.054$).

Compared with controls, the COPD group included more current smokers (35.6% vs. 26.8%, $p=0.037$). For those with an established COPD index, the respiratory rate was higher, but the forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1), and FEV1/FVC were significantly lower in the COPD group than in the control group (all $p=0.000$), indicating poor lung function in patients with COPD. No differences were found in other factors examined between the two study groups.

The genotype and allele frequencies of the *RAGE* (−374T/A, −429T/C, and G82S) polymorphisms in patients with COPD and control individuals are summarized in Table 2. The genotype distributions of these two polymorphisms among the cases and controls were in agreement with the Hardy–Weinberg equilibrium, and no deviations were observed ($p>0.05$). A significant difference was found between COPD patients and control subjects in the distribution of the G82S variation (Table 2). The G versus S allele frequencies of the *RAGE* G82S polymorphism were 69.7% versus 30.3% among the patient cohort and 76.6% versus 23.4% among the control subjects ($p=0.023$). The prevalence of the *RAGE* G82S GS genotype and S allele frequencies was significantly higher in patients than in controls (OR=1.70, 95% CI: 1.15–2.50, $p=0.0098$ and OR=1.42, 95% CI: 1.06–1.91,

TABLE 2. GENOTYPE AND ALLELE FREQUENCIES OF *RAGE* POLYMORPHISMS BETWEEN COPD PATIENTS AND CONTROLS, AND CORRESPONDING ORs FOR COPD

Genotype & allele	COPD patients (n=216)	Healthy controls (n=239)	OR (95% CI)	p-Value
−374T/A				
TT	158 (73.1)	171 (71.5)	1.00	
TA	52 (24.1)	59 (24.7)	0.95 (0.62–1.47)	0.92
AA	6 (2.8)	9 (3.8)	0.72 (0.25–2.07)	0.73
T allele	368 (85.2)	401 (83.9)	1.00	
A allele	64 (14.8)	77 (16.1)	0.91 (0.63–1.30)	0.65
−429T/C				
TT	170 (78.7)	190 (79.5)	1.00	
TC	43 (19.9)	47 (19.7)	1.02 (0.63–1.67)	0.93
CC	3 (1.4)	2 (0.8)	1.68 (0.19–2.26)	0.57
T allele	383 (88.7)	427 (89.3)	1.00	
C allele	49 (11.3)	51 (10.7)	1.07 (0.69–1.66)	0.75
G82S				
GG	101 (46.8)	142 (59.4)	1.00	
GS	99 (45.8)	82 (34.3)	1.70 (1.15–2.50)	0.0099^b
SS	16 (7.4)	15 (6.3)	1.50 (0.71–3.17)	0.38
G allele	301 (69.7)	366 (76.6)	1.00	
S allele	131 (30.3)	112 (23.4)	1.42 (1.06–1.91)	0.023^a

Data are presented as number (%).

The bold value indicates statistical significance.

^a $p<0.05$.

^b $p<0.01$.

OR, odds ratios.

$p=0.023$, respectively). No differences in the −374T/A and −429T/C genotype and allele frequencies were observed between COPD cases and control subjects (Table 2). For the −374T/A polymorphism, the prevalence of T and A allele was 85.2% and 14.8% in COPD patients, and 83.9% and 16.1% in controls ($p=0.65$), respectively. The *RAGE* −374T/A AA genotype and A allele frequencies did not show any significant difference between COPD patients and controls (OR=0.72, 95% CI: 0.25–2.07, $p=0.73$ and OR=0.91, 95% CI: 0.63–1.30, $p=0.65$, respectively). Similarly, for the *RAGE* −429T/C polymorphism, the prevalence of the T and C allele was 88.7% and 11.3% in COPD patients and 89.3% and 10.7% in controls ($p=0.75$), respectively. The *RAGE* −429T/C CC genotype and C allele frequencies did not show any significant difference between COPD patients and controls (OR=1.68, 95% CI: 0.19–2.26, $p=0.57$ and OR=1.07, 95% CI: 0.69–1.66, $p=0.75$, respectively).

The allele and genotype distributions were compared in current smokers, past smokers, and never smokers (COPD patients and controls) to determine whether the prevalence of different alleles or genotypes was associated with tobacco exposure. The results are presented in Table 3. In current smokers, the prevalence of the *RAGE* G82S GS genotype was significantly higher in COPD patients than in controls (OR=0.49, 95% CI: 0.24–0.99, $p=0.044$). By comparison, in past smokers and never smokers, the prevalence of the *RAGE* G82S genotype was not statistically significant between the COPD patients and the controls (OR=0.63, 95% CI: 0.25–1.61, $p=0.33$ and OR=0.66, 95% CI: 0.38–1.13, $p=0.13$, respectively). There was also no statistically

TABLE 1. BASELINE DEMOGRAPHIC AND CLINICAL CHARACTERISTICS

Variable	COPD patients	Healthy controls	p-Value
Number of subjects	216	239	
Age, years	60.9 ± 8.9	62.1 ± 10.1	0.181
Gender (male), n (%)	140 (64.8)	142 (59.4)	0.247
Body mass index, kg/m ²	24.9 ± 3.6	24.6 ± 3.7	0.382
Education, years	7.0 ± 2.1	7.4 ± 2.3	0.054
Smoking status, [n (%)]			
Current smokers	77 (35.6)	64 (26.8)	0.037^a
Past smokers	40 (18.6)	37 (15.5)	
Never smokers	99 (45.8)	138 (57.7)	
Lung function			
Respiratory rate, breaths/min	23.5 ± 5.1	16.8 ± 3.8	0.000^b
FVC, % predicted	66.8 ± 10.9	96.8 ± 11.2	0.000^b
FEV1, % predicted	43.2 ± 7.8	98.1 ± 10.6	0.000^b
FEV1/FVC, %	50.1 ± 6.5	80.7 ± 9.8	0.000^b

Values are mean (SD), except where indicated. Continuous data are expressed as the mean $\pm$ SD.

The bold value indicates statistical significance.

^a $p<0.05$.

^b $p<0.01$.

COPD, chronic obstructive pulmonary disease; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 s.

TABLE 3. GENOTYPE AND ALLELE FREQUENCIES OF RAGE POLYMORPHISMS BETWEEN COPD PATIENTS AND CONTROLS, AND CORRESPONDING ORs FOR COPD BY SMOKING STATUS

Gene genotype	Current smokers				Past smokers				Never smokers			
	COPD patients	Healthy controls	OR (95% CI)	p-Value	COPD patients	Healthy controls	OR (95% CI)	p-Value	COPD patients	Healthy controls	OR (95% CI)	p-Value
- 374T/A												
TT	54 (70.1)	46 (71.9)	1.00		29 (72.5)	26 (70.3)	1.00		69 (69.7)	98 (71.0)	1.00	
TA	20 (26.0)	16 (25.0)	0.94 (0.44-2.02)	0.87	10 (25.0)	10 (27.0)	1.12 (0.40-3.11)	0.83	26 (26.3)	35 (25.4)	0.95(0.52-1.72)	0.86
AA	3 (3.9)	2 (3.1)	0.78 (0.13-4.89)	0.79	1 (2.5)	1 (2.7)	1.12 (0.07-18.75)	0.94	4 (4.0)	5 (3.6)	0.88 (0.23-3.40)	0.85
T allele	128 (83.1)	108 (84.4)	1.00		68 (85.0)	62 (83.8)	1.00		164 (82.8)	231 (83.7)	1.00	
A allele	26 (16.9)	20 (15.6)	0.91 (0.48-1.72)	0.78	12 (15.0)	12 (16.2)	1.09 (0.46-2.62)	0.84	34 (17.2)	45 (16.3)	0.94 (0.58-1.53)	0.80
- 429T/C												
TT	62 (80.5)	52 (81.2)	1.00		32 (80.0)	30 (81.1)	1.00		77 (77.8)	109 (79.0)	1.00	
TC	14 (18.2)	12 (18.8)	1.02 (0.43-2.40)	0.96	8 (20.0)	7 (18.9)	0.93 (0.30-2.89)	0.90	20 (20.2)	26 (18.8)	0.92 (0.48-1.76)	0.80
CC	1 (1.3)	0	—	0.36	0	0	—	—	2 (2.0)	3 (2.2)	1.06 (0.17-6.49)	0.95
T allele	138 (89.6)	116 (90.6)	1.00		72 (90.0)	67 (90.5)	1.00		174 (87.9)	244 (88.4)	1.00	
C allele	16 (10.4)	12 (9.4)	0.89 (0.41-1.96)	0.78	8 (10.0)	7 (9.5)	0.94 (0.32-2.73)	0.91	24 (12.1)	32 (11.6)	0.95 (0.54-1.67)	0.86
G82S												
GG	33 (42.8)	38 (59.4)	1.00		18 (45.0)	21 (56.7)	1.00		49 (49.5)	81 (58.7)	1.00	
GS	39 (50.6)	22 (34.4)	0.49 (0.24-0.99)	0.044^a	19 (47.5)	14 (37.8)	0.63 (0.25-1.61)	0.33	45 (45.5)	49 (35.5)	0.66 (0.38-1.13)	0.13
SS	5 (6.5)	4 (6.2)	0.69 (0.17-2.80)	0.61	3 (7.5)	2 (5.4)	0.57 (0.09-3.81)	0.56	5 (5.0)	8 (5.8)	0.97 (0.30-3.13)	0.96
G allele	105 (68.2)	98 (76.6)	1.00		55 (68.8)	56 (75.7)	1.00		143 (72.2)	211 (76.4)	1.00	
S allele	49 (31.8)	30 (23.4)	0.66 (0.39-1.12)	0.12	25 (31.2)	18 (24.3)	0.71 (0.35-1.44)	0.34	55 (27.8)	65 (23.6)	0.80 (0.53-1.22)	0.30

Data are presented as number (%).
The bold value indicates statistical significance.
^a $p < 0.05$.

significant difference within the G allele or S allele of the *RAGE* G82S polymorphism in the COPD group compared with the controls ($p > 0.05$). We did not find any significant association of the $-374T/A$ or $-429T/C$ genotypes and alleles with the risk of developing COPD for either current smokers or past smokers or never smokers.

Discussion

The results of the present study provide evidence that a genetic variant of the *RAGE* gene (G82S) is associated with the risk of developing COPD, in contrast with the $-374T/A$ and $-429T/C$ polymorphisms. To the best of our knowledge, these results are the first to suggest an association of the G82S polymorphism of the *RAGE* gene with COPD in a Chinese population. The frequency of individuals with the GS genotype and S allele was significantly higher in patients with COPD than in controls. Additionally, smokers with the G82S polymorphism of *RAGE* may run a higher risk of acquiring COPD. Our findings suggest that the G82S polymorphism of *RAGE* may serve as a genetic marker for predicting the occurrence of COPD in high-risk subjects.

RAGE is a multiligand member of the immunoglobulin superfamily of cell surface molecules expressed constitutively in relatively large amounts in the lung (Cheng *et al.*, 2005). RAGE is a primary cell surface receptor for the S100/calgranulin ligand superfamily and EN-RAGE (extracellular newly identified RAGE-binding protein) (Nawroth *et al.*, 1999). Cellular RAGE can bind with EN-RAGE in the endothelium on mononuclear phagocytes or lymphocytes to trigger cellular activation with the generation of key proinflammatory mediators such as TNF- α and CRP (Hofmann *et al.*, 1999). An analysis of RAGE in bronchial biopsies by immunohistology demonstrated its abundance in the bronchial epithelia of patients with COPD. The conditional upregulation of RAGE expression in the bitransgenic adult mouse lung leads to the manifestation of key features of COPD, including pronounced inflammation and loss of parenchymal tissue (Robinson *et al.*, 2012a). RAGE-deficient mice under hyperoxic conditions survived longer than wild-type controls. These mice also exhibited less cellularity of the airways and diminished alveolar damage compared with wild-type controls (Reynolds *et al.*, 2010). Taken together, this evidence indicates that RAGE plays an important role in the pathogenesis of COPD.

The prevailing mechanisms underlying the pathogenesis of COPD include chronic inflammation, imbalances involving proteases/antiproteases, oxidative stress, and elevated apoptosis mediated by RAGE (Robinson *et al.*, 2012a). Inflammation is produced predominantly by neutrophil infiltrates, which are found in abundance in the bronchoalveolar lavage and sputum of COPD patients. Chemoattractants recruiting neutrophils and other potent inflammatory mediators are also found at increased levels in COPD, including IL-1 β , CXCL1, CXCL5, and TNF- α , among others. (Robinson *et al.*, 2012a). As a well-known factor for airborne pollutant exposure, smoking can trigger inflammation that leads to an impairment of lung function through its influence on TNF- α expression, which suggests that smoking may interact with RAGE to enhance inflammation (Tanni *et al.*, 2010; Diez Pina *et al.*, 2012). Notwithstanding, only one-fourth of cigarette smokers develop

clinically detectable airflow limitation and other symptoms of COPD, suggesting an important role for genetic susceptibility (Stockley *et al.*, 2009).

Two previous large genomewide association (GWA) studies identified that *RAGE* polymorphism was associated with lung function (Hancock *et al.*, 2010; Repapi *et al.*, 2010). Cheng *et al.* (2013) investigated 21 SNPs in the *AGER* locus with sRAGE levels and found that the G82S polymorphism of the *RAGE* gene was associated with circulating levels of sRAGE levels in COPD patients. Young *et al.* (2011b) found that the minor allele (T allele or CT/TT genotype) of the G82S polymorphism of *RAGE* was more commonly found in smokers who did not have COPD compared with those with COPD in a Western population. In our case-control study, we found that the G82S polymorphism of *RAGE* was associated with the risk of developing COPD. Carriers of the GS genotype and S allele of *RAGE* showed a significantly greater prevalence of COPD than subjects with the GG or SS genotype. This is the first report demonstrating an association of the G82S polymorphism of *RAGE* with COPD in a Chinese population. Our results indicated that subjects with the minor S allele of G82S polymorphism of *RAGE* had an increased risk for COPD compared with those bearing the GG genotype, which is in contrast to Young's report (Young *et al.*, 2011b) that found that the minor S allele played a protective role in COPD. Interestingly, the prevalence of G82S polymorphism of *RAGE* in our study was different from that previously reported in the white population. In our population, the G82S frequency was 59.4% for the GG genotype and 34.3% for the GS genotype, which were significantly different from the G82S frequencies of GG (85%) and GS (14%) in the white population. This difference may result from profound ethnic differences. In addition, differences in the sample size, patient selection criteria, and research strategy for these studies may help explain this difference. The S allele of this SNP results in glycine being changed to serine at position 82 of the third exon encoding the RAGE protein (a nonsynonymous change altering the polarity of the amino acid at this position) and has been shown to be associated with both reduced serum levels of sRAGE and increased sRAGE signaling compared with the more common G allele (Smith *et al.*, 2011). This change in sRAGE signaling affects downstream gene expression through mitogen-activated protein kinases and NF- κ B, both of which have been implicated in the inflammatory responses in COPD (Young *et al.*, 2011a).

The pathogenesis of COPD involves the combined effects of factors such as chronic smoke exposure and the presence or absence of protective or susceptible genetic variants. Cigarette smoking is currently the greatest risk factor for the development of COPD. RAGE and two of its ligands (amphoterin and S100A12) were elevated in pulmonary epithelial cells and macrophage (RAW) cells cultured with cigarette smoke extract (CSE) (Reynolds *et al.*, 2008). Moreover, cigarette smoke exposure significantly upregulated the expression of RAGE and its ligand (S100A6) in both the airways and lungs of rats (Zhang *et al.*, 2008, 2009). The siRNA inhibition of RAGE expression decreased CSE-induced NF- κ B activation and cytokine secretion (Reynolds *et al.*, 2011). Further evidence indicated that elevated levels of RAGE in alveolar macrophages was accompanied by an increased

expression of active Ras and proinflammatory cytokines, which were reduced in RAGE-deficient alveolar macrophages (Robinson *et al.*, 2012b; Chen *et al.*, 2013). Together, these findings suggest that RAGE may mediate the effects of cigarette smoking or CSE on airway and pulmonary inflammation. In our study, we classified the patients with COPD into smoking and nonsmoking groups. We found that the smoking individuals carrying the GS genotype or S allele of *RAGE* may run a higher risk of COPD compared with those with the GG or SS genotype. Cigarette smoke is an important exogenous source of reactive glycation products capable of promoting the formation of AGEs, which are irreversibly glycosylated proteins that efficiently bind RAGE (Cerami *et al.*, 1997). Other studies have shown that serum AGEs and apolipoprotein B-linked AGE levels are significantly elevated in cigarette smokers relative to nonsmokers (Nicholl *et al.*, 1998). It is conceivable that smoking may promote the binding of RAGE with its ligands, thereby exacerbating the progression of COPD.

Our pilot study has certain limitations. First, this study was based upon a cross-sectional design; as a result, we cannot rule out the possibility of recall and selection bias. However, the genotype distribution of controls was in agreement with the Hardy–Weinberg equilibrium for all three polymorphisms, and the allele frequencies were in the range of those previously reported (Lindholm *et al.*, 2006; Peng *et al.*, 2009; Lu *et al.*, 2011; Kawai *et al.*, 2012; Ng *et al.*, 2012). Second, information about important COPD risk factors, such as smoking, was gathered from a self-reporting questionnaire, which may introduce information bias. However, these factors are major health issues, and participants' awareness of these conditions was assessed in the questionnaire by asking whether their condition had been diagnosed by physicians. Third, this study included only 216 patients with COPD and may not have sufficient statistical power to assess the associations of *RAGE* polymorphisms with risk of COPD, particularly for grouped analyses, but we did find significant results for G82S polymorphism of *RAGE* and the risk of COPD. However, our results should be interpreted with caution. A sample size with sufficient statistical power is critical to the success of genetic association studies to detect causal genes of human complex diseases. The sample size for detecting associations between disease and SNP markers is known to be highly affected by disease prevalence, disease allele frequency, linkage disequilibrium, inheritance models (e.g., additive, dominant, and multiplicative models), and effect size of the genetic variants (e.g., OR, relative risk). Particular attention must be paid to sample sizes when more than one variable is studied simultaneously, such as multiple imperfectly correlated traits, intergenic interactions (epistasis), or gene–environment interactions. Cardon and Bell (2001) indicated that sample sizes involving 1000 to 10,000s of individuals might be required to generate robust data. Pooling case–control studies in complex diseases requires very high accuracy; however, genuine differences in allele frequencies between cases and controls might be quite small. Thus, the comparatively small sample size, together with other environmental risks may mask the genuine differences in allele frequencies between cases and controls. Too small a sample size to detect true evidence for an association increases false-negative rates and reduces the reliability of a study.

False-negative rates are increased by multiple factors that cause systematic biases and such biases reduce statistical power. Hong and Park (2012) suggested that a lower sample size for testing more common SNPs with stronger effect sizes and increased LD between marker alleles and disease alleles might contribute to achieve adequate statistical power. In addition, neither the levels of RAGE nor those of sRAGE were evaluated. However, the primary purpose of our study was to establish a genetic reference for future studies. Thus, this investigation focused on assessing the association of different gene polymorphisms of *RAGE* on the risk of developing COPD. Larger patient and control cohorts will be needed to confirm the association of the *RAGE* gene polymorphism with COPD in other populations.

In conclusion, our study is the first to describe the existence of an association of *RAGE* gene polymorphism with the risk of developing COPD in a Chinese population. Our findings support the notion that the G82S polymorphism of *RAGE* contributes to the development of COPD. In particular, smokers carrying the GS genotype of *RAGE* may have a greater risk of developing COPD. Our study may provide clues for use in the evaluation of individual susceptibility to COPD and to explore effective measures for the control and prevention of COPD.

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Disclosure Statement

The authors have no actual or potential conflicts of interest related to this article. Appropriate approval and procedures were used concerning human study subjects.

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