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Research article

sRAGE levels are decreased in plasma and sputum of COPD secondary to biomass-burning smoke and tobacco smoking: Differences according to the rs3134940 AGER variant

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ABSTRACT

The receptor for advanced glycation end products (RAGE) and its gene (*AGER*) have been related to lung injury and inflammatory diseases, including chronic obstructive pulmonary disease (COPD). We aimed to evaluate the association of rs2071288, rs3134940, rs184003, and rs2070600 *AGER* single-nucleotide variants and the soluble-RAGE plasma and sputum levels with COPD secondary to biomass-burning smoke (BBS) and tobacco smoking. Four groups, including 2189 subjects, were analyzed: COPD secondary to BBS exposure (COPD-BBS, n = 342), BBS-exposed subjects without COPD (BBES, n = 774), tobacco smoking-induced COPD (COPD-TS, n = 434), and smokers without COPD (SWOC, n = 639). Allelic discrimination assays determined the *AGER* variants. The sRAGE was quantified in plasma (n = 240) and induced-sputum (n = 72) samples from a subgroup of patients using the ELISA technique. In addition, a meta-analysis was performed for the association of rs2070600 with COPD susceptibility. None of the studied genetic variants were found to be associated with COPD-BBS or COPD-TS. A marginal association was observed for the rs3134940 with COPD-BBS (p = 0.066). The results from the meta-analysis, including six case-control studies (n = 4149 subjects), showed a lack of association of rs2070600 with COPD susceptibility (p = 0.681), probably due to interethnic differences. The sRAGE plasma levels were lower in COPD-BBS compared to BBS and in COPD-TS compared to

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SWOC. The sRAGE levels were also lower in sputum samples from COPD-BBS than BBES. Subjects with rs3134940-TC genotypes exhibit lower sRAGE plasma levels than TT subjects, mainly from the COPD-BBS and SWOC groups. The *AGER* variants were not associated with COPD-BBS nor COPD-TS, but the sRAGE plasma and sputum levels are related to both COPD-BBS and COPD-TS and are influenced by the rs3134940 variant.

1. Introduction

The receptor for advanced glycation end products (RAGE) is a protein from the immunoglobulin superfamily that can bind the end products of non-enzymatic glycation and oxidation of protein/lipids, which are the advanced glycation end products and a variety of other molecules derived from stressed or damaged cells, such as amyloid β peptide, high-mobility group box 1 (HMGB1), cell adhesion molecules, and molecules from bacteria, viruses, and parasites [1]. RAGE can be mainly found as membrane-bound and soluble RAGE (sRAGE). The former comprises three domains: an extracellular domain that recognizes and binds RAGE ligands, a hydrophobic transmembrane domain, and a cytoplasmic domain involved in intracellular signaling. Meanwhile, sRAGE contains only the extracellular domain and is a product of alternative splicing events (esRAGE) or the proteolytic cleavage (cRAGE) for the membrane form [2].

RAGE is expressed in different tissues, such as the brain, kidney, liver, heart, and several cell types, including smooth muscle cells, endothelium, T-lymphocytes, neurons, and monocytes/macrophages [3]. It is expressed at high levels in the lung, mainly at the basal cell membrane of alveolar type I cells [4]. When RAGE binds to the multiple ligands, a signaling pathway induces inflammation and can internalize its ligands via the endocytic pathway [5,6]. sRAGE may act as a dominant negative isoform and block RAGE signaling by functioning as an extracellular decoy receptor by inhibiting RAGE ligand binding [7].

AGER, the gene encoding RAGE, is located on the short arm of chromosome 6 (6p21.3), the same locus of the major histocompatibility complex III. It presents many alternatively spliced transcript variants encoding different isoforms, from which the predominant has 11 exons and encodes a 420 amino acid protein. This gene is highly polymorphic [8], and several single-nucleotide variants (SNV) have been related to diseases such as diabetic retinopathy [9], breast cancer [10], and gastric cancer [11].

Regarding pulmonary diseases, the *AGER* variants have been associated with idiopathic pulmonary fibrosis [12], cystic fibrosis [13], and chronic obstructive pulmonary disease (COPD) [14]. Moreover, RAGE has been reported as a promising biomarker of lung epithelium injury. Differential RAGE levels have been related to acute respiratory distress syndrome (ARDS) [15] and idiopathic pulmonary fibrosis [16].

The SNV rs2070600 has been controversially associated with COPD susceptibility related to tobacco smoking and sRAGE plasma levels [17–19]. Nevertheless, other *AGER* SNVs have been less studied. For instance, the rs2071288 is related to sRAGE levels in patients with COPD [18,20], and rs184003 with COPD risk [21]. Meanwhile, the rs3134940 is located in the regulatory binding site influencing the production of sRAGE, but it has been poorly studied in patients with COPD [8,22]. In addition, all of these studies have included patients with COPD secondary to tobacco smoking, and there are also patients with COPD due to biomass-burning smoke (BBS) chronic exposure, and differences could be observed in the genetic and protein association. Therefore, we aimed to evaluate the association of rs2071288, rs3134940, rs184003, and rs2070600 AGER variants with COPD secondary to biomass-burning smoke and tobacco smoking. In addition, a meta-analysis for the association of the rs2070600 with COPD susceptibility was performed, and the evaluation of sRAGE plasma and sputum levels in a subgroup of patients with COPD and control subjects.

2. Methods and subjects

2.1. Patients with COPD and control subjects

The study was approved by the Ethics in Research Committee of the Instituto Nacional de Enfermedades Respiratorias Ismael Cosio Villegas (INER) in Mexico City (protocol numbers B11–19, C38-19, and B14-17), and it accomplished the Declaration of Helsinki. All the subjects included in the study filled out a hereditary and pathology survey. Data about smoking and BBS chronic exposure were considered. Before phlebotomy, participants signed an informed consent letter. They were Mexican mestizos, ≥40 years old, and both sexes were included. Cases or controls were excluded whether they presented autoimmune and/or other inflammatory diseases.

We included patients with COPD secondary to household BBS exposure (COPD-BBS, n = 342) and tobacco smoking-induced COPD (COPD-TS, n = 434). COPD was diagnosed through a clinical examination and pulmonary function test results, considering an FEV_1/FVC ratio <70% after bronchodilator administration, according to the specifications for Mexican patients [23]. All patients were stable, with no history of exacerbations in the previous three months or another lung disease, and they were not under supplementary oxygen, antibiotics, and/or systemic corticosteroid treatment at the enrollment time.

The control group for COPD-BBS included household BBS-exposed subjects without COPD (BBES, n = 774) who reported a BBS exposure at least 100 h/year. COPD-BBS and BBES groups were recruited from rural villages in the Oaxaca highlands and suburban areas in the Tlalpan mayoralty, where they used biomass for cooking and heating. In both cases, they were non-smokers, and they were part of the National Program for Equality between women and men with COPD timely diagnostic campaign [24].

Tobacco-smoking subjects without COPD (SWOC, n = 639) were included as the control group of COPD-TS. They were considered smokers if they consumed ten cigarettes per day for at least ten years. The COPD-TS and SWOC participants were enrolled from the

Tobacco Smoking and COPD Research Department (DITABE), COPD clinic, and clinical service 5 of the INER, and there was no history of exposure to BBS in both cases.

2.2. Blood sampling and DNA isolation

Peripheral blood samples were collected in tubes with EDTA and were centrifuged at 4500 rpm for 5 min. Plasma was separated using micropipettes and stored at −80 °C until assayed; meanwhile, the blood cells were further processed for DNA isolation. According to the supplier recommendations, genomic DNA was isolated with the Blood DNA Preparation - Solution Kit, Jena Bioscience (Jena, Germany). The purity and concentration of DNA were evaluated through a NanoDrop™ 2000 spectrophotometer, Thermo Fisher Scientific (Massachusetts, USA).

2.3. Induced-sputum collection and preparation

A nebulization with sterile 4.5% saline solution was performed for the induced-sputum collection samples. Each patient was asked to wash their mouth with water. Nebulization was conducted for 5 min with a 5-min rest period between each. This procedure was performed a maximum of 3 times according to the protocol established by the ERS [25]. The sputum sample was collected in a sterile recipient. The saliva in the sputum sample was removed using a transfer pipette; subsequently, the mucus was disintegrated in isotonic saline solution using needles of 0.9 and 0.7 mm in diameter. This mix was filtered with a 70 μm nylon filter. The fluid was centrifuged at 3500 rpm for 10 min, and the induced sputum supernatant was separated from the pellet by decantation and stored in cryotubes at −80 °C. Induced-sputum samples were only available from some patients with COPD-BBS and the BBES controls that agreed to participate in the nebulization and expectoration procedures.

2.4. Genotyping of AGER single-nucleotide variants

The selection of candidate *AGER* variants was based on an exhaustive literature review performed in February 2022 in science databases and genomic browsers (i.e., PubMed, ClinVar, dbSNP, and ENSEMBL) through the search terms 'AGER variants' 'COPD genetics' 'AGER and diseases', 'AGER and COPD'. The main selection criteria were their functional implication in the soluble protein (sRAGE) and/or their relevance in COPD. The rs2071288, rs3134940, rs184003, and rs2070600 variants were genotyped for allelic discrimination with TaqMan® SNP Genotyping Assays (C_15861169_10, C__3293840_10, C__2412456_10, and C_15867521_20, respectively) in a StepOnePlus™ Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA), according to the supplier instructions.

2.5. Meta-analysis for the association of rs2070600 AGER variant with COPD susceptibility

The association of rs2070600 variants with COPD susceptibility has been controversially described. Therefore, we conducted a meta-analysis for the association of this *AGER* variant and COPD susceptibility. The search was performed in June 2023 in the PubMed database of the National Library of Medicine, according to the search terms: 'AGER polymorphism AND COPD' and 'rs2070600 AND COPD'. The search retrieved 16 and 15 results, respectively. We included studies if they: (1) were case-control studies including the COPD susceptibility as the primary outcome, (2) were independent studies reporting original data, (3) included essential information to calculate OR (absolute numbers of genotypes), and (4) report a genotype distribution accomplishing with HWE in the control population. Studies were excluded when absolute numbers of CC genotypes were not reported. Nine studies were duplicated and removed. Finally, five studies were considered for the meta-analysis after applying inclusion and exclusion criteria.

The meta-analysis was performed in JASP [26] through the effect size analysis. The effect size and standard error were calculated according to the JASP recommendations [26,27]. The heterogeneity between studies was assessed by the I^2 measure and τ^2, and Cochran's Q test with n-1 degrees of freedom (n = number of studies included) through the restricted maximum likelihood model [26]. A random-effect model was preferred when the heterogeneity was significantly different from zero. The Wald test calculated the associated P value for the estimated effect size. The forest and funnel plots were performed in JASP, and the publication bias was evaluated through Egger's test.

2.6. Quantification of sRAGE plasma and sputum levels

The quantification of the soluble receptor for advanced glycation end products (sRAGE) was performed in plasma by an ELISA assay (soluble Receptor for Advanced Glycation End product ELISA Kit, My BioSource, catalog number MBS766075, San Diego, CA, USA), following the manufacturer's instructions. A subgroup of 80 subjects of each COPD (COPD-BBS, COPD-TS) and non-COPD (BBES, SWOC) comparison was included in the protein analysis. The plasma samples were selected considering firstly the *AGER* genotypes of the studied variants to assure a heterogeneous genotype distribution (COPD-BBS: TT = 44 and TC = 36; BBES: TT = 52 and TC = 28; COPD-TS: TT = 40 and TC = 40; SWOC: TT = 38 and TC = 42), and secondly the plasma sample availability. In addition, the sRAGE was quantified through the same ELISA kit in 72 induced sputum supernatant samples, which were only available from patients with COPD-BBS (n = 36) and BBES (n = 36).

2.7. Statistical analysis

Categorical data are presented as frequencies (percentage), and continuous data are shown as the median and interquartile range (IQR 25–75). The Kolmogorov-Smirnov test was employed to assess the normal distribution of continuous data. The genetic association study and logistic regression analyses were performed with PLINK v1.07[28]. The Hardy-Weinberg equilibrium and the linkage disequilibrium analysis were conducted in Haploview [29]. The chi-squared test was utilized to compare categorical variables, while Mann-Whitney U or Kruskal-Wallis tests were performed to analyze continuous variables. The univariate comparison of the protein levels in the study groups was evaluated through the Mann-Whitney *U* test, and the adjustment for co-variables was assessed with linear regression. Tests were performed with RStudio version 4.1.2 [30].

3. Results

3.1. Demographic and clinical data of patients included in the study

This study involved the analysis of 2189 subjects, classified according to the associated environmental risk factors and the diagnosis of COPD at the sampling time. Table 1 shows the patients' and control subjects' demographic and clinical data. Besides the differences in clinical variables, the BBES group comprised more males than COPD-BBS; they also were younger and presented less biomass exposure than the patients' group. Nevertheless, the body mass index (BMI) was similar between groups. Similarly, the age, male proportion, BMI, smoking index, and smoking years differed between the COPD-TS and SWOC groups.

3.2. Association study of AGER single-nucleotide variants with COPD susceptibility

The genotype and allele frequencies of the studied *AGER* variants according to the different groups are presented in Table 2. The genotype distribution was accomplished with the Hardy-Weinberg Equilibrium except for the rs2071288 and rs184003 in the BBES group. We found no significant difference in the frequencies between the COPD groups and their respective group controls. However, the rs3134940 genotypes and alleles frequency showed a marginally significant difference between the COPD-BBS group and BBES ($p = 0.066$ and 0.075, respectively). We wondered if any co-variate was affecting the association of genetic variants; thus, a logistic regression analysis was performed to adjust for co-variables different between groups according to Table 1 (COPD-BBS: age, sex, and BEI exposure. COPD-TS: Age, sex, BMI, SI, Years smoking). The adjusted p-values according to the combination of co-variates are presented in Supplementary Tables 1 and 2 For the COPD-BBS association study, no adjusted p-values were found to be significant (Supplementary Table 1).

Nevertheless, we observed a significant difference when age was considered a co-variate in the analysis association of the rs2070600 and COPD secondary to tobacco smoking (Supplementary Table 2, $p = 0.021$). The statistical significance remained in all the models as long as age was included as co-variate (Supplementary Table 3, $p < 0.05$ in all cases). The CT rs2070600 genotype was found to be a low-risk variant ($p = 0.026$, OR = 0.150, CI 95% = 0.002–0.699) when co-variates were considered (Supplementary Table 4). It is worth mentioning that the results should be carefully assessed since the CT frequency in the four studied groups is low, which could underpower the findings.

We performed a linkage disequilibrium analysis for the COPD-BBS + BBES and COPD-TS + SWOC groups. We observed a moderate linkage disequilibrium of the rs3134940 with rs2071288 and rs184003 variants (D' = 64 and 76 respectively, Supplementary Fig. 1a) in COPD-BBS analysis, but a complete linkage disequilibrium for the rs3134940 and rs184003 in COPD-TS analysis (Supplementary

Table 1
Demographic and clinical data of the subjects included in the genetic association study.

Variable	COPD-BBS (n = 342)	BBES (n = 774)	p-value	COPD-TS (n = 434)	SWOC (n = 639)	p-value
Age, years	73 (66–79)	58 (50–68)	<0.0001	67 (61–73)	54 (48–60)	<0.0001
Sex Male	9.94%	20.43%	<0.0001	72.04%	48.91%	<0.0001
BMI kg/m^2	26.9 (23.3–45.0)	27.6 (24.8–30.9)	0.0754	25.5 (22.4–28.8)	27.3 (24.6–29.9)	<0.0001
BEI, hours/year	270 (138–400)	186 (127–240)	<0.0001	NA	NA	NA
TI	NA	NA	NA	40 (22.5–57.8)	20.7 (9.5–35)	<0.0001
Years smoking	NA	NA	NA	40 (30–50)	31 (25–39)	<0.0001
FVC % pred	77 (64–91.8)	92 (80–103)	<0.0001	75 (61–90)	96 (85–107)	<0.0001
FEV_1 % pred	58 (46–76)	98 (83.2–109.7)	<0.0001	49 (34.8–72.2)	96 (83–108)	<0.0001
FEV_1/FVC	60 (51.5–68.4)	83.8 (78.2–93.9)	<0.0001	53 (40–62.4)	79 (75–83.5)	<0.0001
GOLD I	18.9%	NA	NA	9.7%	NA	NA
GOLD II	58.9%	NA	NA	45.6%	NA	NA
GOLD III	20.0%	NA	NA	29.5%	NA	NA
GOLD IV	2.2%	NA	NA	15.2%	NA	NA

The categorical variables are presented as percentages. The continuous variables are expressed as median (first quartile-third quartile). All lung function values are post-bronchodilator. BBS, biomass-burning smoke; BBES, subjects exposed to BBS without COPD; BEI, biomass-burning smoke exposure index; BMI, body mass index; COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in the first-second; FVC, forced vital capacity; GOLD, Global Initiative for Chronic Obstructive Lung Disease; NA, not applicable; TI, tobacco index; SWOC, smokers without COPD; TS, tobacco smoking.

Table 2
Association study of *AGER* variants with COPD by biomass-burning smoke exposure and tobacco-smoking subjects.

SNV	COPD-BBS (n = 342)	BBES (n = 774)	p-value	COPD-TS (n = 434)	SWOC (n = 639)	p-value
rs2071288	n = 341	n = 771		n = 433	n = 637	
CC	334 (0.979)	754 (0.978)	0.801	420 (0.970)	618 (0.970)	0.985
CT	7 (0.020)	16 (0.021)		13 (0.030)	19 (0.030)	
TT	0	1 (0.001)		0	0	
C	675 (0.990)	1524 (0.988)	0.771	853 (0.985)	1255 (0.985)	0.985
T	7 (0.010)	18 (0.012)		13 (0.015)	19 (0.015)	
rs3134940	n = 340	n = 767		n = 427	n = 634	
TT	292 (0.859)	688 (0.897)	0.066	360 (0.843)	550 (0.867)	0.355
TC	48 (0.141)	79 (0.103)		67 (0.157)	83 (0.131)	
CC	0	0		0	1 (0.002)	
T	632 (0.929)	1455 (0.948)	0.075	787 (0.921)	1183 (0.933)	0.317
C	48 (0.071)	79 (0.051)		67 (0.078)	85 (0.067)	
rs184003	n = 342	n = 774		n = 430	n = 639	
CC	309 (0.903)	702 (0.907)	0.172	349 (0.811)	532 (0.832)	0.527
CA	33 (0.096)	65 (0.084)		77 (0.179)	99 (0.155)	
AA	0	7 (0.009)		4 (0.009)	8 (0.013)	
C	651 (0.952)	1469 (0.949)	0.781	775 (0.901)	1163 (0.910)	0.491
A	33 (0.048)	79 (0.051)		85 (0.099)	115 (0.090)	
rs2070600	n = 342	n = 770		n = 434	n = 635	
CC	342 (1.000)	767 (0.996)	0.596	431 (0.993)	625 (0.984)	0.312
CT	0	3 (0.004)		3 (0.007)	10 (0.016)	
TT	0	0		0	0	
C	684 (1.000)	1537 (0.998)	0.248	865 (0.996)	1260 (0.992)	0.197
T	0	3 (0.002)		3 (0.003)	10 (0.008)	

Genotypes were missed in some cases; therefore, the n included in each study is specified in the table. Data is presented as absolute numbers (frequency). BBS, biomass-burning smoke; BBES, subjects exposed to BBS without COPD; COPD, chronic obstructive pulmonary disease; SWOC, smokers without COPD; TS, tobacco smoking.

Fig. 1b). We performed the analyses for haplotype association, and no differences were observed in the haplotype frequencies among the COPD cases and control groups (Supplementary Table 5); however, the marginal association in haplotypes, including the rs3134940-C, was also observed in the COPD-BSS analysis (rs3134940/rs184003 CC, $p = 0.074$).

3.3. Meta-analysis results for the association of rs2070600 AGER variant with COPD susceptibility

The meta-analysis was conducted with the five studies in the literature, and the results are reported in this manuscript. Only studies including COPD secondary to tobacco were found; therefore, no COPD-BBES reports can be observed in the meta-analysis. The information collected for the meta-analysis and particular data are described in Table 3. These studies included 4149 patients and healthy subjects from Korea, China, Egypt, and Mexico.

The heterogeneity estimated by the restricted maximum likelihood method differed significantly from zero ($I^2 = 90.165\%$, $\tau^2 = 0.407$, $Q = 25.73$ with degree of freedom = 5 and $p < 0.001$). Therefore, a random-effect (RE) model was assumed. Fig. 1 shows the forest plot, including the effect sizes of the studies. The Wald test suggested that the rs2070600 *AGER* variant could not be associated with COPD susceptibility (estimated effect size = 0.119; $z = 0.411$; $p = 0.681$). Egger's test showed no evidence of publication bias ($p = 0.699$). The performance of further multivariable analyses was limited due to the small number of studies. However, the relevance of the age could be of great interest to explore as a covariate in the meta-analysis.

3.4. Levels of soluble RAGE in plasma and induced-sputum supernatant in COPD patients and control subjects

As mentioned before, the plasma levels of sRAGE were assessed in subgroups of participants of each group included in the study. Table 4 shows the demographic and clinical data of the subjects evaluated. The age, BMI, and exposure risk factors (BEI and TI) differed

Table 3
Studies included in the meta-analysis for the association of the rs2070600 *AGER* variant with susceptibility to COPD secondary to tobacco.

Study	Publication year	Patients with COPD		Non-COPD subjects		Studied population	Mean age (cases)	Smokers cases (%)	Males (%)
		CC	CT + TT	CC	CT + TT				
This study	2024	431	3	625	10	Mexican	67	100	72
Sin [17]	2022	194	87	93	63	Korean	72.3	63.6	72.5
Yang [31]	2014	405	275	391	296	Chinese	62.7	66.9	71
Kamel [32]	2022	17	43	25	15	Egyptian	62.65	90	83.3
Niu [21]	2019	72	33	359	168	Chinese	57.73	0	55.24
Guo [33]	2012	213	118	100	113	Chinese	61	100	90

COPD: chronic obstructive pulmonary disease.

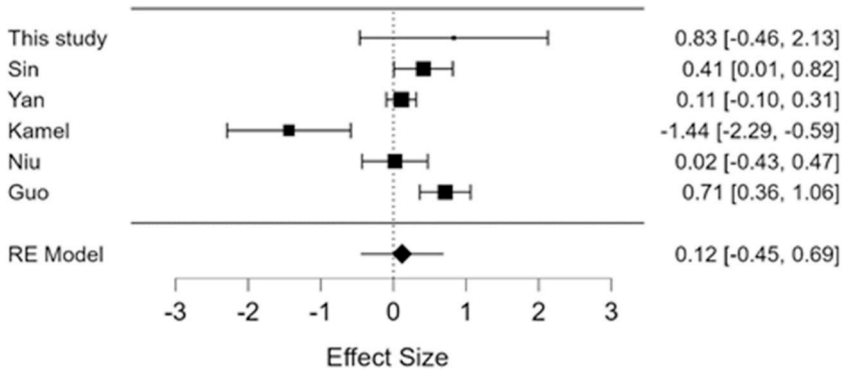

Fig. 1. Forest plot for the effect size of the meta-analysis for the association of rs2070600 *AGER* variant with COPD susceptibility. RE: random-effect model.

Table 4
Demographic and clinical data of subjects included in the sRAGE plasma levels study.

	COPD-BBS (n = 80)	BBES (n = 80)	p-value	COPD-TS (n = 80)	SWOC (n = 80)	p-value
Age (years)	73 (67–79)	60 (53.7–67.0)	<0.001	69 (62–76)	53 (49–60)	<0.001
Sex male (%)	7 (8.7)	4 (5.0)	0.267	53 (66.2)	78 (97.5)	0.097
BMI kg/m^2	33.39 (29.2–37.4)	28.45 (25.8–31.1)	<0.001	24.59 (21.59–26.84)	26.67 (24.03–29.93)	0.002
BEI hours/year	264 (100–380)	271 (147.5–388.8)	0.019	NA	NA	NA
TI	NA	NA	NA	37.75 (25–54)	28.55 (20–32)	0.001
FEV_1%	70 (55–97)	103 (95.5117.5)	<0.001	57 (44.0–68.5)	102 (83–113.5)	<0.001
FVC%	87 (70–101)	100 (90–110)	<0.001	84 (70.5–99.0)	100 (82.5–111.0)	0.003
FEV_1/FVC%	65.7 (55.1–69.3)	82.1 (78.25–87.15)	<0.001	65.7 (55.1–69.3)	80 (75.85–83.02)	<0.001
GOLD I	22.0%	NA	NA	10.0%	NA	NA
GOLD II	52.0%	NA	NA	50.0%	NA	NA
GOLD III	25.0%	NA	NA	26.0%	NA	NA
GOLD IV	0.0%	NA	NA	14.0%	NA	NA

The categorical variables are presented as percentages. The continuous variables are expressed as median (first quartile-third quartile). All lung function values are post-bronchodilator. BBS, biomass-burning smoke exposure; BBES, subjects exposed to BBS without COPD; BEI, biomass-burning smoke exposure index; BMI, body mass index; COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in the first-second; FVC, forced vital capacity; GOLD, Global Initiative for Chronic Obstructive Lung Disease; NA, not applicable; TI, tobacco index; SWOC, smokers without COPD; TS, tobacco smoking.

among COPD and control groups of biomass-burning and tobacco-smoking exposures.

We observed significantly lower levels of the sRAGE plasma levels among patients with COPD-BBS and COPD-TS (227.3 pg/mL [157.4–348.8 pg/mL] and 187.3 pg/mL [110.3–306.3 pg/mL], respectively), than their corresponding control subjects (BBES 363.39

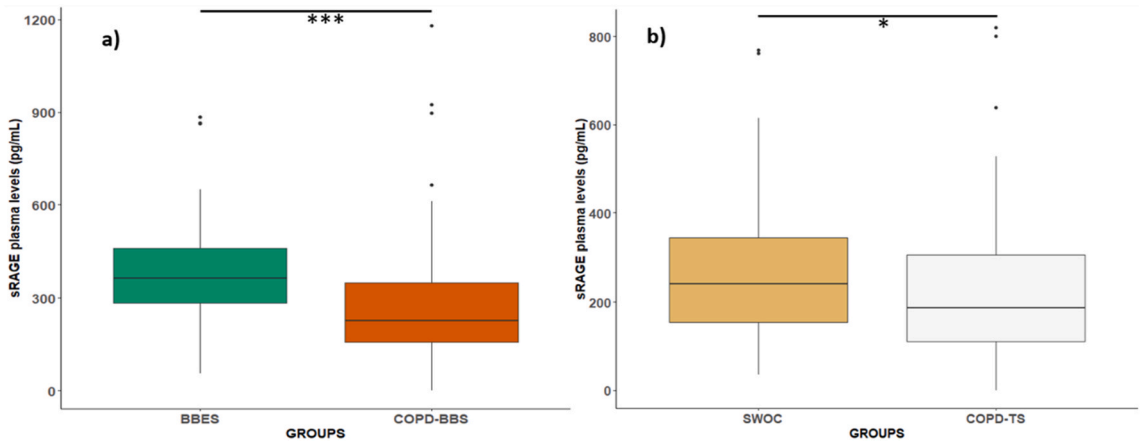

Fig. 2. Plasma levels of sRAGE in COPD patients and exposed to BBS or smokers subjects without COPD. (a) COPD secondary to biomass-burning smoke (COPD-BBS, n = 80) and biomass-burning smoke-exposed subjects without COPD (BBES, n = 80, ***p < 0.001, Mann-Whitney *U* test). (b) Tobacco smoking-induced COPD (COPD-TS, n = 80) and smokers without COPD (SWOC, n = 80, *p < 0.05, Mann-Whitney *U* test).

pg/mL [283.8–458.8 pg/mL], and SWOC 240.9 pg/mL [152.9–344.0 pg/mL]) (Fig. 2a and b), which suggests that low levels of the sRAGE can be found in patients with COPD, regardless the origin of the environmental risk factor exposure. The difference in sRAGE plasma levels between COPD-BBS and BBES remains in a regression model adjusting for age, BMI, and BEI ($p < 0.001$, Supplementary Table 6), and in the COPD-TS and SWOC groups when age, BMI, and smoking index were considered ($p = 0.001$, Supplementary Table 7).

We wondered if the rs3134940 *AGER* variant could influence the plasma levels of sRAGE due to the marginal association observed in Table 2. We found that the sRAGE plasma levels were lower in patients with COPD-BBS carrying the TC genotype (163.5 pg/mL [0–294.8 pg/mL]) when compared to subjects with TT genotypes (278 pg/mL [208–365 pg/mL]) (Fig. 3a, $p = 0.004$). It was not observed when subjects from the BBES (Fig. 3b, TC 374.0 pg/mL vs. TT 356.8 pg/mL, $p = 0.260$) and COPD-TS (Fig. 3c, TC 147.0 pg/mL vs. TT 211.7 pg/mL, $p = 0.089$) were classified by the genotypes, but it did when the group of SWOC was analyzed (Fig. 3d, TC 229.3 pg/mL vs TT 291.3 pg/mL, $p = 0.034$).

In addition, the sRAGE levels were assessed in induced sputum supernatant only in the COPD-BBS ($n = 36$) and BBES ($n = 36$) groups due to the clinical characteristics of these subjects and the sample availability. The clinical and demographical characteristics of the participants in this study are included in Supplementary Table 8. In agreement with the findings of sRAGE plasma levels, the sputum sRAGE levels were significantly lower in patients with COPD-BBS (6 pg/mL [0–102.9 pg/mL) than BBES (278.9 pg/mL [174.3–365.2 pg/mL]) (Fig. 4). A significant difference was also observed in the regression model adjusting for age, sex, and BEI ($p = 0.036$, Supplementary Table 9). The analysis according to other *AGER* genotypes could not be performed due to the low minor allele frequencies of the studied SNVs.

Finally, we evaluated whether the sRAGE plasma and sputum levels differed according to clinical and demographical variables. The correlation plots showed weak correlations with age, BMI, exposure risk factors indexes, and pulmonary function tests with plasma or sputum levels of sRAGE (Supplementary Figs. 2–4).

4. Discussion

AGER has been considered a COPD risk gene since it has been identified in different association studies performed in patients from several countries [21,32–35], but contradictory results have been obtained. Herein, we assessed the *AGER* and sRAGE variability in COPD induced by tobacco smoking and, for the first time, in patients with COPD secondary to biomass-burning smoke. We observed

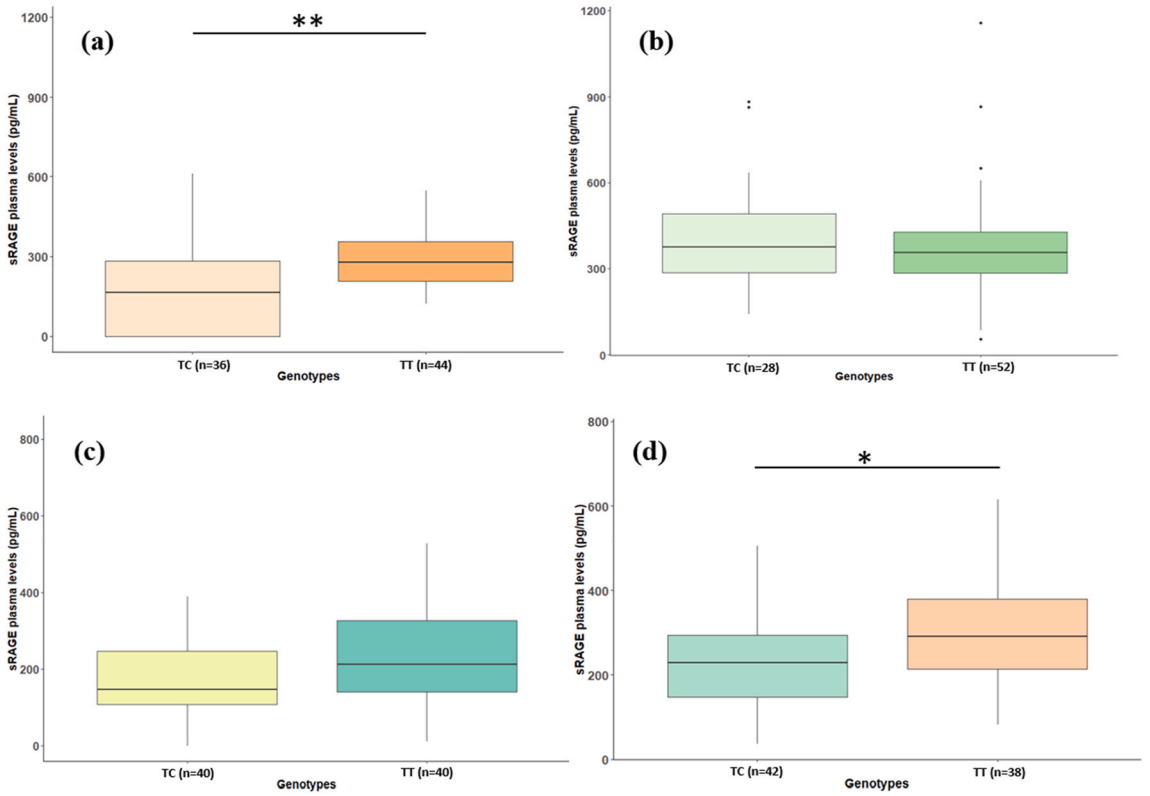

Fig. 3. Plasma levels of sRAGE according to the SNV rs3134940 genotypes in *AGER*. (a) COPD secondary to biomass-burning smoke (COPD-BBS, $n = 80$, $^{**}p < 0.01$, Mann Whitney *U* test); (b) biomass-burning smoke-exposed subjects without COPD (BBES, $n = 80$); (c) tobacco smoking-induced COPD (COPD-TS, $n = 80$); (d) smokers without COPD (SWOC, $n = 80$, $^{*}p < 0.05$, Mann Whitney *U* test).

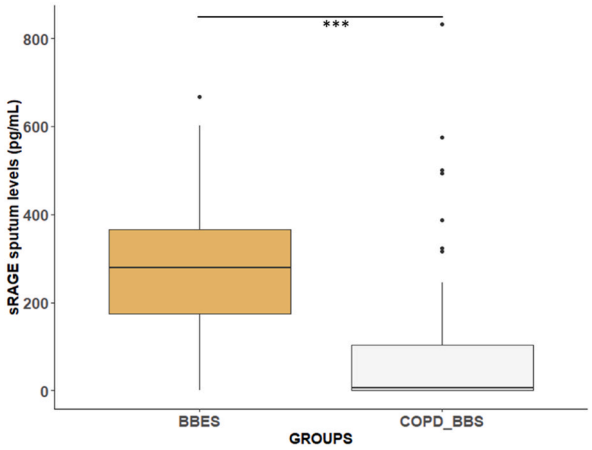

Fig. 4. Levels of sRAGE in induced sputum supernatant evaluated in COPD patients secondary to biomass-burning smoke (COPD-BBS, n = 36) and biomass-burning smoke-exposed subjects without COPD (BBES, n = 36) ***p < 0.001, Mann Whitney *U* test.

that the decrease of sRAGE in plasma and sputum is related to COPD regardless of the environmental risk factors origin (tobacco smoking or exposure to biomass-burning smoke). Moreover, the influence of the rs3134940 *AGER* variant in the sRAGE plasma levels was observed.

The rs3134940 variant, also known as 2184A/G, is located in the intron 8–9 of *AGER* [8], in the regulatory binding site influencing the sRAGE production [22]. In agreement, we observed a difference in the sRAGE plasma levels according to the CC and TC genotypes, with the lower levels observed for the heterozygous genotype, which was also more frequent among patients with COPD than control subjects, although non-significant statistically. The rs3134940 has been previously studied in patients with acute respiratory distress syndrome (ARDS) [36], asthma [37], and other diseases such as diabetes [38] and its complications [39], but not with COPD.

The rs2071288 and rs2070600 have been previously associated with sRAGE levels [14], COPD risk [40], pulmonary function parameters [20], emphysema, and pulmonary fibrosis [41]. We did not observe any association for the rs2071288; however, the CT genotype of the rs2070600 was found to have a low COPD risk strongly influenced by age as a covariate. The main difference lies in the minor allele frequencies of the population studied. Herein, we observed frequencies of the minor alleles <0.10, and the lowest was observed for the rs2070600 (0.000–0.008), which directly affects the association results. Contrarily, this variant is common among Asian populations, in whom the variant has been identified as a COPD risk factor [42]. In the meta-analysis, we could not observe an association of the rs2070600 variants with COPD susceptibility either; however, it was limited by the small number of studies and the lack of the representation of other worldwide populations; therefore, the result driven by the analysis shown the strong influence of the ethnicity in the association of this *AGER* variant with COPD susceptibility, and its association in other COPD clinical outcomes should be further studied.

Likewise, the effect of ethnic origin in the association results of *AGER* variants with COPD was also observed in this study. The same four *AGER* variants were evaluated in two groups of patients from Mexico, and different distributions of the alleles were observed. The rs3134940 was marginally associated with COPD-BBS risk, but this was not detected for the COPD-TS group. The groups exposed to BBS are mainly from indigenous groups with relevant ancestry differences from mestizos included in the smoking subjects with and without COPD, previously demonstrated by our group [43]. Therefore, it is clear that the association of *AGER* variants with COPD risk presents a relevant inter-ethnic and intra-ethnic variability, and further studies on the worldwide population are required to establish a more robust conclusion about its consideration as a COPD risk gene.

Nevertheless, the relevance of the RAGE axis in the innate immune response and as a mediator of pro-inflammatory processes has been widely described [44]. The regulatory RAGE ligands are considered to be increased in inflammatory processes, which upregulates the membrane-bound RAGE expression [45]. The binding of ligands to RAGE induces the cleavage of the receptor to release cRAGE, one of the isoforms of sRAGE. Meanwhile, genetic variants in *AGER* could affect the amounts of the endogenous isoform of sRAGE (esRAGE) since this receptor results from alternative splicing of RAGE pre-mRNA, accounting for less than 25% of the total sRAGE [44]. Hypothesizing that the patients with COPD included in the study also present increased levels of RAGE ligands, which could lead to a higher expression of membrane RAGE. However, whether this could decrease the soluble form of the receptor is not well understood. Although higher plasma levels of sRAGE have been associated with mortality in patients with ARDS [36] and patients with COVID-19 treated with dexamethasone [46], lower sRAGE plasma levels have been found in patients with COPD secondary to tobacco smoke [17,47,48] and emphysema [47], even the sRAGE plasma levels has been related with the rs2070600 genotype [17], similarly to our finding with the rs3134940 *AGER* variant. We observed slightly lower sRAGE plasma levels than in other studies, but the findings about the decrease of the sRAGE in patients with COPD and smokers agree with previous reports [47,49].

Probably, the lack of protective effect exerted by sRAGE affects the regulation of the inflammatory process of COPD. In agreement, a beneficial effect of exogenous sRAGE administration in animals with different inflammatory diseases in which RAGE ligands are accumulated has been reported [50,51]. sRAGE may sequester RAGE ligands, blocking their interaction with RAGE and, probably, other cell surface receptors, which would attenuate inflammation and cellular stress [45].

Thus, we observed the decrease in sRAGE plasma and sputum levels related to COPD regarding environmental risk factors (biomass-burning exposure or tobacco smoking) and the influence of the rs3134940 *AGER* variant in the receptor levels. However, none of the *AGER* variants was associated with BBS or tobacco-induced COPD, except for the rs2070600 variant when the model was adjusted for age in the tobacco-induced COPD study.

Our study is not exempt from limitations. Due to limited funds and sample availability, the sRAGE levels could not be determined in the totality of subjects included in the study. The quantification of relevant RAGE ligands would have deepened the study of sRAGE levels in plasma and sputum. In addition, we lack information about inflammation biomarkers that could correlate with the levels of sRAGE in plasma and sputum and the differential levels of esRAGE and cRAGE. Further information is warranted to unravel the specific mechanism for the decrement of sRAGE in the inflammation process of COPD.

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Data availability statement

Clinvar accession numbers SCV004041890, SCV004041891, SCV004041892, SCV004041893. Organization ID: 507267.

CRediT authorship contribution statement

Ingrid Fricke-Galindo: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Salvador García-Carmona:** Writing – original draft, Software, Methodology, Investigation, Formal analysis. **Jesús Alanis-Ponce:** Validation, Methodology, Investigation, Formal analysis. **Gloria Pérez-Rubio:** Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Alejandra Ramírez-Venegas:** Visualization, Validation, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Francisco Montiel-Lopez:** Visualization, Software, Resources, Methodology, Investigation. **Robinson Robles-Hernández:** Supervision, Resources, Methodology, Investigation, Formal analysis. **Rafael de Jesús Hernández-Zenteno:** Visualization, Software, Resources, Methodology, Investigation, Conceptualization. **Daniela Valencia-Pérez Rea:** Validation, Methodology, Investigation, Formal analysis. **Brandon Bautista-Becerril:** Visualization, Validation, Supervision, Resources, Methodology, Investigation, Data curation. **María Elena Ramírez-Díaz:** Validation, Supervision, Resources, Methodology, Investigation. **Filiberto Cruz-Vicente:** Validation, Supervision, Resources, Methodology, Investigation. **María de Lourdes Martínez-Gómez:** Supervision, Resources, Methodology. **Raúl Sansores:** Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition. **Ramcés Falfán-Valencia:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e28675.

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