Single-nucleotide polymorphism rs2070600 regulates AGER splicing and the sputum levels of the COPD biomarker soluble receptor for advanced glycation end-products

Faiz, Alen; Rathnayake, Senani N. H.; ten Hacken, Nick H. T.; Guryev, Victor; van den Berge, Maarten; Pouwels, Simon D.

Published in:
ERJ Open Research

DOI:
10.1183/23120541.00947-2020

ORIGINAL RESEARCH LETTER

Single-nucleotide polymorphism rs2070600 regulates *AGER* splicing and the sputum levels of the COPD biomarker soluble receptor for advanced glycation end-products

To the Editor:

Several studies have found that systemic soluble receptor for advanced glycation end-products (sRAGE) levels are decreased in COPD patients and strongly correlate with lung function decline, emphysema and disease progression [1–6]. Therefore, the COPD Biomarker Qualification Consortium and the Biomarker Development Center recently put sRAGE forward as the most promising biomarker for COPD [7]. sRAGE is anti-inflammatory decoy receptor for the pro-inflammatory innate immune receptor RAGE, which is transcribed from the *AGER* gene. It was shown that one particular single nucleotide polymorphism (SNP) decreases the circulating levels of sRAGE in COPD patients [3]. This SNP, rs2070600, causes an amino acid change from glycine to serine at the 82nd amino acid of RAGE, increasing the glycation-rate of one of the two glycation sites at the ligand-binding domain and subsequently increases the ligand-binding capacity of RAGE [8]. Recently, we showed that *AGER* expression was lower in lung tissue from smokers compared with never-smokers, and that smoking of three cigarettes severely decreased the serum levels of sRAGE within 2 h [4]. Furthermore, we showed that smokers display increased alternative splicing of *AGER* into the endogenous soluble splicing form [9]. Interestingly, we also showed that unlike plasma sRAGE levels, the levels in induced sputum did not correlate with COPD status [5]. To date, no studies have been performed investigating the differences between sRAGE levels in serum *versus* induced sputum. Here, we measured sRAGE in serum and induced sputum of healthy individuals and investigated if these levels were affected by age, smoking or the presence of the minor allele of rs2070600. Additionally, we studied whether rs2070600 can influence the production of sRAGE by affecting splicing of *AGER*.

In the present study, we obtained serum, induced sputum, and bronchial biopsies from 37 active smokers and 40 never-smokers without airway obstruction, which were matched based on age, sex, body mass index, and lung function (ClinicalTrials.gov identifier: NCT00848406) [10]. The study was approved by the medical ethics committee of the University Medical Center Groningen (UMCG), Groningen, The Netherlands, and all subjects provided written informed consent. sRAGE levels were measured in serum and sputum supernatant using ELISA (Human RAGE Duoset ELISA, DY1145; R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocol. mRNA levels and splicing of *AGER* was measured in bronchial biopsies as described previously [9]. In short, mRNA expression analysis was performed using raw counts of *AGER* and analysed using the R-package DESeq2 [9, 11]. For every read spanning an intron in the alignment, the first and last intron base were recorded. Next, the number of reads for all observed splice junction positions across alignment files from all samples were determined. The genotype for rs2070600 was determined from RNA-Seq mapped reads, with each patient requiring >5 reads

@ERSpublications
The COPD susceptibility SNP rs2070600 affects the levels of the COPD biomarker sRAGE in sputum as well as splicing of *AGER*. Moreover, @PouwelsScience *et al.* demonstrate large differences in sRAGE levels between serum and sputum. https://bit.ly/3t0pJtK

Cite this article as: Faiz A, Rathnayake SNH, ten Hacken NHT, *et al.* Single-nucleotide polymorphism rs2070600 regulates *AGER* splicing and the sputum levels of the COPD biomarker soluble receptor for advanced glycation end-products. *ERJ Open Res* 2021; 7: 00947-2020 [https://doi.org/10.1183/23120541.00947-2020].

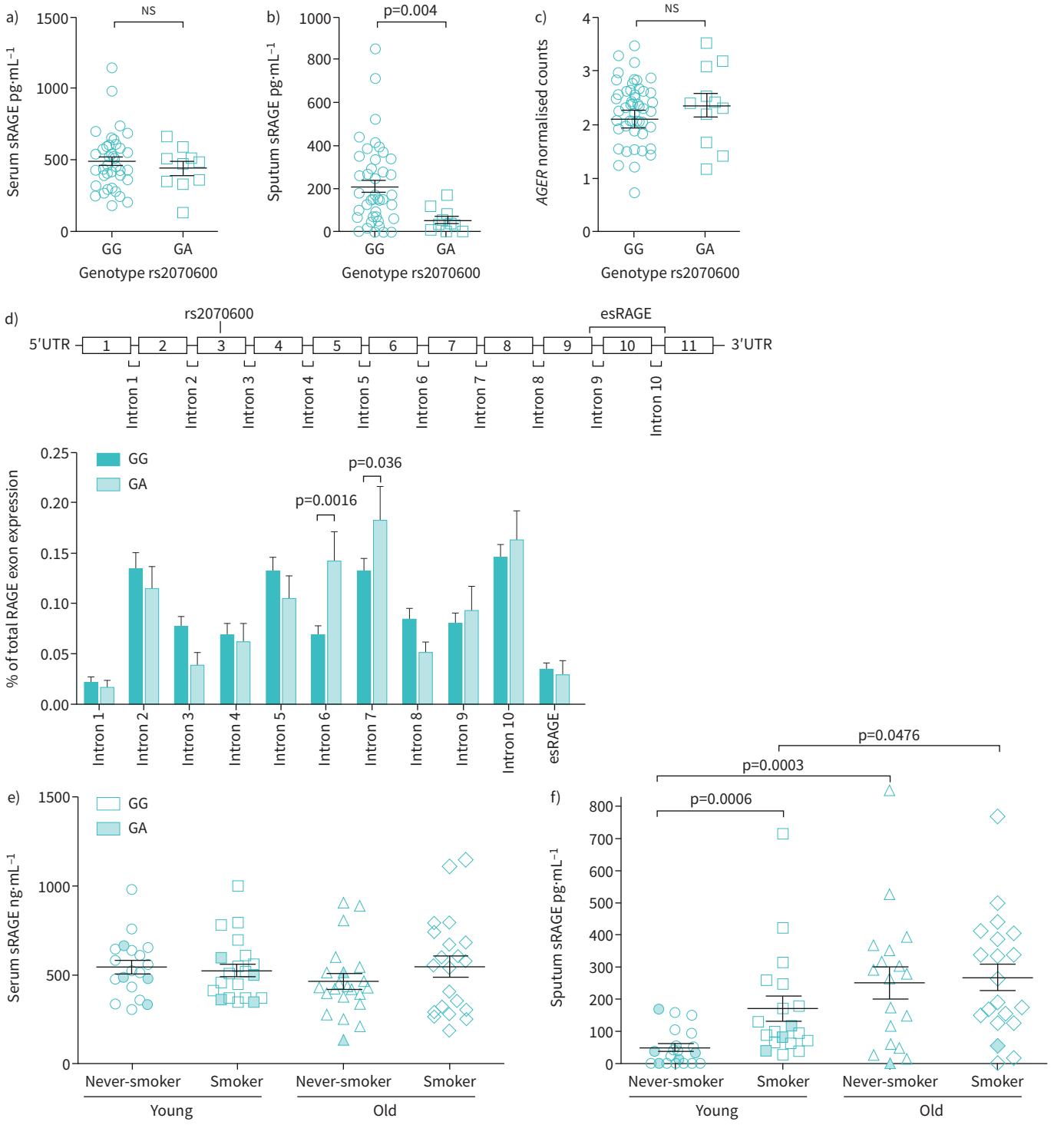

FIGURE 1 rs2070600 affects sputum soluble receptor for advanced glycation end-products (sRAGE) levels as well as the splicing of *AGER*. The levels of sRAGE were measured using ELISA in a) serum and b) sputum of healthy individuals with either the homozygous GG (n=37) or the heterozygous GA genotype (n=10). c) mRNA expression levels of *AGER* were measured in lung tissue of healthy individuals with either the homozygous GG (n=49) or the heterozygous GA genotype (n=11). The effect of rs2070600 on *AGER* splicing was measured by quantifying specific splice events within the *AGER* gene, panel d) depicts a schematic representation of the *AGER* gene with its introns, exons and the alternative splicing event leading to the formation of endogenous sRAGE (esRAGE) as well as the quantification of the most abundant splice sites. The levels of sRAGE were measured in e) serum and f) sputum of healthy individuals who were either active smokers or never-smokers and were either young (<40 years of age) or old (>40 years of age). All data is shown as individual data points or as mean±SEM. Statistical significance was tested using a Mann–Whitney U-test, where $p<0.05$ is considered statistically significant. NS: nonsignificant.

to be successfully genotyped. The GA heterozygous genotype for rs2070600 was present in five smokers and six never-smokers, and no subjects displayed the homozygous AA genotype. Clinical characteristics were similar for smokers and never-smokers: mean±SEM age 38±2 years for smoker and 41±3 years for never-smokers; and mean±SEM forced expiratory volume in 1 s % pred 99±1 for smoker and 100.9±2 for never-smokers.

In the current study, we observed that the serum levels of sRAGE are independent of subjects being either homozygous for the major allele of rs2070600 or heterozygous (p=0.67, figure 1a). Interestingly, the levels of sRAGE in sputum are strongly affected by rs2070600 (p=0.004, figure 1b). Here, heterozygous individuals showed significantly lower levels of sRAGE compared with individuals homozygous for the major allele. This indicates a location-dependent effect, which is not reflected in the circulation. Interestingly, the mRNA expression of *AGER* in bronchial biopsies is not affected by rs2070600 (p=0.59, figure 1c). Next, the impact of rs2070600 on alternative splicing of *AGER* in bronchial biopsies was studied. The splicing out of intron 6 and 7 was significantly higher in heterozygous individuals (p=0.016, p=0.036, figure 1d) compared to subjects homozygous for the major allele, indicating an increased presence of splice variants possessing exons 6–8. Interestingly, the splice variant leading to the production of endogenous sRAGE, lacking the trans-membrane domain, which accounts for ∼10% of all soluble RAGE, was not altered by rs2070600.

Next, we performed an exploratory analysis to identify whether sRAGE in serum and sputum were affected by age or smoking status. Here, it was found that the serum sRAGE levels are not different between healthy individuals who are either young (<40 years of age) or old (>40 years of age) and who are active smokers or never-smokers (figure 1e). The sRAGE levels in sputum were significantly higher in young individuals who were active smokers compared with the never-smokers (figure 1f). Furthermore, the old never-smokers showed significantly higher levels of sRAGE compared with young individuals. However, these levels did not further increase in the old smoking group. These data indicate that both smoking and ageing can increase the sputum sRAGE levels without smoking having an additive effect in subjects older than 40 years. Of note, individuals having the GA genotype for rs2070600 were dispersed between the groups (figure 1e–f).

Previously, it was shown that serum and plasma sRAGE levels are decreased in COPD patients, making it a potential biomarker for COPD [5, 6]. The serum sRAGE levels in COPD patients were also decreased in individuals possessing the minor allele of rs2070600 [1]. Although, it is known that rs2070600 is associated with serum sRAGE levels, it is possible that the SNP does not directly affect differential splicing because: 1) the endogenous sRAGE splice variant only accounts for 10% of all sRAGE produced; or 2) a SNP in linkage disequilibrium with rs2070600 may influence the splicing. In the current study serum sRAGE levels were not affected by rs2070600, smoking status or age in healthy individuals, but these factors did influence the sputum sRAGE levels. Therefore, sputum sRAGE levels may be a more sensitive biomarker compared with serum sRAGE levels. However, these results need to be validated in a large cohort including COPD patients. A limiting factor of our study may be that the power of our study may be too low to detect differences between the groups in serum samples.

This is the first study to show that rs2070600 is affecting sputum sRAGE levels. Moreover, rs2070600 affects splicing of the *AGER* gene, but does not alter the production of endogenous soluble RAGE or the expression of *AGER* in bronchial biopsies. Furthermore, this study shows that the effects of smoking, age and rs2070600 on sRAGE levels in healthy individuals are location dependent. Strong and significant effects of smoking status, age and rs2060700 on sputum sRAGE levels were observed, while no effects were found on serum sRAGE levels and *AGER* mRNA expression in bronchial biopsies. Together these results indicate that rs2070600 is a major driver for sputum sRAGE levels and future research should investigate whether the sputum sRAGE levels are a more sensitive biomarker for COPD progression compared with serum sRAGE levels.

Alen Faiz[1], Senani N.H. Rathnayake[1], Nick H.T. ten Hacken[2], Victor Guryev[3], Maarten van den Berge[2,4] and Simon D. Pouwels[2,4,5]

[1]Respiratory Bioinformatics and Molecular Biology Group, University of Technology Sydney, Sydney, Australia. [2]Dept of Pulmonary Diseases, University Medical Center Groningen, Groningen, The Netherlands. [3]European Research Institute for the Biology of Ageing, Groningen, The Netherlands. [4]GRIAC Research Institute, University of Groningen, Groningen, The Netherlands. [5]Dept of Pathology and Medical Biology, University Medical Center Groningen, Groningen, The Netherlands.

Correspondence: Simon D. Pouwels, Dept of Pulmonary Diseases, University Medical Center Groningen, Groningen Research Institute for Asthma and COPD, Hanzeplein 1, 9713 GZ, Groningen, The Netherlands. E-mail: s.d.pouwels@umcg.nl

Received: 16 Dec 2020 | Accepted: 24 April 2021

This study is registered at ClinicalTrials.gov with identifier: NCT00848406. Samples were used from the 'A Study to Obtain Normal Values of Inflammatory Variables From Healthy Subjects (NORM)' study which has been registered on ClinicalTrials.gov with the identifier code: NCT00848406 and the medical ethic committee approval code: METc2009007. All study protocols were approved by the medical ethic committee of the University Medical Center Groningen (UMCG), Groningen, The Netherlands and all subjects provided written informed consent. Furthermore, all clinical procedures were performed according to the standards set by the latest Declaration of Helsinki.

Conflict of interest: None declared.

References

1 Serban KA, Pratte KA, Bowler RP. Protein biomarkers for COPD outcomes. *Chest* 2021; 159: P224–2253.
2 Cazzola M, Puxeddu E, Ora J, *et al.* Evolving concepts in chronic obstructive pulmonary disease blood-based biomarkers. *Mol Diagn Ther* 2019; 23: 603–614.
3 Cheng DT, Kim DK, Cockayne DA, *et al.* Systemic soluble receptor for advanced glycation endproducts is a biomarker of emphysema and associated with AGER genetic variants in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2013; 188: 948–957.
4 Pouwels SD, Klont F, Kwiatkowski M, *et al.* Cigarette smoking acutely decreases serum levels of the chronic obstructive pulmonary disease biomarker sRAGE. *Am J Respir Crit Care Med* 2018; 198: 1456–1458.
5 Hoonhorst SJM, Lo Tam Loi AT, Pouwels SD, *et al.* Advanced glycation endproducts and their receptor in different body compartments in COPD. *Respir Res* 2016; 17: 46.
6 Zemans RL, Jacobson S, Keene J, *et al.* Multiple biomarkers predict disease severity, progression and mortality in COPD. *Respir Res* 2017; 18: 117.
7 Klont F, Pouwels SD, Hermans J, *et al.* A fully validated liquid chromatography-mass spectrometry method for the quantification of the soluble receptor of advanced glycation end-products (sRAGE) in serum using immunopurification in a 96-well plate format. *Talanta* 2018; 182: 414–421.
8 Park SJ, Kleffmann T, Hessian PA. The G82S polymorphism promotes glycosylation of the receptor for advanced glycation end products (RAGE) at asparagine 81: comparison of wild-type rage with the G82S polymorphic variant. *J Biol Chem* 2011; 286: 21384–21392.
9 Faiz A, van den Berge M, Vermeulen CJ, *et al.* AGER expression and alternative splicing in bronchial biopsies of smokers and never smokers. *Respir Res* 2019; 20: 70.
10 Imkamp K, Berg M, Vermeulen CJ, *et al.* Nasal epithelium as a proxy for bronchial epithelium for smoking-induced gene expression and expression Quantitative Trait Loci. *J Allergy Clin Immunol* 2018; 142: 314–317.
11 Faiz A, Heijink IH, Vermeulen CJ, *et al.* Cigarette smoke exposure decreases CFLAR expression in the bronchial epithelium, augmenting susceptibility for lung epithelial cell death and DAMP release. *Sci Rep* 2018; 8: 12426.