Published in final edited form as:

J Allergy Clin Immunol. 2022 June ; 149(6): 1992–1997.e12. doi:10.1016/j.jaci.2021.11.027.

A genomic approach identifies sRAGE as a putatively causal protein for asthma

Helena Bui, BA[a,b], **Amena Keshawarz, PhD**[a,b], **Shih-Jen Hwang, PhD**[a,b], **Chen Yao, PhD**[a,b], **Gha Young Lee, BS**[a,b,c], **Kathryn Recto, BS**[a,b], **George T. O'Connor, MD**[d], **Daniel Levy, MD**[a,b]

[a]Population Sciences Branch, Division of Intramural Research, National Heart, Lung, and Blood Institute, National Institutes of Health, 31 Center Drive, Ste 10-7C114, Bethesda, MD 20891, USA

[b]Framingham Heart Study, 73 Mt. Wayte Avenue, Framingham, MA 01702, USA

[c]Harvard Medical School, 25 Shattuck Street, Boston, MA 02115, USA

[d]Boston University School of Medicine, 72 E. Concord Street, Boston, MA 02118, USA

Abstract

Background: Asthma is a complex respiratory condition caused by environmental and genetic factors. Although lower concentrations of the anti-inflammatory protein sRAGE have been associated with asthma in humans and mouse models, it is uncertain whether sRAGE plays a causal role in asthma.

Objective: We designed a two-stage study of sRAGE in relation to asthma with i) association analysis in FHS participants and ii) causal inference testing using MR.

Methods: We measured plasma levels of sRAGE and performed cross-sectional analysis to examine the association between plasma sRAGE concentration and asthma status in 6,546 FHS participants. We then used sRAGE pQTLs derived from a GWAS of plasma sRAGE levels in ~7,000 FHS participants with UK Biobank asthma GWAS in MR to consider sRAGE as a putatively causal protein for asthma. We also performed replication MR using an externally-derived sRAGE pQTL from the INTERVAL study. Last, we conducted colocalization using *cis*-pQTL variants at the *AGER* locus with variants from the UK Biobank asthma GWAS.

Results: Association analysis revealed that each 1 SD increment in sRAGE concentration was associated with a 14% lower odds of asthma in FHS participants (95% CI 0.76–0.96). MR identified sRAGE as putatively causal for and protective against asthma based on self-reported

Corresponding Author: Daniel Levy, MD, Framingham Heart Study, 73 Mt. Wayte Avenue, Framingham, MA 01702, levyd@nih.gov, 508-935-3458.

Supplemental Materials:

Please note that all supplemental tables, figures, text, and files referred to in this article have been uploaded into an Online Repository on the *Journal of Allergy and Clinical Immunology* website, http://www.jacionline.org.

(OR [per 1 SE increment in inverse rank-normalized sRAGE]=0.97, 95% CI 0.95–0.99; p=0.005) and doctor-diagnosed asthma (OR=0.97, 95% CI 0.95–0.99; p=0.011).

Conclusion: Through this genomic approach, we identified sRAGE as a putatively causal, biologically important, and protective protein in relation to asthma. Functional studies in cell/animal models are needed to confirm our findings.

Capsule Summary:

By identifying sRAGE as a putatively causal and protective protein in relation to asthma, it can be studied as a targeted therapeutic for asthma and related inflammatory diseases.

Keywords

asthma; Mendelian randomization; sRAGE; causal inference; Framingham Heart Study; GWAS; protein-trait association; proteomics; genomics

Introduction

Asthma is a chronic inflammatory disease of the lung airways, characterized by variable airflow obstruction and symptoms including wheeze, cough, chest tightness, and shortness of breath. It is the most common chronic, non-communicable disease in children and adolescents in the United States(1), and it currently affects more than 339 million people worldwide.(2) Asthma is a multi-faceted and complex condition with underlying environmental and genetic factors contributing to its pathogenesis. While effective therapies exist to control symptoms and prevent exacerbations, asthma cannot be cured. Insights into causal mechanisms of this disease may lead to new therapeutic strategies.

The mRAGE protein is encoded by the *AGER* gene. Its transmembrane receptor binds to ligands such as HMGB1— a chromatin-binding molecule that also operates as a pro-inflammatory damage-associated molecular pattern protein in response to cellular stress.(3) mRAGE binding of pro-inflammatory ligands, particularly HMGB1, has been shown to play a role in the progression of respiratory inflammatory responses.(4,5) mRAGE is notably expressed in lung tissue, specifically in AT-1 epithelial cells, and its expression increases in the presence of bound ligands.(5)

In contrast, sRAGE is a truncated form of RAGE that lacks the transmembrane domain and serves as a circulating competitive regulator of mRAGE—preventing the initiation of downstream inflammatory responses.(4,6) In previous association studies, sRAGE concentration in the circulation and in bronchoalveolar lavage fluid is lower in asthmatic patients compared to healthy controls(7–11) and in a mouse model of asthma compared to control mice.(12) Functional models of asthma have also shown that locally administered sRAGE reduces HMGB1 expression, airway inflammation, and hyperreactivity.(12) Thus, there is a growing body of evidence that sRAGE is not only associated with asthma but may play a causal and protective role against asthma. A causal relation of sRAGE in relation to asthma remains to be established with certainty.

In the context of these findings, we conducted a multistage study, first testing the association of plasma sRAGE level with asthma in over 6,500 FHS participants and second, testing for causal inference by performing MR using genetic variants associated with circulating sRAGE levels in two UK Biobank GWAS of asthma. Thus, we aimed to investigate a specific protein-trait association based on reports in the literature. We also performed supplementary colocalization analyses using *cis*-pQTL variants at the *AGER* gene locus. Overall, this study examined whether sRAGE may be a causal factor that protects against the development of asthma, highlighting the *AGER*/mRAGE/sRAGE axis as a potential therapeutic target for the prevention and treatment of asthma.

Methods

Study population

This study included FHS participants who attended Offspring cohort, Exam 7 (1998–2001) and Third Generation cohort, Exam 1 (2002–2005).(13) The asthma status of all 7,266 FHS participants who attended a baseline exam was ascertained using data from questionnaires and face-to-face interviews conducted by a physician. Participants also provided complete information on health history and medication use for asthma as well as a blood sample for measurement of plasma sRAGE concentration (methods for protein collection appear below). Asthma was defined as 1) a self-reported history of asthma and 2) self-reported use of asthma medication. In FHS Offspring cohort participants (Exam 7) medication use was captured by questions inquiring about current use of inhaled corticosteroids, bronchodilators, or aerosols for asthma. In Third Generation cohort participants (Exam 1), information on medication use was collected using the WHO ATC coding system, with asthma medication use defined as the report of using codes R03AC (selective beta-2-adrenoreceptor agonists) or R03BA (inhaled glucocorticoids). The control (or “free of asthma”) group consisted of participants who reported both no history of asthma and no asthma medication use.

Spirometry testing was performed on FHS Offspring (Exam 7) and Third Generation (Exam 1) participants using standardized methods with measurement of FEV1, FVC, and the FEV1/FVC ratio. Percent predicted values for FEV1 and FVC were calculated using Hankinson reference equations.(14)

All participants provided informed consent, and the study protocol received approval from the Boston University Medical Campus Institutional Review Board. The primary study design is shown in Figure I.

Protein collection

Plasma sRAGE protein concentration was measured in FHS participants as a part of the Systems Approach to Biomarker Research in Cardiovascular Disease Initiative, a project aiming to generate extensive biomarker data using high-throughput “omics” platforms including discovery proteomics, targeted protein immunoassays, metabolomics/lipomics, gene expression, and microRNA profiling.(13) Fasting blood plasma samples were obtained at the baseline clinical visit (Offspring cohort, Exam 7 [1999–2003] and Third

Generation cohort, Exam 1 [2002–2005]). Samples were stored at −80°C. Plasma sRAGE was quantified using a modified ELISA sandwich approach multiplexed on a Luminex xMAP platform (Sigma-Aldrich, St. Louis, MO) as described previously.(13) All targets were initially developed as singleton assays before compatible targets were combined to form multiplex panels to avoid cross-reactivity. The sRAGE assay used MAB11451 (R&D Systems, Minneapolis, MN) as the detection antibody, product 1145-RG-050 (R&D Systems, Minneapolis, MN) as the capture antibody, and BAF1145 (R&D Systems, Minneapolis, MN) as the reference protein. The mean inter- and intra-assay coefficients of variation for sRAGE were 5.6% and 14.5%, respectively.(13)

Serum IgE levels were measured in FHS Offspring (Exam 7) and Third Generation (Exam 1) participants using the Phadia ImmunoCAP 100 system (Phadia, Uppsala, Sweden) with high precision and reproducibility, as described previously.(15)

Regression analyses

We tested for association between baseline plasma sRAGE level and asthma at the baseline exam using a logistic regression model that adjusted for age, sex, BMI, pack-years of cigarette smoking, and familial correlation using a generalized estimating equation. We also conducted additional age- and sex-adjusted analyses of sRAGE levels associated with inhaled corticosteroid use among FHS participants with asthma. We defined $p<0.05$ as statistically significant for these analyses.

Instrumental variables in MR for causal inference

MR is an analytical approach that mimics randomized control trials.(16) MR can estimate the causal relationship between an exposure and an outcome by using SNPs as instrumental variables. A two-sample MR approach was used to infer a causal association between plasma sRAGE level as the exposure and asthma as the outcome.(17)

In a separate study by our team, GWAS of sRAGE was conducted using plasma sRAGE levels in conjunction with genome-wide genotype data on 6,861 FHS participants to identify pQTLs; independent external replication of pQTL variants was carried out using data from the INTERVAL study as previously outlined.(18) Supplemental Table I provides additional details regarding the pQTL variants used in MR analyses; Supplemental Table II provides details regarding plasma sRAGE levels in 6,861 FHS participants by rs2070600 variant genotype measured using the Illumina Exome Chip (Illumina Inc., San Diego, CA).

sRAGE *cis*-pQTL variants(18) were pruned at LD $r^2 <0.01$, leaving three sRAGE *cis*-pQTL variants (rs2070600, rs6923504, and rs9266529) as instrumental variables for MR analysis. The inverse variance weighted estimate method was used to conduct the multi-SNP MR, and the Wald ratio method was used to conduct single-SNP MR. MR analysis was conducted using two asthma GWAS from UK Biobank as described below (Asthma GWAS).(19,20)

Replication MR analysis was performed using the same UK Biobank asthma GWAS in conjunction with an externally-derived sRAGE pQTL from the INTERVAL study, a United Kingdom-based cohort created by the Universities of Cambridge and Oxford in collaboration with the National Health Service Blood and Transport division.(21) Sensitivity

tests for heterogeneity and horizontal pleiotropy were performed. The heterogeneity test operates under the null assumption that the degree of causal effect from each instrumental variable is identical(22) while the horizontal pleiotropy test aims to detect when a genetic variant affects the outcome through a pathway other than, or in addition to, the exposure.(23) Statistical significance for MR and sensitivity analyses was defined as $p<0.05$ because only one exposure was tested in causal inference. All MR analyses were conducted in R (ver. 4.0.3)(24) using the "TwoSampleMR" package(20) from University of Bristol MRC-IEU.

Asthma GWAS

Results of two asthma GWAS from UK Biobank that are available from the UK MRC-IEU were used in integrative genomic analyses. MRC-IEU has derived and streamlined GWAS data from UK Biobank—a large-scale biomedical database containing health and genetic information from over half a million United Kingdom-based participants.(19,20)

The first GWAS contained data for self-reported asthma and included 53,598 asthma cases and 409,335 controls.(19,20) Asthma by self-report was defined as participants who self-reported a history of asthma in a medical history questionnaire followed by confirmation by a trained nurse in an interview. The second GWAS considered cases of asthma that were doctor-diagnosed and included 14,283 asthma cases and 98,300 controls.(19,20) Participants with doctor-diagnosed asthma were defined as those who answered "yes" to the question: "Has a doctor ever told you that you have asthma?" Answers were also verbally confirmed with a trained nurse in an interview. Supplemental Table III summarizes the overlap of FHS-derived sRAGE *cis*-pQTL variants used as instrumental variables in MR with self-reported and doctor-diagnosed asthma from the UK Biobank GWAS.

Colocalization analyses

Colocalization compares and integrates association signals from GWAS variants and pQTL variants on a locus-by-locus (i.e., unique chromosomal location defining the position of a gene) basis to identify instances in which both traits share a comment sentinel variant in a given region. High probability of colocalization may suggest that there is a single variant that affects both the protein expression and the clinical trait of interest.(25)

Colocalization analysis was performed using the "coloc" package in R (ver. 4.0.3)(24),(26) and the two UK Biobank asthma GWAS.(19,20) *Cis* sRAGE GWAS variants residing within 1 Mb (upstream and downstream) of the protein coding gene *AGER* were utilized in analysis.(18) Evidence of colocalization was defined as a PPH4 of 0.8 or greater of the two traits sharing the same sentinel variant.

Results

Study population

A total of 375 (5.7%) FHS participants fulfilling criteria for asthma and 6,171 participants free of asthma were eligible for analyses (total sample size 6,546). Table I displays the clinical characteristics of the participants by asthma status. We observed similar distributions of age, BMI, and pack-years of cigarette smoking between the participants with asthma

and those free of asthma; the asthma group included a higher proportion of women (65.9% versus 52.3%). Further information on medication use is provided in Supplemental Table IV.

Spirometry data were available in 315 out of 375 (84%) asthma cases and 5,381 out of 6,171 (87%) participants free of asthma; FEV1 values below 80% predicted were more than three times more common in participants with asthma than among non-asthmatic participants (35% versus 10%, respectively; Supplemental Table V). Similarly, FVC values below 80% predicted were three times more common in participants with asthma compared to non-asthmatic participants (15% versus 5%, respectively). A clinically relevant ratio of FEV1/FVC below 0.70 was observed in 43% of asthmatic participants and 16% of those without asthma.

Serum total IgE values were available in 366 out of 375 (98%) asthma cases and 6,022 out of 6,171 (97%) non-cases. A higher mean IgE levels was observed in participants with asthma than in those without asthma (213 ± 378 versus 86 ± 270 kU/L, respectively; Supplemental Table VI).

Regression analyses

After adjusting for relevant covariates, a higher plasma sRAGE level was associated with lower odds of asthma (OR per 1 SD increment of sRAGE=0.86, 95% CI 0.76–0.96, p=0.0075). Results are summarized in Table II. Plasma sRAGE level did not differ between participants with asthma who used corticosteroids compared to those who did not (p=0.348; Supplemental Table VII).

MR and colocalization analyses

Mendelian randomization revealed sRAGE to be putatively causal and protective in relation to both self-reported (OR per 1 SE increment in inverse rank-normalized sRAGE levels=0.97, 95% CI 0.95–0.99) and doctor-diagnosed (OR=0.97, 95% CI 0.95–0.99) asthma. These MR results are summarized in Table III.

There was significant heterogeneity among *cis*-pQTL variants used in MR analyses (Supplemental Table VIII, but single SNP analysis revealed that all three SNPs used in MR (i.e., rs2070600, rs9266529, and rs6923504) shared similar (i.e., protective) directionality (Supplemental Table IX). Of note, no horizontal pleiotropy was detected (Supplemental Table X).

Replication of MR analysis using a single sRAGE pQTL variant (rs2070600) from the INTERVAL study(18) validated sRAGE as putatively causal and protective in relation to UK Biobank self-reported asthma (OR per 1 SE increment in inverse-rank normalized sRAGE levels=0.974, 95% CI 0.969–0.978) and doctor-diagnosed asthma (OR per 1 SE increment in inverse-rank normalized sRAGE levels=0.97, 95% CI 0.96–0.98) (Supplemental Table XI).

Last, no colocalization was observed between sRAGE pQTL variants and asthma GWAS variants ($PPH4_{self\text{-}reported}$=1.89E-17; $PPH4_{doctor\text{-}diagnosed}$=5.69E-20; Supplemental Table XII).

Discussion

The key findings of our study are twofold. First, protein-trait association analysis revealed that each 1 SD increment in plasma sRAGE concentration was associated with a 14% lower odds of asthma. Second, causal inference testing using two-sample MR indicated that sRAGE may be causal for and protective against asthma.

Both mRAGE and sRAGE have been studied in relation to asthma in clinical studies and mouse models. In murine models, Perkins et al. and Oczypok et al. reported that pulmonary mRAGE is a critical contributing factor to the asthma airway inflammatory response.(27,28) Functional studies in mouse models of asthma have suggested that sRAGE plays a significant role in alleviating airway inflammation. Following overexpression of sRAGE in neutrophilic asthmatic mice through the use of a viral vector, Zhang et al. (2021) found that sRAGE suppressed mucus hypersecretion and expression of mucus-producing genes.(29) A similar study by Zhang et al. (2017) showed that locally administered sRAGE lowered HMGB1 expression, neutrophilic inflammation, and Th17-type responses in asthmatic mice.(12) A recent study by Raita et al. has provided support for our findings of a putatively causal association between sRAGE and asthma, although the genetic instruments (i.e., sRAGE pQTLs) differed from the ones identified in both FHS and the INTERVAL study.(30)

sRAGE is a truncated circulating form of RAGE, a transmembrane protein coded by the *AGER* gene on chromosome 6. While the intact membrane-bound mRAGE protein plays an important pro-inflammatory role, sRAGE has the opposite effect. sRAGE has been studied as an inflammation-related biomarker in other immune-mediated diseases in addition to asthma. For example, previous studies have revealed that decreased sRAGE levels are observed in patients with Crohn's disease,(31) ulcerative colitis,(31) multiple sclerosis,(32,33) and Hashimoto's thyroiditis.(34) One sRAGE variant evaluated in this study, rs2070600, is a missense SNP in exon 3 of *AGER* causing a glycine-to-serine amino acid substitution at position 82 of the protein.(35) This substitution promotes mRAGE glycosylation, modifying ligand-binding structure and increasing receptor affinity for ligands while also decreasing mRAGE proteolytic cleavage, which has the effect of decreasing sRAGE levels.(35) Although few studies have examined the relationship between this SNP and asthma, Miller et al. reported that rs2070600, the T allele of which was associated with lower sRAGE levels in FHS participants (Supplemental Table II), was associated with lower FEV_1, lower FEV_1/FVC ratio, and lower serum sRAGE concentration in smokers.(36) rs2070600 has also been associated with lower sRAGE concentration in the settings of emphysema, chronic obstructive pulmonary disease,(37) and idiopathic pulmonary fibrosis.(38)

While single SNP analysis revealed three causal and protective *AGER* locus variants associated with asthma, rs6923504 does not map to the *AGER* gene but instead to the nearby *HLA-DRB9* gene. *HLA-DRB9* is one gene of a family of HLA gene complexes that code for major histocompatibility complex class II cell surface receptors; these receptors are responsible for presenting peptide antigens to the immune system in order to elicit or suppress T-helper cell immune response.(39) Although rs6923504 has been associated

with several autoimmune diseases such as type 1 diabetes, ulcerative colitis, systemic lupus erythematosus, and primary biliary cholangitis,(40) this particular SNP has not been examined before in the context of asthma. Few studies of the rs9266529 variant are available. Both rs6923504 and rs9266529 displayed significant associations with self-reported and doctor-diagnosed asthma in the UK Biobank GWAS (Supplemental Table III).

It is also important to acknowledge the potential effects of asthma medication on sRAGE concentration. Medications such as corticosteroids have anti-inflammatory properties through activation or repression of target genes as well as non-genomic mechanisms such as receptor-binding modifications.(41) The existing literature does not clearly establish a causal effect of corticosteroids or beta-2-adrenergic agonists on sRAGE in the setting of asthma. Feng et al. reported that plasma sRAGE levels were significantly increased after six months of treatment with inhaled corticosteroids in patients with asthma-COPD overlap syndrome. (42) In contrast, other COPD studies examining the effects of beta-2 adrenergic agonist and corticosteroid treatment revealed no effect of therapy on systemic level of sRAGE after three months of treatment.(43) Our analyses also showed that use of inhaled corticosteroids was not significantly associated with plasma sRAGE levels (Supplemental Table VII).

This study has several limitations. First, the study participants in FHS and in the UK Biobank asthma GWAS were predominantly of European ancestry; the results of our study may not be generalizable to other racial or ethnic groups in which the prevalence of asthma and its environmental and genetic predispositions may differ. Second, sRAGE levels were measured in plasma, which may not reflect tissue- or organ-specific effects. Third, while our definition of asthma was based on both self-report of asthma diagnosis and use of asthma medication, we acknowledge that the observational nature of our population-based data prevents additional clinical classification. We have provided additional clinical data in FHS participants including spirometry testing and serum IgE levels. The clinical data reveal higher prevalence of impaired spirometry values and higher serum IgE levels in FHS participants with asthma compared with those without asthma which is consistent with what is found in the literature.(44) Our study also has several strengths. We conducted a large protein-trait association analysis of plasma sRAGE with asthma in more than 6,500 FHS participants. MR results were fully consistent with the protein-trait association findings. We were also able to externally validate our MR findings using an sRAGE pQTL variant from the INTERVAL study that supported our hypothesis that sRAGE is putatively causal for asthma.

In conclusion, through both protein-trait association and MR analyses, our findings suggest that sRAGE may play a causal, biologically important, and protective role in relation to asthma and may serve as a promising therapeutic use in its prevention and treatment. Additional functional studies in cell and animal models are needed to further evaluate the molecular role of sRAGE in the pathogenesis of asthma and to determine its potential therapeutic utility.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Funding Sources, Competing Interests:

The Framingham Heart Study laboratory work for this project was funded by National Institutes of Health contract N01-HC-25195. The analytical component of this project was funded by the Division of Intramural Research, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD (D. Levy, Principal Investigator). All authors declare no other competing interests.

Disclosure:

The National Heart, Lung, and Blood Institute and Ionis Pharmaceuticals entered into a Cooperative Research and Development Agreement (CRADA) to conduct research targeting the *AGER* gene in relation to lung disease. Dr. Daniel Levy is the NHLBI principal investigator on the CRADA. Neither Dr. Levy nor the NHLBI is receiving any funding from Ionis Pharmaceuticals in conjunction with the CRADA. Dr. Chen Yao has contributed to this project and its completion as an employee of the National Heart, Lung, and Blood Institute; Dr. Yao is currently an employee of Bristol Myers Squibb. There are no other relevant financial, personal, or professional relationships with other people or organizations to disclose.

Abbreviations:

AGER	Advanced Glycosylation End-Product Specific Receptor [Gene]
AT-1	[Pulmonary] Alveolar Type 1
BMI	Body Mass Index
CI	Confidence Interval
COPD	Chronic Obstructive Pulmonary Disease
ELISA	Enzyme-Linked Immunosorbent Assay
FEV1	Forced Expiratory Volume in 1 Second
FHS	Framingham Heart Study
FVC	Forced Vital Capacity
GWAS	Genome-Wide Association Study
HLA	Human Leukocyte Antigen
HMGB1	High Mobility Group Protein Box 1
IgE	Immunoglobulin E
LD	Linkage Disequilibrium
MR	Mendelian Randomization
mRAGE	Membrane-Bound Receptor for Advanced Glycation End-Products
MRC-IEU	Medical Research Council Integrative Epidemiology Unit
OR	Odds Ratio
PPH4	Posterior Probability of Hypothesis 4
pQTL	Protein Advanced Glycation End-Products

RAGE	Receptor for Advanced Glycation End-Products
SD	Standard Deviation
SE	Standard Error
SNP	Single Nucleotide Polymorphism
sRAGE	Soluble Receptor for Advanced Glycation End-Products
WHO ATC	World Health Organization Anatomical Therapeutic Coding

References

1. Centers for Disease Control and Prevention. CDC Healthy Schools: Asthma [Internet]. 2018. Available from: https://www.cdc.gov/healthyschools/asthma/
2. Organisation WH. Word Health Organization - 2014 - Global status report on alcohol and health 2014(2). Vol. 24, Research on Economic Inequality. 2016.
3. Yang H, Wang H, Andersson U. Targeting Inflammation Driven by HMGB1. Vol. 11, Frontiers in Immunology. 2020.
4. Lanati N, Emanuele E, Brondino N, Geroldi D. Soluble RAGE-Modulating Drugs: State-of-the-Art and Future Perspectives for Targeting Vascular Inflammation. Curr Vasc Pharmacol. 2010;8(1).
5. Hudson BI, Lippman ME. Targeting RAGE Signaling in Inflammatory Disease. Annu Rev Med. 2018;69.
6. Myles A, Viswanath V, Singh YP, Aggarwal A. Soluble receptor for advanced glycation endproducts is decreased in patients with juvenile idiopathic arthritis (ERA category) and inversely correlates with disease activity and S100A12 levels. J Rheumatol. 2011;38(9).
7. El-Seify MYH, Fouda EM, Nabih ES. Serum level of soluble receptor for advanced glycation end products in asthmatic children and its correlation to severity and pulmonary functions. Clin Lab. 2014;60(6).
8. Liang Z, Liu L, Zhao H, Xia Y, Zhang W, Ye Y, et al. A systemic inflammatory endotype of asthma with more severe disease identified by unbiased clustering of the serum cytokine profile. Med (United States). 2016;95(25).
9. Li Y, Wu R, Tian Y, Bao T, Tian Z. Fraction of exhaled nitric oxide and soluble receptors for advanced glycation end products are negatively correlated in children with recurrent wheezing. Asian Pacific J Allergy Immunol. 2017;35(1).
10. Lyu Y, Zhao H, Ye Y, Liu L, Zhu S, Xia Y, et al. Decreased soluble RAGE in neutrophilic asthma is correlated with disease severity and RAGE G82S variants. Mol Med Rep. 2018;17(3).
11. Sukkar MB, Wood LG, Tooze M, Simpson JL, McDonald VM, Gibson PG, et al. Soluble RAGE is deficient in neutrophilic asthma and COPD. Eur Respir J. 2012;39(3).
12. Zhang F, Su X, Huang G, Xin XF, Cao EH, Shi Y, et al. sRAGE alleviates neutrophilic asthma by blocking HMGB1/RAGE signalling in airway dendritic cells. Sci Rep. 2017;7(1).
13. Ho JE, Lyass A, Courchesne P, Chen G, Liu C, Yin X, et al. Protein biomarkers of cardiovascular disease and mortality in the community. J Am Heart Assoc. 2018;7(14).
14. Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general U.S. Population. Am J Respir Crit Care Med. 1999;159(1).
15. Granada M, Wilk JB, Tuzova M, Strachan DP, Weidinger S, Albrecht E, et al. A genome-wide association study of plasma total IgE concentrations in the Framingham Heart Study. J Allergy Clin Immunol. 2012;129(3).
16. Lawlor DA, Harbord RM, Sterne JAC, Timpson N, Smith GD. Mendelian randomization: Using genes as instruments for making causal inferences in epidemiology. Stat Med. 2008;27(8).
17. Deng L, Zhang H, Yu K. Power calculation for the general two-sample Mendelian randomization analysis. Genet Epidemiol. 2020;44(3).

18. Yao C, Chen G, Song C, Keefe J, Mendelson M, Huan T, et al. Genome- wide mapping of plasma protein QTLs identifies putatively causal genes and pathways for cardiovascular disease. Nat Commun. 2018;9(1).
19. Elsworth B, Lyon M, Alexander T, Liu Y, Matthews P, Hallett J, et al. The MRC IEU OpenGWAS data infrastructure. bioRxiv [Internet]. 2020;2020.08.10.244293. Available from: 10.1101/2020.08.10.244293
20. Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, et al. The MR-base platform supports systematic causal inference across the human phenome. Elife. 2018;7.
21. Sun BB, Maranville JC, Peters JE, Stacey D, Staley JR, Blackshaw J, et al. Genomic atlas of the human plasma proteome. Nature. 2018;558(7708).
22. Burgess S, Bowden J, Fall T, Ingelsson E, Thompson SG. Sensitivity analyses for robust causal inference from mendelian randomization analyses with multiple genetic variants. Vol. 28, Epidemiology. 2017.
23. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. Nat Genet. 2018;50(5).
24. R Core Team. R: A language and environment for statistical computing [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2020. Available from: https://www.r-project.org/.
25. Cano-Gamez E, Trynka G. From GWAS to Function: Using Functional Genomics to Identify the Mechanisms Underlying Complex Diseases. Vol. 11, Frontiers in Genetics. 2020.
26. Giambartolomei C, Vukcevic D, Schadt EE, Franke L, Hingorani AD, Wallace C, et al. Bayesian Test for Colocalisation between Pairs of Genetic Association Studies Using Summary Statistics. PLoS Genet. 2014;10(5).
27. Perkins TN, Oczypok EA, Dutz RE, Donnell ML, Myerburg MM, Oury TD. The receptor for advanced glycation end products is a critical mediator of type 2 cytokine signaling in the lungs. J Allergy Clin Immunol. 2019;144(3).
28. Oczypok EA, Milutinovic PS, Alcorn JF, Khare A, Crum LT, Manni ML, et al. Pulmonary receptor for advanced glycation end-products promotes asthma pathogenesis through IL-33 and accumulation of group 2 innate lymphoid cells. J Allergy Clin Immunol. 2015;136(3).
29. Zhang X, Xie J, Sun H, Wei Q, Nong G. sRAGE Inhibits the Mucus Hypersecretion in a Mouse Model with Neutrophilic Asthma. Immunol Invest. 2021;
30. Raita Y, Zhu Z, Freishtat RJ, Fujiogi M, Liang L, Patregnani JT, et al. Soluble receptor for advanced glycation end products (sRAGE) and asthma: Mendelian randomisation study. Pediatric Allergy and Immunology. 2021.
31. Ciccocioppo R, Imbesi V, Betti E, Boccaccio V, Kruzliak P, Gallia A, et al. The Circulating Level of Soluble Receptor for Advanced Glycation End Products Displays Different Patterns in Ulcerative Colitis and Crohn's Disease: A Cross-Sectional Study. Dig Dis Sci. 2015;60(8).
32. Glasnović A, Cvija H, Stojić M, Tudorić Eno I, Ivčević S, Romić D, et al. Decreased level of sRAGE in the cerebrospinal fluid of multiple sclerosis patients at clinical onset. Neuroimmunomodulation. 2014;21(5).
33. Damasiewicz-Bodzek A, Łabuz-Roszak B, Kumaszka B, Tadeusiak B, Tyrpień-Golder K. The assessment of serum concentrations of ages and their soluble receptor (Srage) in multiple sclerosis patients. Brain Sci. 2021;11(8).
34. Ruggeri RM, Barbalace MC, Cristani MT, Alibrandi A, Giovinazzo S, Giuffrida G, et al. Serum levels of advanced glycation end products (AGEs) are increased and their soluble receptor (sRAGE) reduced in Hashimoto's thyroiditis. J Endocrinol Invest. 2020;43(9).
35. Serveaux-Dancer M, Jabaudon M, Creveaux I, Belville C, Blondonnet R, Gross C, et al. Pathological implications of receptor for advanced glycation end-product (AGER) gene polymorphism. Vol. 2019, Disease Markers. 2019.
36. Miller S, Henry AP, Hodge E, Kheirallah AK, Billington CK, Rimington TL, et al. The Ser82 RAGE variant affects lung function and serum RAGE in smokers and sRAGE production in vitro. PLoS One. 2016;11(10).
37. Cheng DT, Kim DK, Cockayne DA, Belousov A, Bitter H, Cho MH, 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(8).

38. Yamaguchi K, Iwamoto H, Horimasu Y, Ohshimo S, Fujitaka K, Hamada H, et al. AGER gene polymorphisms and soluble receptor for advanced glycation end product in patients with idiopathic pulmonary fibrosis. Respirology. 2017;22(5).
39. Holling TM, Schooten E, Van Den Elsen PJ. Function and regulation of MHC class II molecules in T-lymphocytes: Of mice and men. Hum Immunol. 2004;65(4).
40. Dorak Tevflik M Causality Assessment of HLA Region Associations in GWAS for Autoimmune Diseases [Internet]. Liverpool Hope University. 2015 [cited 2020 Nov 17]. Available from: http://www.dorak.info/tbgk2015.pdf
41. Horvath G, Wanner A. Inhaled corticosteroids: Effects on the airway vasculature in bronchial asthma. Vol. 27, European Respiratory Journal. 2006.
42. Feng JX, Lin Y, Lin J, He SS, Chen MF, Wu XM, et al. Relationship between fractional exhaled nitric oxide level and efficacy of inhaled corticosteroid in asthma-copd overlap syndrome patients with different disease severity. J Korean Med Sci. 2017;32(3).
43. Celli BR, Anderson JA, Brook R, Calverley P, Cowans NJ, Crim C, et al. Serum biomarkers and outcomes in patients with moderate COPD: A substudy of the randomised SUMMIT trial. BMJ Open Respir Res. 2019;6(1).
44. Beeh KM, Ksoll M, Buhl R. Elevation of total serum immunoglobulin E is associated with asthma in nonallergic individuals. Eur Respir J. 2000;16(4).

Key Messages:

- Cross-sectional association analyses in FHS revealed that each 1 standard deviation increment in plasma sRAGE concentration corresponded to a 14% lower odds of asthma.
- Causal inference analysis using Mendelian randomization revealed a putatively causal and protective relation of sRAGE to asthma.

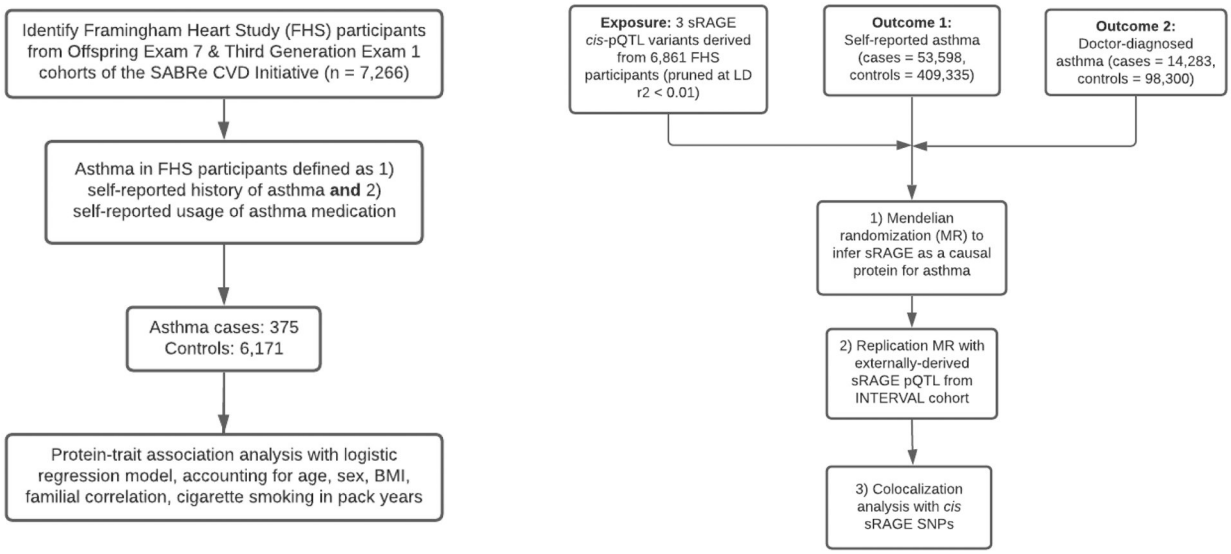

Figure 1. Study design

The figure on the left-hand side details the steps for identifying FHS participants from Offspring and Third Generation cohorts with and without asthma to be used in cross-sectional protein-trait association analysis. The figure on the right-hand side details the exposures (i.e., instrumental variables) and outcomes for Mendelian randomization and colocalization analyses.

Table I.

Clinical characteristics of FHS participants

Characteristic (Mean ± SD)	All participants (n=6,546)	Participants with asthma (n=375)	Participants free of asthma (n=6,171)
Age, years	49.6 ± 13.8	49.8 ± 14.3	49.6 ± 13.7
Women, n (%)	3474 (53.1%)	247 (65.9%)	3227 (52.3%)
BMI, kg/m ^{2}	27.4 ± 5.4	28.4 ± 6.1	27.3 ± 5.4
Smoking, pack years	10.7 ± 18.1	12.8 ± 20.9	10.6 ± 17.9
sRAGE, pg/mL	3574.0 ± 1167.7	3391.4 ± 1089.0	3585.1 ± 1171.5

Abbreviations: BMI = Body Mass Index (kg/m^2); FHS = Framingham Heart Study; SD = Standard Deviation; sRAGE = Soluble Receptor for Advanced Glycation End-Products

Table II.

Association of plasma sRAGE level with asthma in FHS participants: Results of multi-variable adjusted logistic regression analysis

Parameter	OR* (95% CI)	p
sRAGE	0.856 (0.763, 0.959)	0.0075

Covariates adjusted for include age, sex, BMI, pack-years of cigarette smoking, and familial correlation.

* Odds Ratio of asthma per 1 standard deviation (SD) increment in sRAGE. The SD of sRAGE was 1,171.5 pg/ml.

Abbreviations: FHS = Framingham Heart Study; OR = Odds Ratio; sRAGE = Soluble Receptor for Advanced Glycation End-Products; 95% CI = 95% Confidence Interval

Table III.

Mendelian randomization results using inverse variance weighted method for 3 sRAGE variants in relation to UK Biobank asthma GWAS

Exposure	Outcome	# of SNPs	Beta	SE	OR (95% CI)	p
sRAGE	Self-reported asthma	3	−0.027	0.009	0.973 (0.955, 0.992)	0.005
	Doctor-diagnosed asthma	3	−0.029	0.011	0.972 (0.950, 0.993)	0.011

Instrumental variables used in Mendelian randomization analysis were three *cis*-pQTLs derived from 6,861 Framingham Heart Study participants: rs2070600, rs6923504, rs9266529. Two GWAS of asthma were used in two-sample MR analyses: one for self-reported asthma and one for doctor-diagnosed asthma (see Methods for details).

Mendelian randomization results revealed sRAGE to be putatively causally implicated with a slight protective effect in both self-reported and doctor-diagnosed asthma; results are reported in odds ratios (OR) per 1 standard error (SE) increment in inverse rank-normalized sRAGE levels.

Abbreviations: OR = Odds Ratio; SE = Standard Error; SNP = Single Nucleotide Polymorphism; sRAGE = Soluble Receptor for Advanced Glycation End-Products; 95% CI = 95% Confidence Interval